UNIVERSITAT POLITÈCNICA DE VALÈNCIA

COMAV
Instituto de Conservación y Mejora de la Agrodiversidad Valenciana

DOCTORAL THESIS

Selection of traditional varieties and breeding of new Cucurbit varieties adapted to organic production

Author:

Alejandro Flores León

Advisor:

Dra. María Belén Picó Sirvent

Valencia, marzo de 2023

Agradecimientos

Quisiera comenzar agradeciendo en primera instancia, a la que ha sido mi directora durante el desarrollo de toda mi tesis, Dña. Belén Picó. En estos años me has enseñado las cualidades que tiene que tener un buen investigador, como es la disciplina, la curiosidad, la pasión, el entusiasmo, la persistencia, y que no hay que asustarse cuando uno se enfrenta a nuevos retos. Si nos surgen dudas a lo largo del camino, no hay que vacilar en preguntar a aquellos que más saben y aprender de las lecciones recibidas. Espero haber cumplido con las expectativas que tenías de este bio-informático recién llegado, que había trabajado con microsatélites en Córdoba.

Quisiera también dar las gracias a Jaime, por todo su trabajo y apoyo, por ayudarme a navegar en el mundo de los ácidos, azúcares y volátiles. Ha sido tu guía, la que ha ayudado a que el barco llegue a buen puerto.

A todos mis compañeros de laboratorio, daros las gracias por haberme acompañado en esta andanza. A Gorka y Cristina, ambos habéis sido faros que habéis ayudado a despejar hasta la más densa de las nieblas. A Eva y Alicia, por ayudarme y escucharme en todo momento. A María y Clara, espero haberos enseñado tanto como yo he aprendido de vosotras.

A mis amigos Jorge, Fernanda, Emilio, Laura y Alberto, que durante todos estos años en Valencia habéis sido como una familia, además de compartir las inquietudes y la vida del doctorando. Solo puedo deciros, gracias por todos los buenos momentos que hemos pasado y por los venideros.

A mi familia, primas, tíos, tías, sobrino y especialmente a mi abuela y a mis padres. Solo espero poder ser una persona de la que podáis estar orgullosos. Me habéis dado todo y más si habéis podido, habéis sido mi bastón donde apoyarme. Que sepáis que parte de este trabajo también es vuestro, espero así lo consideréis.

Alejandro Flores León is a recipient of a grant PROMETEO/2017/078 funded by Conselleria d'Educació, Investigació, Cultura i Esports (Generalitat Valenciana, Spain). The present works in this Thesis have been supported by grants PROMETEO/2017/078 and PROMETEO/2021/072 (to promote excellence groups) funded by Conselleria d'Educació, Investigació, Cultura i Esports (Generalitat Valenciana, Spain), grant PID2020-116055RB-C21 funded by Spanish Ministerio de Ciencia, Innovación y Universidades, Agencia Estatal de Investigación (AEI) 10.13039/501100011033 and AGL2017-85563-C2-1-R-AR funded by Spanish Ministerio de Ciencia, Innovación y Universidades, Agencia Estatal de Investigación (AEI) 10.13039/501100011033 and "ERDF: A way of making Europe", RTA2017-00061-C03-03 (INIA) cofunded by the Spanish Ministerio de Ciencia, Innovación y Universidades and FEDER funds.

Index

List of Abbreviations

AATs: Alcohol Acyltransferases
ADHs: Alcohol Dehydrogenase
AL: Alficoz
AM: Amarillo
AUDPC: Area Under the Disease Progress Curve
BL: Blanco
CABYV: *Cucurbit aphid borne yellow virus*
CHA: Chate
CMV: *Cucumber mosaic virus*
CVYV: *Cucumber vein yellowing virus*
CYSDV: *Cucurbit yellow stunting disorder virus*
DAI: Days After Inoculation
EU: European Union
FC: Fruit cavity
FD: Fruit diameter
FF: Flesh firmness
F1PAT81: *Cucumis melo* subsp. *agrestis* "Pat 81" x *Cucumis melo* subsp. *melo* "Piel de sapo" rootstock
FIAN: *Cucumis ficifolius* x *Cucumis anguria* rootstock
FIMY: *Cucumis ficifolius* x *Cucumis myriocarpus* rootstock
FL: Fruit Length
FW: Fruit Weight
GBS: Genotype-by-Sequencing
GC: Gas Chromatography
GC-MS: Gas Chromatography-Mass Spectrometry
GWAS: Genome Wide Association Study
LD: Linkage Disequilibrium
MAF: Minor Allele Frequency
MNSV: *Melon necrotic spot virus*
NG: non-grafted
PCA: Principal Component Analysis
PRSV-W: *Papaya ringspot virus W*
PS: Piel de Sapo
QTLs: Quantitative Trait Loci
RC: Rochet
RF: Rind Firmness
SIM: Selected Ion Monitoring
SNP: Single Nucleotide Polymorphism
SPE: Solid Phase Extraction
SSC: Soluble Solids Content
SSR: Simple Sequence Repeat

TN: Tendral
ToLCNDV: *Tomato leaf curl New Delhi virus*
VOCs: Volatile Organic Compounds
WMV: *Watermelon mosaic virus*
ZYMV: *Zucchini yellow mosaic virus*

Resumen

El melón (*Cucumis melo* L.) es uno de los principales cultivos pertenecientes a la familia de las cucurbitáceas, del cual España representa uno de los centros secundarios de diversificación, donde todavía se cultivan melones no dulces (Flexuosus) y dulces (Ibericus). En la actualidad, un gran número de variedades tradicionales de melón se han ido sustituyendo por variedades comerciales F1 de los subgrupos Piel de Sapo y Amarillo. Una forma de fomentar y recuperar el cultivo de estas variedades seria mediante prácticas de cultivo ecológico. Existen una serie de factores limitantes para el cultivo de estas variedades tradicionales, entre las que se incluyen factores bióticos y abióticos. El uso de injertos podría suponer un método para ayudar frente a estos estreses. Tanto el uso de injertos como el estrés salino pueden afectar a los distintos parámetros de calidad de los frutos. Es por ello que en la presente tesis doctoral se ha analizado la genética, morfología y el perfil de ácidos, azucares y volátiles de una colección de variedades tradicionales de melones españoles, tanto dulces como no dulces. Además, se ha visto el efecto sobre factores agronómicos, morfológicos y metabólicos, tanto de la salinidad como de diferentes patrones. Los análisis genéticos diferenciaron entre los materiales exóticos, las accesiones de Flexuosus e Ibericus. En el caso de los Ibericus no fue posible encontrar una clara distinción entre los distintos subgrupos. Se encontró así mismo diferencias morfológicas entre las distintos Subgrupos entre sí y dentro de los mismos. Los principales compuestos orgánicos volátiles (VOCs) detectados en los frutos no dulces fueron aldehídos y alcoholes, siendo capaces de diferenciar entre ellos. En cuanto a los VOCs de los Ibericus dulces, se observó un alto nivel de variabilidad. El desempeño tanto de los cultivares de alficoz y melones dulces injertados, se evaluó bajo condiciones de cultivo ecológico. En ambos casos se encontró que los principales factores limitantes fueron los patógenos de suelo y virus. En cuanto a los virus, el principal fue el virus del mosaico de la sandía (WMV), seguido por el virus del mosaico amarillo del calabacín (ZYMV), virus del rizado del tomate de Nueva Delhi (ToLCNDV) y virus del amarilleo de las cucurbitáceas, transmitido por pulgón (CABYV). En cuanto a los hongos de parte aérea, el principal fue el oídio, causado por *Podosphaera xanthii*. En cuanto a los hongos patógenos de suelo, tanto *Macrophomina phaseolina* como las especies de *Fusarium, Neocosmospora keratoplastica* y *N. falciformis*, redujeron los rendimientos, en sinergia con la alta salinidad. En este caso los patrones de *Cucurbita* tuvieron un peor desempeño que los de *Cucumis*. Así mismo, los patrones de *Cucumis* obtuvieron características de fruto similares a los frutos sin injertar o mejoraron la calidad de algunos parámetros. Así mismo se ha evaluado la resistencia de una variedad de alficoz español a 4 patógenos (*M. phaseolina*, *Monosporascus cannonballus*, *N. keratoplastica* y *N. falciformis*). En cuanto al efecto sobre los metabolitos, el uso de injertos afectó a los perfiles de ácidos, azúcares y VOCs de alficoz. Esto perjudicó la percepción durante las catas, dando como resultado sabores extraño, especialmente en los injertados en *Cucurbita*, mientras que los patrones de *Cucumis* no dieron sabores raros, sino más bien parecidos a los sin injertar. En el caso de los melones dulces, el principal factor que cambiaba los perfiles de metabolitos fue la el cultivar, tanto para ácidos como de azúcares. El efecto del uso de injerto fue mínimo, mientras que la salinidad si tuvo un efecto mayor. El efecto sobre los VOCs también resultó muy dependiente de la combinación patrón-variedad, con el mismo patrón presentando diferentes efectos dependiendo de la variedad sobre la que se encontrase.

Abstract

Melon (*Cucumis melo* L.) is one of the main crops belonging to the Cucurbitaceae family, of which Spain represents one of the secondary centres of diversification, where unsweet (Flexuosus) and sweet (Ibericus) melons are still cultivated. Nowadays, cultivation of traditional melon landraces has been replaced by commercial F1 varieties of the Piel de Sapo and Amarillo subgroups. In order to promote and recover these traditional landraces, would be through organic farming. There are a number of factors which limit the cultivation of these traditional landraces, both biotic and abiotic factors. The use of grafting could be a method to help with these stresses. Grafting and abiotic stresses can have an effect different quality fruit parameters. For this reason, the genetics, morphology and acid, sugar and volatile profiles of a collection of traditional Spanish melon landraces, both sweet and non-sweet, have been analysed in this doctoral thesis. In addition, the effect of salinity and different rootstocks on agronomic, morphological and metabolic factors has been studied. Genetic analyses differentiated between exotic materials, Flexuosus and Ibericus accessions. In the case of Ibericus, it was not possible to find a clear distinction between the different subgroups. Morphological differences between and within subgroups were also found. The main volatile organic compounds (VOCs) detected in the non-sweet fruits (Flexuosus and Chate) were aldehydes and alcohols, being able to differentiate between them. A high level of variability was observed in the VOCs profile of sweet Ibericus. The performance of both grafted alficoz and grafted sweet melon cultivars was evaluated under organic farming conditions. In both cases, soil pathogens and viruses were found to be the main limiting factors. Regarding viruses, the main one was *Watermelon mosaic virus* (WMV), followed by *Zucchini yellow mosaic virus* (ZYMV), *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Cucurbit aphid-borne yellows virus* (CABYV). The main airborne fungus detected was powdery mildew caused by *Podosphaera xanthii*. As for soilborne pathogenic fungi, *Macrophomina phaseolina* and *Fusarium* species, *Neocosmospora keratoplastica* and *N. falciformis*, reduced yields, in synergy with high salinity. In this case, *Cucurbita* rootstocks performed worse than *Cucumis* rootstocks. Likewise, *Cucumis* rootstocks obtained fruit characteristics similar to ungrafted fruit or improved the quality of some parameters. The resistance of a Spanish alficoz variety to four pathogens (*M. phaseolina*, *Monosporascus cannonballus*, *N. keratoplastica* and *N. falciformis*) has also been evaluated. Regarding the effect on metabolites, the use of grafting affected the acid, sugar and VOCs profiles of alficoz. Cucurbita grafted fruits resulted in lower perception during tasting, due to odd flavours, while *Cucumis* did not have that effect, resulting in similar scores to ungrafted fruits. In sweet Ibericus melons, the main factor changing the metabolite profiles was the cultivar, both for acids and sugars. The effect of grafting was minimal, whereas salinity had a greater effect. The effect on VOCs was also highly dependent on the rootstock-variety combination, with the same rootstock showing different effects depending on the variety on which it was grown.

Resum

El meló (*Cucumis melo* L.) és un dels principals cultius pertanyents a la família de les cucurbitàcies. Espanya representa un dels centres secundaris de la seua diversificació, on encara es cultiven melons no dolços (Flexuosus) i dolços (Ibericus). Actualment, un gran nombre de varietats tradicionals de meló ha sigut substituït per varietats comercials F1 dels subgrups "Piel de Sapo" i Groc. Una manera de fomentar i recuperar el cultiu d'aquestes varietats seria mitjançant pràctiques de cultiu ecològic. Existeixen una sèrie de factors biòtics i abiòtics. L'ús d'empelts podria suposar un mètode per ajudar front a estos estressos. Tant l'ús d'empelts com l'aparició d'estressos associats a la salinitat poden afectar als distints paràmetres de qualitat dels fruits. És per això que en la present tesi doctoral s'ha analitzat la genètica, morfologia i el perfil d'àcids, sucres i volàtils d'una col·lecció de varietats tradicionals de melons espanyols, tant dolços como no dolços. A més, s'ha vist l'efecte sobre factors agronòmics, morfològics i metabòlics, tant de la salinitat com de diferents patrons. Les anàlisi genètiques varen diferenciar entre els materials exòtics, les accessions de Flexuosus i les d'Ibericus. En els cas dels Ibericus no va ser possible trobar una clara diferència entre els diferents subgrups. Així mateix, es varen trobar diferències morfològiques en els diferents subgrups entre si mateix i dins dels mateixos. Els principals compostos orgànics volàtils (VOCs) detectats en els fruits no dolços varen ser aldehids i alcohols, sent diferenciables entre ells. Quant als VOCs dels Ibericus dolços, es va observar un alt nivell de variabilitat. El comportament dels cultivars d'alficòs i melons dolços empeltats es va avaluar sota les condicions del cultiu ecològic. En ambdós casos es va trobar que els principals factors limitants varen ser els patògens del sòl i els virus. Quant als virus, el principal va ser el virus del mosaic del meló d'Alger (WMV), seguit pel virus del mosaic groc del carabassí (ZYMV), el virus de l'arrissat de la tomaca de Nova Delhi (ToLCNDV) i el virus de l'engroguiment de les cucurbitàcies, transmés pels àfids (CABYV). Quant als fongs de les parts aèries, el principal va ser l'oïdi, causat per *Podosphaera xanthii*. Quant als fongs patògens del sòl, tant *Macrophomina phaseolina* com les espècies de *Fusarium, Neocosmospora, keratoplastica* i *N. falciformis*, varen reduir els rendiments, establint una sinèrgia amb l'alta salinitat. En aquest cas els patrons de *Cucurbita* varen tindre un pitjor comportament que els de *Cucumis*. Així mateix, els patrons de *Cucumis* varen obtindre característiques del fruit similars als fruits sense empeltar o varen millorar la qualitat d'alguns paràmetres. També s'ha avaluat la resistència d'una varietat d'alficòs espanyol a 4 patògens (*M. phaseolina, Monosporascus cannonballus, N. keratoplastica* i *N. falciformis*). Quant a l'efecte sobre els metabòlits, l'ús d'empelts va afectar als perfils d'àcids, sucres i VOCs de l'alficòs. Aquest fet va perjudicar la percepció durant el tast, la qual cosa va donar com a resultat uns sabors estranys, especialment en els alficossos empeltats en *Cucurbita,* mentre que els patrons de *Cucumis* no varen donar sabors estranys, sinó més bé semblants als sense empeltar. En el cas dels melons dolços, el principal factor que va provocar un canvi en els perfils dels metabòlits va ser el del cultivar, tant per a àcids com per a sucres. L'efecte de l'ús de l'empelt va ser mínim, mentre que la salinitat sí va tindre un efecte més elevat. L'efecte sobre el VOCs també va resultar molt dependent de la combinació patró-varietat, presentant el mateix patró diferents efectes depenent de la varietat sobre la que estigués empeltat.

Chapter 1: General Introduction

Chapter 1: General Introduction

1.1. Economic importance of melon

Melon (*Cucumis melo* L.) is a crop belonging to the Cucurbitaceae family, of great global importance. In 2020, global melon production reached 27.5 million tons, with 1.04 million hectares. The main global melon producers are China, Turkey, India, Iran, Kazakhstan and Afghanistan, with China being the main producer with a total 13.8 million tonnes in 2020 (Figure 1).

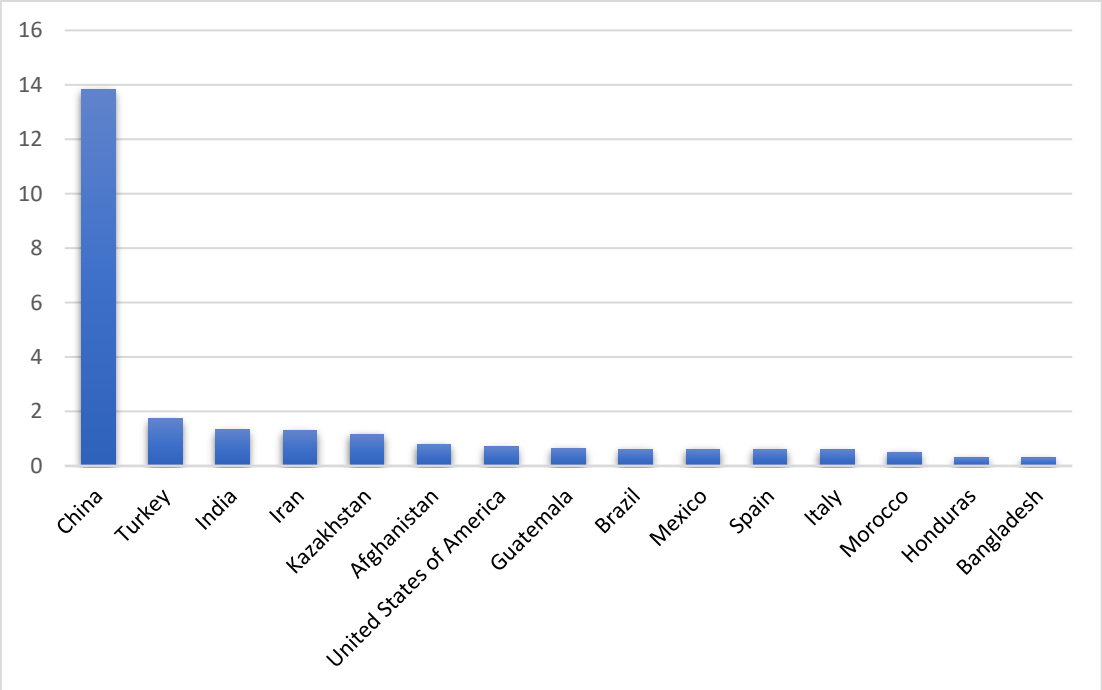


Figure 1. Melon Production (Million tonnes) of the top 15 World melon productors (FAO,2020).

At the European Union (EU) level, Spain stands out as the main producer, with a total of 660 thousand tonnes and crop production area of 19.7 thousand hectares (Eurostats, 2021; FAO, 2020). Spanish melon production is mainly destined for export, with 66% of melon production being exported to countries such as France, Germany, Netherland and Portugal (FEPEX, 2021). In Spain the main melon producers are the Region of Murcia, Andalucía, Castilla La-Mancha, Comunitat Valenciana and Extremadura (MAPA,2021) (Figure 2).

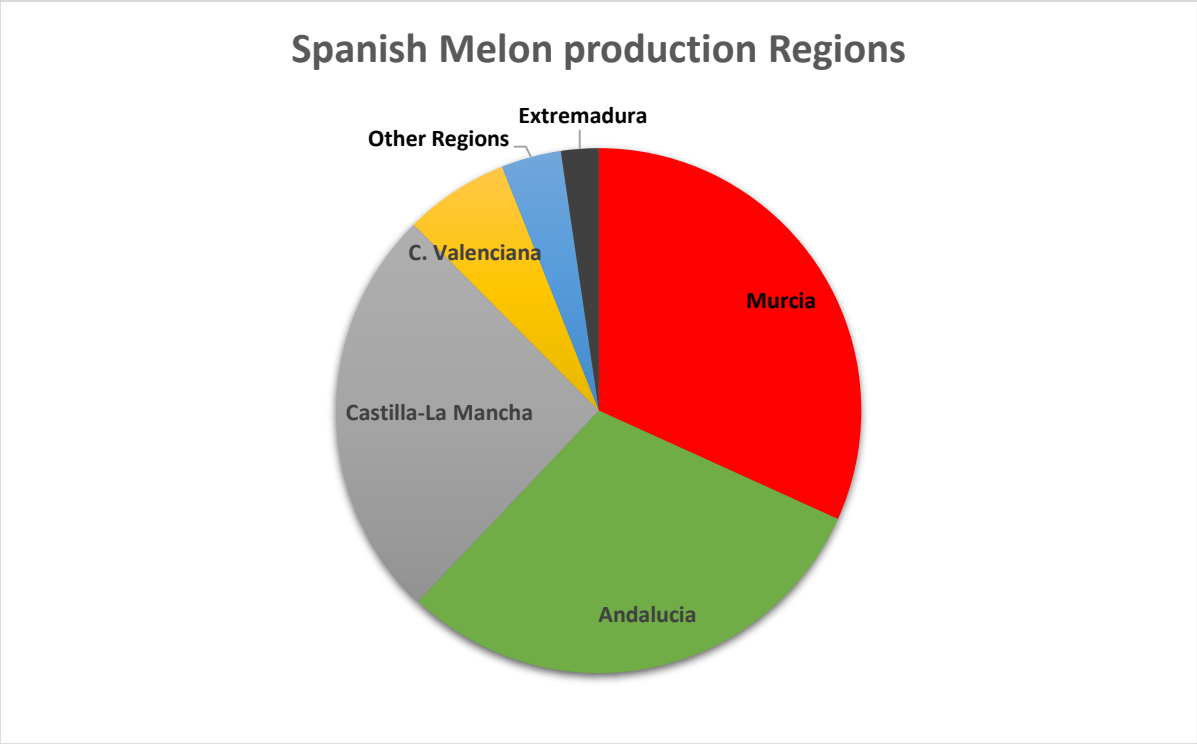


Figure 2. Main Spanish melon producers (% of total production) (MAPA, 2021)

In the region of Murcia, 100% of melon is cultivated under irrigation conditions, with 98.3% of cultivated crop surface being in open fields, and the rest under greenhouse conditions. In the Region of Castilla-La Mancha, the province of Ciudad Real produces 82% of the total of the Region. The cultivated crop surface in Castilla-La Mancha is under open field irrigated conditions (94.5%) with the rest being under rainfed conditions. In Andalucia, 97.7% of cultivated crop surface is in irrigation conditions, being a 47.3% is done in open fields and 57.3% is in greenhouse production conditions, mainly in the region of Almeria. In the Comunitat Valenciana, 88.1% of crop surface is characterized for open field irrigation conditions, with Alicante being the main producer province.

1.2. Origin and taxonomy of melon

Melon is a crop belonging to the Cucurbitaceae family, which also includes other important crops such as cucumbers (*Cucumis sativus* L.), watermelons (*Citrullus lanatus* (Thun.) Matsum & Nakai), pumpkins and zucchinis (*Cucurbita* spp.), bitter melon (*Momordica charantia* L.) or gourds (*Lagenaria siceraria* (Molina) Standl.) (Chomicki et al., 2019). Cucurbits have been used since ancient times, based on the remnants of fruits, seeds and even leaves (Piperno et al., 2000; Wasylikowa and Van Der Veen, 2004).

The *Cucumis* Genus, to which both cucumber and melon belong, also include less important crops such as gherkins (*Cucumis anguria* L.) and kiwano (*Cucumis metuliferus* E Mey Ex Naudin) (Chomicki et al., 2019).This genus accounts for approximately 66 species (Sebastian et al., 2010), such us *Cucumis myriocarpus* L., a weed affecting crops, *Cucumis ficifolius* A. Rich, a plant used in traditional medicine in Ethiopia, or *Cucumis dipsaceus* Ehrenger ex Spach, a plant used in ornaments (Esteras et al., 2012; Shaik et al., 2017; Araya et al., 2019).

Melon domestication presents different theories. Based upon archaeological findings, it was suggested that melon domestication occurred in the Middle East or Egypt (Zohary, 2000). Recently, in a study by Endl et al., (2018), it is suggested that melon domestication occurred in 2 instances, one in Africa and the other in Asia, resulting the African in the traditional melon cultivars from Sudan, and the Asian in the commercial melon cultivars. Complementary but nor contradictory research by Zhao et al., (2019), found 3 independent domestication events in melon, 1 in Africa and 2 in India, and in the case of the Indian domestications, the process was achieved through different routes, achieving similar phenotypes. In the present day, India is considered as the primary diversification centre for commercial melon cultivars, while countries such as Afghanistan, Iran, Turkey, Portugal and Spain, are considered secondary diversification centres (Robinson and Decker-Walters, 1997; Gonzalo et al., 2019).

Already in Ancient Egypt, melons was consumed in an immature state, such as Adzhur (*chate*) or snake-shape melons belonging to the *flexuosus* group (Janick et al., 2007). Snake-shaped melons were mentioned by the classical sources, in Greek *sikyos*, in Latin *cucumis* and in Hebrew *qishu'im* (Paris, 2012). Roman author Lucius Junius Moderatus *Columela* in the first century B.C. already mentioned in his works "*De re rustica*" snake-shaped "cohombros" (Hammer and Gladis, 2014). Another roman author, Gaius Plinius Secundus, known as Pliny the Elder, mentions *cucumis* in his works "*Naturalis historia*", alluding to the snake-shaped melons of the *flexuosus* group. It would not be until the Islamic Conquest during the Middle Ages that sweet melons were introduced in the Iberian Peninsula (Paris et al., 2012a)

Nowadays, *C. melon* is separated into 2 different subspecies, depending in the hypanthium's hairiness, i.e. in subspecies *agrestis*, with short hairs and subspecies *melo*, with long hairs (Jeffrey, 1980). Melon botanical classification has changed through history since the "tribes" proposed by Naudin (1859). Pitrat, (2008) classified melon by

their subspecies and if they were sweet, non-sweet or fragant, resulting in 15 botanical melon groups, 5 for the *agrestis* subspecies (*acidulus*, *conomon*, *momordica*, *makuwa* y *chinensis*) and 10 for the *melo* subspecies (*chate*, *flexuosus*, *tibish*, *adana*, *ameri*, *cantalupensis*, *chandalak*, *reticulatus*, *inodorus* y *dudaim*). Most recently, Pitrat, (2016) performed an intraspecific reclassification based upon the descriptors of previous authors (Naudin, 1859; Pangalo, 1958; Grebenščikov, 1986; Pitrat et al., 2000) proposing new groups and subgroups. This results in the present 19 groups: Agrestis, Kachri, Chito, Tibish, Acidulus, Momordica, Conomon, Makuwa, Chinensis, Flexuosus, Chate, Dudaim, Chandalak, Indicus, Ameri, Cassaba, Ibericus, Inodurus and Cantalupensis (Figure 3).

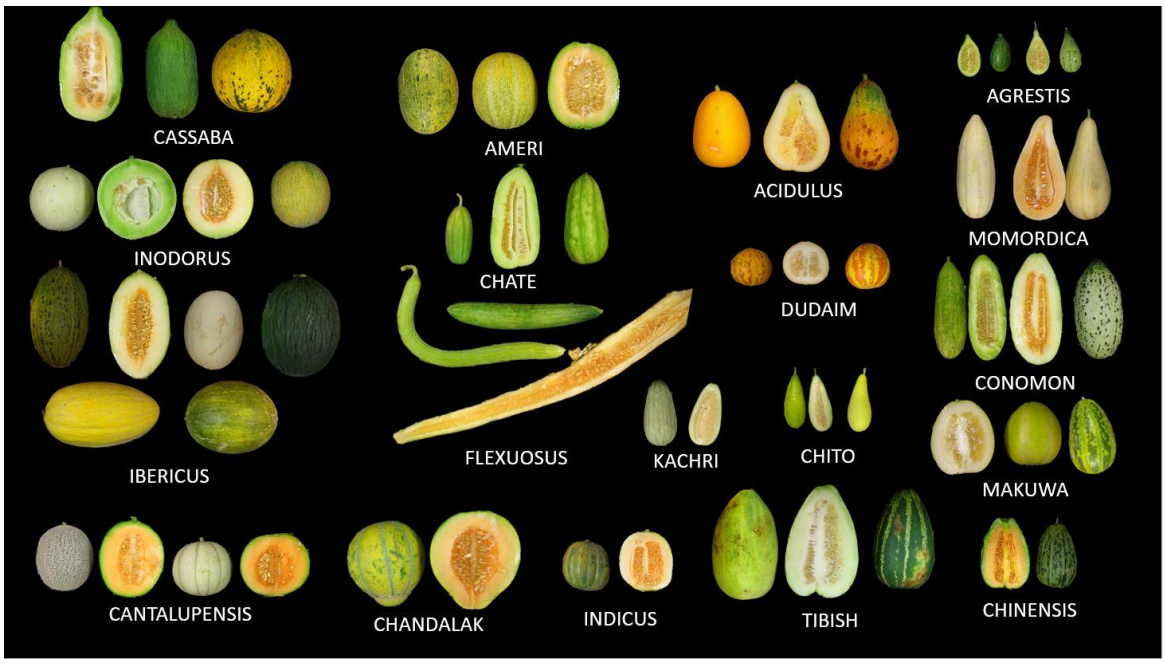


Figure 3. Melon botanical groups as proposed by Pitrat (2016).

Melon biodiversity is not considered at risk, although this specie has not been evaluated by the International Union for the Conservation of Nature (IUCN, 2021). Even so, a great number of GeneBanks exist in Russia, China, United States of America, France or Turkey (Pitrat, 2008; Sari et al., 2008) with the aim of preserving the great biodiversity of this specie. According to the GeneSys system (https://www.genesys-pgr.org) (Genesys PGR) the main germplasm collections are located in the United States (3398 accessions), Spain (1914 accessions), Brazil (652 accessions), Ukraine (511 accessions) and Germany (449 accessions), although several other countries and repositories not currently documented in GeneSys include those found in China, Japan, Rusia and India among others (Grumet et al., 2021). In Spain, numerous different organizations maintain collections with the

main purpose of studying and preserving the biodiversity of this species, such as the Group of Cucurbit Genetics of the Institute for the Conservation and Breeding of Valencian Agro-diversity (COMAV-UPV). This Genebank has 997 accessions belonging to the *C. melo* specie from around the world, with the intent of representing the wide diversity of this crop.

1.3. Groups Flexuosus and Ibericus

Melons belonging to the Flexuosus group are of great importance and highly appreciated in countries of the Mediterranean Basin, Asia Minor, North Africa and the Middle East, where they are known by different names such as alficoz, cucumeru, faqqous, hiti or armenian cucumber (Merheb et al., 2020).

The Flexuosus melon plants are characterized for their monoecious flowers, with long or short hypanthium's hairiness. The fruits can be extremely big, some even reaching 2m in length. The fruit colour tends to be in shades of green or light green in an immature state, and when they reach maturity, shades of cream or orange, with green or light orange flesh. The fruits are non-sweet, barely present any aroma and have a very short shelf-life (Pitrat, 2016). The Flexuosus group presents 3 different subgroups (Pitrat, 2016):

- **Adjour**: they present ribs, without vein tracks. This is the most common subgroup, being cultivated from Morocco to India.
- **Tara**: they present wrinkled exocarp, with longer and thinner fruits than Adjour subgroup. They are mostly cultivated in Afghanistan, India and Pakistan.
- **Arya**: fruits are smooth without wrinkles or ribs, and ovaries present short hairs. These fruits are cultivated in India.

Numerous studies have been performed to analyse the biodiversity of this crop, specially focused in the Middle East and Turkey (Dastranji et al., 2017; Abu Zaitoun et al., 2018; Merheb et al., 2020). As for fruit quality parameters, this has also been studied. Omari et al., (2018) analysed 43 different local varieties of Flexuosus melons, including fruit tastings. Their results revealed the great biodiversity within this crop, including the characteristics that provide better fruit tasting parameters. Both Tang et al., (2015) and Chen et al., (2016) analysed the volatile organic compounds (VOCs) identifying the main VOCs in this group of melons. Esteras et al., (2018) also analysed the VOCs of Flexuosus melons, but in this case of mature fruits, not at the commercial maturity state (i.e.,

immature). As for different pathogen resistances, Ambrósio et al., (2015) reported susceptibility of Flexuosus melons to *Macrophomina phaseolina.* Solmaz et al., (2016) evaluated 15 Turkish Flexuosus melon varieties for resistance to Fusarium races 1 and 2, mildew, powdery mildew and viruses, such as *Zucchini Yellow Mosaic Virus* (ZYMV) and *Cucumber Mosaic Virus* (CMV), and as a result, found that the grand majority are susceptible to all of these pathogens.

Figure 4. Representatives of the different Ibericus Group melons, Piel de Sapo, Amarillo, Branco, Rochet and Tendral.

The melons belonging to the Ibericus Group (Figure 4) are mainly cultivated in the Iberian Peninsula, but it is also possible to see them cultivated in other countries of the Mediterranean Basin (Pitrat, 2016). The plants belonging to this group present andromonoecious flowers, with long hypanthium's hairiness. Fruits are characterized for a size variation range from medium to large, rounded or elliptical shape, that can be slightly wrinkled, without ribs, thick exocarp, light green (sometimes light-orange) juicy flesh, 3 white or orange placentas with big seed and without gelatinous covering. The fruits present a high sugar content, low aroma and long shelf-life (Pitrat, 2016). This Group presents 5 different Subgroups based upon their exocarp colour (Pitrat, 2016):

- **Piel de Sapo**: fruit exocarp is green with yellow specks and dark green spots. Fruits can present netting or fine wrinkling.
- **Amarillo**: fruit exocarp is yellow, sometimes with some wrinkling. Fruits can also present rounded shape.

- **Branco**: fruit exocarp presents a white colour with wrinkling. Fruit flesh present a white, light green or light orange colour.
- **Rochet**: fruit exocarp present a green colour with green-yellow specks. Fruits can sometimes also present netting.
- **Tendral**: fruit exocarp present a dark green colour, with deep wrinkles. These fruits are specially characterized for their very long shelf-life.

The diversity of the Ibericus group has previously been studied in multiple occasions (López-Sesé et al., 2003; Escribano et al., 2007; Escribano and Lázaro, 2012; Esteras et al., 2013; Lázaro et al., 2017). Numerous studies have been focussed on the quality fruit parameters most appreciated by consumers of Ibericus melons (Escribano et al., 2010; Escribano and Lázaro, 2012; Bianchi et al., 2016). VOCs analysis of Ibericus melons has also been carried out, highlighting their unique aroma profile, with a separation of the aroma profile of traditional cultivars and more commercial cultivars. When comparisons between traditional and commercial varieties of melons are performed, consumers have a clear preference for the more traditional melon cultivars (Escribano and Lázaro, 2012). Ample trials for pathogens resistance have been performed on the susceptibility of Ibericus melons, resulting that they are highly susceptible to soilborne pathogens such as *Monosporascus cannonballus*, *M. phaseolina* and *Fusarium oxysporum* f sp. *melonis* races 0, 1, 2 (Alvarez et al., 2005; Oumouloud et al., 2009; Castro et al., 2020; de Sousa Linhares et al., 2020). Resistance to other fungi such as *Podosphaera xanthii* (previously classified as *Sphaerotheca fuliginea*), responsible for powdery mildew, has been evaluated in varieties of melons Ibericus (Alvarez et al., 2005). As for viruses, Díaz et al., (2003) evaluated resistance of 253 melon varieties (146 Spanish Ibericus melons) against CMV, ZYMV, *Papaya ringspot virus* W (PRSV-W) and *Watermelon mosaic virus* (WMV), without detecting any resistances to these viruses, with the majority of resistances having been detected in other more exotic melon Groups such as Momordica, Agrestis, Conomom, Chinensis or Acidulus (Martín-Hernández and Picó, 2020).

1.4. Grafting

Grafting is the horticultural technology by which 2 genetically different plants parts are joined, the upper being the scion and the lower being the rootstock, and are able to growth together (Fallik and Ziv, 2020). Rootstocks have been used since ancient times, as there are already references to this practice in Ancient Greece by Aristoteles (384–322 AC), in

Ancient China and in Ancient Rome (Lee and Oda, 2003; Mudge et al., 2009). The use of grafts in vegetables is relatively new, but in pomology, this practice has been extensively used for thousands of years (Maurya et al., 2019). The first use of grafted vegetables at a commercial level was the use of resistant rootstocks of pumpkin (*Cucurbita moschata*) for watermelon (*Citrullus lanatus*) in the late 1920s in Japan and Korea (Leonardi, 2016). Nowadays, the use of grafted vegetables is extensively used in Asia, Europe, North and Central America, North of Africa and the Middle East. In terms of usage of grafted Cucurbits, both Japan and Korea present a high percentage of grafted plant use, with 93-98% of both watermelon and cucumber being grafted, while melon only in Japan is 32-45% and in Korea 83-95% (Miguel and Camacho Ferre, 2014). In Spain, Miguel and Camacho Ferre (2014) state that only 3% is grafted, much lower than the watermelon (98%). In the case of vegetables, including both Cucurbitaceae and Solanaceae species, the use of grafts has been done with the objective of conferring resistances to soilborne diseases, increase yields and improvement of the fruit's quality (Kubota et al., 2008). Each method of grafting is selected depending on different factors, which included both the experience and preferences of the farmer, the number of required grafted plants, the object of grafting and the access to machinery and infrastructures (Lee et al., 2010). Moreover, although machines and robots capable of performing grafts do exist, manually performing the grafting process still remains as the most popular method for this practice (Lee et al., 2010). The different grafting methods and why they are used are as follows (Bie et al., 2017; Maurya et al., 2019) (Fig. 5):

- **Cleft grafting**: the scion is cut at the 1-3 true leave stage at an angle, and then wedged into the rootstock, with a clip placed at the scion-rootstock junction. This method is relatively difficult to perform in vegetables compared to woody species, so it is confined to only some Solanaceae crop (Johnson et al., 2011).
- **Hole insertion grafting**: Hollow hypocotyls are preferred in this method. First, the true leaves and growing point of the rootstocks are removed just below or just above the cotyledon leaves, performing a hole in the area. The hypocotyl of the scion is the cut to leave a point, as is then inserted into the hole, carefully avoiding inserting in to hypocotyl cavity of the rootstock (this would negate the graft as adventitious roots could grow form the scion). This type of graft results in a very strong union, vascular connection and reduces the work-load as it does not require for the removal of clips (Lee, 1994).

- **Tongue approach grafting**: this type of grafting requires that both the rootstock and the scion present a similar stem size. The true leaves of the rootstock are removed leaving the cotyledon leaves, and in the hypocotyl area a 30-40º cut is performed (with respect to the perpendicular axis). In the scion the same cut is performed but in an ascending direction. Then both the scion and rootstock are joined by inserting the cut from one into the other. A clip is then placed in the junction zone to maintain them until transplant. If the union is not performed correctly either the rootstock or the scion will present reduced growth or death, while a successful graft will show a correct growth. This method requires both higher man-hours and workspace, but permits for a higher survival rate. For this reason, it is extensively used by both farmers and low scale plant producers. This method is not the best for rootstocks or scion that present hollow hypocotyls (Maurya et al., 2019).
- **Splice grafting / one cotyledon splice grafting**: this method is quite popular for both grafted plant producers and growers. This method can also be performed by hand and grafting machines or robots as well. To perform this method, a 35-45º cut is done, removing one cotyledon and the growing point of the rootstock. The scion is then cut so that it matches the rootstock cut. A pin or clip is used to hold the graft junction.
- **Pin grafting:** this method is very similar to the splice grafting method, but specially designed pins are employed for the scion-rootstock union. These pins are made of natural ceramic material, which can remain in the plant without causing problems. This method saves time, as there is no need for the removal of the clips. But on the other hand, it is by far more expensive, as these pins are one-use only, and cannot be reused in the future. More recently, pins made from wood or bamboo can be used to replace the ceramic pins. This method also requires important control during the healing and field conditions, as offshoots from the rootstock can emerge.

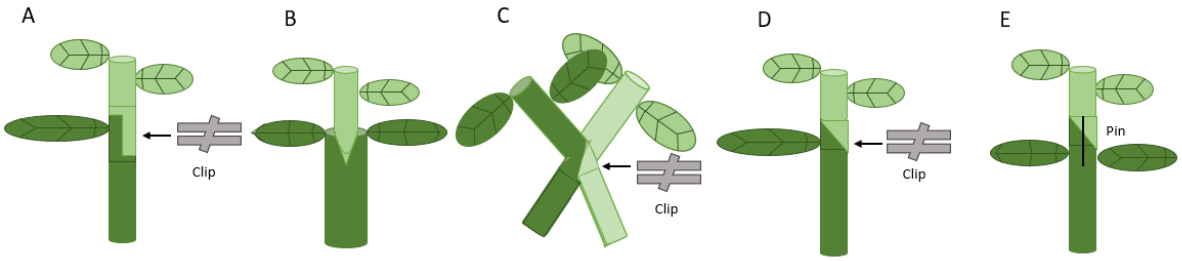


Figure 5. Grafting methods employed in vegetables. Cleft grafting (A), Hole insertion (B), Tongue approach (C), Splice grafting (D) and Pin grafting (E). Adapted from Bie et al., (2017).

Grafted plants must undergo a series of post-graft healing processes to ensure a higher graft success rate. The loss of water by the scion during the first 48h leads to wilting and the failure of the graft. For this reason, high humidity (95%) and a temperature 27-28ºC is required for the first 48h, after which plants can be placed in standard greenhouse conditions (Guan and Zhao, 2014). After that, the grafted plants are covered by a black plastic sheet or black 0.01mm film for 5-7 days to increase and maintain humidity, reduce luminosity and promote the healing process (Denna, 1962). It is important to note that the overuse of these type of covering can lead to future plant problems, due to stem elongations or spindly plants, hence proper measure must be taken to prevent this (Bie et al., 2017).

In the case of Cucurbits, the main objective of grafting has been to provide resistance against nematodes, fungal soilborne pathogens and viruses such as *Melon necrotic spot virus* (MNSV), due to the emergence of pesticide resistant pathogens and the prohibition of active substances such as methyl bromide (Davis et al., 2008b; Lee et al., 2010; Suansia and Samal, 2021). The use of grafts has provided with a far more ecological way to fight against different stresses which affect crops, both biotic ,like soilborne pathogens, or abiotic such as saline or heavy metals (Pico et al., 2017). In the case of Cucurbits, it is possible to find different species used as potential rootstocks in the Cucurbitaceae Family. This includes those species of the Genus *Cucurbita*, *Lagenaria*, *Benincasa*, *Luffa*, *Cucumis* or *Citrullus*. In the majority of cases, grafting increases plant growth and better yields under stress conditions, but in some cases, they do not present specific tolerances and can even lead to negative effects (Rouphael et al., 2010, 2012; Pico et al., 2017):

- *Cucurbita* rootstocks: they represent the most popular in the cultivation of cucurbits. The use of interspecific crosses has allowed for the combined qualities of the different species of the *Cucurbita* Genus. Most commonly, *C. maxima* x *C.*

moschata have been used as rootstocks, mainly those developed in Korea, China and Japan (Pico et al., 2017). These interspecific hybrids are capable of providing unspecific but efficient protection against a number of abiotic stresses, like salinity and biotic stresses, such as soilborne pathogens (Buller et al., 2013; Orsini et al., 2013; Zhou et al., 2014; Ulas et al., 2020). To a lesser extent, other *Cucurbita* species have been employed as rootstocks, with some compatibility essays having been done with watermelon and cucumber (Huang et al., 2010; Bekhradi et al., 2011; Guan et al., 2020). The use of *Cucurbita* rootstocks has been associated to negative effects in the fruit quality (Koutsika-Sotiriou and Traka-Mavrona, 2002; Colla et al., 2006; Davis et al., 2008a).

- *Cucumis* rootstocks: one of the problems associated with the use of rootstocks of different species of the scion is the excess of vigour, which reduce plant survival after grafting, and problems associated with fruit quality. The use of resistant rootstocks of the same species as the scion would be the most desirable, as it could reduce the previously mentioned problems. This is of special interest in the cultivation of melon and watermelon (Pico *et al*., 2017). In the case of melon, screenings have been performed in search for resistances against nematodes, soilborne pathogens and salinity (Kusvuran et al., 2007; Park et al., 2013; Ambrósio et al., 2015; Dasgan et al., 2015; Guilherme et al., 2016). In some cases, rootstocks have been developed resistant to soilborne pathogens (Fita et al., 2007; Jang et al., 2014). Another source of potential rootstocks could be found among the different species of the *Cucumis* Genus, and exploit the genetic variability among them in search of resistances to different stresses (Trionfetti Nisini et al., 2002; Matsumoto et al., 2011; Thangamani et al., 2018). The use of interspecific hybrids developed from wild *Cucumis* species is rather difficult, but some have already been developed. To this respect, 2 different rootstocks "Fimy" and "Fian" were developed as a result of the interspecific cross between *C. ficifolius* x *C. anguria* and *C. ficifolius* x *C. myriocarpus* (Cáceres et al., 2017).
- *Benincasa* y *Luffa* rootstocks: both loofah (*Luffa cylindrica* M. Roem) and wax gourd (*Benincasa hispida* (Thunb.) Cogn.) are members of the Cucurbitaceae Family which can potentially be used as resistant rootstocks. In the case of *L. cylindrica*, resistance to soilborne pathogens has been tested, especially against *Meloidogyne incognita*, as well as the compatibility with melon cultivars (Galatti et al., 2013). Resistance in loofah against *Fusarium* has also been tested

(Tamilselvi et al., 2016). In cucumber, *L. cylindrica* has been seen to provide resistance to heat (Li et al., 2014). As for *B. hispida*, resistance to *Fusarium*, nematodes (*M. incognita* and *Meloidogyne javanica*) and *Verticillium dahliae* and compatibility with melon scion has also been evaluated (Trionfetti Nisini et al., 2002; Ito et al., 2014; Wimer et al., 2015).

Scion-rootstock compatibility is an issue to take into consideration, as the behaviour of grafted plants on the field depends not only in the cultivation and environmental conditions but also the scion and rootstock genotypes and their compatibility (Pina et al., 2017). In the case of melon-pumpkin grafting, Aloni et al., (2008) suggests 2 stages, in the first one vascular connection of the both the scion and the rootstock are reunited (14 Days After Grafting) and in the second stage in which graft compatibility or incompatibility manifests (24 Days After Grafting). This incompatibility resulted in lower root sugar concentration, water uptake peroxidase and superoxide dismutase activities and higher hydrogen peroxide and superoxide levels (Aloni et al., 2008). Calatayud et al., (2013) also document a decrease of photosynthetic activity in the graft union area. Aloni et al., (2008) propose that the main cause of this incompatibility is due to a hormonal imbalance (auxin and ethylene) in the root after vascular union is formed. Moreover, they showed that that by exposing the grafted plants to high temperatures (32ºC day/ 28ºC night) after the grafting establishment, can lead to the inhibition of root and shoot development of the incompatible grafted plants when compared to compatible graft development. The effect of these incompatibilities affecting to the quality of fruits is of great importance, not only to their ability to resist different stresses

1.5. Salinity

Soil salinization refers to the accumulation of salts in soils at such a proportion that it impacts production, environmental health and economic welfare (Rengasamy, 2006). It is estimated that approximately 15% of the World Total Land Area has been degraded by physical, chemical degradation, soil erosion and soil salinization (Wild, 2003). At a global level, (Chang et al., 2019) reported that approximately 20% of the total irrigated areas are being negatively affected by soil salinization and waterlogging problems. Salinity has a negative impact on plant growth due to the high osmotic potential of soil solution and nutritional imbalance (Munns and Tester, 2008). For starters, high levels of soil salinity can significantly hinder seed germination as a result of the effects of high osmotic

potential and specific ion toxicity (Safdar et al., 2019). In the case of melon, it has been shown that high levels of salinity (upwards of 13.5 dS/m) can have a detrimental effect on the germination of melon seeds (Sivritepe et al., 2003) although this can be palliated by priming the seeds previously with 18 dS/m NaCl solution for 3 days, and a 20°C temperature. Salinity can cause several problems in plants, especially as it diminishes access to water, raising the salt reserves in the plant, causing it to raise to poisonous points in several tissues of plants (Munns et al., 1995). A reduction of photosynthesis rates has been usually associated with salinity, as this can primarily be caused by several different factors such as the lower stomatal conductance, depression in specific metabolic processes associated to carbon uptake, or a combination of the previous factors (Flexas et al., 2004; Zhang et al., 2009). One possible solution to the problem of salinity is the use of grafts as they have been shown to improve photosynthesis via a better management of the stomatal parameters (He et al., 2009; Rouphael et al., 2012).

1.6. Fruit Quality and the effect of grafting

The concept of fruit quality is broad and varied, since it includes a large number of factors such as size, shape, colour, texture, aroma, nutritional content or high value-added compounds (Monforte and Alvarez, 2006; Obando et al., 2008; Obando-Ulloa et al., 2010). Moreover, in the case of melon this is even more complicated, due to the great genetic diversity of this specie, which includes a multitude of shapes, colours, forms of consumption or post-harvest life. Thus, it is not logical to establish the same criteria for melons of the Flexuosus Group, consumed unripe with a short post-harvest life, with Piel de Sapo melons, consumed ripe as dessert with a long post-harvest life. Furthermore, if we look at the Ibericus Group, it is difficult to establish a base criterion for all, since their shape, size and even colour can change within Subgroups. In general, the quality parameters of a melon fruit can be summarized by the following characteristics (Monforte and Alvarez, 2006):

- **External characteristics**: parameters such as shape, weight, rind colour.
- **Internal characteristics:** size of the seed cavity, thickness of the rind and flesh, flesh colour.
- **Biochemical composition**: content of acids, sugars, volatile aromatic compounds, vitamins.
- **Shelf-life**: post-harvest life, cracking, vitrescence.

In the case of fruits from grafted plants, quality is something complex that must also include the perception of different consumers, since quality also includes factors resulting from sensory stimuli such as flavour or texture (Leonardi et al., 2017). These arise as problems have been detected in fruits of grafted melon plants, causing problems of reduced soluble solids content, fruit fermentation, fibrous flesh texture and even off-flavours (Davis et al., 2008a). The effect of grafting and salinity to different fruit quality characteristics will be reviewed.

1.6.1. External fruit characteristics

Fruit size is one of the most visible parameters for consumers and is directly related to yield and production. It is not only important for consumers, but also throughout the distribution and sales chain. There is a great diversity of sizes within the *C. melo* species, with wild melons presenting small sizes (30-50g), and commercial melons of the Ibericus Group reaching weights 3kg or higher (Monforte and Alvarez, 2006; Pitrat, 2016). Fruit shape is defined as the ratio between equatorial and longitudinal diameters. The fruit shape can go from very round, almost spherical in shape, to elongated snake shape, as those that of Flexuosus (Pitrat, 2016). Ibericus melons tend to present round and elongated shapes. More recently, a gene *CmOFP13* (MELO3C025206, ovate family proteins) located in QTL *fsqs8.1* (chromosome 8) was identified, which induced round fruit shape in the introgression line CALC8-1 (PI 124,112 (CALC) x Piel de Sapo (PS)) (Martínez-Martínez et al., 2022).

1.6.2. Internal fruit characteristics

Among the different internal aspects of the fruit, the texture and firmness of the flesh are of great importance, since they are directly related to the post-harvest life of the fruit (Monforte and Alvarez, 2006). Hoberg et al., (2003) already defined the importance of aroma and texture in distinguishing among melon varieties. Firmness is related to Ca concentration, and to a series of more complex mechanisms such as transpiration and wax layers, cell-cell adhesion, cell wall architecture, cell wall protein status among others (Huxham et al., 1999; Saladié et al., 2007).

The seed cavity in melon fruits houses the seeds of the fruit. This area of the fruit is not consumed, so an increase in the size of the seed cavity would ultimately reduce the potentially consumable flesh.

Fruit flesh colour is one of the most important trait in improving fruit quality, with 3 basic colours (salmon, green and white), although a range of intermediate colours can be found (Monforte and Alvarez, 2006). This trait is controlled by two major genes: green flesh (*gf*) (Hughes, 1948) and white flesh (*wf*) (Iman et al., 1972), which interact epistatically (Clayberg, 1992), and these genes have already been mapped (Monforte et al., 2004; Galpaz et al., 2018). Recently the *gf* gene has been cloned, localized in chromosome 9 and responsible for β-carotene accumulation *CmOr* (Tzuri et al., 2015). Gene *wf* has been associated by Galpaz et al., (2018) with *CmPPR1* (MELO3C003069, pentatricopeptide repeat-containing family protein), while Zhao et al., (2019) suggests MELO3C003097 (Protein SLOW GREEN 1, chlroplastic), both located very near each other in chromosome 8.

1.6.3. Biochemical composition

Increasing the sugar content of fruits is of particular interest in breeding plans for the species, especially those consumed as dessert. For the measurement of soluble solids content of fruits, breeders use refractometers to evaluate the sugar concentration in pulp extracts (Monforte and Alvarez, 2006). In melon, the major sugars are fructose, glucose and sucrose. Sucrose accumulation in fruits of sweet genotypes is determined by the recessive major gene *suc*, although other *loci* are also involved (Burger et al., 2002). Argyris et al., (2017) detected in populations derived from "Piel de Sapo" x Trigonus accession "Ames 24294" (Agrestis Group), several Quantitative Trait Loci (QTLs) for Soluble Solids Content in chromosome 3,4, 8 and 12; in chromosome 3 for fructose content; and sucrose content in chromosomes 4, 5, and 10. Pereira et al. (2018) identified QTLs located in chromosomes 8, 9 and 10 related to Soluble Solids Content in a Recombinant Inbred Line (RIL) population derived from "Védrantais" (Group Cantalupensis) x Piel de Sapo "T111" (Group Ibericus). The sugar content evolves during fruit development, with an increase in sucrose content, without variations in glucose and fructose content (Burger et al., 2000). Likewise, the acid content of melon fruits also contributes to good fruit flavour (Kader, 2008), with the main acid in melon being citric acid, followed by malic acid (Tang et al., 2010). Cohen et al., (2014) already identified the *CmPH* (MELO3C025264, auxin efflux carrier family protein) gene with a major effect on fruit acidity. The more acidic melons, such as Flexuosus or wild accession lack a four amino acid duplication, which can be found in the non-acidic accessions of the melon species, such as Ibericus melons. Fruit acidity is expressed by titratable acid

content, expressed as the equivalent percentage of the predominant acid. Both sugar and acid content can be affected by grafting, as well as by rootstock-scion combinations and salinity.

Another important aspect of the organoleptic fruit quality of melon are the Volatile Organic Compounds (VOCs). Different compounds participate in melon fruit aroma, such as esters, alcohols, and carbonyl compounds (Németh et al., 2020). The composition and content of VOCs in melon varies between climacteric and non-climacteric varieties, due to the synthesis of ethylene (Monforte and Alvarez, 2006). Esteras et al., (2018) analysed the flesh aroma profile of 71 representative melon accession finding that 2 main genotype clusters based of their aroma (high or low), but a large diversity of profile was found within each cluster. Another interesting but seldom studied aspect of VOCs, would be the rind aroma profile. To this respect, Esteras et al., (2020), studied the 72 representative melon accessions, found interesting combinations, with Spanish Ibericus having high ester content, exotic accessions presenting high content of specific compounds or wild Agrestis with unexpected high content of specific esters. Again, the aroma profile of melons can be affected by the use of rootstocks.

1.6.4. Shelf life

In melon we can distinguish two types of maturation. Some melons, such as those belonging to the Cantalupensis Group, present climacteric maturation whereas those such as from the Ibericus or Inodorus Groups do not present climacteric maturation. In climacteric fruits, maturation is characterized by change of colour, aroma production, dehiscent fruit, lower flesh firmness, leading this to a lower shelf-life (Monforte and Alvarez, 2006). In contrast non-climateric fruits, do not present these drastic changes, which leads to a difficulty in judgment of their optimum maturation point and also present much longer shelf-life than climacteric melons (Monforte and Alvarez, 2006). In climacteric fruits, the ripening process is accompanied by an increase in respiration and by a burst in ethylene production (derived from the autocatalytic ethylene synthesis) (Pech et al., 2008). Ethylene is considered as the major signalling molecule controlling the fruit ripening process (Pech et al., 2012). Several studies focused on this trait in melon have been performed. (Ríos et al., 2017) detected 2 QTLs (*ETHQB3.5* and *ETHQV6.3*) in a collection of Introgression lines “Songwhan Charmi” (Group Conomon) x “Piel de Sapo” (Group Ibericus) (Eduardo et al., 2005) , and that the gene underlying in *ETHQV6.3*

is *CmNAC-NOR* (MELO3C016540) encoding a NAC (NAM, ATAF1,2, CUC2) transcription factor, related to the NOR (non-ripening) gene in tomato. Liu et al., (2022), employed CRISPR/Cas9 to obtain *CmNAC-NOR* knock-out mutants *(nor-3* and *nor-1*) in a climacteric cantaloupe inbred line "Védrantais". The *nor-3* (3bp deletion) resulted in an 8-day ripening delay, while the nor-1 (1bp deletion) resulted in a fully disrupted NAC domain, completely blocking climacteric ripening. Recently, Pereira et al., (2020) performed a Genome Wide Association Study (GWAS) with 211 accessions of the ssp. *melo* identified two regions on chromosome 8, with the main gene candidates being a *CmCTR1*-like (MELO3C024518, serine/threonine kinase) and *CmROS1* (MELO3C024516, protein ROS1).Giordano et al., (2022), performed CRISPR/Cas9 gene editing of both genes in climacteric "Vedrantais" (Group Cantalupensis), finding that both genes where involved in the climacteric nature, and that they could activate climacteric ripening in a non-climacteric background. This aspect of prolonged shelf-life is key in certain aspects of the food chain. The effect of grafting on melon postharvest preservation has also been studied and found to have an effect on it.

1.6.5. Effect of Grafting and Salinity on external fruit characteristics

An increase in mean fruit weight is directly linked to an increase in yield, although this increase in yields can be due to an increase in the number of fruits per plant, rather than the fruit weight. The use of rootstocks has already been proven to directly influence the increase in fruit weight. Verzera et al., (2014) showed that the rootstocks (*C. maxima* × *C. moschata*) "Polifemo" and "RS841" increased the average fruit weight of melon fruit of the variety "Incas" of the Inodorus Group, Honeydew Subgroup, although neither increased the number of fruits per plant. Colla et al., (2010) observed an increase in both the number of fruits and the average weight of each fruit in "Proteo" (Cantalupensis hybrid) melon plants grafted on *Cucurbita* 'P360' rootstock. Condurso et al., (2012) found no difference in fruit size, but found that "Proteo" (*C. melo* L. var. r*eticulatus)* melon plants grafted on the *Cucurbita* rootstock "Polifemo" significantly increased the number of fruits per plant. The opposite effect has also been observed with rootstocks other than the *Cucurbita* spp, with decreases in weight and number of fruits per plant. Trionfetti Nisini et al., (2002) observed that "Proteo" melon plants grafted on *B. hispida* showed both lower number of fruits per plant and lower weight per fruit, as well as those grafted on *C. zeyheri* which showed lower number of fruits per plant, while *Cucurbita* PGM 96-05 increased fruit size Traka-Mavrona et al., (2000) found no differences in any case for

these parameters in Inodorus" Thraki" and Cantalupensis" Kokkini Banana" melons grafted on hybrid rootstocks of *Cucurbita*.

Salinity has also been shown to affect both fruit weight and the number of fruits per plant. Mendlinger and Pasternak, (1992) found that an increase in the salinity decrease mean fruit weight of "Galia" (Group Cantalupensis), "BG3" and "BG5" melon cultivars, although no effect was noted to the nº of fruits per plants. In a study with salinity and the salination level effect on melon, Del Amor et al., (1999) found that the although salination time (earlier exposure to saline stress) and salinity level both reduced the overall yield of "Galia" plants but that reduction of nº of fruits had the bigger impact rather than reduction of fruit weight. Edelstein et al., (2005) in their study with grafted "Arava" (Group Cantalupensis) showed that salinity decreased the yield of both grafted and non-grafted plants by reducing the nº of fruits per plant, as the average fruit weight did not show significant differences. Tedeschi et al., (2011) reported that salinity reduced the yield by both reduction of number and average weight of the marketable fruit. As for fruit shape, Visconti et al., (2019) did not report fruit length changes in "Piel de Sapo" due salinity but that the diameter did seem to be reduced due to the drip irrigation system, by which the saline water was supplied.

1.6.6. Effect of Grafting and Salinity on internal fruit characteristics

Different rootstocks affect melon fruit flesh firmness in different ways. Colla et al., (2006) observed that fruits of the cultivar "Cyrano" (Group Cantalupensis) grafted on the *Cucurbita* rootstock "P360" increased flesh firmness. Jang et al., (2014) also found that some of the grafting rootstock combinations influenced firmness, in the case of "Shintozwa" (commercial hybrid of *Cucurbita*) decreased firmness of fruits of melon Inodorus "Homerunstar" while other rootstocks of *Cucumis melo* "Cairo 6" and "Kırkağaç 637 Altınbaş" increased firmness. Cáceres et al., (2017) observed an increase in flesh firmness in "Finura" (Group Ibericus) grafted on a *Cucumis* hybrid "UPV-FA" (*Cucumis ficifolius* x *Cucumis anguria* hybrid) but this did not occur in "Vedrantais" (Group Cantalupensis) fruits grafted on the same rootstock. As for the seed cavity, Schultheis et al., (2015) also observed that, in general, grafting the different melon varieties onto the hybrid rootstock of *Cucurbita* "Carnivor" did not affect flesh firmness significantly, except in the case of the cultivar "Athena" (Group Cantalupensis), resulting in a decrease in firmness.

The effect of grafting on seminal cavity size was studied by Colla et al., (2006), although they did not observe an increase in seminal cavity between grafted and ungrafted fruits, but a decrease in the percentage of flesh in the total fruit. Cáceres et al., (2017) observed an increase in the size of the seminal cavity in "Finura" (Group Ibericus) fruits grafted on commercial *Cucurbita* hybrid "Cobalt" and *Cucumis* hybrid "UPV-FA" (but this did not occur in "Vedrantais" fruits, which showed similar size between grafted and ungrafted ones.

In regards to flesh colour, Colla et al., (2006) observed that Hunter's Lab values of flesh of ”Cyrano” (Group Cantalupensis) melons grafted on 'P360' *Cucurbita* hybrid rootstock varied with respect to ungrafted fruit. Similarly, Cáceres et al., (2017) also observed that the rootstocks "Cobalt" Cucurbita hybrid rootstock and *Cucumis melo* "64-376RZ" altered the internal colour values of grafted “Finura” (Group Ibericus) fruits, but neither Cucumis hybrids "UPV-FMy" (*Cucumis ficifolius* x *Cucumis myriocarpus*) nor "UPV-FA" did so, although the latter did affect the fruits of "Vedrantais". San Bautista et al., (2011) also observed a variation in the flesh colour of grafted Piel de Sapo "Ricura" (Group Ibericus) melon fruits, specifically in the values of a (green-red gradient) and those of b (blue-yellow gradient). On the other hand, Crinò et al., (2007) did not observe differences in the internal colour of grafted fruits with respect to non-grafted ones. San Bautista et al., (2011) reported in Piel de Sapo cultivar “Ricura” grafted onto ‘Shintoza’ and double grafted (grafting by means of a mutually compatible intermediate rootstock between the target rootstock and our scion of interest) (using ‘Sienne’ Cantalupensis cultivar as intermediary), that the doble grafted fruit flesh had more yellowness (‘b’ values) than non-grafted plants and more redness (‘a’ values) than both single grafted and non-grafted plant.

Salinity can also affect the internal fruit characteristics. The flesh firmness of “Galia” melons was affected negatively by both the salinity levels and the time of the salt application, reducing its value, as well as the flesh thickness, which was significantly reduced by increase of salinity (Del Amor et al., 1999). Visconti et al., (2019) , did report that Flesh firmness of “Piel de Sapo” (Group Ibericus) melons was affected by the saline irrigation method (Subsurface irrigation) but that the flesh colour was not affected by the irrigation method of saline water. Botía et al., (2005) found that “Amarillo Oro” (Group Ibericus) and “Galia” (Group Cantalupensis) melon cultivars behaved differently under saline conditions, with “Amarillo Oro” firmness being higher under saline conditions but

"Galia" Flesh % and flesh firmness was affected by the saline treatment. Colla et al., (2006) also studied the effect of salinity on the grafted plants, and found that salinity reduced the % of Flesh of "Cyrano" (Group Cantalupensis) melon cultivars, while increasing the fruit firmness. Colla et al., (2006) also reported that salinity did not affect the fruit flesh colour of "Cyrano" (Group Cantalupensis).

1.6.7. Effect of Grafting and Salinity on affecting Biochemical composition

The effect of grafting on the biochemical composition of melons has been analysed. Soteriou et al., (2016) observed a general improvement in the SSC content of grafted cultivar "Elario" (Galia type) with respect to the non-grafted, while for cultivar "Raymond" (Ananas type) observed a decrease of the SSC with *Cucurbita* hybrid rootstock "30900" while the others did not affect the SSC. As for the sugars, Soteriou et al., (2016) also displayed that the sugar profile varied with grafting, with rootstock "N101" decreasing the glucose content but increasing the sucrose content of cultivar "Elario", while graft "Raymond" cultivar on *Cucurbita* hybrid rootstock "30900" fruits presented lower glucose and sucrose content. Park et al., (2013) did not find that grafting affected the SSC content of both "Earl's elite" (reticulatus type) or "Homerunstar" (Group Inodorus) cultivars. Ozbahce et al., (2021) in their study with grafted "Edalı F1" melons ("hıdır" type melon, Group Cassaba) found that at different irrigation levels, different rootstocks both increased or decreased the SSC and sugar profile of the melon fruits. Condurso et al., (2012) studied the effect of grafting onto 7 different rootstocks (2 *C. melon* and 5 *C. maxima* x *C. moschata*) onto cultivar "Proteo" (Group Cantalupensis). The study found that fruits from grafted plants presented higher content of certain compounds such as alcohols 1-hexanol, 2-methyl-1-butanol, ethanol, 1-octanol, aldehydes (E)-2-butenal and octanal, and esters ethyl decanoate and ethyl dodecanoate, while had lower contents of other such as, ethyl 2-methylbutanoate, ethyl butanoate, 6-methyl-5-epten-2-one and eucalyptol. It is interesting to note that Condurso et al., (2012) did not detect (Z)-3-nonen-1-ol and 2-phenyl ethanol (typical of "Proteo") in the grafted samples, with (E)-3-hexen-1-ol was only detected on the grafted plants and not the non-grafted control. Verzera et al., (2014) also studied how cultivar "Incas" of *C. melo* L. subsp. *melo* var. *inodorus* was affected by being grafted onto 6 different rootsotkcs (2 *C. melon* and 4 *C. maxima* x *C. moschata*). Verzera et al., (2014) found that fruits from grafted plants tended to have lower content than the ungrafted although the presence of certain VOCs (nonanal, (Z)-6-nonenal, E)-2-nonenal, (E,Z)-2,6-nonadienal, 1-nonanol,

(Z)-3-nonen-1-ol and (Z,Z)-3,6-nonadien-1-ol) still prevailed. Based in the key aroma compounds, the "Incas" grafted melons onto 4 of the rootstocks (*C. melo* "Energia", *C. maxima* x *C. moschata* "RS841", "P360" and "Polifemo") presented a similar profile to the non-grafted control. Verzera et al., (2014) also indicated that C6-straight chain aldehydes (typical of *C. moschata*) were below the detection limit in the fruits from *Cucurbita* hybrid grafted plants.

Salinity has been seen to also affect the biochemical composition of melons. Huang et al., (2012) reported an increase in the Total Soluble Sugar of melon cultivar "Huanghemi" under saline conditions. Botía et al., (2005) observed that Galia melons under saline conditions presented higher Total Soluble Solids as well as acidity (% of citric acid), while Amarillo melons presented higher Total Soluble Solids while irrigation with saline water was performed at the begging of the crop cycle or during fruiting, and that acidity decreased when saline water was supplied during flowering and fruiting. Colla et al., (2006) also observed in "Cyrano" fruits that salinity increased the Total Soluble Solids and also the titratable acidity. Again, Del Amor et al., (1999) observed in "Galia" melons that salinity increased Total Soluble Solids (TSS), reducing sugars and acidity, with duration of salination (Days after transplant when saline water was added) also increasing TSS. Visconti et al., (2019) found that in "Finura" (Group Ibericus) melons, salinity and the method of saline water application changed both the SSC and Titratable acidity, with drip irrigation presenting a 6% lower than Flood irrigation, and subsurface drip irrigation having 9% lower acidity compared to normal drip. Akrami et al., (2019) when comparing the effect of salinity on a "Galia" (Group Cantalupensis) and 16 different traditional melon cultivars indigenous of Iran, found that TSS, in general, increased under saline conditions ≈31%, on average, and that this response varied between cultivars.

1.6.8. Effect of Grafting and Salinity on Shelf Life

In melon, differences can be observed in the type of maturation. Some melons such as those belonging to the Cantalupensis Group present climacteric maturation whereas those such as from the Ibericus or Inodorus Groups do not present climacteric maturation. In climacteric fruits, maturation is characterized by change of colour, aroma production, dehiscent fruit, lower flesh firmness, leading this to a lower shelf-life (Monforte and Alvarez, 2006). In contrast non-climateric fruits, do not present these drastic changes, which leads to a difficulty in judgment of their optimum maturation point and also present

much longer shelf-life than climacteric melons (Monforte and Alvarez, 2006). This aspect of prolonged shelf-life is key in certain aspects of the food chain. The effect of grafting on melon postharvest preservation has also been studied, and found to have an effect on it.

Zhao et al., (2011) studied the effect of grafting on the postharvest ripening, a key fruit quality trait, of "Athena" (Group Cantalupensis). The fruits were treated with 1-methylcyclopropene (1-MCP), an ethylene antagonist chemical shown to improve shelf-life in melon. The study revealed that non-grafted "Athena" and fruits from plants grafted onto "Strong Tosa" (*C. maxima* x *C. moschata*) had a 31/22-day shelf life (first/second harvest) while self-grafted and grafted onto "Tetsukabuto" (*C. maxima* x *C. moschata*) presented 6/3 days less shelf life (first/second harvest). This could be due to earlier higher ethylene production and high respiratory rate of "Tetsukabuto" (Zhao et al., 2011).

1.7. Organic Farming

Organic farming was first developed both in Switzerland by Hans Müller, Maria Biegler and Hans Peter Rusch and in the United Kingdom by Lady Eve Balfour and Sir Albert Howard in the 1940s, stemming from the concepts of biodynamic farming initiated by Rudolf Steiner in the 1920s in Germany (Darnhofer et al., 2010). It was not until the 1980s when the negative impact of more intensive farming methods were seen, that organic farming practices started to become widespread (Darnhofer et al., 2010). According to the International Federation of Organic Agriculture Movements (IFOAM - Organics International) organic farming is defined as a production system that sustains ecosystems, soil health and people, relying on ecological processes, biodiversity and cycles adapted to the local agroconditions, rather than the use of inputs that present potential adverse effects. It combines tradition, innovation and science in order to benefit the shared environment and promote fair relationships and good quality of life for all. The European Union also defines organic farming as an agricultural method aimed to produce food using natural substances and processes and encourages:

- Responsible use of natural resources and energy
- Maintain biodiversity
- Preserve regional ecological balances
- Enhance soil fertility
- Maintain water quality

For this reason, the European Union has clear guideline and structure for the production of organic foods and on organic farming production. The EU grants the Organic logo to those that follow the set of regulations provided by it. Hence a more simplified or alternative definition of what organic farming is, could be:

"Organic farming is a **certified farming system**, which **responsibly maximizes** local resources, **avoids** the use of **agrochemical**, promotes **biodiversity** and **forgoes** the use of **Genetically Modified Organisms** (GMOs)"

As of 2020 (Eurostats,2021) Spain has the highest totally converted certified organic surface of the European Union, followed by France and Italy (Fig. 5). Spain is also the first producer organic vegetables cultivated for their fruit, followed by Germany and the Netherlands. (Eurostats, 2021) (Fig. 6)

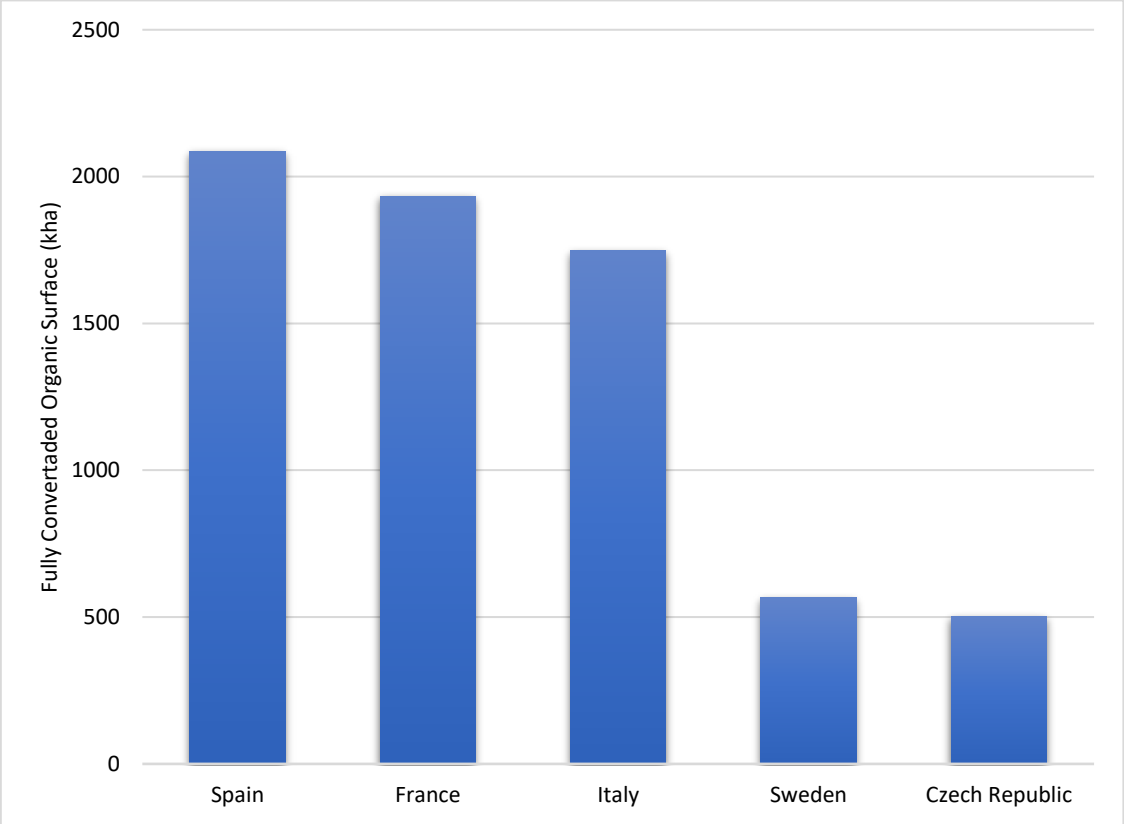


Figure 6. Top 5 countries with the highest fully converted organic farming area (Eurostats,2021)

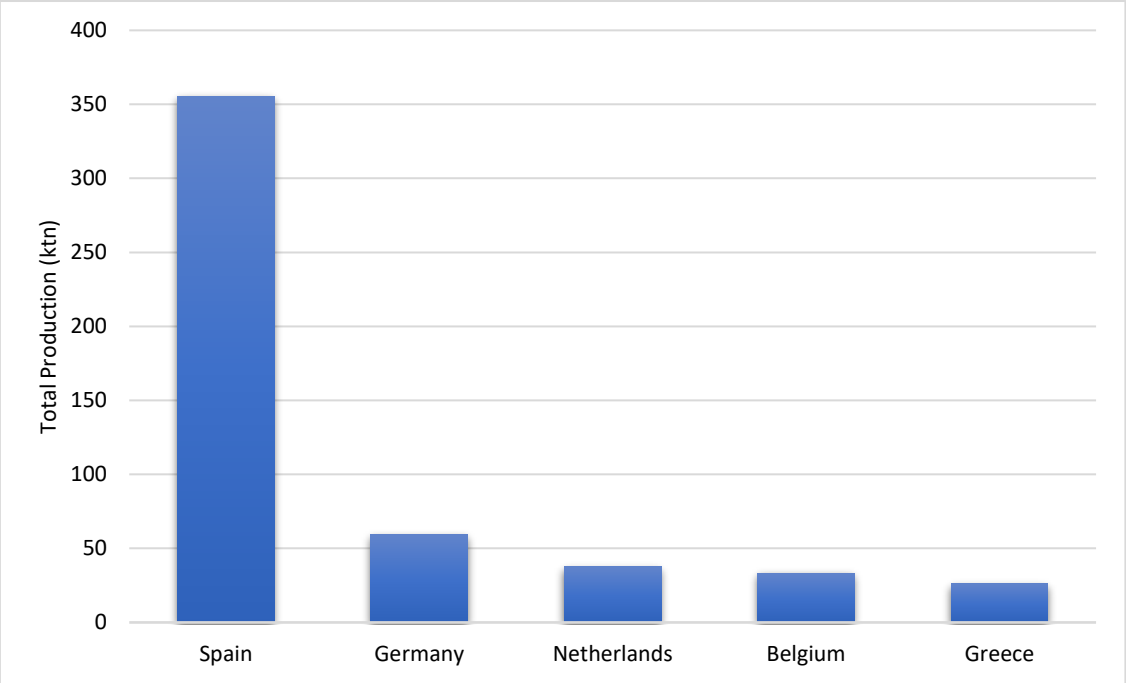


Figure 7. Top 5 European Union countries producers of organic fresh vegetables (Eurostats, 2021)

As previously mentioned, in the European Union, organic farming has a set of legislation, in this case Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007. This new regulation provides transitional periods for implementations of new rules, specifically for trade. Section 2 of Chapter IX of Regulation (EU) 2018/848, indicate which provision of previous Regulations (EC) 834/2007 and 889/2008 still apply for a limited time.

In the EU, to ensure profits for the use of these methods and maintain consumer confidence, strict control is maintained and guaranties the correct application of organic farming guidelines. Organic farming is only a small step in the supply chain ladder, which includes, transformation, distribution and retail, also subjected to regular controls. To this effect the UE has the following the rules through the whole supply chain:

- Each UE member can designate “Authorities” to inspect and control organic food operators. Producers, distributors and marketers are obligated to register in their local control authority, even before being allowed to market their food as organic products.
- Upon inspection and correct check-ins, the operators will be awarded a certification, which confirms their products as organic

- All operators will be inspected at least 1 per year, to ensure they continue to follow the organic production guidelines
- The importation of organic food is also controlled to ensure they are also produced and shipped following the organic principles.

Organic products produced and sold in the UE all share a common identity in the form of the organic logo. This logotype facilitates the identification by consumers of organic products and allows for the commercialization of products across the whole EU area. This logotype is only assigned to organic products certified by the authorized control authorities. This certification confirms that the products follow the guidelines for production, transport and storage.

Figure 8. European Organic Logo.

For the last decade the consumption of organic product has steadily risen. At the beginning, the main reasons for consumers to choose organic were for food safety and eco-friendliness reasons (De Magistris and Gracia, 2008; Cicia et al., 2009), now the trend tends to be for food safety, human health, environmental impact, wage fairness, animal welfare, visual appeal, taste, freshness and nutritional value (Furno et al., 2021).

However, several problems can arise from the use of organic production. One problem facing organic farming production is the reduction of yields, with organic crops producing an average 80% of conventional crops (De Ponti et al., 2012). This yield reduction contributing factors are the availability of Nitrogen, weed control, pest and disease control, cultivar choice and water limitations, although this last one is a rather minor one (Seufert, 2019). With respect to Nitrogen availability, it has often been considered as one

of the main factors contributing to the yield gap (Forster et al., 2013; Palmer et al., 2013). Only small portions of N is available for the crops from inputs such as crop residues or animal manure, as most is dependent on microbial mineralization (Seufert, 2019). It is also important to considerer the imbalance of other nutrients such as Potassium (K), Sulphur (S) or Phosphorus (P), often due to the tendency to focus on Nitrogen (Watson et al., 2002; Zikeli et al., 2017).

The presence of weeds in the field and their effect on the yield gap is also important to consider. Often weeds are eliminated through mechanical removal, but this can become detrimental as the usage of weeds is also associated to pest control. Weeds can be used to sustain beneficial insect species, by providing their floral resources, they can keep those insects in a field between flowering events, as these insects are especially important in pollination reliant crops, allowing for bigger yields, and reducing costs (Kleiman et al., 2020). Still, weed competition against crops for nutrient resources should also be considered, as in some cases these weeds can serve as reservoirs for potentially harmful viruses (Aguiar et al., 2018; Desbiez et al., 2020). In the case of pests and diseases, they do have an important yield limiting capability, especially in organic farming where an organic method of control is difficult to be implemented or does not exist (Östman et al., 2003; Finckh et al., 2006). Some authors argue that if organic was scaled up, pests would further limit yields, as organic benefit from pest suppression due to conventional farming (Avery, 2001). But this is far from the reality, as Gosme et al., (2012) found that the presence of organic field in the neighbourhood decreased the presence of aphids in organic and conventional fields and decreased disease incidence only in conventional fields.

The choice of cultivars for organic crop is of great importance. Some estimations suggest that approximately 95% of cultivars employed in organic farming were originally bred to be used in conventional farming systems (Lammerts Van Bueren et al., 2011). This is therefore a problem, as these cultivars are especially bred to achieve maximum outputs in high input conditions, and therefore are ill adapted to lower input or organic farming conditions. Therefore, a targeted breeding program for cultivars specifically for organic farming is of great importance. Murphy et al., (2007) showed the importance of targeted breeding in wheat, where organic varieties performed better under organic agriculture conditions that regular varieties.

1.8. Doctoral Thesis Structure

The present doctoral thesis is a recompilation of 2 published articles, and another 2 in the process of publication. The present works have been performed by the Cucurbits Breeding Group, of the Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV), at the Universitat Politècnica de València (UPV). This work has been supervised by Dr. Maria Belén Picó Sirvent. Collaborating members from this Insitute in the present thesis include, Dr. Ana Pérez de Castro, Dr. María José Diez, Dr. Carmelo López, Dr. José Vicente Valcárcel, Dr Gorka Perpiñá, Dr. Carmina Gisbert and Dr. Alicia Sifres. Through the whole thesis, collaborations with other groups have been done. Collaboration with the Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández, with both Dr. Juan Jose Ruiz and Dr. Santiago García Martínez from the Department of Applied Biology. Another collaborating group is the Plant Protection Unit and Horticulture Unit of the AgriFood Institute of Aragon (IA2), from the Universidad of Zaragoza, Dr. Vicente González, Dr. Ana Garcés-Claver and Dr. Carmen Julián. Dr. Maria Ferriol from Instituto Agroforestal Mediterráneo (IAM-UPV) also performed a collaboration. From the Joint Research Unit UJI/UPV, collaborating Dr. Jaime Cebolla Cornejo, Dr. Raúl Martí and Dr. Salvador Roselló, and from the Research Institute for Pesticides and Water of the Universitat Jaume I (IUPA-UJI), Dr. Joaquin Beltran.

The present thesis is structured in an Introduction, followed by 4 chapters and a general discussion. Throughout the whole document, it is intended to study and select traditional Spanish melon cultivars, both sweet and non-sweet, and how their quality is affected under organic farming conditions. The first chapter includes a published article in the **International Journal of Molecular Sciences**. In this paper a genomic, morphologic and metabolic characterization of traditional Spanish melons was carried out. In it, a genomic characterization of a total of 47 cultivars, including sweet Ibericus melons, non-sweet Flexuosus and Chate and Exotic Momordica, Agrestis, Ameri and Kachri is presented. A Genotype-by-Sequencing (GBS) approach employing MsII was used to obtain the Single Nucleotide Polymorphisms (SNPs), to study the population structure, linkage disequilibrium and the phylogeny on key melon genes. The morphologic analysis of the traditional melons and non-sweet was also performed at commercial maturity (ripe of Ibericus and immature for Flexuosus and Chate). The acid and sugars profiles were characterized as also the Volatile Organic Compounds. The results obtained for the

morphological traits showed the main parameters of differentiation between subgroups. The acid and sugar profiles were different between the non-sweet and sweet melons, with different tendencies between Subgroups. The VOCs profiles provided information about the main compounds in non-sweet fruits, and for sweet Ibericus melons the trends could be identified, although variation between cultivars was detected.

The second chapter consist of an article published in the journal **Frontiers in Plant Science**. In this article, the effect of grafting on a traditional snake melon cultivar under organic farming conditions was evaluated. In this study different localizations were employed, as to observe the effect of biotic and abiotic stresses. The pests and pathogens affecting the snake melon, including an evaluation of the resistance of snake melon to two different fungal pathogens. The effect of grafting on both the agronomic performance and metabolic profiles, including the consumer acceptability is studied. The results showed the main viruses and soilborne pathogens affecting snake melons. Salt stress had a relatively minor effect on yield, but when combined with soilborne pathogens a synergic effect was found, severely decreasing the yields. Grafting did affect the yields and the metabolic profile of the snake melons. Grafting also influenced the consumer acceptance, with fruits from *Cucurbita* grafted plants having a consumer acceptance lower than those from ungrafted plants, and with those from *Cucumis* grafted plant having a less effect on this aspect.

The third chapter goal is to study of the effect of grafting on 19 Ibericus melon cultivars, employing a total of 5 different rootstocks, both *Cucurbita* and wild *Cucumis* hybrids, under organic farming conditions. A total of 3 different localizations with different conditions were employed, including salinity and soilborne pathogens affected fields. The results showed the effect on yield that both grafting and the presence of soilborne pathogens cause. The effect on morphological characteristics of the fruits was also observed. This chapter is currently under preparation, with the intention of being submitted to **Agronomy for Sustainable Development**

Finally, the last chapter deals with the effect of grafting on the metabolic profile of Ibericus melon cultivars, with both *Cucurbita* and wild *Cucumis* hybrids rootstocks. The acid and sugars profile were measured and compared between the different localizations as well as the effect that grafting had on each rootstock-scion combination. The Volatile Organic Compounds of both grafted and non-grafted plant fruits was also analysed. The

results showed how grafting affected the different cultivars and each rootstock-scion combination. This chapter has been sent for publication in **Food Chemistry**.

Objectives

1.9. General Objective

Evaluate a collection of traditional Spanish melon landraces, including both sweet melons, Ibericus, and non-sweet, Flexuosus, melons and select those best adapted for organic farming conditions.

1.9.1. Specific Objectives

1) Characterization of a traditional Spanish melon collection, including both sweet and non-sweet melons.

2) Evaluate the performance of traditional melon landraces cultivated under organic farming conditions.

3) Ascertain the main limiting factors of melon cultivation under organic farming conditions, both abiotic and biotic.

4) Evaluate the effect of grafting on agronomic and fruit quality of traditional melon cultivars.

Chapter 2. Spanish Melon Landraces: Revealing Useful Diversity by Genomic, Morphological, and Metabolomic Analysis

Chapter 2. Spanish Melon Landraces: Revealing Useful Diversity by Genomic, Morphological, and Metabolomic Analysis.

This chapter consist on a genomic, morphologic and metabolomic characterization of a collection of 47 cultivars, including sweet Ibericus melons, non-sweet Flexuosus and Chate and Exotic Momordica, Agrestis, Ameri and Kachri. A GBS approach was performed to analyse the genomic data, which included population structure, linkage disequilibrium, phylogeny and effect of the SNPs. The morphological and metabolic (acids, sugars and VOCs) features were also analysed to better characterize each melon Subgroup. Chapter 2 was published in the **International Journal of Molecular Science** Journal**.**

This work was performed as part of the projects PROMETEO/2017/078 and PROMETEO/2021/072 funded by Conselleria d'Educació, Investigació, Cultura i Esports (Generalitat Valenciana, Spain), as well as the AGL2017-85563-C2-1-R-AR project, with grants from the Agencia Estatal de Investigación (AEI) and "ERDF: A way of making Europe".

This work was performed by the Cucurbits Breeding Group of the COMAV, in collaboration with Dr. Jaime Cebolla Cornejo, Dr. Raul Martí and Dr. Salvador Roselló from the Joint Research Unit UJI/UPV as well as with Dr. Joaquin Beltran from the Research Institute for Pesticides and Water of the Universitat Jaume I (IUPA-UJI).

Chapter 2. Spanish Melon Landraces: Revealing Useful Diversity by Genomic, Morphological, and Metabolomic Analysis.

Alejandro Flores-León[1], Clara Peréz Moro[1], Raul Martí[2], Joaquin Beltran[3], Salvador Roselló[4], Jaime Cebolla-Cornejo[2*] and Belen Picó[1]

1 COMAV, Instituto de Conservación y Mejora de la Agrodiversidad, Universitat Politècnica de València, Cno. de Vera, s.n, València, 46022, Spain
2 Joint Research Unit UJI/UPV - Improvement of Agri-Food Quality, Universitat Politècnica de València, Cno. de Vera, s.n, València, 46022, Spain
3 Instituto Universitario de Plaguicidas y Aguas (IUPA), Universitat Jaume I, Campus de Riu Sec, Avda. Sos Baynat s/n, Castellón, 12071, Spain
4 Joint Research Unit UJI/UPV - Improvement of Agri-Food Quality, Department de Ciències Agràries i del Medi Natural, Universitat Jaume I, Avda. Sos Baynat s/n, Castellón, 12071, Spain
* Correspondence: jaicecor@upv.es

2.1. Abstract

Spain is a secondary centre of the diversification of the melon (*Cucumis melo* L.), with high diversity represented in highly appreciated landraces belonging to the Flexuosus and Ibericus groups. A collection of 47 accessions of Flexuosus, Chate, Piel de Sapo, Tendral, Amarillo, Blanco, and Rochet was analysed using a Genotyping-By-Sequencing (GBS) approach. A total of 66,971 quality SNPs were identified. Genetic analysis differentiated Ibericus accessions and exotic materials (Ameri, Momordica, Kachri, and Agrestis), while Flexuous accessions shared ancestry between them. Within the Ibericus group, no clear genomic distinction could be identified for the different landraces evaluated, with accessions of different landraces showing high genetic similarity. The morphological characterization confirmed that the external colour and fruit shape had been used as recognition patterns for Spanish melon landraces, but variability within a landrace exists. Differences were found in the sugars and acid and volatile profiles of the materials. Flexuosus and Chate melons at the immature commercial stage accumulated malic acid and low levels of hexoses, while Ibericus melons accumulated high contents of sucrose and citric acid. Specific trends could be identified in the Ibericus landraces. Tendral accumulated low levels of sugars and citric acid and high of malic acid, maintaining higher firmness, Rochet reached higher levels of sugars, and Amarillo tended to lower malic acid contents. Interestingly, high variability was found within landraces for the

acidic profile, offering possibilities to alter taste tinges. The main volatile organic compounds (VOCs) in Flexuosus and Chate were aldehydes and alcohols, with clear differences between both groups. In the Ibericus landraces, general trends for VOCs accumulation could be identified, but, again, a high level of variation exists. This situation highlights the necessity to develop depuration programs to promote on-farm in situ conservation and, at the same time, offers opportunities to establish new breeding program targets and to take advantage of these sources of variation.

Keywords: GBS; SNPs; *Cucumis melo* L.; flavour; breeding; fruit quality

2.2. Introduction

The melon (*Cucumis melo* L.) is an important crop belonging to the Cucurbitaceae family, with a total global production of 28.5 million tons for the year 2020, with a considerable increase (>40%) over the last two decades (FAO, 2020). Spain is the leading producer in Europe with more than 610.000 t (FAO, 2020), but more importantly, it represents a valuable centre of diversification. Early studies based on archaeological findings suggested Egypt or the Middle East as the domestication centres of the species (Zohary, 2000). However, recent molecular studies propose two independent domestication events in Africa and Asia, with most of the present melon types being derived from the Asian lineages (Endl et al., 2018). Accordingly, India is widely accepted as the primary diversification centre of the species, and Mediterranean and Far-East diversity would have been derived by divergent diversification (Gonzalo et al., 2019). Among secondary centres of diversity, Spain stands out with the Ibericus group of the former Inodorus classification (Pitrat, 2016). Nonetheless, the first melon type cultivated in the area belonged to the Flexuosus group, non-sweet melons, which were cultivated since Roman times as mentioned by roman author Columella (Hammer and Gladis, 2014; Flores-León et al., 2021). Sweet melons were later introduced into the Iberian Peninsula by Muslims, who arrived in the VIII century, where they were documented as being grown by the second half of the XI century (Paris et al., 2012a). A great diversity of sweet melons was generated during centuries of cultivation, but these landraces have become progressively replaced by F1 hybrids mainly belonging to the Piel de Sapo and Amarillo market classes. Few works have analysed in depth a wide collection of Spanish landraces. In some cases, such as in Piel de Sapo commercial cultivars, melons resemble externally the corresponding landraces, but in general, a wider diversity in morpho-agronomical traits

is found in Spanish landraces (Escribano and Lázaro, 2009). Nonetheless, despite the external resemblance, consumers appreciate the differences between them and value the sensory characteristics of the traditional landraces over the commercial cultivars available (Escribano and Lázaro, 2012).

Few studies have analysed the diversity present in different populations of the same landrace. One example would be the simple sequence repeats (SSRs) analysis, performed by (Escribano et al., 2012), of several Spanish landraces. This study revealed a higher degree of homogeneity in some types, e.g., Piel de Sapo, while others, including Rochet or Amarillo, showed higher diversity and population diversification. Other molecular studies have focused on the evaluation of a wide diversity representing worldwide landraces. Lázaro et al., (2017) studied the variability of 62 Spanish melon landraces employing both SSR and morphology and identified several non-climacteric landraces, i.e., Piel de Sapo, Mochuelo, Tendral, Amarillo (Yellow)/Blanco (White), and Negro (Black), and a set of highly variable climacteric ones. Lázaro et al., (2017) also pointed out that many accessions could not be classified, attesting to the great variability in Spanish melons. Other molecular studies have focused on the evaluation of a wide diversity representing worldwide landraces. With this approach, a resequencing of the transcriptome was performed of 67 melon genotypes representing the diversity grown in the World and including specific populations of specific Spanish landraces (Blanca et al., 2012). The SNP analysis revealed that higher levels of variation are still present in landraces compared to the commercial types.

Esteras et al., (2013) characterized with 768 Single Nucleotide Polymorphisms (SNPs) a collection of 68 accessions, representing global diversity and revealed that genomic regions distinguished two main groups of accessions belonging to Inodorus and Cantalupensis, the former being more variable. The group of Spanish Inodorus landraces displayed a substantial degree of polymorphism, with Blanco and Tendral being more variable than Amarillo and Piel de Sapo. Rochet accessions were separated from the rest of the accessions and Tendral was more similar to Eurasian landraces.

Genotyping-By-Sequencing (GBS) technology has recently provided deeper insight into the study of melon molecular diversity and relationships, as the number of SNP studies increased dramatically. This technology has been widely employed as it is low-cost, simple, and efficient when compared to other genotyping techniques (Pereira et al., 2018). In the last decade, the evolution of GBS has enabled the number of SNPs detected and

studied to multiply by 30 and has offered a complete view of almost the whole genome. Gonzalo et al., (2019) and Hyun et al., (2021) obtained sets of informative SNPs of 6,158 and 6,406, respectively, while Moing et al., (2020) identified a global collection of 23,931 polymorphisms. A similar level of polymorphism can be detected with a local set of diversity. That would be the case of the study performed by Pavan et al., (2017), which detected 25,422 SNPs in a set of Italian landraces from Apulia. All these studies used ApeKI as restriction enzyme, thus the use of an alternative enzyme would enable the analysis of different genomic regions.

The objective of the present study is to apply GBS technology to perform an in-depth study on the characteristics of a melon germplasm collection, representing the wide biodiversity of Spanish landraces, both at the intervarietal and intravarietal levels. An exhaustive characterization of morphological traits and internal quality, including the sugar and acid and volatile profiles, was also developed in order to combine data from the molecular and metabolomic point of view. The results obtained offer valuable information to establish targets for the development of next-generation cultivars with outstanding quality.

2.3. Results

2.3.1. GBS results

The results for the GBS mapping indicated a correct alignment (Supplementary Table 1). On average each accession had a total of 3M reads, with an average mapping rate of 73.95%, with the lowest mapping rate obtained with the Chate accession 41CHA (71.93%) and the highest with the introgression line calc8-1 (75.07%). The Freebayes software revealed a total of 96,267 raw SNP positions, resulting in 66,971 after filtering with Vcftools for minor allele frequency (maf) of 1%, and a maximum missing count of 4. The SnpEff program revealed that 95 SNPs presented a high impact (e.g., SNPs resulting in a stop codon, loss or gain, and affects splice acceptor and donor sites), 2116 SNPs presented a moderate impact (e.g., SNPs codon changes resulting in a different amino acid), and 2858 SNPs presenting a low impact (e.g., synonymous changes) (Supplementary Table 1).

2.3.2. Population Structure Analysis

The principal component analysis (PCA) biplot (Figure 1), with 29.45% of the variance explained, revealed three groups. Accessions from exotic melon groups were clearly separated. Accessions from Ibericus landraces appeared grouped with low levels of variability. Higher variability was observed in Flexuosus accessions, with accession 39AL plotting close to the Chate accession and the Ameri accession I156, with low values of the second PC.

The population structure analysis performed with ADMIXTURE software revealed that the best 2 K subpopulations values were K=2 and K=3 (Figure 2, Figure 3). The bar graph showing the ancestry of each subpopulation can be seen in Figure 3. With K=2, 2 clear subpopulations one representing the exotic types (blue) and the other with the Spanish sweet Ibericus melon accessions (red). Flexuosus and Chate accessions showed a mixed percentage (around 50%) of each subpopulation. This result agreed with the PCA results, leading to the idea that these materials stand between cultivated sweet melons and exotic wild-type melons. Accession I156 of the Ameri group also displayed a mixed ancestry pattern. Interestingly, the accession 21AM of the Amarillo landrace also displayed around 15% exotic ancestry. When a value of K=3 was applied sweet melons and exotic materials were still recognized as different subpopulations and this time a new group was formed by the Flexuosus accessions. Exotic melon accessions still remain as part of the same group, but some changes occur. The accessions 41CHA of Chate and 39AL of Flexuosus showed a mixed ancestry between Flexuosus and sweet melons (30-35%). Specific Ibericus accessions from different landraces, 30BL and 27BL from Blanco, 07PS from Piel de Sapo, and 20AM and 21AM from Amarillo showed low levels of ancestry of the Flexuosus group. In the case of 21AM accession which previously displayed 15% exotic ancestry, this time presented a 30% ancestry from the Flexuosus group. Finally, I156 displayed again a mixed ancestry, but mainly of the sweet melon and Flexuosus group. In the PCA, the three subpopulations appeared clearly distinguished, while the Chate accession, the Algerian Flexuosus accession, and the Ameri accession plotted between the three groups, the first two closer to the Flexuosus group (Figure 1).

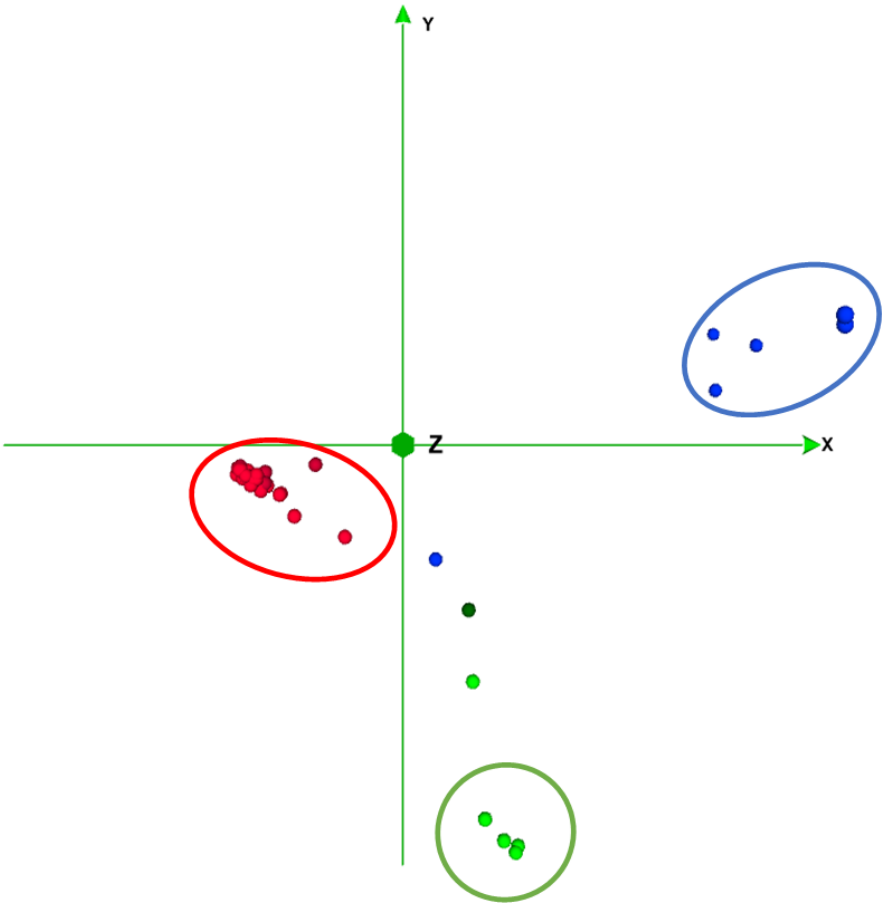


Figure 1. Principal component analysis (PCA) results for the Ibericus (red), exotic (blue), Flexuosus (green), and Chate (dark green) accessions studied. Subpopulations identified with ADMIXTURE (K=3) are encircled.

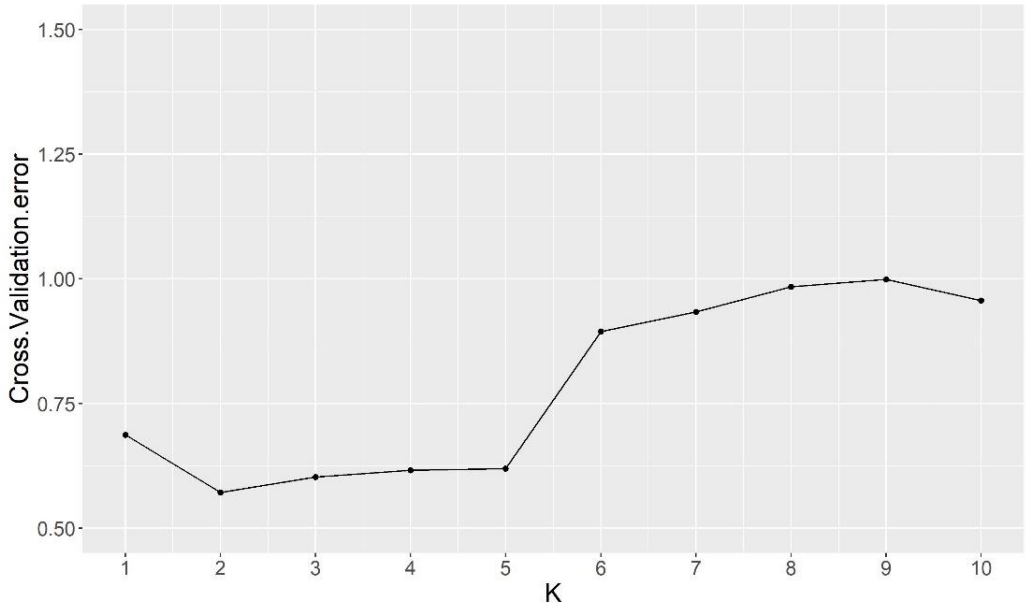


Figure 2. ADMIXTURE analysis results for the best grouping number based on the cross-validation error.

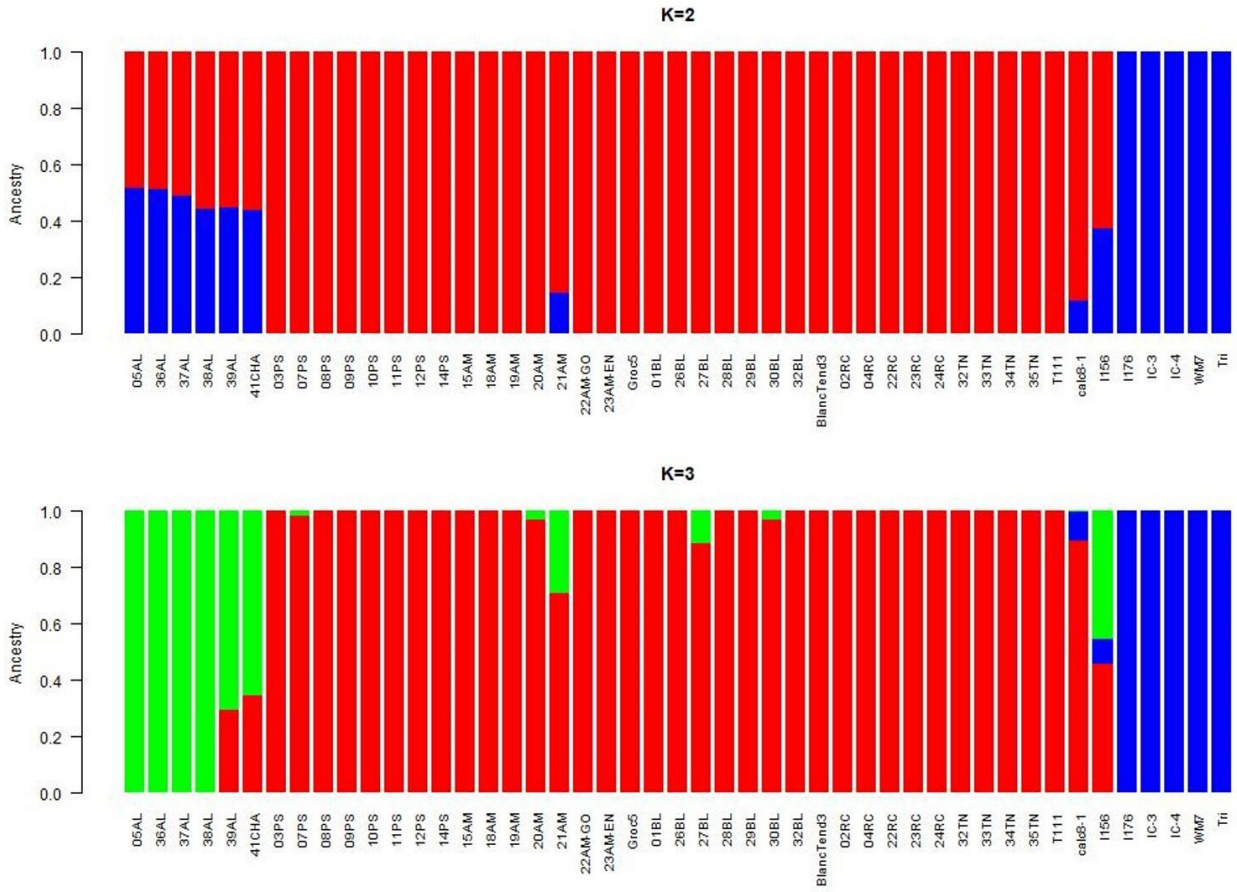


Figure 3. ADMIXTURE results assuming K=2 and K=3 populations. Each colour represents the ancestry component. Stacked bars represent each of the accessions studied.

2.3.3. Linkage Disequilibrium Decay

The linkage disequilibrium (LD) decay was calculated for the complete accession collection and separately, in three different groups, Ibericus, exotic and Flexuosus, and Chate melons (Fig 4). The LD was variable among the different groups. Overall, the results showed a much faster decay in exotic melons. When the whole collection was analysed, the LD decay distance (r2=0.2) was approximately 100kbp. Within groups, the decay distance of exotic melons LD (r2=0.25) remained around 100kbp, but it was higher for Ibericus melon (300kbp; r2=0.30) and Flexuosus and Chate melons (300kbp; r2=0.35).

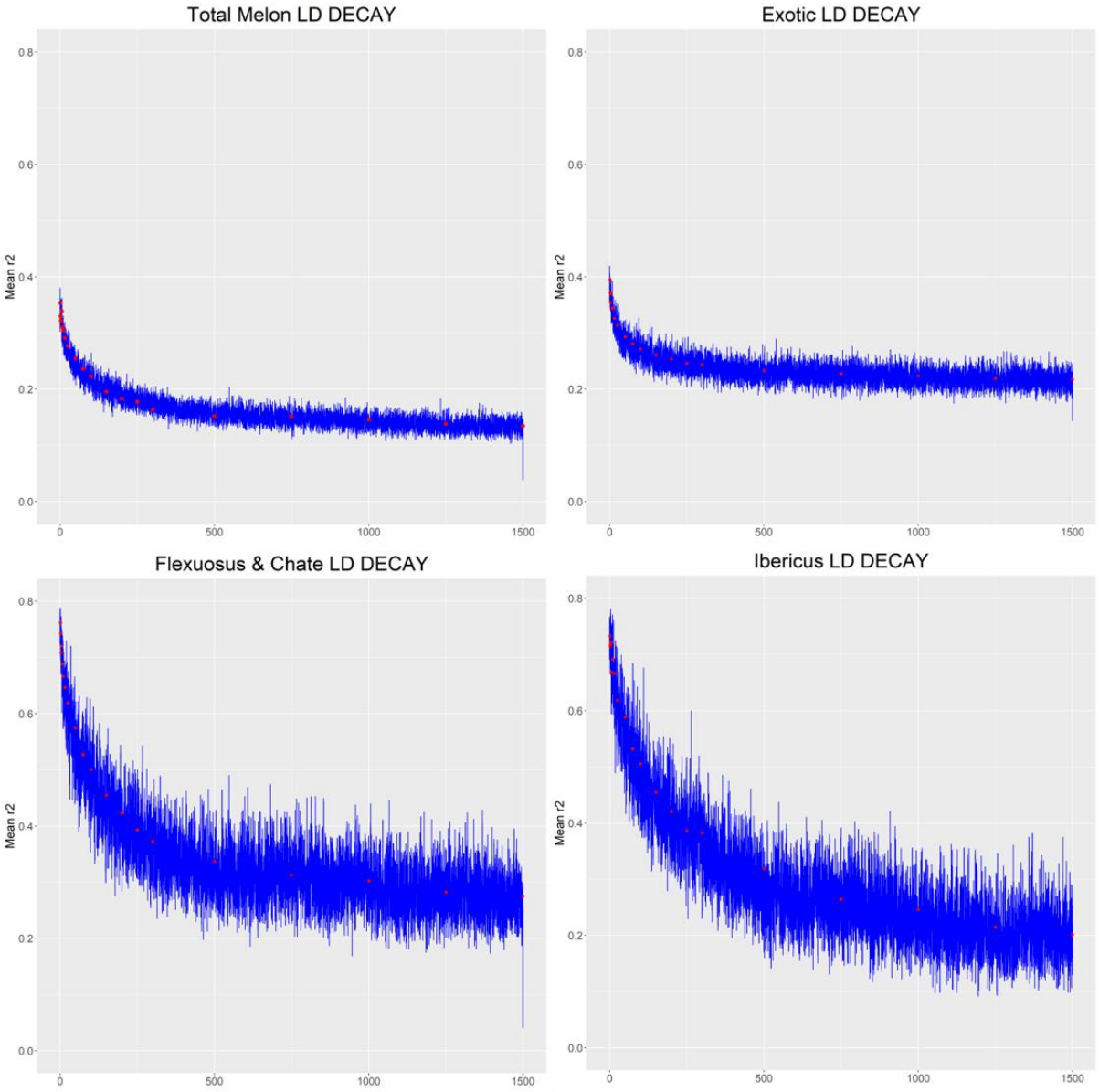


Figure 4. Graphs for the linkage disequilibrium in the melon populations analysed based on their distance (kb). The decay linkage disequilibrium (LD) is up to 1500kbp. Separate graphs were created for the complete melon population, the Ibericus melons, the Flexuosus and Chate melon, and the exotic accessions.

2.3.4. Phylogeny

The consensus phylogenetic tree (Figure 5) separated Agrestis and Kachri melons from the rest of the accessions. Momordica accessions IC-3 and IC-4 were closely grouped and at a certain distance from the remaining Momordica accession I176. The exotic accession I156 from the Ameri group and the Spanish landraces were grouped separately and formed four clades, one with the Chate accession, one with I156, another one with the Flexuosus accessions, and the remaining one with the Ibericus accessions. Within the Flexuosus clade, Spanish landraces appeared grouped together and at a certain distance from the Algerian accession 39AL. Within the Ibericus clade, accessions from different

landraces appeared intermixed. Only in some cases, some accessions of certain landraces configured specific groups. For example, 15AM, 19AM, Groc5, and 22AM-GO from the Amarillo landrace were grouped together and at a certain distance of 21AM and 18AM. In the rest of the cases, Tendral, Blanco, Rochet, and Piel de Sapo accessions appeared intermixed. Only certain couples of accessions of the same landrace appeared grouped with high bootstrap values and low genetic distance.

2.3.5. Fruit Characterization

Fruits are characterized at their commercial state. Consequently, Flexuosus and Chate fruits were collected whilst immature, while Ibericus fruits were harvested when ripened. High variability was present between and within groups (Figure 6, Figure 7, Supplementary Table 2). Nonetheless, the principal component analysis (Figure 6) revealed, as expected, a clear differentiation between Flexuosus and Chate accessions and those belonging to the Ibericus group. The former had long and narrow green fruits with no seed cavity and a minimum rind and these variables differentiated both groups. As these fruits are eaten immature, flesh firmness was also higher than in the Ibericus group. The overall fruit weight of the Flexuosus–Chate group ranged between 170 and 450g. In general, Chate could be differentiated as having an intermediate weight (309g) and shorter fruits, with the smallest L/D ratio. Among Flexuosus accessions, the only ribless accession, 39AL from Algeria, presented the highest weight but these fruits were shorter than the rest of the Flexuosus accessions. The fruit pH of Flexuosus and Chate melons was similar (4-4.7), but Chate accession showed higher SSC values compared to Flexuosus accessions (3.1°Brix vs. 1.8-2.7°Brix).

Landraces of the Ibericus group were mainly differentiated by rind colour (Figure 6, Figure 7, Supplementary Table 2), being white for Blanco melons, yellow for Amarillo, and greenish for Piel de Sapo, Tendral, and Rochet. Regarding size, fruits from the Ibericus group showed similar sizes with mean fruit weight ranging between 1800 and 2200g. Within each landrace, weight was similar, although Blanco accessions showed higher variability. In this landrace, accessions 26BL and 01BL (1250-1650g) presented significantly lower fruit weight than 30BL (2815.5g). Tendral and, to a lesser extent, Rochet melons tended to show a lower length to diameter ratio with a more rounded shape than the rest of the landraces, which were more elongated, especially Piel de Sapo. General trends were identified for each landrace, though in some cases (e.g., 23AM-EN

in Amarillo or 34TN in Tendral) certain accessions tended to show differences from the rest of the group. In the case of Piel de Sapo and Rochet the differences were less marked, and the spectra of variation overlapped.

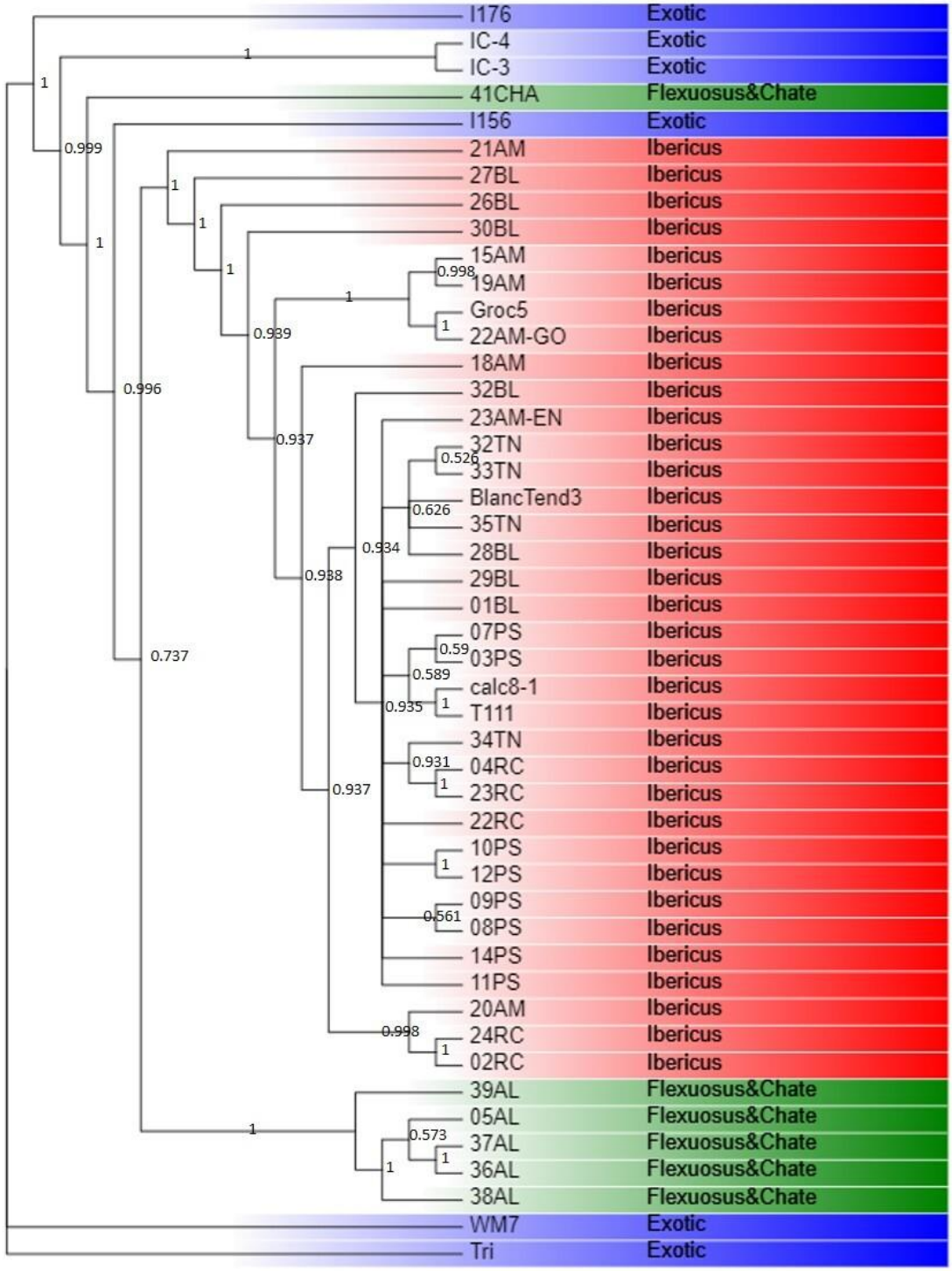


Figure 5. Consensus maximum likelihood phylogenetic tree (50% cut-off), representing the phylogenetic relationships of the Ibericus melon (Red), exotic (Blue), Flexuosus, and Chate (Green). Trigonus serves as the outgroup of the tree. Bootstrap values are based on 1000 iterations.

Nonetheless, Rochet melons tended to be less firm (Flesh Firmness = 2.2kg/cm2), Piel de Sapo, Amarillo, and Blanco showed intermediate values and Tendral fruits were firmer (3 kg/cm2). Within each landrace, variability was found in Piel de Sapo and Blanco for flesh firmness (accessions 12PS and 30BL being firmer). On the other hand, no significant differences were detected for SSC between landraces with values ranging between 11 and 12.25°Brix. Nonetheless, within Piel de Sapo and Blanco landraces significant differences were detected between accessions with 08PS, 03PS, and 27BL, reaching 1.5-2°Brix values lower than 11PS and 28BL.

The rind colour varied between the greenish landraces. Piel de Sapo accessions presented green colour with darker green spots, Rochet displayed a light green external colour, and Tendral presented a darker green rind colour. In Amarillo melons, external colour was rather uniform, but differences were found within landrace flesh Hunter colour coordinates (red–green gradient). In the case of the Blanco landrace, accessions 28BL and 29BL displayed green coloured stripes in sections of their rind of varying lengths. In the last two landraces, significant differences were found between accessions for seed cavity diameter.

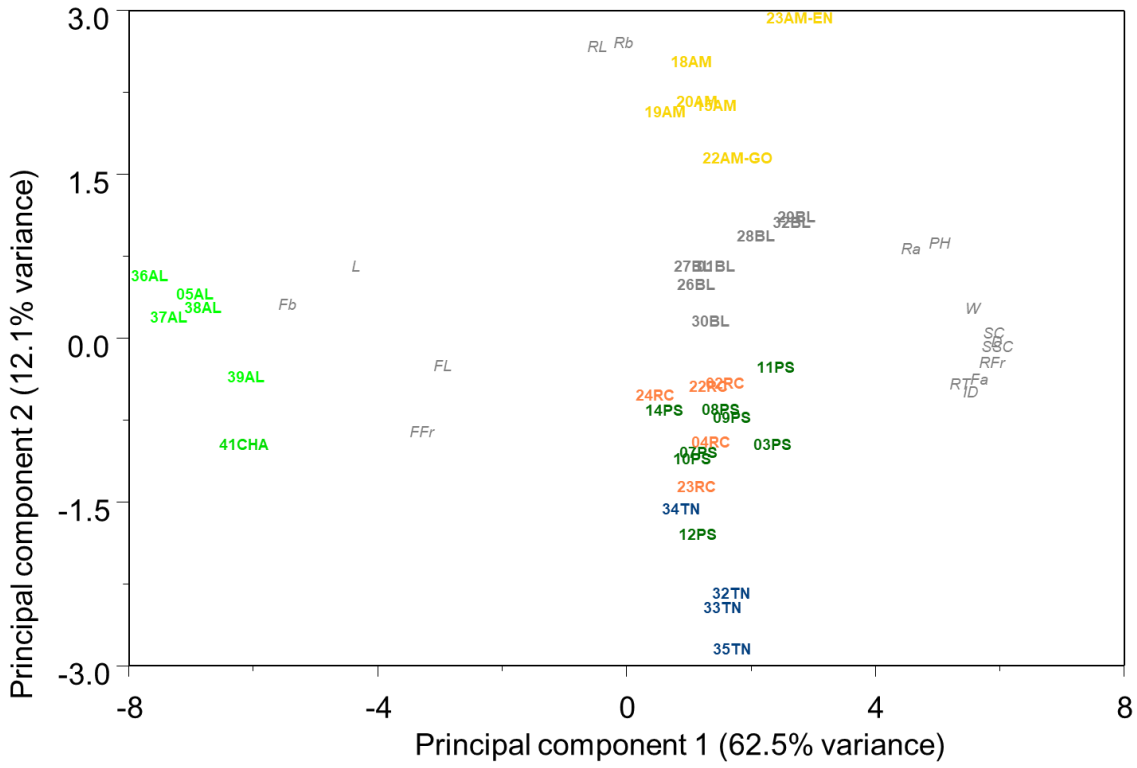


Figure 6. Biplot of principal component analysis of morphological traits. AL: Flexuosus; CHA: Chate; PS: Piel de Sapo; TN Tendral; AM: Amarillo; BL: Blanco; RC: Rochet. Variables indicated in italics. F: Fruit; R: Rind; I: Internal; W: Weight; L: Length; D: Diameter; Fr: Firmness; SC: Seed cavity; SSC: Soluble solids content; L, a, b: Hunter colour coordinates.

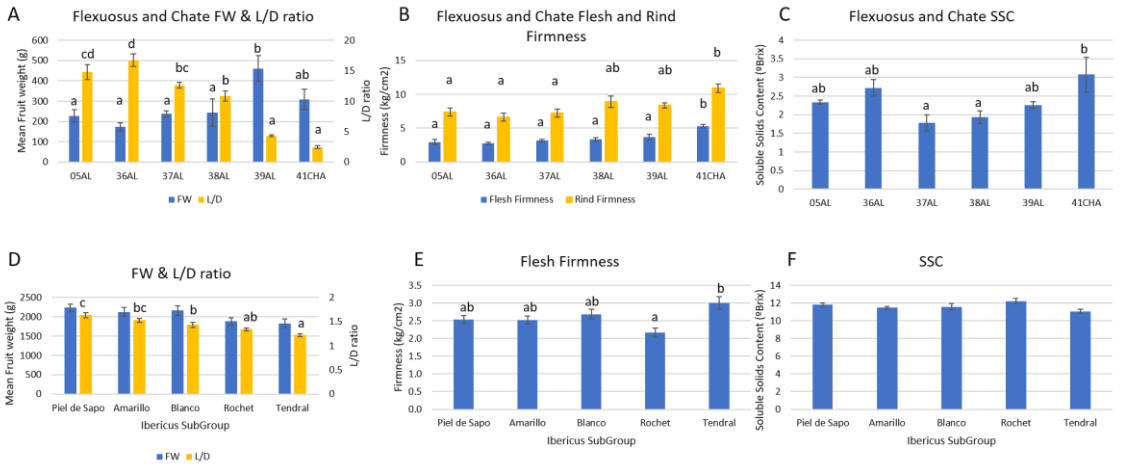


Figure 7. Fruit Weight, L/D ratio for the Flexuosus and Chate fruits (A) and the Ibericus Subgroups (D); flesh and rind firmness for the Flexuosus and Chate fruits (B) and Ibericus landraces (E); and soluble solids content for the Flexuosus and Chate fruits (C) and Ibericus landraces (F). Different letters indicate significant differences (Tukey's test, $P \leq 0.05$). Those bars without letters had no significant differences in the ANOVA test ($P=0.05$) and no differences were found between landraces.

2.3.6. Sugars and Acids Content

The main difference between the Flexuosus–Chate and Ibericus groups was that the former accumulated lower levels of sugars and did not accumulate significant contents of sucrose (Figure 8 and 9, Supplementary Table 3). Additionally, in the former group, the predominant acid was malic acid, while in the Ibericus group it was citric acid, and glutamic acid levels in the former were close to the limit of quantification. Within the Flexuosus–Chate group, a trend towards higher SSC was confirmed for Chate, though no significant differences were found between this population and 36AL from Flexuosus. Nonetheless, no significant differences were found for hexoses and acid accumulation between accessions of the group.

In the case of the Ibericus group (Figure 9, Supplementary Table 3), differences were found between landraces for both the acid and sugar contents. Tendral presented the highest malic acid content and the lowest citric acid levels. On the other hand, Amarillo tended to offer low malic acid contents. In the case of sugar content, as expected, the main sugar was sucrose, with lower contents of fructose and glucose. Rochet and Piel de Sapo had the highest sucrose levels, but the higher accumulation of fructose of the former resulted in higher values of total sugars and sucrose equivalents. Within landraces, special considerable differences were found in acid contents (Supplementary Table 3). Overall, no significant differences were observed in the content of both fructose and glucose

between the different accessions of each landrace but in the Rochet landrace, accessions 02RC and 22RC offered significantly lower content than the others.

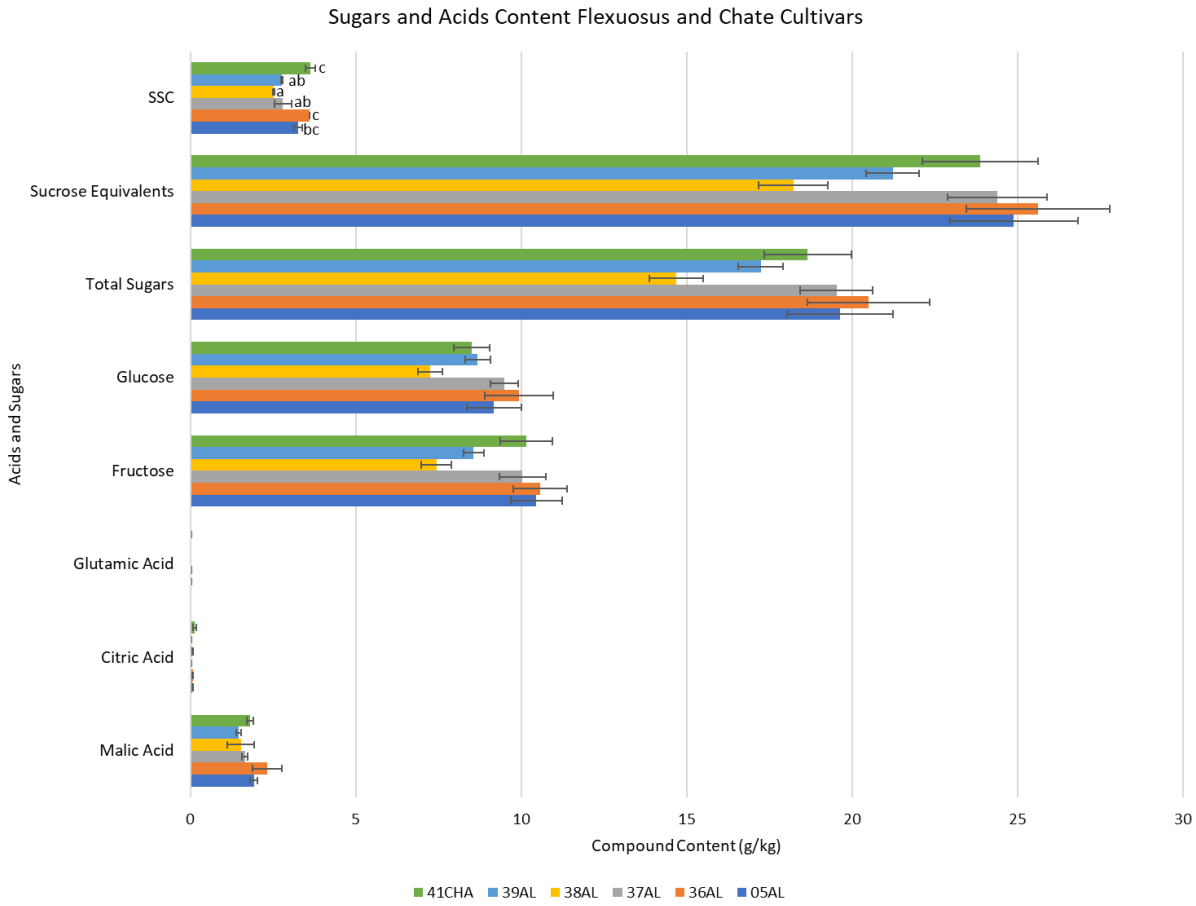


Figure 8. Mean acids and sugar content for the Flexuosus and Chate accessions. Different letters indicate significant differences (Tukey's test, P≤0.05). Those bars without letters had no significant differences in the ANOVA test (P=0.05) and no differences were found between landraces.

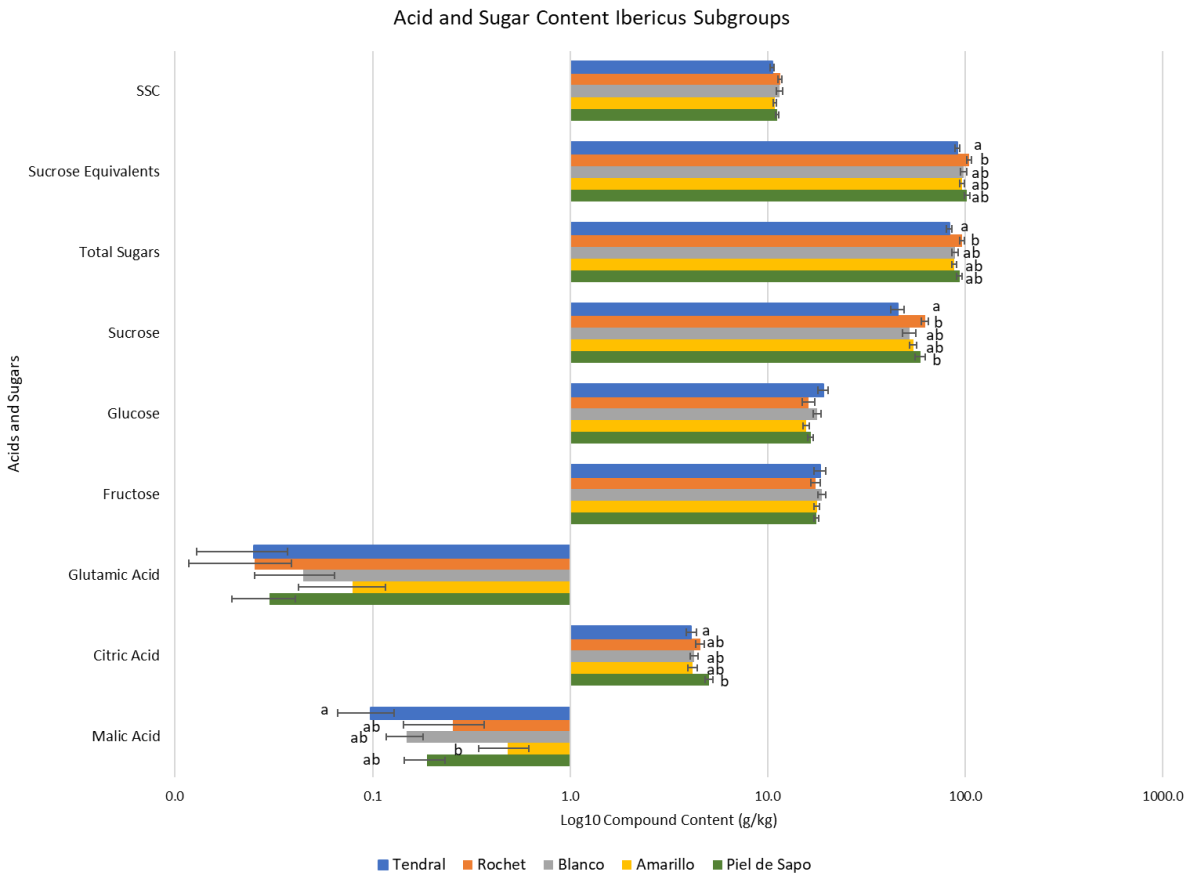


Figure 9. Mean acids and sugar content for the Ibericus landraces. Different letters indicate significant differences (Tukey's test, P≤0.05). Those bars without letters had no significant differences in the ANOVA test (P=0.05) and no differences were found between landraces.

2.3.7. Volatile Organic Compounds (VOCs) Content

Important differences were found between the Flexuosus–Chate and the Ibericus group in the VOC profile. In brief, accessions from the former had increased levels of aldehydes and lower levels of esters (Figure 10, 11, 12 and 13, and Supplementary Table 4) In fact, aldehydes represented the main compounds of the Flexuosus and Chate melons aroma profile, followed by alcohols, and alkyl esters. Smaller contents of apocarotenoids and acetate esters were also detected. A small content of phenylpropanoids and monoterpenoids was also found. Among aldehydes, (E,Z)-2,6- nonadienal, (E)-2-nonenal, nonanal, and (Z)-6-nonenal showed the highest levels, while 1-nonanol, 2-phenylethanol, and (Z)-3-nonen-1-ol were the most abundant alcohols. Among this group of accessions, 41CHA and 38AL outstood for higher accumulation of aldehydes, reaching 4900.82 ng g−1 and 4363.67 ng g−1, respectively (Figure 10) contents that doubled those of the rest of the accessions of the group (1660-2300 ng g−1). The Chate accession

41CHA also doubled the accumulation of alcohols of Flexuosus accessions (512.95 ng g−1 vs. 161-256 ng g−1).

The PCS of VOCs contents confirmed the different volatile profiles of Chate and Flexuosus accessions, mainly explained by the differential accumulation of aldehydes and alcohols (Figure 11). Within the Flexuosus group variability was observed with two subgroups of accessions. One of them is formed by 05AL, 36AL, and 39AL, characterized by a higher content of alkyl esters, such as ethyl hexanoate or ethyl (E)-2-butenoate, acetate esters, (Z)-3-hexen-1-ol-acetate, or 2-methylpropyl acetate, and the other formed by 37AL and 38AL, with lower ester accumulation. Between these two last accessions, important differences were found though, as 38AL reached high accumulation levels of aldehydes.

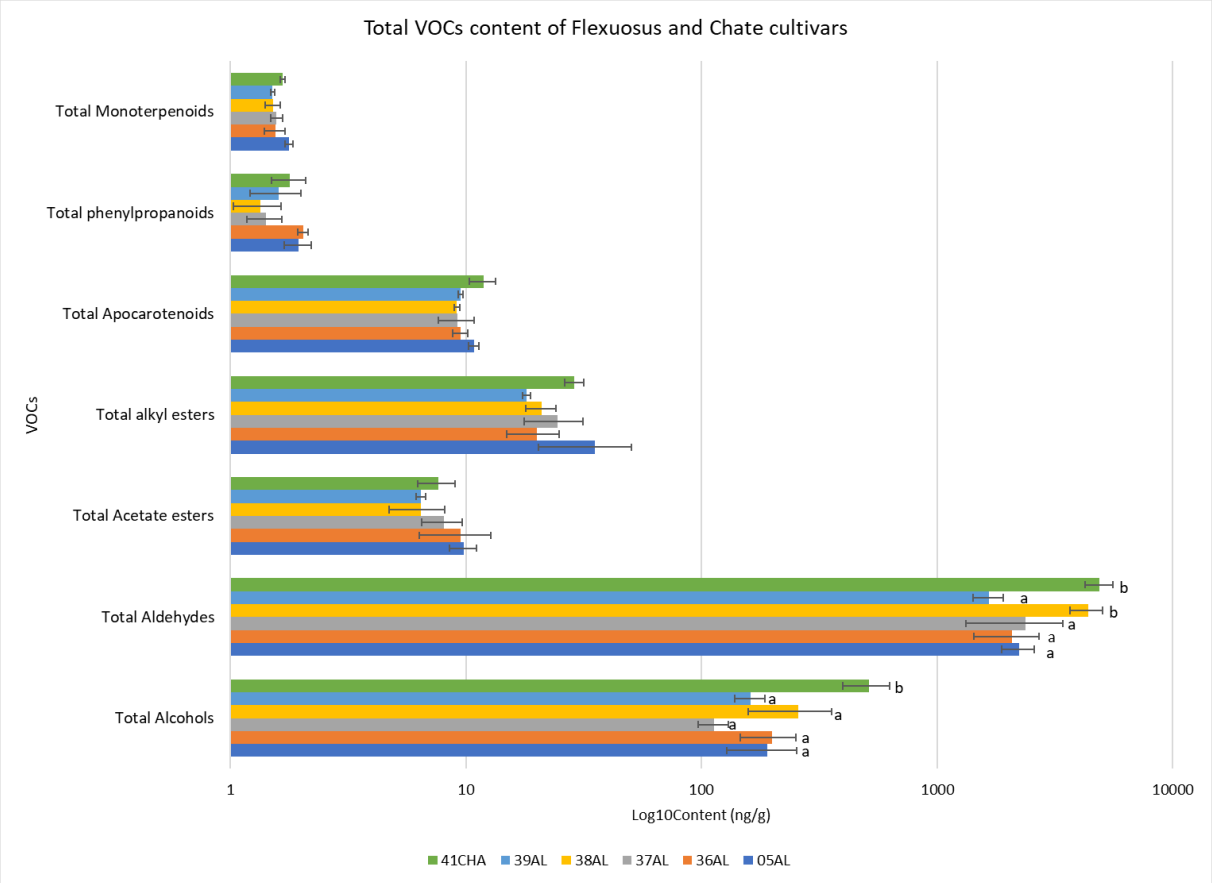


Figure 10. Total VOCs content for the Flexuosus and Chate accessions. Different letters indicate significant differences (LSD test, P≤0.05). Those bars without letters had no significant differences in the ANOVA test (P=0.05) and no differences were found between landraces.

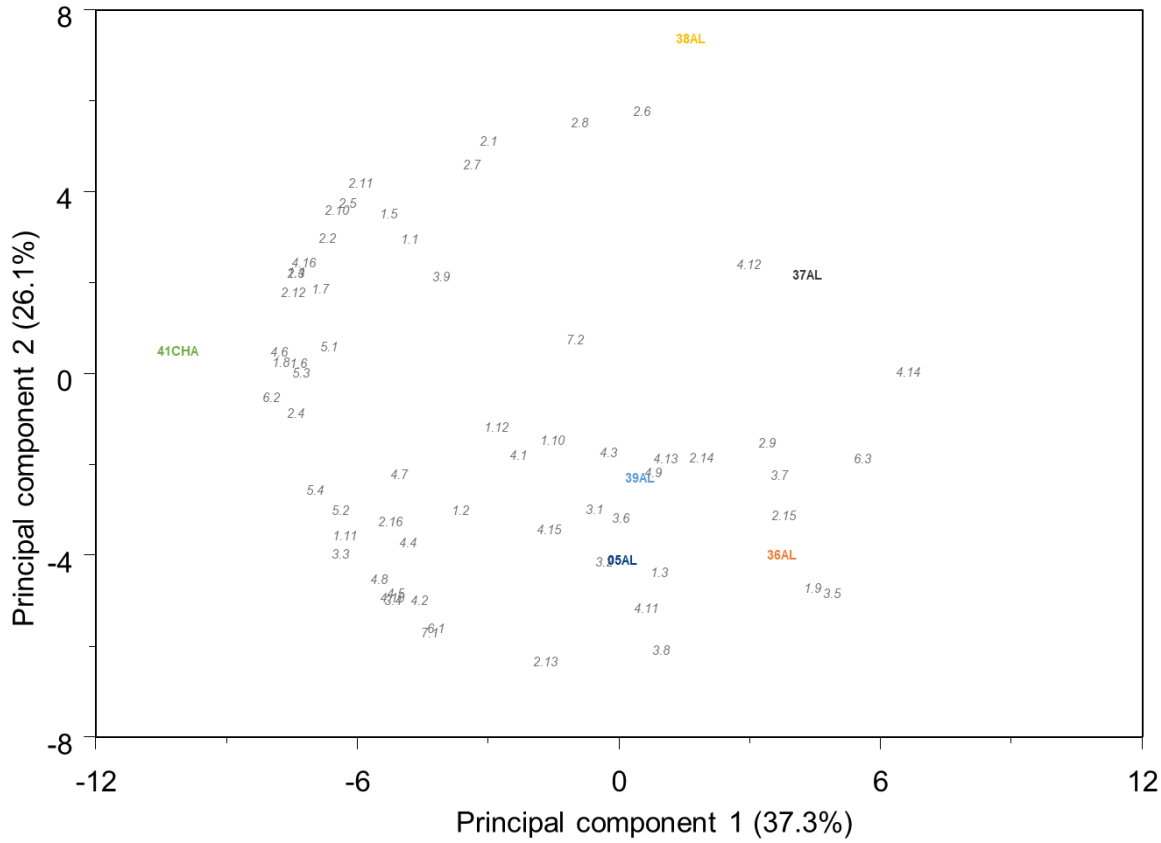


Figure 11. Principal component analysis biplot for the VOCs profile of the Flexuosus and Chate melon accessions. 1_1(1-pentanol); 1_2(1-hexanol); 1_3((Z)-3-hexen-1-ol); 1_4(1-octanol); 1_5(1-nonanol); 1_6((Z)-3-nonen-1-ol); 1_7((Z)-6-nonen-1-ol); 1_8((E,Z)-2,6-nonadien-1-ol); 1_9(1-decanol); 1_10(benzyl alcohol); 1_11(2-phenylethanol); 1_12(phenol); 2_1(Hexanal); 2_2(Heptanal); 2_3((E)-2-heptenal); 2_4((E,E)-2,4-heptadienal); 2_5((E)-2-octenal); 2_6(octanal); 2_7(nonanal); 2_8((Z)-6-nonenal); 2_9(benzaldehyde); 2_10((E)-2-nonenal); 2_11((E,Z)-2,6-nonadienal); 2_12((E,E)-2,4-nonadienal); 2_13(Decanal); 2_14((E,E)-2,4-decadienal); 2_15(phenylacetaldehyde); 2_16(2-hydroxybenzaldehyde); 3_1(amyl acetate); 3_2(butyl acetate); 3_3(benzyl acetate); 3_4(hexyl acetate); 3_5((Z)-3-hexen-1-ol-acetate); 3_6(heptyl acetate); 3_7(octyl acetate); 3_8(2-methylpropyl acetate); 3_9(phenethyl acetate); 4_1(methyl butyrate); 4_2(methyl 2-methylbutyrate); 4_3(ethyl butyrate); 4_4(ethyl 2-methylbutyrate); 4_5(propyl butyrate); 4_6(butyl isobutyrate); 4_7(isobutyl butyrate); 4_8(ethyl (E)-2-butenoate); 4_9(butyl butyrate); 4_10(isoamyl butyrate); 4_11(ethyl pentanoate); 4_12(methyl hexanoate); 4_13(ethyl hexanoate); 4_14(ethyl heptanoate); 4_15(ethyl 3-(methylthio)propanoate); 4_16((E,E)-2,4-hexadienoic acid, ethyl ester); 5_1(6-methyl-5-hepten-2-one); 5_2(beta-cyclocitral); 5_3(geranylacetone); 5_4(beta-ionone); 6_1(guaiacol); 6_2(eugenol); 6_3(isoeugenol); 7_1(linalool), 7_2(Eucalyptol).

In the landraces of the Ibericus group, the most important VOCs accumulated were alcohols, esters, and aldehydes (Figure 12 and 13, Supplementary Table 4). Accessions of Piel de Sapo and Rochet landraces had significantly higher total alcohol contents than Amarillo and Blanco, but no significant differences were observed for the aldehyde or ester contents. Piel de Sapo presented the lowest total apocarotenoids and monoterpenoids, but high contents of phenylpropanoids. Tendral and Rochet presented high contents of phenylpropanoids and monoterpenoids, and Blanco presented low levels of both compounds.

The PCA of VOCs contents in the Ibericus revealed that, despite existing general trends, the spectrum of variation of sweet melon landraces overlapped (Figure 13). One of the

more variable landrace was Amarillo, which showed two types of behaviour. Accessions 20AM and 22AM-GO had higher contents of certain aldehydes and alcohols, such as (E)-2-octenal, (E,Z)-2,6- nonadienal, and (E,Z)-2,6-nonadien-1-ol, while 15AM and 23AM-EN stood out regarding ester accumulation. Specifically, 23AM-EN presented high contents of alkyl esters and, particularly, ethyl 2-methylbutyrate and ethyl 3-(methylthio) propanoate.

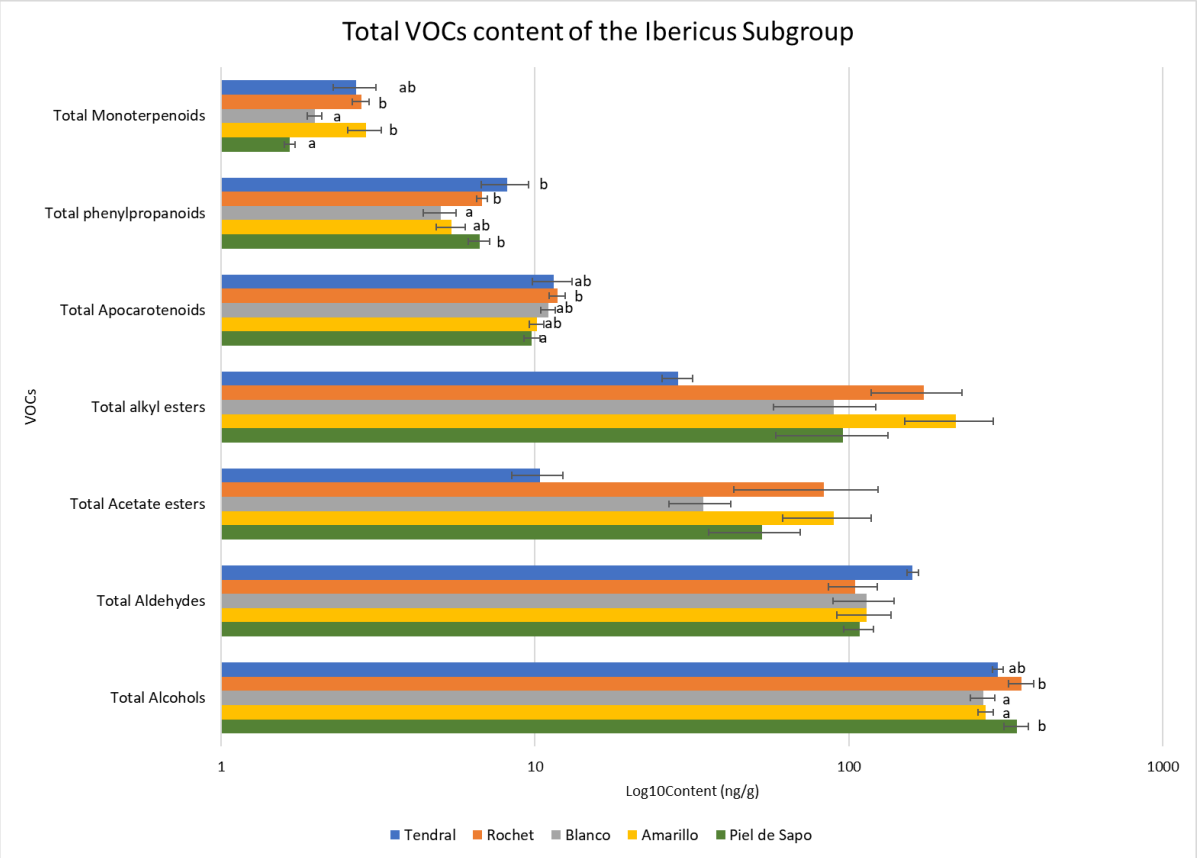


Figure 12. Total VOCs content for the Ibericus landraces. Different letters indicate significant differences (LSD test, P≤0.05). Those bars without letters had no significant differences in the ANOVA test (P=0.05) and no differences were found between landraces.

Figure 13. Principal component analysis for VOCs profile of Ibericus melon accessions. 1_1(1-pentanol); 1_2(1-hexanol); 1_3((Z)-3-hexen-1-ol); 1_4(1-octanol); 1_5(1-nonanol); 1_6((Z)-3-nonen-1-ol); 1_7((Z)-6-nonen-1-ol); 1_8((E,Z)-2,6-nonadien-1-ol); 1_9(1-decanol); 1_10(benzyl alcohol); 1_11(2-phenylethanol); 1_12(phenol); 2_1(Hexanal); 2_2(Heptanal); 2_3((E)-2-heptenal); 2_4((E,E)-2,4-heptadienal); 2_5((E)-2-octenal); 2_6(octanal); 2_7(nonanal); 2_8((Z)-6-nonenal); 2_9(benzaldehyde); 2_10((E)-2-nonenal); 2_11((E,Z)-2,6- nonadienal); 2_12((E,E)-2,4-nonadienal); 2_13(Decanal); 2_14((E,E)-2,4-decadienal); 2_15(phenylacetaldehyde); 2_16(2-hydroxybenzaldehyde); 3_1(amyl acetate); 3_2(butyl acetate); 3_3(benzyl acetate); 3_4(hexyl acetate); 3_5((Z)-3-hexen-1-ol-acetate); 3_6(heptyl acetate); 3_7(octyl acetate); 3_8(2-methylpropyl acetate); 3_9(phenethyl acetate); 4_1(methyl butyrate); 4_2(methyl 2-methylbutyrate); 4_3(ethyl butyrate); 4_4(ethyl 2-methylbutyrate); 4_5(propyl butyrate); 4_6(butyl isobutyrate); 4_7(isobutyl butyrate); 4_8(ethyl (E)-2-butenoate); 4_9(butyl butyrate); 4_10(isoamyl butyrate); 4_11(ethyl pentanoate); 4_12(methyl hexanoate); 4_13(ethyl hexanoate); 4_14(ethyl heptanoate); 4_15(ethyl 3-(methylthio)propanoate); 4_16((E,E)-2,4-hexadienoic acid, ethyl ester) 5_1(6-methyl-5-hepten-2-one); 5_2(beta-cyclocitral); 5_3(geranylacetone); 5_4(beta-ionone); 6_1(guaiacol); 6_2(eugenol); 6_3(isoeugenol); 7_1(linalool), 7_2(Eucalyptol).

In order to analyse the metabolomic profile of VOC accumulation, two correlation networks were obtained, considering the different volatile profiles of the Flexuosus-Chate group and Ibericus. In the Flexuosus–Chate group, strong correlations were found, in general, within alcohols, aldehydes, and alkyl esters and between these groups of volatiles (Figure 14). Within acetate esters, the correlations within group were not solid, though some of them correlated with specific alcohols and aldehydes. Apocarotenoids correlated among themselves and with certain alcohols and the phenylpropanoid eugenol. Interestingly, phenylpropanoids eugenol and guaiacol did not correlate with each other, but guaiacol correlated with several alcohols, esters and the phenylpropanoid linalool. Monoterpenoids eucalyptol and linalool did not correlated. The former correlated positively with ethyl butyrate and negatively with ethyl hexanoate, while the latter correlated with guaiacol and several esters.

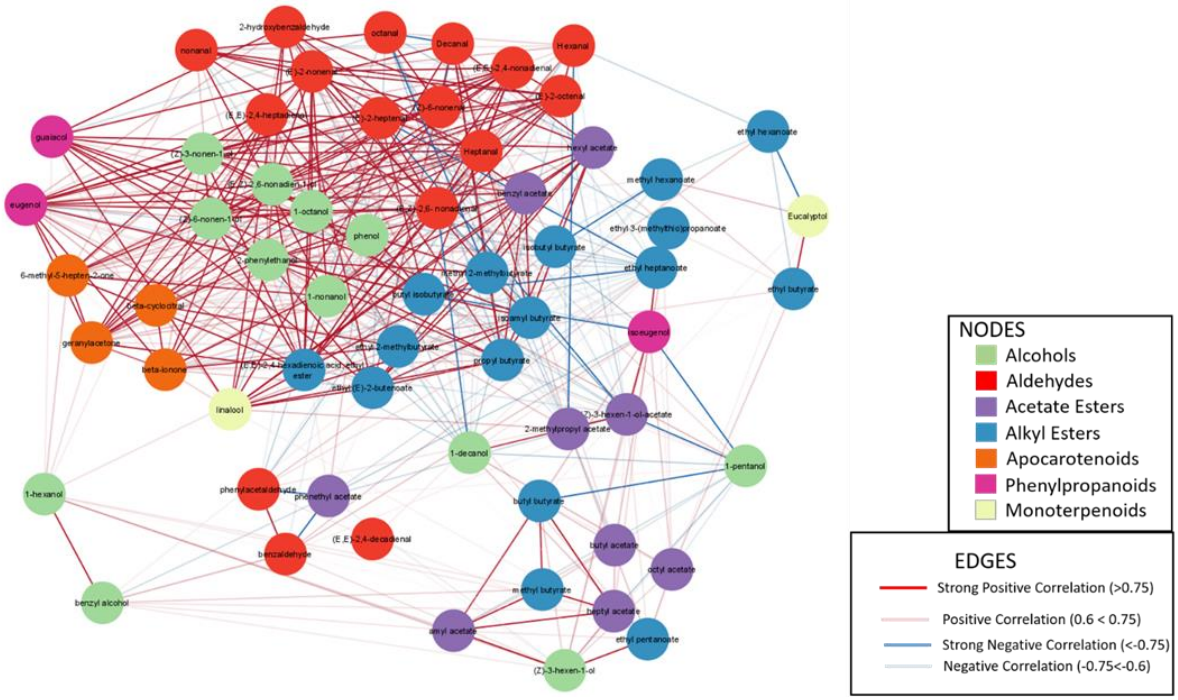


Figure 14. Correlation network analysis of the VOCs profile of the Flexuosus and Chate group. Each node represents one VOC. Positive and negative correlations are indicated (red for positive, blue for negative) and line thickness increases for higher correlation values.

The correlation network of the Ibericus group had a different pattern (Figure 15). Two main subgroups of aldehydes could be identified, while decanal and benzaldehyde appeared dispersed among other VOCs. Alcohols also appeared scattered in relationships with aldehydes and esters. Alkyl and acetate esters, on the other hand, appeared more correlated within and between each other. The relationships between apocarotenoids were not as clear as in the Flexuosus–Chate group, but some relationships were maintained, for example with 2-methybutyrate and (E,E)-2,4-hexadienoic acid ethyl ester.

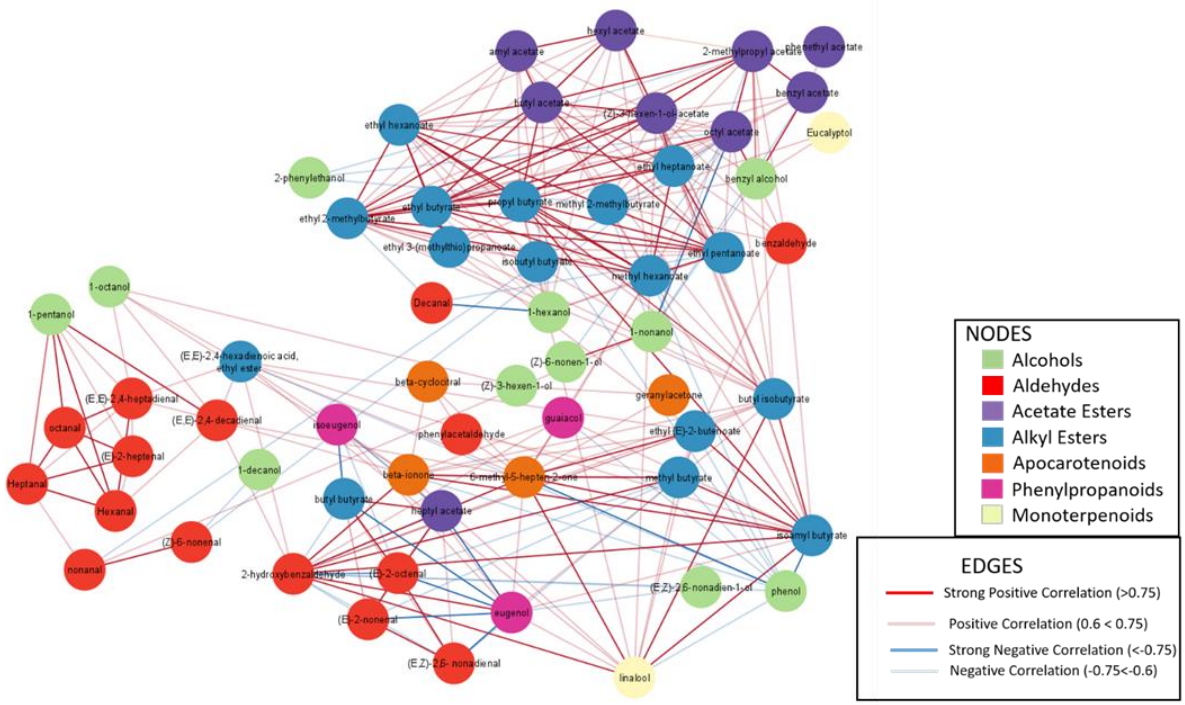


Figure 15. Correlation network analysis of the VOCs profile of the Ibericus group. Each node represents one VOC. Positive and negative correlations are indicated (red for positive, blue for negative) and line thickness increases for higher correlation values.

2.4. Discussion

The use of GBS technology to study and characterize melon germplasm has been widely employed with one of the most recently performed assays, employing 2083 world accessions (Wang et al., 2021). These studies all used the *Ape*KI restriction enzyme, which has permitted the comparison between different datasets, as well as usage of versions 3.5.1 or 3.6.1 of Melon DHL92. The present study was performed employing a different enzyme *Ms*II, which allowed the study of different areas of melon genome, as well as the usage of the most recent Genome v4. Despite this change, the genomic analysis offered further details but remains consistent with previous studies.

Our present study with 47 melon accession revealed a total of 96,267 raw SNP positions, after filtering resulted in 66,971 high quality SNPs. Previous studies (Gur et al., 2017; Kishor et al., 2021) have achieved different results, with a higher quantity of both raw and quality filtered SNPs. The criteria of SNP filtering do vary in different previous studies (Jung et al., 2020; Hyun et al., 2021; Wang et al., 2021), with the use of different minor allele frequency (maf) of either 0.01 or 0.05, as well as different maximum missing data. With more restrictive criteria, the resulting filtered SNPs will be less in quantity. Our criteria for maf and maximum missing data would serve as to conserve SNPs, which

are rare in the accessions employed, while the maximum missing enables us to have more genomic data for each position. The 66,971 SNPs filtered, as well as the use of accessions of a more exotic nature, allowed for a better study of melon landraces.

Our study showed a clear grouping of the exotic, Ibericus, and Flexuosus melons, although the I156 Ameri melon, 41CHA Chate, and 39AL melon displayed more distance from their respective groups. Our population structure analysis corroborated our PCA results, showing K=2 and K=3 to be the best and second best, similar to those obtained by other authors (Leida et al., 2015; Abu Zaitoun et al., 2018; Hyun et al., 2021; Kishor et al., 2021). Leida et al., (2015) found that Ibericus melons formed their own subpopulation (K=7, second best), while Flexuosus and Chate had mixed subpopulations without forming their own group. Abu Zaitoun et al., (2018) studied the 88 Palestinian snake melon local landraces and found two different subpopulations differentiated by their geographical origin. Our results show that the Spanish Flexuosus formed a tight-knit group with the Algerian 39AL being more distant from them.

The results obtained for the phylogenetic relationships of the melon accessions clearly revealed a distancing from the more exotic accessions, including a certain proximity between the Flexuosus and the Ibericus melons, similarly to results obtained by the population structure analysis. Our results also showed that the Ameri accession I156 was phylogenetically close to the cultivated melons, which has been previously observed in other works, e.g., by Nimmakayala et al., (2016) and Moing et al., (2020). In their study, Sabato et al., (2019) found, using 179 melon accessions and archaeological melon seeds, that the population containing the archaeological seeds also included Ameri melons and Italian Chate, as well as Flexuosus melons. This also coincides with our results as both the Ameri and Chate melons had high genomic similarity as compared to other groups. In the Ibericus clade, some of the melons appear to be more distant than the others. These seem to be those that contain a small percentage of ancestry belonging to the Flexuosus subpopulation, again validating the results obtained in the population structure analysis. Overall, our results show no clear grouping, according to the classification performed by Pitrat (2016), with some cultivars of different subgroups grouping together. The subgroups proposed by Pitrat (2016) only consider the exocarp of the fruits to differentiate between them. This simplification is quite useful in a commercial sense, but not from a genetic point of view. Esteras et al., (2013), in their study of 93 diverse melon accessions, examined the variability within the Spanish Inodorus landraces (which today comprise

the Ibericus Group) and found that some landraces did form clear groups, such as the Piel de Sapo, but in some cases, clusters could be found which contained melons of a different type, again finding that geographical origin can play an important role, as can their morphological type. Lázaro et al., (2017) were able to obtain seven groups based on their morphology, but the SSR analysis revealed two major groups, the first and largest containing Piel de Sapo, Tendral, and Winter, with some Yellow/White (with Tendral characteristics), while the second included Black, Mochuelo, and Yellow/White melons. In general, the present study, performed with a considerably higher number of SNPs, agrees with previously published information. The Ibericus accessions appeared mixed and, although some accessions of Piel de Sapo, Amarillo or Tendral tended to form groups, the truth is that accessions from different landraces appeared in the same subclusters, probably denoting a high level of shared genomes, with subtle genomic differences defining the landrace typical attributes.

The use of snpEff permitted the discovery of several interesting SNPs with a high impact on specific genes. For example, accessions 41CHA and 39AL, presented a SNP causing a stop gain in MELO3C018465, a Glycosyltransferase, which play an important role in maintaining cell homeostasis and regulates plant growth and development (Fu et al., 2018). MELO3C024563 (Putative UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase SPINDLY), MELO3C024565 (mRNA-decapping enzyme-like protein), and MELO3C015904 (SWR1-complex protein 4) have been detected to have modifications in our study, with the last one having a SNP which changes an amino acid in the sequence, mainly in the Ibericus and some of the Flexuosus melons. These genes have been previously reported by Kishor et al., (2021) to have an effect on sex expression in Oriental melon (*Cucumis melo* L.var. *makuwa*). Another interesting SNP detected in all the essayed accessions was located in gene MELO3C020760, a SAUR20-like aux-in-responsive protein. These genes (SAUR) are part of an important family related to auxin signal transduction, usually employed as marker genes (Zhang et al., 2017). Recent analysis have identified in cotton, an SNP *locus* (Gh_D08G1308) associated to plant salt tolerance which resulted to belong to SAUR-like auxin-responsive protein family (Zheng et al., 2021). Tzuri et al., (2015) found that SNPs *CmOr* (MELO3C005449) was related to fruit quality and responsible for fruit flesh colour, however this SNP was not detected in our sample. Other SNPs in important genes, such as MELO3C010779 (*CmACS-11*), an androecy gene which controls female flower

development (as can be seen in Boualem et al., (2015)), also do did not appear in our sample. Natarajan et al., (2016) identified several SNPs associated with defence genes against powdery mildew that have been observed in our samples (moderate change A/G), such as MELO3C002352 (Arginino succinate synthase). Moderate change A/C was detected in accessions 41CHA and 39AL on MELO3C022146 (TMV resistance protein N-like). Another moderate effect change T/C was detected on this gene, being detected on most exotic materials but also on some Ibericus melons, i.e., 32BL, 03PS, and the Spanish Flexuosus melons, which also Natarajan et al., (2016) detected. Another SNP change was detected in MELO3C022339 (Glutaredoxin protein) with a moderate change in Trigonus (G/T) and a low effect change (C/T) in some Flexuosus, Ibericus 21AM, Trigonus, and WM7. This last change has already been linked to resistance DEGs (differential expressed genes) associated with the response to *Tomato leaf curl New Delhi virus* (ToLCNDV), with the SNP being present in resistant WM7 but not in the susceptible Piel de Sapo variant Piñonet (Sáez et al., 2022).

Previous studies characterizing Spanish landraces revealed that most part of the variation was focused on the external colour, shape (globular to elongate), and rind patterns (Escribano and Lázaro, 2009). In general, all of them had large sizes. As reported by Lázaro et al., (2017), it seems that farmers selected large sizes that usually require longer growing cycles. Indeed, external appearance has been used in this and other crops, such as the tomato, as a key factor for the recognition of landraces by farmers and consumers, resulting in a clear differentiation in basic morphological traits (Casals et al., 2011). In this sense, our results revealed that although some level of variation exists within landrace, they can easily be distinguished in most cases. This is evident for the Flexuosus and Chate group compared to the Ibericus landraces, as in the former, fruits are long and elongated and lack seed cavities. However, even in this group, the Chate accession evaluated presented differences with most of the Flexuosus accessions, with shorter and wider fruits. Within the Flexuosus group, however, some degree of variability was found. Soltani et al., (2010) observed a high degree of variability in ribless accessions from Iran but not in the ribbed ones. In our case, only one ribless accession 39AL was evaluated and, in fact, presented a distinct phenotype compared to the ribbed materials in other traits. Nonetheless, the variability found seems to be more restricted than that described by Ali-Shtayeh et al., (2017) in Palestine with different landraces. In Spain, snake melons were already described by Columella (Janick et al., 2007), but, nowadays, after a strong

genetic erosion process, its cultivation is highly limited, which would explain the lower variability compared to that found in the Middle East, where they are still widely cultivated, well-known, and appreciated.

In the case of the Ibericus melon, landraces are mainly distinguished by external colour, even within greenish landraces. However, despite these general trends, it is possible to recognize specific accessions with special differentiation, as it would be the case of 23AM-EN (with a more intense yellow rind colour, longer, and more seed cavity) in Amarillo or 34TN (with a lighter green rind colour) in Tendral. Apart from external appearance, long-term conservation also can be used as a key differentiating factor. In the present study, the winter melon Tendral, with long-term conservation clearly differing from other landraces by its high flesh firmness, confirming a slow ripening pattern, which Barreiro et al., (2001) linked to limiting steps in the synthesis of ethylene. Artés et al., (1993) also found the highest firmness values in Tendral melons compared to other Spanish landraces, and, as concurred by de Barreiro et al., (2001), they described lower SSC values in this landrace. In our case, SSC values were not significantly different, though a trend towards lower values seemed evident. Apart from the evident difference in the shape of Flexuosus–Chate and Ibericus melons, the other main difference is related to their sugar and acid profile. Flexuosus are not sweet melons, and accordingly, SCC obtained in these accessions were much lower than the Ibericus accessions. In general, the values obtained were lower than those described in the similar Italian landraces Carosello and Barattiare that reach up to 3.6-4°Brix (Buttaro et al., 2009). Nonetheless, these results could be a consequence of environment and genotype x environment interactions, as similar Spanish accessions grown in different environments also offered SSC values ranging from 3.3 to 4.0°Brix (Flores-León et al., 2021). Burger et al., (2003) described in the Flexuosus melon Faqqous the genotypic combination *Suc*/*Suc* that prevents sucrose accumulation in the fruit. Accordingly, in our analysis sucrose levels in the Flexuosus–Chate melons remained under quantification limits.

The acidic profile also differed in this group. Both citric and malic acid were detected, but the predominant acid was malic in the Flexuosus-Chate group, while in Ibericus melons citric acid was predominant. Burger et al., (2003) suggested that the *so*/*so* mutation responsible for a high acidic profile was fixed in melons before the fixation of the *suc*/*suc* mutation that led to sweet melons. Cohen et al., (2014) also described that the *CmPH* allele is present in non-sweet melons and leads to substantial increases in the

acidic profile at the mature stage. In our case, the levels of acids at the immature commercial stage are lower than those of Ibericus landraces at the mature stage, but the predominant acid changes. This change in the profile would be justified by the trends of acid accumulation in melon, as in the immature stage malic acid is predominant and its concentration is progressively reduced increasing that of citric acid (Tang et al., 2010; Cohen et al., 2014).

In the Ibericus landraces, some specific trends in the acidic profile could be identified. Tendral tended to accumulate higher levels of malic acid and lower levels of citric, Amarillo tended towards lower malic acid contents. This trend might not be generalizable, as Albuquerque et al., (2006) did not identify significant differences in the citric and malic acid contents in Portuguese accessions of Tendral and Pele de Sapo. In any case, it seems evident that within each landrace it is possible to identify accessions with differing levels of acid accumulation, which would be useful for the development of breeding programs of sweet melon cultivars with an acidic profile, leading to a very unique sensorial profile (Burger et al., 2003, 2006).

Differences were also found in the sugar profile of the landraces. As expected, due to the aforementioned lower SSC values of Tendral melons, sucrose and hexoses contents were lower, a configuration that might be related to the ripening process, as it has been shown that hexoses content in Tendral melons increases during long-term conservation due to starch and sucrose hydrolysis (Barreiro et al., 2001). Interestingly, higher sucrose and hexoses accumulation was found in Rochet melons leading to higher sucrose equivalents values. This variable that weighs the sweetening power of each sugar has, in other crops, a higher relationship with sweetness perception (Baldwin et al., 1998), a key factor defining melon taste (Burger et al., 2006). In this context, further analysis of this profile would enable the establishment of breeding program targets in the future.

Regarding the VOCs profile, the Flexuosus and Chate melons exhibit a completely different profile compared to the Spanish Ibericus landraces, with high contents of aldehydes and alcohols and low contents of esters. This profile is highly related to the moment of evaluation, as fruits were harvested following commercial practices in the area, at im-mature state, and in melons, VOCs accumulate during the ripening process (Beaulieu and Grimm, 2001). Other works have evaluated the Flexuosus VOC profile at the mature stage and, considering the climacteric nature of these fruits, they were

characterized by a moderate to high content in ethyl esters (Esteras et al., 2018). Nonetheless, Tang et al., (2015) and Chen et al., (2016) both analysed the VOCs of the "Cai Gua" Flexuosus melon, harvested the fruits at commercial maturity, and found a similar VOCs profile, rich in aldehydes. Flores-León et al., (2021) analysed the aroma of a Spanish Flexuosus melon at a commercial maturity, finding that the main VOCs were aldehydes, followed by alcohols, and that the aldehydes were (E,Z)-2-6-nonadienal, followed by E-2-nonenal, hexanal, and benzaldehyde. In our study, among the main aldehydes found at the immature stage (E,Z)-2,6- nonadienal, (E)-2-nonenal, nonanal, and (Z)-6-nonenal stood out. Among them (E,Z)-2,6- nonadienal and (E)-2-nonenal have an important impact on melon aroma (Gonda et al., 2016). (E,Z)-2,6- nonadienal is reported to contribute to a cucumber-like/green odour, while (E)-2-nonenal provides a fresh/green odour. Only one Chate accession was evaluated, and some level of variability might be expected, as in the case of Flexuosus. Nonetheless, our results seem to point out that Chate melons would tend to show a richer VOC profile compared to Flexuosus melons, especially in the accumulation of aldehydes and alcohols.

The differences in the VOC profile between the Flexuosus, Chate, and Ibericus groups were also evident in the different network correlation analyses obtained with each group. Esteras et al., (2018) obtained a general network using a wide spectrum of melon variability where acetate and ethyl esters were highly correlated, a result also obtained by Freilich et al., (2015) with a RILs collection derived from a cross between the Momordica and Cantalupensis group or by Perpiñá et al. (2021) in an ILs collection introgressing the Makuwa genome, using Cantalupensis as the recurrent parent. This intercorrelation was also found in Ibericus melons, but in the Flexuosus and Chate melons acetate esters and butyrate esters, despite showing high correlation values within groups that are less intercorrelated. In this group, alcohols and aldehydes show high within-group and between-groups correlations, but the between-group correlation in the Ibericus melons is lost. These differences are probably related to the different harvesting dates for each material: immature in the case of Flexuosus and Chate and mature in the case of Ibericus melons.

The levels of esters detected in the Ibericus landraces were low, a characteristic typical of non-climacteric melons. Previous works have shown that Ibericus melons have levels of esters in the rind similar to Cantalupensis climacteric melons, but the contents are considerably reduced in the flesh (Esteras et al., 2020). Indeed, when wide collections of

melon germplasm have been analysed for their VOC profile, Inodorus melons, which include the Ibericus group, are grouped with sweet and non-sweet non-climacteric melons with low VOCs accumulation and non-sweet climacteric melons, such as Flexuosus, Chate, Ameri, and Momordica. In a study by Esteras et al. (2018), Amarillo, Tendral, and Blanco landraces were grouped in a cluster characterized by fewer lipid-derived VOCs and higher acetate esters, while Piel de Sapo melons appeared in another subcluster characterized by higher amounts of linoleic acid derivatives, such as pentanal and hexanal. Our results, with a higher number of populations per landrace, confirm these trends. Although the spectra of variation in the VOC profile of different landraces tend to overlap due to the high variability found, it seems clear that Amarillo, Rochet, and Blanco tend to accumulate higher amounts of esters, while Piel de Sapo melons are richer in aldehydes.

Interestingly, within each landrace a high level of variation is present. For example, in Piel de Sapo accessions, 8PS, 10PS, 09PS, 12PS, and 07PS are rich in aldehydes, especially (E,Z)-2,6-nonadienal, as compared to 11PS and 03PS, which accumulate lower levels of these compounds and higher levels of alkyl and acetate esters. (E,Z)-2,6-nonadienal provides green and cucumber-like aromatic notes (http://www.thegoodscentscompany.com/), while esters are typical of aromatic climacteric melons. Indeed, esters tend to contribute to fruity, sweet, and melon-like notes, whereas aldehydes and alcohols tend to contribute green, fresh, and cucumber-like notes (Gonda et al., 2016).

In other crops, such as tomato, an autogamous species, it has been demonstrated that spontaneous cross-pollination and seed-mixing during centuries of cultivation would generate variability, which is then reduced by farmer selection in external morphological traits, defining each landrace, but the variability would be maintained in internal attributes, such as those related to fruit quality (Cortés-Olmos et al., 2015). Indeed, in tomatoes it has been proven that the spectra of variation of landraces tend to overlap, identifying general trends in each landrace but maintaining a high level of variability (Cebolla-Cornejo et al., 2013). As in our case, accessions of different landraces appear clustered together in genomic analysis, suggesting a common genetic background. In the case of the tomato, it is known that most morphological traits defining the external appearance of the landrace are controlled by a highly limited number of genes (Cebolla-Cornejo et al., 2013). Consequently, despite detecting clear morphological differences,

genetic differences are not evident in genomic analysis. Spontaneous crosses and consequent strong farmer selection pressures applied to redirect segregating populations have been described in an autogamous species, such as the tomato. Therefore, it would be reasonable to expect a similar evolution in a cross-pollinating species, such as the melon. Indeed, our data suggest that the different Ibericus landraces share a common genomic background with subtle differences, and that, although general trends can be identified, a high degree of variation is observed from a metabolomic point of view. This profile is consistent with the application of strong selection pressures to recover the external traits defining landrace morphology, following spontaneous crossings between different landraces grown in the same area. As in other crops, such as tomato, it would be necessary to develop depuration programs in order to tackle on-farm in situ conservation strategies focused on high quality markets. Indeed, it would be crucial to select and promote those accessions with a sugar, acid, and volatile profile that maximizes the potential quality of the landrace.

2.5. Materials and Methods

2.5.1. Plant Material

A collection of 47 melon accessions representing Spanish diversity were included in this study (Supplementary Table 5). They belonged to landraces of the Ibericus (33), Flexuosus (5), and Chate groups, and were provided by Universitat Politècnica de València germplasm bank (http://www.upv.es/contenidos/BGCOMAV/indexc.html). Several external controls were also included: "T111", a Piel de Sapo breeding line (Semillas Fito S.A.); the introgression line "calc8-1" (Díaz et al., 2014), derived from an ILs population of a Momordica ac-cession into a PS cultivar; the Chate accession (Chate-Car (Esteras et al., 2013), coded as "41CHA") as an example of another traditional non-sweet melon cultivar; the accession 39AL, a Flexuosus melon from Algeria representing an alternative morphological variant; one accession from the Ameri group (Am-NesviGeor (Leida et al., 2015), coded as I156), three accessions of Momordica group (Mom-PI414 (Esteras et al., 2013), coded as I176; two individuals of IC274006 (Dhillon et al., 2007)(coded as IC-3 and IC-4); one of the Kachri group (WM7 (Roy et al., 2012)); and one from the Agrestis group (Trigonus (Díaz et al., 2017), coded as Tri).

2.5.2. Experimental Design

Melon plants were cultivated in the greenhouse facilities located in the "Fundación Cajamar" in Paiporta, Valencia (39°25′05.8′N 0°25′03.4′W). A total of 3 plants per accession were transplanted at the start of April. The plants were transplanted onto substrate bags of 29kg with a 3:7 coconut chips to coconut fibre ratio. Irrigation was performed using drip systems. Nutrients were supplied to the plants through the irrigation water supply. The plants were pruned so as to regulate the vegetative growth and flowering of the plants. Melon fruits were collected when fruits achieved commercial maturity. A total of 5 fruits per accession were collected and characterized.

2.5.3. DNA Extraction and Genotyping-by-Sequencing Libraries

Prior to transplantation, leaf tissue from each melon accession was collected and frozen with liquid Nitrogen and stored at -80°C. The DNA extraction was performed following the CTAB protocol (Doyle, 1991). The extracted DNA was resuspended in MilliQ water. GBS libraries were then prepared, employing the restriction enzyme MsII, using the Illumina NovaSeq 6000 SP FC platform (Illumina Inc, San Diego, CA, USA) in the LGC Genomics GmbH (Berlin, Germany), following the procedure by Elshire et al., (2011). They raw reads were then quality-filtered, adapted, enzyme-clipped, and processed (2 × 150bp).

2.5.4. SNP Calling and Analysis

The high quality paired-end reads were first mapped to the latest version of Melon reference genome (v4.0), available at melonomics.net (Castanera et al., 2020), employing Bowtie2 v2.3.4.1 (Langmead and Salzberg, 2012) with the "--very-sensitive" option. This ensures a slower but more sensitive and accurate mapping. The resulting mapping files (SAM format) were then converted into BAM format with Samtools v1.11 (Li et al., 2009). Freebayes v1.3.4 (Garrison and Marth, 2012) was used to call the SNPs, setting a mini-mum mapping quality cut-off of 40, minimum base quality of 20, minimum base count of 10, and eliminating indels. This raw SNP file was further filtered using Vcftools (Danecek et al., 2011), with a minor allele frequency (--maf 0.01), and maximum missing count (--max-missing-count 4). The variant calling file was further analysed using the SnpEff program (Cingolani et al., 2012) to view the SNPs with the highest effect.

2.5.5. Population Structure

Firstly, a principal component analysis (PCA) was performed employing Tassel 5 software for Windows (Bradbury et al., 2007) and the results visualized using CurlyWhirly Software for Windows. To investigate the population structure of the 47 accessions, an ADMIXTURE (Alexander et al., 2009) analysis was performed. The admixture-linux-1.3.0 was run by employing the default parameters with an unsupervised mode with K=1 to 10. The cross-validation error for each K was obtained with the -cv option, identifying the best suitable modelling. The cross-validation error K graph and Ancestry Q files were plotted using R.

2.5.6. Phylogenetic Relationship

All SNPs were concatenated into a single pseudo-sequence for all the 46 accessions. ClustalW 2.1 (Larkin et al., 2007) for Linux was employed to perform an alignment of the sequences. PAUP* (Swofford, 2002) version 4 for Windows was used to select the best substitution model, resulting in the choosing of model GTR+G nucleotide substitution model. Finally, the aligned sequences were introduced in RAxML v8.2.12 (Stamatakis, 2014) to create the maximum-likelihood tree, inputting the substitution model selected (-m GTRGAMMA), indicating the outgroup, in this case being Trigonus accession (-o “Tri”), and a bootstrap of 1000 (-N 1000). Finally, the program MEGAX (Kumar et al., 2018a) for Windows was employed to visualize and perform a cut-off low scoring bootstrap branches (cut-off at 50% bootstrap). Evolview (Subramanian et al., 2019) was used to edit the tree.

2.5.7. Linkage Disequilibrium Decay

To understand the variability between the different melons, a pairwise estimate of the linkage disequilibrium (LD) was performed employing the program PopLDdecay (Zhang et al., 2019). The LD was analysed for the total melons studied, as well as 3 different subgroups, the Ibericus melons, Flexuosus, and Chate melons, and the exotic germplasm. LD values (r2) with respect to the genetic distance (kbp) were plotted. A maximum distance between loci of 1500kbp was used to study the LD decay.

2.5.8. Melon Characterization

The collection of Spanish accession was characterized. A total of 5 melons per accession were harvested at their commercial maturity state. Fruits were characterized for fruit weight (FW in g, measured with a digital scale), fruit length, diameter, and cavity (FL, FD, and FC, in cm, measured with a ruler), rind thickness (in mm, with a Vernier calliper), rind and flesh firmness (RF and FF, measured with a penetrometer in kg/cm^2), fruit pH (universal pH indicator paper), and soluble solids content (SSC, °Brix, measured with some drops of juice using a hand-held Pocket refractometer (PAL-α), Atago CO., LTD, To-kyo, Japan). Finally, both the fruit flesh and rind colours were measured in Hunter L, a, and b coordinates (CR-400 colorimeter, Konica Minolta, Inc., Tokyo, Japan). Additionally, out of the 5 fruits, 3 were sampled for their sugar and acid content.

2.5.9. Metabolomic Analysis

Accessions of the Spanish landraces were also analysed by determining sugar and acid and volatile profiles. For that purpose, three fruits were randomly selected. In the Ibericus group, a 5cm cross-section of the fruit in the equatorial area was obtained, rind was discarded, and the edible flesh was homogenized (Silent Crusher M; Heidolph, Schwabach, Germany) and frozen at -80°C until analysis. In the case of the Flexuosus and Chate groups, the whole fruit was sampled after discarding the rind. Aliquots were used to measure sugars (glucose, fructose, and sucrose) and organic acids (malic, citric, and glutamic acids) employing capillary electrophoresis and volatile organic compounds (VOCs).

For sugar and acid analysis, the methodology used in (Martí et al., 2019) was followed using an Agilent 7100 system (Agilent Technologies, Waldbronn, Germany). Samples were defrosted in the dark and centrifuged at 510 revolutions g^{-1} for 5 min. The upper phase was diluted with deionized water at a 1:20 ratio. It was then filtered employing 0.22µm membranes (Costar® Spin-X®, Corning, Amsterdam). Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with dimensions of 50 µm internal diameter, 363 µm external diameter, 67 cm total length, and 60cm effective length were used for the separation process. Before using for the first time, the capillaries were conditioned by flushing 1 mol L-1 NaOH at 95 kPa for 5 minutes at 50°C, 0.1 mol L−1 NaOH for 5 minutes at 20°C, and then 10 minutes with deionized water. At the beginning of each sequence, the capillary was flushed for 30 minutes at 20°C with the running

buffer, which consisted of a 20 mmol L−1 2,6-pyridine dicarboxylic acid and 0.1% w:v hexadimethrine bromide solution adjusted at pH 12.1. Between samples, the capillary was flushed with 58 mmol L−1 sodium dodecyl sulphate (2 min) and running buffer (5 min). Between runs, the capillary was flushed with 58 mmol L−1 SDS (2 min) and BGE (5 min). Samples were injected hydrodynamically at 3400 Pa for 10 s and separations were performed at −25 kV and 20°C. Absorbance was measured at 214 nm. Results were expressed in g kg^{-1} of fresh weight. Sucrose equivalents were calculated as the sum of sugar contents weighed with their sweetening power (Koehler and Kays, 1991).

2.5.10. Volatile Organic Compound Analysis

The analysis of VOCs (Supplementary Table 6) was adapted from that described in (Perpiñá et al., 2021). Solid Phase Extraction (SPE) cartridges used for retention were conditioned with 5 mL of diethyl ether and 5 mL of n-hexane and then dried for 10 min. Frozen samples were defrosted in the fridge. Once thawed, 30 g of the sample were weighed into a 150 mL Erlenmeyer flask with a stopper. The extraction process was carried out employing a Purge and Trap headspace system, with the SPE cartridge for the outlet tube and N2 gas for the inlet tube. The samples were extracted for 49 min at 40°C using magnetic agitation and nitrogen flow of 1.6 mL min−1. Afterwards, 5 ml of each diethyl ether–hexane 1:1 (v:v) solution and diethyl ether were used to elute the cartridges. Finally, the collected elution solvents were evaporated to 0.5 mL at 35°C under a nitrogen flow. Resulting extracts were divided into two aliquots in sealed gas chromatography (GC) vials and frosted at -20ºC until analysis. VOCs chromatographic analysis was performed employing a TQ-GC gas chromatography system from Waters (Milford, MA, USA), equipped with a Supelcowax column of 30 m x 0.25 mm x 0.25 µm (Sigma-Aldrich, San Luis, MO, USA). Helium was used as carrier gas at a flow of 1 mL/min. The injection was performed in splitless mode, injecting 1 µL of sample at 280°C. The temperature program started at 40°C (5 min), was then raised to 160°C (4°C min^{-1}), and continued to 250°C (30°C min−1), which was maintained for 2 min. The mass spectra were acquired in selected ion monitoring (SIM) mode using the characteristic ions for each compound. Electron ionization in positive mode was used at a temperature of 250°C and 230°C for the interphase and the ion source, respectively.

2.5.11. Statistical Analysis

StatGraphics Centurion version 17.2.04 for Windows and IBM SPSS Statistics 25 for Windows were used to perform the analysis. R v4.1.2 for Windows, with usage of packages “ggplot2” (Wickham, 2016). Correlation networks analysis was conducted with the Expression Correlation plug-in (www.baderlab.org/Software/ExpressionCorrelation) for the Cytoscape software v3.9.1. (Shannon et al., 2003). Nodes represent each individual volatile compound. Positive correlations were indicated in red edges and negative in blue. Principal component analysis (PCA) of morphological data and VOCs accumulation were performed using S-Plus v. 8.01 for Windows (Insightful Corp., Seattle). A biplot representation was then obtained, including the scores of data points and the loadings of each variable for each principal component.

Supplementary Files

All Supplementary files (1-6) are available for download at: https://www.mdpi.com/article/10.3390/ijms23137162/s1.

Chapter 3. Grafting Snake Melon [*Cucumis melo* L. subsp. *melo* var. *flexuosus* (L.) Naudin] in Organic Farming: Effects on Agronomic Performance; Resistance to Pathogens; Sugar, Acid, and VOC Profiles; and Consumer Acceptance

Chapter 3. Grafting Snake Melon [*Cucumis melo* L. subsp. *melo* var. *flexuosus* (L.) Naudin] in Organic Farming: Effects on Agronomic Performance; Resistance to Pathogens; Sugar, Acid, and VOC Profiles; and Consumer Acceptance

This chapter consists on the effect grafting has on a snake melon cultivar, and its performance under organic farming conditions. Several different localizations were employed, with different limiting factors both biotic and abiotic. The main pests and pathogens affecting snake melon were noted, and the resistance to 2 different pathogens was performed. The effect of 5 different rootstocks (2 *Cucurbita* commercial hybrids and 3 *Cucumis*) on the agronomic performances, as well as their metabolic profiles and the consumer acceptability was also evaluated. Also, in this chapter is included the First Report of *Neocosmospora falciformis* Causing Wilt and Root Rot of Muskmelon in Spain. Chapter was published in the **Frontiers in Plant Science** Journal and in **Plant Disease.**

This work was performed as part of the projects PROMETEO/2017/078 funded by Conselleria d'Educació, Investigació, Cultura i Esports (to promote excellence groups), as well as the project nos. AGL2017-85563-C2-1-R and RTA2017-00061-C03-03 (INIA) cofunded by the Spanish Ministerio de Ciencia, Innovación y Universidades and FEDER funds.

This work was performed by Cucurbits Breeding Group of the COMAV, in collaboration with Dr. Santiago Garcia and Dr. Juan Jose Ruiz from the Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández. Also, collaboration with thePlant Protection Unit and Horticulture Unit of the AgriFood Institute of Aragon (IA2), from the Universidad of Zaragoza, Dr. Vicente González, Dr. Ana Garcés-Claver and Dr. Carmen Julián. Collaborating also with this work was Dr. Maria Ferriol from the Instituto Agroforestal Mediterráneo (IAM-UPV), and with Dr. Jaime Cebolla Cornejo and Dr. Raul Martí from the Joint Research Unit UJI/UPV.

Chapter 3. Grafting Snake Melon [*Cucumis melo* L. subsp. *melo* var. *flexuosus* (L.) Naudin] in Organic Farming: Effects on Agronomic Performance; Resistance to Pathogens; Sugar, Acid, and VOC Profiles; and Consumer Acceptance

Alejandro Flores-León[1], Santiago García-Martínez[2], Vicente González[3], Ana Garcés-Claver[4], Raúl Martí[1], Carmen Julián[3], Alicia Sifres[1], Ana Pérez-de-Castro[1], María José Díez[1], Carmelo López[1], María Ferriol[5], Carmina Gisbert[1], Juan José Ruiz[2], Jaime Cebolla-Cornejo[1] and Belén Picó[1*]

1 Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain,
2 Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández, Orihuela, Spain,
3 Plant Protection Unit/Instituto Agroalimentario de Aragón-IA2, Centro de Investigación y Tecnología Agroalimentaria de Aragón, Universidad de Zaragoza,Zaragoza, Spain,
4 Horticulture Unit/Instituto Agroalimentario de Aragón-IA2, Centro de Investigación y Tecnología Agroalimentaria de Aragón, Universidad de Zaragoza, Zaragoza, Spain,
5 Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Valencia, Spain

Correspondence: mpicosi@btc.upv.es

3.1. Abstract

The performance of snake melon [*Cucumis melo* var. *flexuosus* (L.)] in organic farming was studied under high biotic and salt stress conditions. Soilborne diseases (mainly caused by *Macrophomina phaseolina* and *Neocosmospora falciformis*), combined with virus incidence [*Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), and *Tomato leaf curl New Delhi virus* (ToLCNDV)] and *Podosphaera xanthii* attacks, reduced yield by more than 50%. Snake melon susceptibility to *M. phaseolina* and *Monosporascus cannonballus* was proved in pathogenicity tests, while it showed some degree of resistance to *Neocosmospora keratoplastica* and *N. falciformis*. On the contrary, salt stress had a minor impact, although a synergic effect was detected: yield losses caused by biotic stress increased dramatically when combined with salt stress. Under biotic stress, grafting onto the melon F_1Pat81 and wild *Cucumis* rootstocks consistently reduced plant mortality in different agroecological conditions, with a better performance compared to classic *Cucurbita* commercial hybrids. Yield was even

improved under saline conditions in grafted plants. A negative effect was detected, though, on consumer acceptability, especially with the use of *Cucurbita* rootstocks. *Cucumis* F_1Pat81 rootstock minimized this side effect, which was probably related to changes in the profile of sugars, acids, and volatiles. Grafting affected sugars and organic acid contents, with this effect being more accentuated with the use of *Cucurbita* rootstocks than with *Cucumis*. In fact, the latter had a higher impact on the volatile organic compound profile than on sugar and acid profile, which may have resulted in a lower effect on consumer perception. The use of *Cucumis* rootstocks seems to be a strategy to enable organic farming production of snake melon targeted to high-quality markets in order to promote the cultivation of this neglected crop.

3.2. Introduction

Melon (*Cucumis melo* L.) is a member of the Cucurbitaceae family, which comprises several economically important vegetables, including cucumber, watermelon, squash, pumpkin, and gourds (Pitrat, 2008). It is a highly variable species with a relevant economic importance worldwide (global production 27 Mt) (FAO, 2018). The top 10 producing countries are in Asia (China, Turkey, India, Kazakhstan, and Iran), Africa (Egypt), Europe (Spain), and America (United States, Guatemala, and Mexico). Spain is the 8th highest melon producer in the world, and the first producer and exporter of the European Union (Eurostats, 2019). *C. melo* has been produced in Spain since Roman times. The Roman author Lucius Junius Moderatus *Columella* already mentioned in his writings the cultivation of a snake-shaped cohombro, a *C. melo* belonging to the botanical variety flexuosus (Hammer and Gladis, 2014), which produced non-sweet fruits. With the arrival of Islam to the Iberian Peninsula, sweet casaba type melons as well as cucumbers (*G* L.) were introduced into Europe from Central Asia (Paris et al., 2012a, 2012b). The preference of sweet casaba melon as fruit and cucumber as vegetable displaced and diminished the consumption of flexuosus-type melons.

Snake melon is also an ancient crop highly appreciated in other Mediterranean, Asia Minor, North Africa, and Near and Middle East countries, where it is known by different local names such as Armenian cucumber, Fakous, Kakri, Cucumaru, Hiti, or Mekte, among others (Merheb et al., 2020). Its long non-sweet, non-aromatic fruits are collected when immature and are consumed as fresh vegetables or pickled (Pitrat, 2016; Solmaz et al., 2016a). They are part of many traditional recipes, used like cucumbers because of

their appearance and taste, and are also used in traditional medicine (Ibrahim, 2017). This is one of the less studied melons, although the genetic diversity of Middle East landraces has started to be known (Dastranji et al., 2017; Abu Zaitoun et al., 2018; Merheb et al., 2020).

Despite the fact that it has been a neglected crop, snake melons are still commonly grown in many Mediterranean, Asian, and African countries. In Spain, they remain under cultivation in eastern coastal regions (Valencia, Alicante, and Murcia), where local farmers have conserved landraces locally known as "alficoz or alficoç." Some of these landraces are cultivated for self-consumption and for local markets as the short shelf life of the fruits, much shorter than that of the cucumbers, hampers their commercialization in distant markets. However, this crop is threatened by severe genetic erosion and Spanish snake melon landraces are maintained *ex situ* in the Universitat Politècnica de València GeneBank.

There exists a global interest for the recovery of heirloom vegetables. Farmers markets, where consumers have access to locally grown vegetables, have continued to rise in popularity. Consumer demands on traditional products are more focused on those produced under sustainable or organic production systems. Organic farming values the use of plant diversity and may constitute the ideal context to promote the cultivation of snake melon (Reganold and Wachter, 2016). The consumer perception of organic food as providing differentiating benefits related to health, nutritional value, and maximum respect to the environment and animals (Massey et al., 2018) can be the basis to revitalize its demand, encourage its cultivation, and promote *in situ* conservation.

Melon organic farming faces several limiting factors that need to be overcome to be economically sustainable. One of the major limitations is the negative effect on yield and yield stability (Seufert et al., 2012; Schrama et al., 2018). Pests and diseases are main factors causing this loss of productivity, as the use of agrochemicals is very limited. Also, the fact that the local production of this crop is often restrained to marginable lands, where not only biotic but also abiotic stressful conditions occur, enhances the challenge.

One way of controlling diseases would be the use of resistant varieties. However, the alficoz, as many heirloom landraces, has been often neglected in melon breeding programs as the main focus was on sweet melons. Some breeding has centered on yield

and earlier production, but no cultivars with introgressed resistances are available (Abdelmohsin et al., 2015). Grafting is an effective approach to deal with soilborne pathogens, increasing yields in stressful environments (Bie et al., 2017). Grafting is used in Cucurbits, mainly in watermelons (about the 90% of the watermelon production in many countries is obtained from grafted plants). Grafting is not so popular in melons due to the lack of appropriated rootstocks. Melon grafting is performed primarily to face soilborne pathogens such as *Fusarium* and *Monosporascus* wilts or nematodes (Kyriacou et al., 2018). Compatibility in rootstock–scion combinations and the lack of negative effect on fruit quality are necessary for the successful performance of grafted plants (Pico et al., 2017; Leonardi et al., 2017).

There are many previous studies that report the use of different rootstocks for sweet melons and describe their impact on their aspect and flavor, which is particularly affected by the sugar/acid content and aroma (Colla et al., 2006; Crinò et al., 2007; Condurso et al., 2012; Verzera et al., 2014). These studies clearly show that grafting can have an impact on the quality profile of sweet melons. Also, the impact of grafting in flavor perception through the accumulation of certain compounds has been reported in watermelons (Guler et al., 2014; Fredes et al., 2017; Tripodi et al., 2020). It seems that these effects would be limited in species harvested before biological maturity such as cucumber (Davis et al., 2008a), as it would be the case for snake melon.

Only few studies have analyzed flavor preferences of snake melons and their relationship with metabolite accumulation. A recent study with accessions from the Middle East suggested that crispy and non-hollow fruits are not necessarily tastier, but softer and hollow fruits are seldom associated with very good taste (Omari et al., 2018). They also highlighted the existence of a high diversity of taste perception within types. Grafting snake melon has been studied (Maroto et al., 2003), but not the impact of grafting on the quality of this crop. The objective of this study is to fill the gap in the knowledge of the effect of grafting on snake melon agronomic performance under organic farming. The effect of biotic and abiotic stressful conditions is evaluated, as well as the impact of the different types of rootstocks, melon, wild *Cucumis*, and *Cucurbita hybrids*, on fruit characteristics, sensory perception, and sugar, acid, and volatile accumulation.

3.3. Materials and Methods

3.3.1. Fields Characteristics

The study was performed in three different fields of the Valencian Community (Eastern Spain) (Supplementary Figure 1). The first was located in Moncada, a small town in the province of Valencia (39°33′26.8″ N, 0°25′06.5″ W), in a field with no previous history of melon cultivation, as it had been a Citrus orchard for the previous 20 years (Supplementary Figure 1A). The second field assay was located in La Punta, a suburban area of the city of Valencia (39°26′41.3″ N, 0°21′'14.9″ W), with a long history of melon cultivation (Supplementary Figure 1B). The third field assay was in the province of Alicante, in the Natural Park of Carrizales (38°08′32.8″ N, 0°42′44.7″ W). The cultivation plots of Carrizales used in 2018 and 2019 were cultivated with alfalfa and oat, respectively, for the three previous years, and then, 6 months before the assay, were fallowed (Supplementary Figure 1C). Climate data were obtained from public databases (La Punta: Agencia Estatal de Meteorología[1]; Moncada and Carrizales: Sistema de Información Agroclimática para el Regadío[2]).

3.3.2. Plant Material

A local snake melon cultivar traditionally known as "Alficoz valenciano" obtained from the GeneBank of the Universitat Politècnica de València (accession number BGV004853) was used for the study. This accession was selected in a previous study considering its good field performance regarding yield and quality and its good consumer acceptance.

In order to analyze the effect of grafting on agronomic performance, fruit quality, and sugar, acid, and volatile profile, five rootstocks were selected and used to graft the snake melon cultivar (Supplementary Figure 2). All graftings were compatible, resulting in fully developed adult plants. Rootstocks included F_1Pat81, an experimental inter-subspecific cross between *C. melo* subsp. *agrestis* Pat 81, resistant to *Monosporascus cannonballus* (Roig et al., 2012), and *C. melo* subsp. *melo* Piel de sapo, two hybrid rootstocks between wild *Cucumis* species, Fian (hybrid *Cucumis ficifolius* × *Cucumis anguria*), and Fimy (hybrid *C. ficifolius* × *Cucumis myriocarpus*), resistant to different soilborne diseases (Cáceres et al., 2017), and two commercial hybrid *Cucurbita maxima* × *Cucurbita moschata* rootstocks (Shintoza and Cobalt). Non-grafted (NG) snake melon plants were

used as controls in all the assays. The grafting technique employed for the experiments was the “Tongue approach grafting” method.

3.3.3. Crop Management

The experiments were conducted under organic production in the three different fields. The three fields represent three different agro-ecological situations, frequent in melon cultivation. All fields have a clay soil (more clay-loam in Moncada). In the latter field, Moncada, the lack of melon cultivation in the 20 previous years provided unstressed conditions. In La Punta, fungal stress had been reported, due to the accumulation of soilborne pathogens after repetitive melon cultivation. In the natural park of Carrizales, the traditional irrigation system uses water coming from a drainage water channel. This water is characterized by its high electrical conductivity, thus resulting in salt stress conditions.

In 2018, the three fields were cultivated. NG plants were cultivated in the three fields. Additionally, in La Punta and Carrizales, snake melon was also cultivated and grafted onto two rootstocks, the experimental melon hybrid F_1Pat81 and the commercial Cucurbita hybrid Cobalt. A randomized complete block design with four plants per treatment and block was used (four blocks).

In 2019, plants were grown in La Punta and Carrizales. This year, the snake melon was cultivated NG and grafted onto five selected rootstocks, the two used in 2018 (F_1Pat81 and Cobalt), the two experimental wild *Cucumis* rootstocks Fian and Fimy, and one additional commercial Cucurbita hybrid, Shintoza, using the same experimental design.

Snake melon plants were transplanted at the 2–3 true leaf stage, between the end of March and the first week of April in the three cultivation sites, in 2018, and the first week of May in 2019. During both years, in the fields, sweet melon Spanish varieties were also cultivated, both grafted and NG.

In Moncada, plants were transplanted onto ridges with black mulch with a separation of 1.1 m between ridges and 1 m between plants. The field was prepared by subsoiling and sheep manure (1 kg m^{-2}) was applied, and afterward, the soil was milled to break down clods of soil and mix the manure. Two applications of azadirachtin with *Equisetum arvense* and diatomaceous earth were performed to control aphids and whiteflies. In La

Punta, a black plastic mulch was also used, with the separation of 2 m between ridges and 0.6 m between plants. The only further treatment performed was the weeding of the soil between ridges. In Carrizales, plants were spaced 2 m between ridges and 0.9 m between plants, with an additional 0.5 m between each treatment and using black plastic mulch. The field was prepared by subsoiling and applying sheep manure (3 kg m^{-2}), and afterward, the soil was milled to break down clods of soil and mix the manure. After transplanting, the plants were covered with a thermal blanket until they reached the appropriate size. The blanket enabled the control of both temperature and humidity, also acting as a barrier against pests. During the crop cycle in Carrizales, two applications were performed with humic and fulvic acids diluted in the water supply. During fruit ripening, one application through the water supply was done of potassium sulfate. Every 15–20 days, sulfur (15–25 kg ha–1) and Vibafusan G (15 kg ha^{-1}) were applied. Finally, two foliar treatments of biostimulator F-Aspir (5 L ha^{-1}) were applied. Finally, in terms of irrigation, drip irrigation was used in Carrizales and Moncada, whereas in La Punta, water was supplied using flood irrigation once every 2 weeks.

3.3.4. Soil and Water Conductivity

For each assayed field, 10 soil samples from different sites were collected, and their conductivity was measured. The soil samples were homogenized and dried at room temperature. The dry soil samples were then sieved (2 mm) and the soil conductivity (dS m^{-1}) was determined using the method described by Primo and Carrasco (1980). The conductivity of the water supply was measured in collected water samples with an electrical conductivity meter (CM35, CRISON, Barcelona, Spain).

3.3.5. Pathogen Detection

All the plants showing virus symptoms were sampled, and the viruses were identified following (Pérez-De-Castro et al., 2020). Root samples were obtained from plants that showed symptoms of soilborne pathogens and they were analyzed to identify the causal agents. Small pieces (0.5–1 cm) from the cortical necrosis of both lower stem and upper root were surface disinfected for 1 min in 1.5% NaClO, washed four times with sterilized bi-distilled water, and plated onto potato dextrose agar (PDA) amended with streptomycin sulfate (0.5 g/L) to avoid bacterial contamination. Plates were incubated at 25°C in the dark for 3–5 days. Then, emerging colonies were transferred to 6 cm Ø PDA plates, and each isolate was subcultured to get pure cultures for subsequent characterization.

Fungal isolates obtained were identified and characterized morphologically on the basis of comparison of their different somatic and/or sexual and asexual reproductive structures. A molecular characterization was made by PCR amplification of the ribosomal ITS fragment for most of the isolates, and TEF-1α and RPB2 gene fragments in the case of certain *Fusarium* species, using ITS1/ITS4 (White et al., 1990), EF1/EF2 (O'Donnell et al., 1998), and fRPB2-7cF/fRPB2-11aR (Reeb et al., 2004) primers, respectively. Sequences obtained allowed the identification of isolates by their comparison with homologous sequences deposited in public databases like GenBank (using BLASTn tool) or Fusarium ID Database3, as well as by performing phylogenetic reconstructions employing Bayesian inference methods from multilocus alignments of combined genomic regions for some of the mentioned Fusarium taxa.

3.3.6. Pathogenicity Tests Against Fungal Pathogens

The degree of susceptibility/tolerance of the snake melon cultivar against four of the most frequently isolated soilborne pathogens, the fungi *Neocosmospora keratoplastica* and *Neocosmospora falciformis*, recently detected causing Fusarium wilt in melon in Spain for the first time (González et al., 2020c, 2020b), and *M. cannonballus* and *Macrophomina phaseolina*, previously reported to cause severe vine decline and charcoal root rot in melons (Castro et al., 2020; de Sousa Linhares et al., 2020), was evaluated. Isolates from Carrizales were used for *Neocosmospora* spp. and from La Punta for *M. cannonballus* and *M. phaseolina*, respectively.

For *N. keratoplastica* and *N. falciformis*, plants of the snake melon cultivar and "Don Quixote" Piel de Sapo (commercial control not previously tested against these pathogens) were grown with a sterilized substrate in plastic trays. When plants reached the 15-day-old stage, they were uprooted and artificially inoculated by root dip for 2 min into a conidial suspension of 5 × 106 conidia ml–1 of each isolate (seven plants with each pathogen and three non-inoculated controls). Then, inoculated plants were planted into pots containing sterilized substrate and maintained in a growth chamber for 30 days at 26°C (González et al., 2020c). Disease severity was evaluated at 30 days after inoculation (DAI), using the following scale: 1 = no symptoms; 2 = beginning of wilting or yellowing on leaves; 3 = all leaves completely wilted, stem standing; and 4 = dead plant (Cothiere et al., 2016). Plants with a disease severity score (at 30 DAI) lower than 2 were considered to be resistant (R); between 2 and 3, moderately resistant (MR); and higher than 3,

susceptible (S). The area under the disease progress curve (AUDPC) was calculated for each inoculation (Perchepied and Pitrat, 2004) with the formula:

$$AUDPC = \sum_{i} [(x_i + x_{i+1} - 2)/2](t_{i+1} - t_i)$$

where i = 1 to 4 scorings, xi = mean disease score of each plant at date i, xi + 1 = mean disease score of each plant at date i + 1, and ti + 1−ti = number of days between scoring date i and scoring date i + 1. The AUDPC value is effective for determining the progress of the disease; it gathers different observations during the epidemic and summarizes all the values in a single one that reflects the severity of disease. Finally, dead or severely wilted plants were removed and processed to check the cause of death and fulfill Koch's postulates.

For both *M. cannonballus* and *M. phaseolina*, six plants of the snake melon accession were inoculated and three were used as non-inoculated controls. Also, six plants of the "Piñonet" Piel de Sapo cultivar were used as commercial susceptible control (Ambrósio et al., 2015; Castro et al., 2020). The inoculum of *M. cannonballus* was prepared as described in Castro et al., (2020). Briefly, inoculated PDA plates were incubated at 25°C for 1 week and used to inoculate autoclaved bottles with hydrated wheat seeds. These bottles were incubated at 25°C for 1 month, being shaken weekly. After this period, the final inoculum was prepared by mixing 200 g of the inoculated wheat seeds kg–1 of peat. Plants were grown in a greenhouse, and 30 DAI roots were washed and extended on an acetate film. Root damage of the primary and lateral roots was scored from 0, healthy with no lesions, to 4, highly damaged roots with root rots, rootlets pruning, etc. The inoculum of *M. phaseolina* was prepared as reported in Ambrósio et al., (2015). Briefly, the isolate was inoculated in PDA + antibiotic (tetracycline 0.05 g/L) plates. This culture was used to inoculate plates containing toothpicks in PDA medium that was incubated at 28°C for 1 week. Inoculated toothpicks were inserted at the base of the stem of plants 20 days after transplanting. Plants were grown in a greenhouse, and the severity of the infection was scored at 7 and 15 DAI, with a severity index, from 0, asymptomatic, to 4, severe lesions on the stem.

3.3.7. Fruit Characterization

All fruits of marketable size were weighted at the time of harvest to estimate total marketable yield per plant (Figures 1A,B). Two fruits per plant were selected for characterization (Figures 1C,D) in order to obtain data for fruit weight (FW, g, measured with a digital scale), fruit length and diameter (FL and FD, in cm, measured with a graduated ruler), rind and flesh firmness (RF and FF, measured with a penetrometer in kg cm^{-2}), fruit pH (universal pH indicator paper), and soluble solids content [SSC, measured as °Brix from drops of juice using a hand-held Pocket refractometer (PAL-α), Atago CO., LTD, Tokyo, Japan]. Both fruit flesh and rind colors were measured with a CR-400 colorimeter (Konica Minolta, Inc., Tokyo, Japan), obtaining Hunter L, a, and b coordinates.

3.3.8. Fruit Sensorial and Metabolomics Analysis

Sensory evaluations were performed employing a modified methodology of Saftner et al., (2006). In 2018, four sensory evaluations using a consumer panel (20 tasters) were performed with snake melon fruits from La Punta (evaluations 1 and 2) and Carrizales (evaluations 3 and 4). In each evaluation, the fruits harvested from different plants of each treatment were arranged in three biological replicates for each of the three treatments, NG and grafted onto F_1Pat81 and Cobalt. The panelists were asked to score the nine samples randomly arranged (three biological replicates of each of the three treatments) in a 1–5 scale (5 representing the highest acceptability), considering flavor, texture, and aroma together. During the evaluations, panelists could add extra comments regarding the scores provided. Panelists were given water and low salt cracker to cleanse the palate. All snake melon samples were collected the day of the evaluation, as snake melons have a short shelf life.

In 2019, five sensory evaluations (20 tasters) were performed, the first three using fruits obtained from La Punta and the last two from Carrizales. The same design as that in 2018 was used, and fruits harvested from different plants of each treatment were arranged in three biological replicates. Evaluations 1 and 5 compared fruits from NG plants and plants grafted onto the two Cucumis rootstocks, F_1Pat81 and Fian. Evaluations 2, 3, and 4 compared fruits from NG plants and grafted onto the Cucumis and Cucurbita rootstocks, F_1Pat81 and Shintoza. Panelists were asked to provide a score between 1 and 5 to flavor, texture, and aroma to each of the nine samples randomly arranged (three biological

replicates of each treatment). Aliquots of fruit tissue from the biological replicates used for the sensory evaluations were used for sugars, organic acids, and volatile organic compounds (VOCs) analysis.

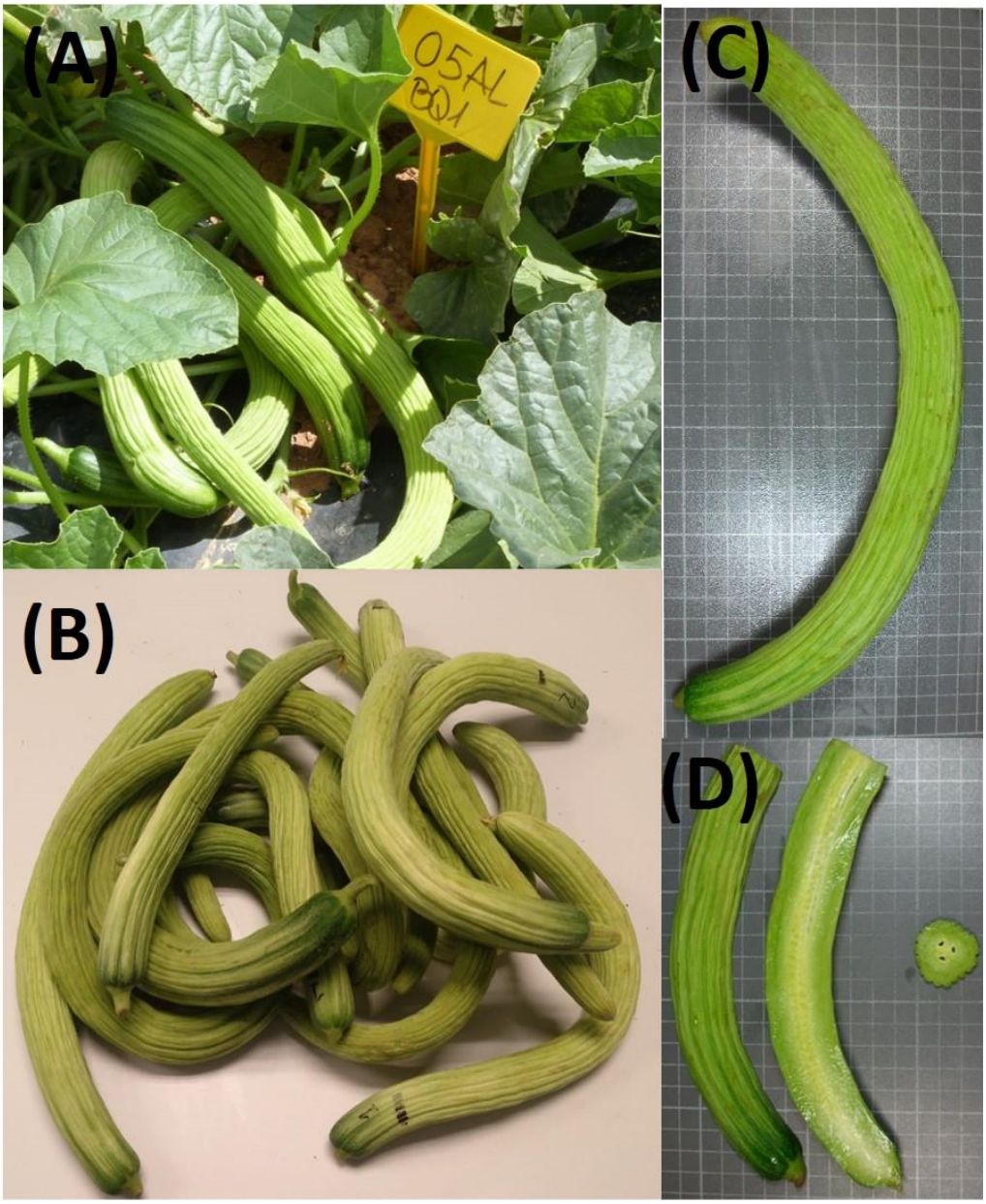


Figure 1. Snake melon fruit in the field ready to be harvested (A) and harvested fruit (B). Characterization of snake melons (C,D).

3.3.9. Sugar and Acid Analysis

The aliquots of fruit tissue from the biological replicates used in sensory evaluations of year 2019 (La Punta and Carrizales, NG plants and plants grafted onto F_1Pat81, Fian, and Shintoza) were homogenized (Silent Crusher M; Heidolph, Schwabach, Germany) and

frozen at −80°C until analysis. A half of each sample was used for sugars (sucrose, glucose, and fructose) and organic acids (citric, malic, and glutamic) analysis. Only sporadic contents of glutamic acid were found, and these data were not included in the results. These compounds were quantified following the methodology described by Cebolla-Cornejo et al., (2012) based in capillary electrophoresis. For that purpose, an Agilent 7100 system (Agilent Technologies, Waldbronn, Germany) was used.

For the analysis, samples were thawed in a refrigerator in complete darkness. Then, they were centrifuged at 510 × g for 5 min. The upper phase was diluted (1:20) with deionized water and filtered using centrifuge tube filters with 0.22-μm membranes (Costar® Spin-X®, Corning, Amsterdam). For the separation, fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, United States) with 50 μm internal diameter, 363 μm external diameter, 67 cm total length, and 60 cm effective length were used. They were previously conditioned with flushes at 95,000 Pa of NaOH 1 mol L–1 at 50°C for 5 min, NaOH 0.1 mol L–1 for 5 min at 20°C, and deionized water (Elix 3, Millipore, Billerica, MA, United States) for 10 min. At the beginning of each sequence, the capillary was flushed at 20°C with the running background electrolyte (BGE) for 30 min. BGE consisted of 20 mmol L–1 2,6-piridin dicarboxylic acid at pH 12.1 and 0.1% w:v hexadimethrine bromide. Between runs, the capillary was flushed with 58 mmol L–1 SDS (2 min) and BGE (5 min). Samples were injected hydrodynamically at 3400 Pa for 10 s, and separations were performed at −25 kV and 20°C. Absorbance was measured at 214 nm. Results were expressed in g kg–1 fresh weight (FW). Sucrose equivalents were calculated by multiplying sucrose, glucose, and fructose contents by their relative sweetening power, 1, 0.74, and 1.73, respectively, and adding them up (Koehler and Kays, 1991).

3.3.10. Analysis of Volatile Compounds

The second half of each homogenized sample used in sugar and acid analysis was employed for the analysis of VOCs listed in Supplementary Table 1. The method described by Fredes et al. (2017) was followed. Solid phase extraction (SPE) cartridges were conditioned with 5 ml of diethyl ether, 5 ml of n-hexane, and dried for 10 min. Frozen samples were defrosted in the fridge. Once thawed, 30 g of the sample was weighed into a 150-ml Erlenmeyer flask with a stopper. The extraction was carried on using a purge and trap headspace system, using nitrogen gas for the inlet tube and the conditioned SPE cartridge for the outlet tube. The samples were extracted for 49 min at

40°C using magnetic agitation and nitrogen flow of 1.6 ml min–1. Subsequently, the cartridges were eluted using 5 ml of a diethyl ether-hexane 1:1 (v:v) solution and 5 ml of diethyl ether. Finally, the collected elution solvents were evaporated to 0.5 ml at 35°C under a nitrogen flow. The resulting extracts were divided into two aliquots in sealed Gas Chromatography (GC) vials, and frosted at −40°C until analysis.

The chromatographic analysis of VOCs was carried on using a Shimadzu GC-2010 Plus system (Shimadzu, Kioto, Japan) coupled with a single quadrupole mass spectrometry system (GCMS QP2010 Ultra, Shimadzu, Kioto, Japan). A Supelcowax 10 column of 30 m × 0.25 mm (Sigma-Aldrich, St. Louis, MO, United States) was used. Helium was used as carrier gas at a flow of 1 ml min–1. The injection was performed in split mode (split ratio 1/50) with a volume of injection of 1 μl at 250°C. The temperature program started at 30°C during 4 min after the injection followed by a rise to 160°C (10°C min–1), and finally, a rise to 250°C (30°C min–1), which was maintained for 3 min. The mass spectra were acquired in Selected ion monitoring (SIM) mode using the m/z for each compound. Electron ionization in positive mode was used at a temperature of 250 and 230°C for the interphase and the ion source, respectively.

From those treatments included in the five sensory evaluations of 2019, NG and F_1Pat81, 10 biological replicates were analyzed for sugar and VOC contents (two of the three biological replicates used per each sensory evaluation), and 7 and 4 biological replicates were independently analyzed for the Shintoza and Fian treatments that were included in three and two of the sensory evaluations performed, respectively.

3.3.11. Statistical Analysis

Fruit characterization, agronomic, sugars, acids, and volatiles data were analyzed using a Dunnett's test. For each location, the effect of each rootstock was compared to the NG control. StatGraphics Centurion version 17.2.04 for Windows and IBM SPSS Statistics 25 for Windows were used for this purpose. Principal component analysis (PCA) of VOCs data were performed using S-Plus v. 8.01 for Windows (Insightful Corp., Seattle, WA, United States). A biplot representation was then obtained, including the scores of data points and the loadings of each VOC for each principal component. Pairwise correlations for metabolite and sensory analysis data were graphically represented as heatmaps using the software heatmapper

3.4. Results

3.4.1. Growth-Limiting Factors

3.4.1.1. Climate, Water, and Soil Properties

Mean temperatures were similar in the three fields (Supplementary Figure 3) except a significantly higher temperature (around 1°C) in April in Carrizales and La Punta compared to Moncada in 2018, and higher temperature in June and July 2019 in Carrizales compared to La Punta. Rainfall was higher in La Punta in the middle of the growing cycle (June) in 2018 and at the beginning (April) of the 2019 assay, and in Carrizales at the end of the growing cycle in 2019.

The irrigation water of Carrizales showed high values of conductivity, reaching 4.5 dS m–1 in 2018 and 5.95 dS m–1 in 2019. Thus, the high salinity in this area of cultivation was confirmed. In fact, soil conductivity reached values of 3.17 ± 0.05 dS m–1 in 2018 and 1.66 ± 0.10 dS m–1 in 2019. The other two fields showed rather standard values for the area, with water conductivity below 2.2 dS m–1 and soil conductivity under 0.7 dS m–1 in both years.

3.4.1.2. Pests and Diseases

Aphids were the main pest in all the fields in the 2 years, with a higher incidence in La Punta and Moncada than in Carrizales (Supplementary Figure 4).

During 2018, in La Punta, some snake melon plants showed mosaic symptoms (Supplementary Figure 4) and the aphid borne potyvirus WMV was detected, with 9% of snake melons affected by this virus. This year in Moncada, the presence of *Cucumber mosaic virus* (CMV), also transmitted by aphids, was not detected in snake melons, although it was present in sweet melons cultivated in the same field. No viruses were detected in the plants grown in Carrizales, likely associated to a reduced presence of the insect vectors. *Podosphaera xanthii*, the fungus responsible for the cucurbit powdery mildew, was detected in La Punta and Moncada, but only caused mild infections to the snake melon plants, whereas the sweet melons were severely affected.

In 2019, WMV was also the most prevalent virus in La Punta, with 26% of the snake melon plants affected by the virus. Other aphid-transmitted potyviruses such as ZYMV and the whitefly transmitted Begomovirus ToLCNDV, both affecting approximately 18%

of the snake melon plants, were also detected in this field. *P. xanthii* (Supplementary Figure 4) affected the snake melon plants in La Punta. Again, the field of Carrizales was free of virus and powdery mildew.

In accordance with the history of fungal stress, we observed some mortality of snake melon plants with vine decay symptoms likely due to fungal soilborne pathogens in 2018 in La Punta. Twelve percent of the NG plants of snake melon died at early developmental stages, and *M. phaseolina* was isolated from their roots. Twelve percent of the snake melon plants grafted onto the Cucurbita rootstock Cobalt died at later stages, with roots affected by *Fusarium oxysporum*, whereas plants grafted on F_1Pat81 were less affected by fungi. Other soilborne pathogens, detected in roots of sweet melon plants cultivated in the same field, but not affecting snake melons, were *Fusarium equiseti*, *N. falciformis*, *Fusarium solani*, *F. solani* f. sp. *cucurbitae*, *M. cannonballus*, *Torula herbarum*, *Fusarium incarnatum*, *Rhizopus* sp., *Alternaria alternata*, *Alternaria* sp., *Rhizoctonia solani*, *Plectosphaerella cucumerina*, *Aspergillus flavus*, *Aspergillus* sp., *Fusarium chlamydosporum*, *Acremonium* sp., *Geotrichum candidum*, *Gibberella fujikuroi*, *Gibberella* sp., *Chaetomium acropullum*, *Chalaropsis radicicola*, *Collariella bostrychodes*, and *Penicillium* sp.

In 2018, no snake melon plants were affected by soilborne diseases in Carrizales, although different soilborne pathogens were detected in the roots of sweet melon plants cultivated in the same field (*M. phaseolina*, *N. falciformis*, *N. keratoplastica*, *F. solani*, *M. cannonballus*, *F. oxysporum*, *Alternaria* sp., *Cladosporium herbarum*, *Fusarium* sp., *C. bostrychodes*, *Mortierella alpina*, and *Acremonium* sp.). No fungal stress was observed in Moncada.

The fungal attack in 2019 was less severe in La Punta, where the lower mean temperature in May, June, and August and higher rainfall by the end of the cycle could have contributed to the reduced fungal damage (Supplementary Figure 3). No snake melon plant died by soilborne pathogens. Although some soilborne pathogens were detected in the sweet melon plants cultivated on the field (*M. phaseolina*, *F. oxysporum*, *F. equiseti*, *F. solani*, *Fusarium* sp., *N. falciformis*, *Alternaria tenuissima*, *A. alternata*, *Gibberella* sp., *Pyxidiophora arvernensis*, *Botryosphaeriaceae*, *C. herbarum*, *Pythium aphanidermatum*, *Gibberella avenacea*, and *Mucor* sp.), they did not affect the snake melon plants.

This year, a more severe fungal attack was observed in Carrizales (Supplementary Figure 5) that might be associated to the different characteristics of the cultivation plot and to the higher average temperatures in July along with a reduced rainfall until the end of the growing cycle compared to the previous year (Supplementary Figure 3). The snake melons showing symptoms of soilborne pathogens were mainly those grafted onto *Cucurbita* rootstocks, Shintoza and Cobalt, and the NG plants (44, 31, and 25% mortality, respectively). The main pathogens detected were *M. phaseolina*, *N. falciformis*, *F. equiseti*, *Gibberella* spp., and *Fusarium longipes*. No symptoms of fungal attack were observed in plants grafted onto *Cucumis* rootstocks, F_1Pat81, Fimy, and Fian. Sweet melons planted on Carrizales were also affected by the same pathogens.

3.4.2. Response of Snake Melon to *M. phaseolina*, *M. cannonballus*, and *Neocosmospora* spp.

The field survey results indicated that the main pathogen associated with dead snake melon plants or plants showing decay symptoms in our conditions is *M. phaseolina*. To test the level of susceptibility to this pathogen under controlled conditions, we conducted an artificial inoculation assay, using a *M. phaseolina* isolate collected in La Punta. Results showed that snake melon is highly susceptible to this pathogen. Symptoms started at 7 DAI and were severe at 15 DAI (Figure 2A). Average damage score was higher in snake melons than in the control sweet melon Piel de Sapo that has also proven to be susceptible to this disease (de Sousa Linhares et al., 2020). All the snake melon plants were dead after 30 DAI, while the mortality of the susceptible control Piel de Sapo was 20%.

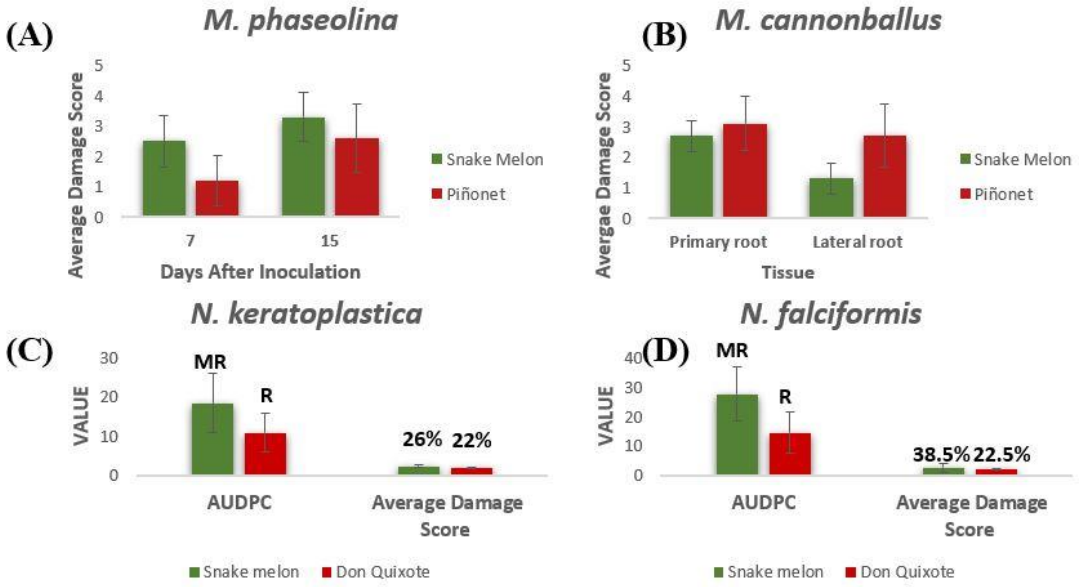


Figure 2. (A) Average damage scores (from 0 to 4) of the stem lesions caused by *M. phaseolina* in snake melon and sweet melon control (Piel de Sapo Piñonet) at 7 and 15 days after inoculation (DAI). (B) Average damage scores (from 0 to 4) caused by *M. cannonballus* in primary and lateral roots of snake melon and sweet melon control at 30 DAI. Average area under the disease progress curve (AUDPC), average damage score, type of reaction (R, resistant; MR, moderately resistant), and disease incidence (%) for snake melon and the sweet melon control plants (Piel de Sapo Don Quixote) inoculated with *N. falciformis* (C) and *N. keratoplastica* (D). Six (A,B) and seven (C,D) biological replicates (plants) were used for each genotype.

We also tested the response to *M. cannonballus*, despite the fact that it was less frequent than *Macrophomina* in our fields, because in many hot and dry regions of melon cultivation, these are two of the main soilborne pathogens affecting melons (Cohen et al., 2016). Snake melon plants were also susceptible to *M. cannonballus* (Figure 2B), with similar average damage scores in primary roots, although less severe lesions in the lateral roots compared to Piel de Sapo.

Field results also showed the importance of *Fusarium* species. Snake melons are known to be highly susceptible to several pathogenic forms of the so-called *F. oxysporum* species complex (Solmaz et al., 2016b; Al-Taae and Al-Taae, 2019), but their response to other species belonging to the *F. solani* species complex (FSSC) (e.g., some of them currently included in the genus *Neocosmospora*) had not been tested yet. For both pathogens, *N. falciformis* and *N. keratoplastica*, snake melon plants showed considerably higher AUDPC than the commercial control sweet melon Piel de Sapo Don Quixote, as well as a higher disease incidence for *N. falciformis* (Figures 2C,D). Supplementary Figures 6, 7 show symptoms on snake melon caused by the different pathogens.

3.4.3. Yield and Fruit Characteristics

The agronomic performance of NG melons in the three fields was compared to analyze the effect of the agro-ecological conditions (Figure 3 and Table 1). Moncada, representing unstressed conditions, could be considered as control, as the lack of previous melon cultivation led to the absence of soilborne diseases, while La Punta and Carrizales represented stressful conditions due to the incidence of diseases and the use of saline water and soil, respectively. The yield of NG snake melon plants in La Punta was reduced compared to Moncada (≈ 4 kg/plant vs ≈ 10 kg/plant, respectively) (Figure 3). This reduction was likely a consequence of the incidence of viruses and soilborne diseases, as stated above. Conversely, the high salinity conditions at Carrizales did not affect yield per plant, which was similar to that of Moncada. No differences were found for most fruit traits among the three fields (Table 1), although the stressful conditions of both La Punta and Carrizales resulted in lower fruit FF. Also, regarding basic quality characteristics, the SSC was higher at Carrizales, which is expected under saline conditions. At this field, fruits showed higher Hunter a value, representing a less greenish color of the flesh.

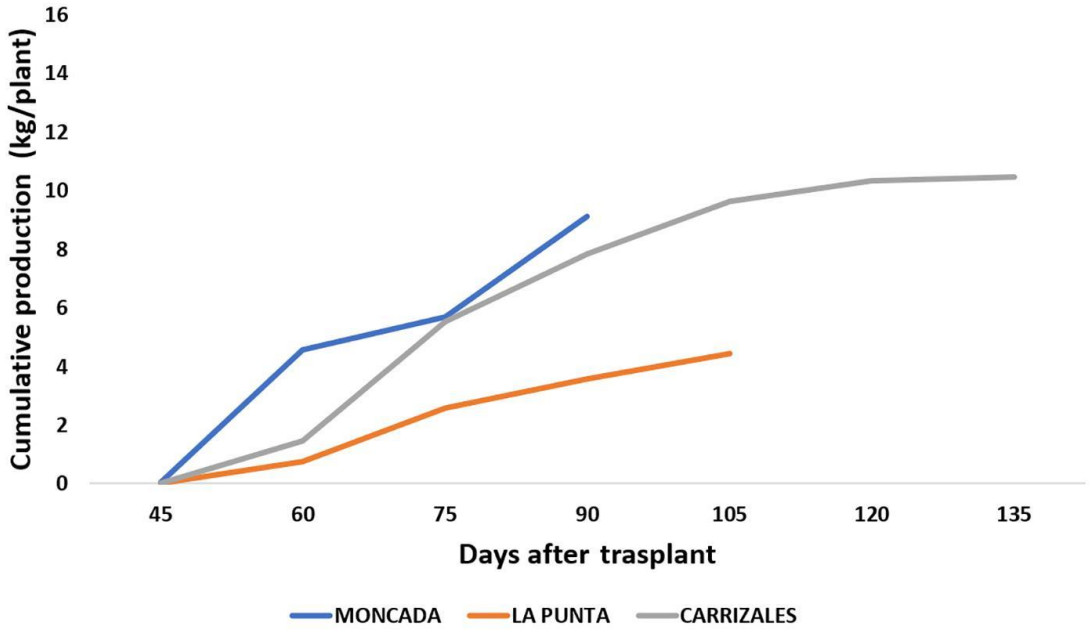


Figure 3. Cumulative production (kg plant–1) of non-grafted (NG) snake melons in 2018, cultivated in the three fields. Sixteen NG plants were cultivated per field. All fruits of marketable size were weighted at the time of harvest to estimate total yield per plant.

Table 1. Agronomic performance and fruit characterization of non-grafted (NG) plants in the three fields (Moncada, La Punta and Carrizales) in 2018. Hunter's L,a,b colour coordinates measured in the flesh (F) and rind (R). Values followed by a (*) are significantly different from that of the unstressed field (Moncada) using Dunnett's test (P≤0.05).

	Field		
	Moncada	**La Punta**	**Carrizales**
Plant mortality (%)	0	12	0
Fruit weight (g)	243.2±18.2	276.4±25.5	251.1±37.6
Fruit length (cm)	48.9±2.0	43.7±2.0	42.7±1.7
Fruit diameter (cm)	3.0±0.1	3.3±0.2	3.0±0.1
Flesh firmness (kg cm^{-2})	4.2±0.3	2.6±0.4*	3.1±0.2*
Rind firmness (kg cm^{-2})	10.0±0.6	9.8±1.1	8.4±0.5
pH	5±0	5±0	5±0
Soluble solids content(ºBrix)	3.3±0.2	3.6±0.2	4.0±0.2*
Hunter L (F)	60.22±1.34	55.56±1.35	65.81±1.79
Hunter a (F)	-11.64±0.27	-11.43±0.39	-10.2±0.27 *
Hunter b (F)	20.56±0.52	19.81±0.73	20.89±1.48
Hunter L (R)	48.02±1.4	51.98±1.56	52.69±1.56
Hunter a (R)	-13.04±0.37	-12.13±0.53	-12.97±0.62
Hunter b (R)	20.49±0.51	19.45±0.82	20.29±0.72

The effect of grafting during 2018 was evaluated in the fields of La Punta and Carrizales using the commercial *Cucurbita* hybrid Cobalt and the experimental *C. melo* hybrid F_1Pat81. Some mortality was observed in grafted snake melon plants in La Punta, with the F_1Pat81 rootstock being the one that displayed the best performance under these epidemiological conditions. The plants grafted onto this rootstock displayed less mortality and higher yield ≈ 6 kg/plant compared to the ≈ 4 kg/plant (Figure 4A) produced by the plants grafted onto Cobalt and NG. The higher susceptibility of snake melon roots and the Cobalt rootstock to the fungal pathogens may account for these differences. Production in Carrizales (Figure 4B) was higher than in La Punta. In this field, fungal stress was lower, and the salt stress had a much less severe impact on snake melon production. Grafting had a favorable effect on snake melon production under saline conditions. The plants grafted onto the melon rootstocks F_1Pat81 were as productive as those grafted onto Cobalt (≈ 14 kg/plant), both much more productive than NG plants (≈ 10 kg/plant).

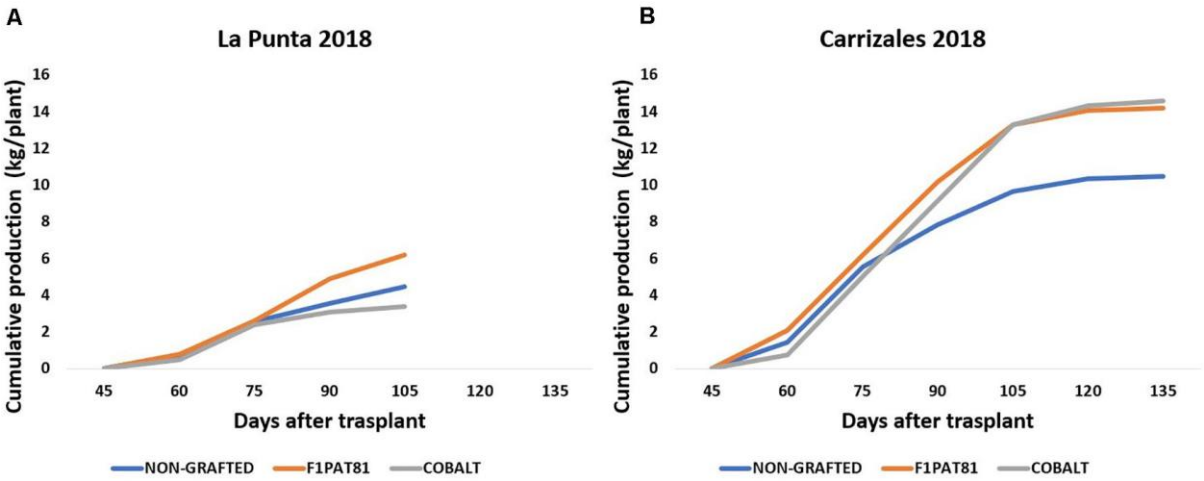


Figure 4. Cumulative production (kg plant–1) of snake melons in 2018 grown in La Punta (A) and Carrizales (B) obtained from non-grafted plants and plants grafted onto the commercial *Cucurbita* hybrid Cobalt and the experimental *C. melo* hybrid F_1Pat81. Sixteen plants were cultivated per field and treatment. All fruits of marketable size were weighted at the time of harvest to estimate total yield per plant.

Regarding snake melon fruit traits, no differences were found between fruits from the NG and those of grafted for fruit shape, firmness, pH, and °Brix (Table 2). Only Hunter L and a values were altered in fruits from plants grafted on F_1Pat81 in La Punta. They were less green with a lighter color. Lower Hunter a values were obtained in fruits from plants grafted onto Cobalt in Carrizales (more greenish color). As stated before, the saline conditions of Carrizales produced snake melon fruits with higher °Brix in all treatments (Table 2).

Table 2. Agronomic performance and fruit characterization of the snake melons produced in plants grafted onto commercial *Cucurbita* hybrid Cobalt and experimental *C. melo* hybrid F_1PAT81 compared to non-grafted in two fields (La Punta and Carrizales) in 2018. Hunter's L,a,b colour coordinates measured in the flesh (F) and rind (R). Values followed by a (*/-) show significant differences compared to the non-grafted control (Dunnett's test; P≤0.05). Values followed by a (-/*) show significant differences compared between the 2 field for the same treatment (P≤0.05). Values followed by (*/*) indicate a significant difference compared to the non-grafted control and between the 2 fields (Dunnett's test; P≤0.05).

		Treatment		
	Field	**NG**	**Cobalt**	**F_1PAT81**
Plant mortality (%)	Carrizales	0	0	0
	La Punta	12	12	0
Fruit weight (g)	Carrizales	251.1±37.6	204.9±26.3	200.3±20.8
	La Punta	276.4±25.5	242.8±29.1	259.9±44.5
Fruit length (cm)	Carrizales	40.7±1.7	38.4±0.8	36.4±0.8
	La Punta	43.7±2.0	38.8±0.7	40.1±0.7
Fruit diameter (cm)	Carrizales	3.0±0.1	2.9±0.2	2.7±0.1
	La Punta	3.3±0.2	3.1±0.1	3.2±0.2
Flesh firmness (kg cm-2)	Carrizales	3.1±0.2	3.7±0.7	3.2±0.4
	La Punta	2.6±0.4	2.7±0.4	2.4±0.2
Rind firmness (kg cm-2)	Carrizales	8.4±0.5	9.8±0.9	8.3±0.9
	La Punta	9.8±1.1	9.8±1.0	8.8±0.5
pH	Carrizales	5±0	5±0	5±0
	La Punta	5±0	4.88±0.13	4.75±0.16
Soluble solids content(ºBrix)	Carrizales	4.1±0.2-/*	4.0±0.2-/*	3.8±0.1 -/*
	La Punta	3.6±0.2-/*	3.5±0.2_/*	3.2±0.2 -/*
Hunter L (F)	Carrizales	65.81±1.79 -/*	58.85±2.1	70.11±2.58 -/*
	La Punta	55.56±1.35 -/*	56.86±1.83	62.03±1.09 */*
Hunter a (F)	Carrizales	-10.22±0.26	-11.66±0.32 */-	-10.38±0.24
	La Punta	-11.43±0.38	-11.70±0.4	-9.75±0.64*/-
Hunter b (F)	Carrizales	20.89±1.48	20.29±0.43	21.62±1.77
	La Punta	18.81±0.73	20.07±0.51	18.76±0.73
Hunter L (R)	Carrizales	52.69±1.56	48.6±0.64	49.87±1.88
	La Punta	51.98±1.56	50.57±1.03	52.1±1.05
Hunter a (R)	Carrizales	-12.97±0.62	-13.39±0.17 -/*	-13.68±0.44 -/*
	La Punta	-12.13±0.53	-12.16±0.34 -/*	-11.07±0.24 -/*
Hunter b (R)	Carrizales	20.29±0.71	20.06±0.38	19.72±0.58
	La Punta	19.45±0.82	19.8±0.5	18.96±0.35

In 2019, the fungal stress in La Punta did not cause plant mortality in snake melons. However, despite lower mortality, the impact on yield was important (Figure 5A). Yield per plant ranged from ≈ 4 to 5 kg plant–1, except for the snake melon plants grafted onto the Fimy rootstock that was the less productive. In Carrizales, the yield in 2019 dropped significantly compared to 2018 (Figure 5B). The fungal attack was more severe. Also, this year, conductivity of irrigation water was higher. Additionally, the fact that an extensive crop like oat, and not a nitrogen fixing crop like alfalfa, was cultivated for the three previous years might have resulted in a poorer and less productive soil. The fungal attack resulted in a higher plant mortality in both NG and grafted onto Cucurbita rootstocks plants, whereas Cucumis rootstocks were again the most tolerant to fungi. Among them, the wild Fian and the cultivated hybrid F_1Pat81 maintained a moderate yield per plant compared to NG plants. Fimy was again the less productive rootstock.

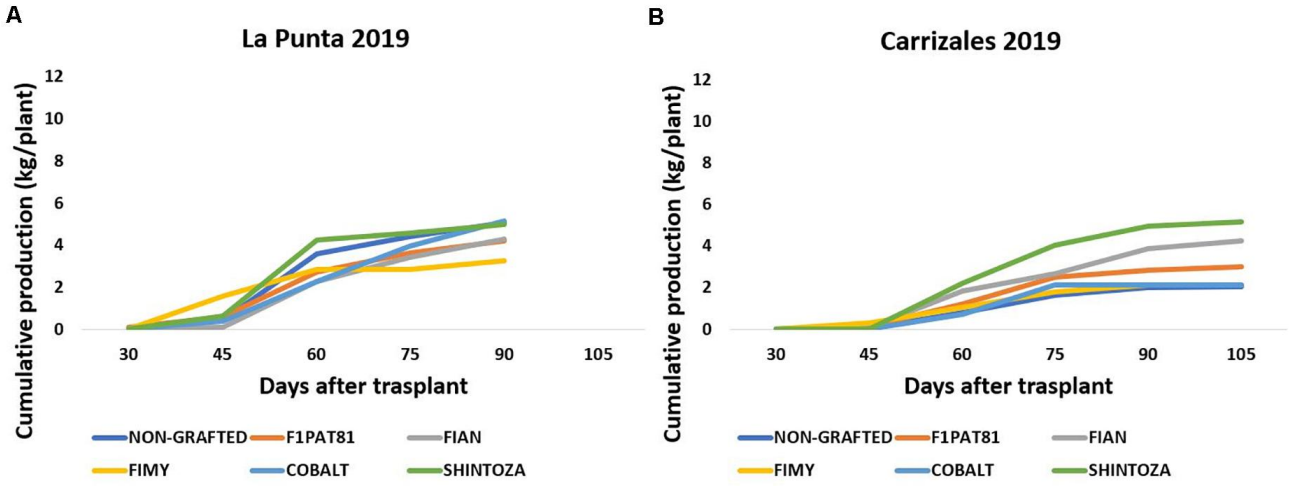


Figure 5. Cumulative production (kg plant–1) of snake melons in 2019 from non-grafted plants and plants grafted on commercial *Cucurbita* hybrids (Cobalt and Shintoza), the *C. melo* hybrid F_1Pat81, and hybrids between *C. ficifolius* and *C. anguria* (Fian) or *C. myriocarpus* (Fimy) in the two sites of cultivation La Punta (A) and Carrizales (B). Sixteen plants were cultivated per field and treatment. All fruits of marketable size were weighted at the time of harvest to estimate total yield per plant.

No significant effects were found regarding fruit traits in grafted plants compared to the NG ones, except for a reduction in °Brix of the fruits harvested from plants grafted onto Shintoza in Carrizales (Table 3). Again, differences between fields were mainly associated with °Brix, higher in Carrizales, and fruit color, with a more yellowish fruit with less firm flesh in La Punta (Table 3).

Table 3. Agronomic performance and fruit characterization of the non-grafted control compared to plants grafted on commercial *Cucurbita* hybrids (Cobalt and Shintoza), the C. melo hybrid F_1Pat81 and hybrids between *C. ficifolius* and *C. anguria* (Fian) or *C. myriocarpus* (Fimy) in the two sites of cultivation (La Punta and Carrizales) used in 2019. Hunter's L,a,b colour coordinates measured in the flesh (F) and rind (R). Values followed by a (*/-) show significant differences compared to the non-grafted control (Dunnett's test; P≤0.05). Values followed by a (-/*) show significant differences compared between the 2 sites for the same treatment (P≤0.05). Values followed by (*/*) indicate a significant difference compared to the non-grafted control and between the 2 sites (Dunnett's tet; P≤0.05).

		Treatment					
		NG	**Cobalt**	**Shintoza**	**F_1PAT81**	**Fian**	**Fimy**
Plant mortality (%)	Carrizales	25	31	44	0	0	0
	La Punta	0	0	0	0	0	0
Fruit weight (g))	Carrizales	241.4±27.0	289.8±46.2	232.6±22.6	264.1±14.1	223.4±27.4	247.2±25.5
	La Punta	260.4±15.8	278.6±25.6	263.4±20.9	300.7±25.2	234.8±21.0	253.0±17.6
Fruit length (cm)	Carrizales	43.5±1.6	47.0±3.5	39.8±2.0	45.7±2.6	39.7±2.1	39.6±1.9
	La Punta	45.6±2.0	44.7±1.8	44.7±2.3	48.7±2.8	42.4±2.5	43.3±1.7
Fruit diameter (cm)	Carrizales	3.2±0.1	3.3±0.2	3.1±0.2	3.4±0.1	3.2±0.1	3.3±0.1
	La Punta	3.3±0.1	3.2±0.2	3±0.1	3.3±0.1	2.9±0.1	3.0±0.1
Flesh firmness (kg cm-2)	Carrizales	4.0±0.3-/*	3.47±0.22	4.32±0.1-/*	4.02±0.15-/*	3.83±0.19	4.18±0.22-/*
	La Punta	3.6±0.1-/*	3.6±0.1	3.7±0.2-/*	3.7±0.2-/*	3.58±0.2	3.4±0.3-/*
Rind firmness (kg cm-2)	Carrizales	10.1±0.2	9.6±0.4 -/*	10.5±0.2	10.7±0.4	10.5±0.3	10.8±0.4
	La Punta	9.4±0.3	11.1±0.4-/*	9.7±0.2	10.0±0.3	10.7±0.3	9.8±0.5
pH	Carrizales	4.11±0.11	4.17±0.17	4.44±0.18	4.56±0.18	4.33±0.17	4.22±0.15
	La Punta	4.89±0.11	4.78±0.15	4.78±0.15	4.11±0.11	4.67±0.17	4.57±0.2
Soluble solids content(ºBrix)	Carrizales	4.8±0.2 -/*	4.0±0.2	3.8±0.2*/-	4.4±0.2-/*	4.5±0.3-/*	4.4±0.3-/*
	La Punta	3.9±0.2 -/*	4.2±0.1	3.8±0.2	3.3±0.3-/*	3.8±0.2-/*	4.0±0.2-/*
Hunter L (F)	Carrizales	57.58±0.8	59.65±1.3	59.78±1.19 -/*	58.36±1.19-/*	56.12±1.53	57.76±0.7
	La Punta	54.35±1.67	58.97±1.09	55.18±1.44 -/*	55.18±1.4-/*	59.88±1.23	57.64±1.95
Hunter a (F)	Carrizales	-11.92±0.4	-11.77±0.45	-11.83±0.42	-12.15±0.23	-11.55±0.39	-11.81±0.38
	La Punta	-12.11±0.35	-11.87±0.38	-12.41±0.25	-11.2±0.43	-12.17±0.28	-11.88±0.24
Hunter b (F)	Carrizales	21±0.22	20.05±0.54	20.96±0.38	21.07±0.31	19.78±0.4 -/*	20.82±0.47
	La Punta	20.44±0.5	21.56±0.51	21.04±0.3	19.79±0.61	21.71±0.42 -/*	21.95±0.85
Hunter L (R)	Carrizales	51.12±0.6	49.81±1.48	50.44±0.51	52.13±0.6	52.02±1.13	50.04±1.42
	La Punta	53.75±0.8	53.02±0.46	51.23±0.95	53.67±1.11	52.72±0.79	50.88±0.93
Hunter a (R)	Carrizales	-12.58±0.46	-12.88±0.26	-13.36±0.21	-13.36±0.4	-13.73±0.41	-12.19±0.41 -/*
	La Punta	-13.08±0.3	-13.18±0.25	-13.74±0.23	-12.91±0.29	-12.82±0.31	-13.56±0.2 -/*
Hunter b (R)	Carrizales	19.33±0.64 -/*	19.67±0.27 -/*	20.48±0.27	20.28±0.49	20.81±0.53	19.27±0.74-/*
	La Punta	21.34±0.42 -/*	21.51±0.35 -/*	21.67±0.37	21.03±0.5	21.19±0.36	20.77±0.56-/*

3.4.4. Sensorial Evaluation

Four sensory evaluations were performed in 2018, two with fruits from La Punta and two with fruits from Carrizales (Figure 6). Except for the second evaluation using fruits from La Punta, which did not result in significant differences among treatments, panelists gave higher scores of flavor acceptability to the fruits from NG plants compared to the fruits from plants grafted onto F_1Pat81 and Cobalt, which had similar

scores. Therefore, an effect of grafting on consumer acceptability was found in fruits from both fields.

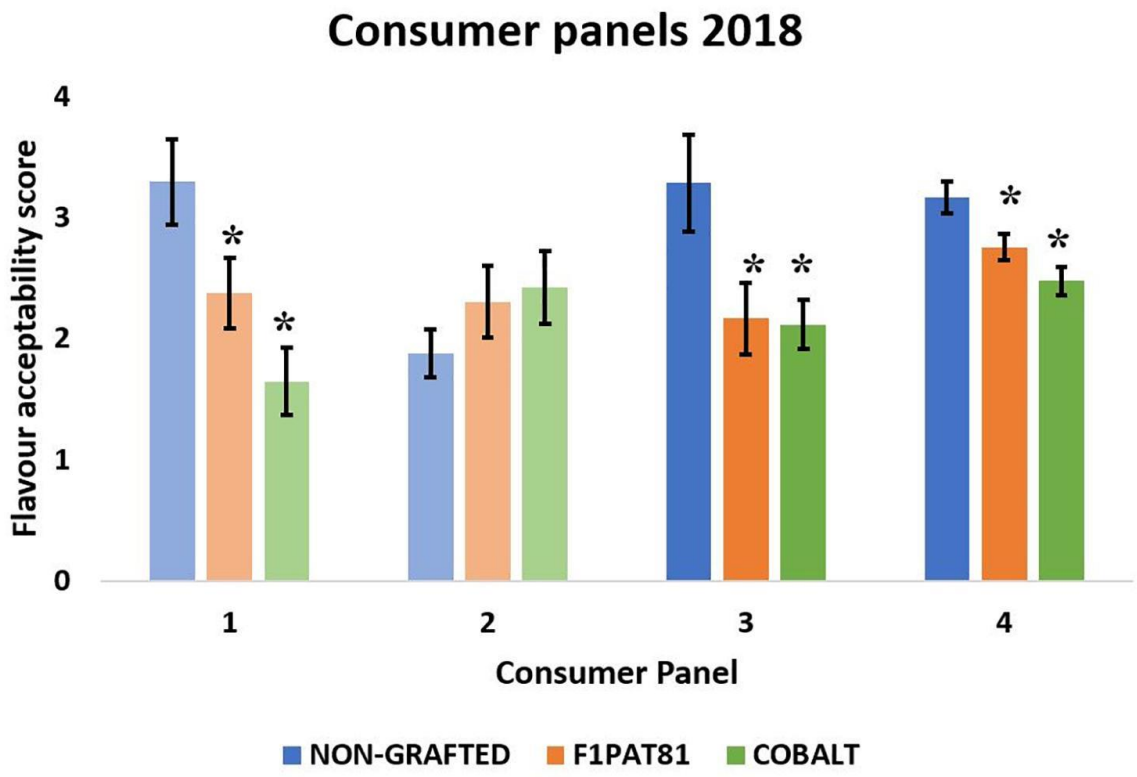


Figure 6. Mean flavor acceptability (1 being the lowest and 5 being the highest) obtained in the sensory evaluations of 2018. Columns of lighter color indicate that the fruit samples were from La Punta and those of darker color indicate that they are from Carrizales. Values followed by (*) show significant differences compared to the NG control (Dunnett's test, $P \leq 0.05$). Averages are calculated with the mean scores of 20 panelists on three biological replicates (fruit samples) of each treatment.

Five sensory evaluations were performed in 2019 (represented as 1–5 in Figure 7), two comparing fruit from the NG control with those from plants grafted onto two Cucumis rootstocks, the melon F_1Pat81 and Fian (sensory evaluations 1 and 5 for La Punta and Carrizales, respectively), and three comparing the NG with the melon F_1Pat81 and the *Cucurbita* rootstock Shintoza (sensory evaluations 2 and 3 for La Punta and 4 for Carrizales). This time, flavor, texture, and aroma were scored independently. Significant differences were found for flavor scores, but not for aroma and texture; the Shintoza rootstocks had consistently lower scores compared to NG and F_1Pat81, and the F_1Pat81 and Fian fruits from Carrizales were less valued than NG snake melons.

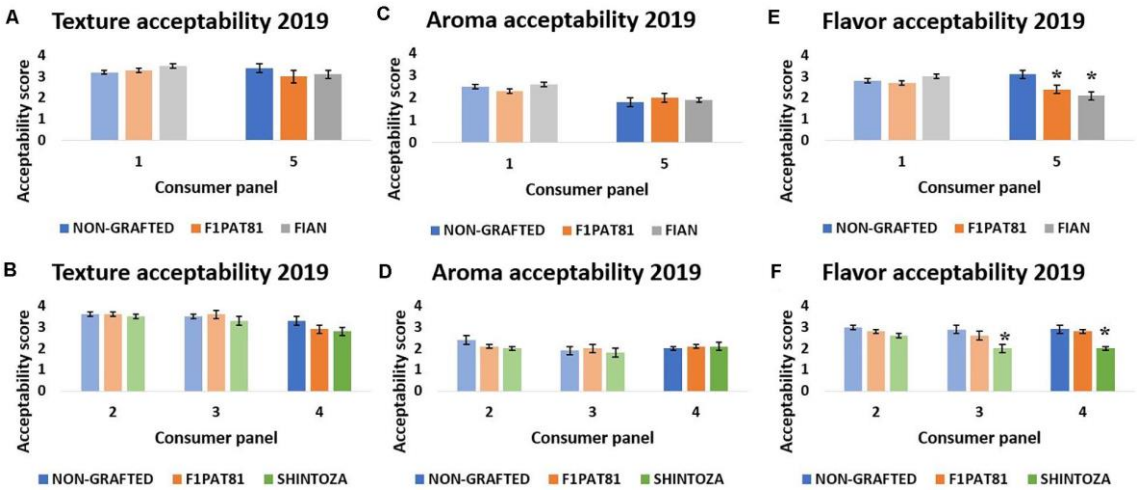


Figure 7. Mean texture, aroma, and flavor acceptability scores (1 being the lowest and 5 being the highest) obtained in the sensory evaluations of 2019. Columns of lighter color indicate that the fruit samples were obtained from La Punta and those of darker color indicate that they were obtained from Carrizales. Columns with (*) show significant differences compared to the NG control (Dunnett's test, $P \leq 0.05$). Averages are calculated with the mean scores of 20 panelists on three biological replicates (fruit samples) of each treatment. (A,C,E) Correspond to sensory evaluations 1 and 5. (B,D,F) Correspond to sensory evaluations 2, 3, and 4.

3.4.5. Accumulation of Sugars, Acids, and Volatiles

Since the sensory evaluations suggested differences in acceptability, we used samples of the evaluation of 2019 to determine sugar and acid accumulation and volatile compounds. The sugar and acid profiles are shown in Figure 8. In general, snake melon fruits have similar levels of fructose and glucose, whereas sucrose levels remained under the limit of detection, expected for a non-sweet fruit. Malic acid was predominant in the samples, with contents up to 20 times higher than those of citric acid.

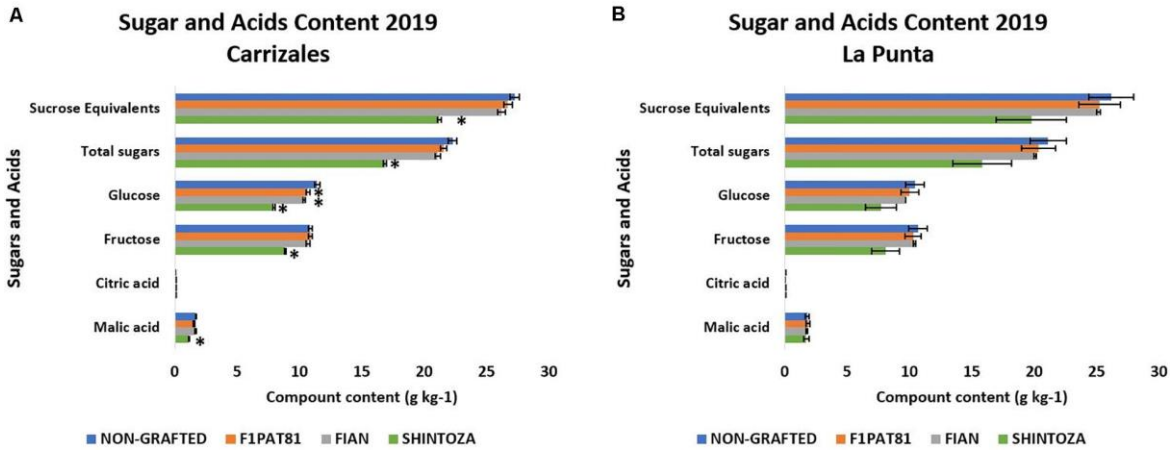


Figure 8. Accumulation of sugars and acids in fruits from non-grafted and grafted (Shintoza, F_1Pat81, and Fian) snake melon plants from Carrizales (A) and La Punta (B). Columns with (∗) show significant differences with respect to the NG control (Dunnett's test, $P \leq 0.05$). Averages were calculated from the biological replicates used in the sensory evaluations. From those treatments included in the five sensory evaluations of 2019, NG and F_1Pat81, averages were calculated from six and four biological replicates, respectively, from the three sensory evaluations of la Punta and the two sensory evaluations of Carrizales. Four and three biological replicates were averaged, respectively, for the Shintoza treatment, included in two sensory evaluations from La Punta and one sensory evaluation from Carrizales. Two biological replicates were averaged for the Fian treatment from each of the two sensory evaluations in which this treatment was included (one from La Punta and one from Carrizales).

Grafting had a higher impact in the sugar content than in the organic acid profile (Figure 8). This effect was significant in Carrizales. Although the same trend seemed evident in La Punta, a higher variability hindered the identification of significance. The rootstock that had a higher impact on sugar accumulation was the *Cucurbita* rootstock Shintoza, with a significantly lower amount of both glucose and fructose; malic acid was also reduced with this rootstock. Sugar reduction also occurred, although to a lesser extent, in *Cucumis* rootstocks, both F_1Pat81 and Fian, but only glucose content was significantly affected. In fact, this reduction did not affect the sucrose equivalents, a variable that accounts for the sweetening power of each sugar and which is more related to sweetness perception.

Regarding VOCs, aldehydes represented, by far, the main compounds of the snake melon aroma profile, followed by alcohols, and a very low amount of esters, as expected for a low-aroma melon, and apocarotenoids (Figure 9). The alcohol profile was rich in 2-phenylethanol, associated to floral odor (Table 4). The ester compounds, which are the major contributors to the aroma of sweet melons, were nearly absent in this immature fruit, with ethyl butanoate, the major ester contributor to melon aroma, being the most abundant, although in very low amounts. The apocarotenoid beta-ionone was the only one consistently detected, but in very low amounts.

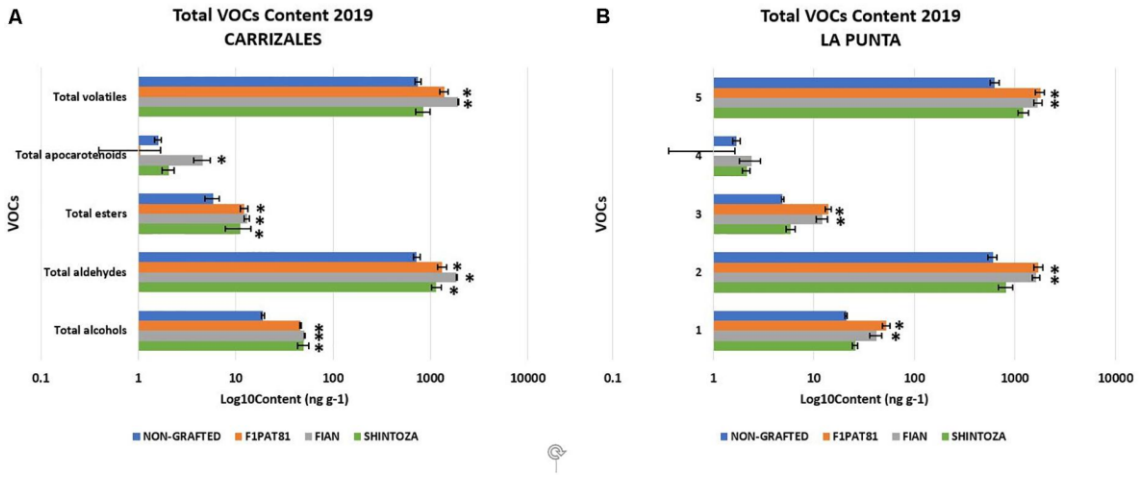


Figure 9. Accumulation of volatile organic compounds in fruits from non-grafted and grafted (Shintoza, F_1Pat81, and Fian) snake melon plants from Carrizales (A) and La Punta (B). Samples obtained from the sensory analyses performed in 2019. Columns with (∗) show significant differences with respect to the NG control (Dunnett's test, $P \leq 0.05$). Averages were calculated from the biological replicates used in the sensory evaluations as described in legend of **Figure 8.**

Table 4. Detailed accumulation of volatile organic compounds (VOCs) in non-grafted (NG) and grafted (F_1PATA81, SHINTOZA, Fian) alficoz fruits for the 2019 campaign. Values followed by a (*/-) show significant differences compared to the non-grafted control (Dunnett's test; P≤0.05). Values followed by a (-/*) show significant differences compared between the 2 sites for the same treatment (P≤0.05). Values followed by (*/*) indicate a significant difference compared to the non-grafted control and between the 2 sites (Dunnett's test; P≤0.05).

			Treatment			
			NG	**Shintoza**	**F_1PAT81**	**Fian**
Alcohols (ng g^{-1})	1-Pentanol	Carrizales	n.d.	2.46±0.54*/*	0.94±0.54 -/*	n.d.
		La Punta	n.d.	n.d. -/*	2.26±0.25*/*	0.98±0.98
	(Z)-3-Hexen-1-ol	Carrizales	0.14±0.14 **-/***	4.03±0.36*/*	2.45±0.4*/-	2.28±0.04*/-
		La Punta	0.82±0.08 **-/***	1.62±0.21*/*	2.89±0.46*/-	1.69±0.41*/-
	1-Nonanol	Carrizales	1.76±0.08 **-/***	2.99±0.4*/*	2.61±0.14*/-	3.23±0.09*/-
		La Punta	1.53±0.05 **-/***	2±0.17 -/*	2.72±0.16*/-	3.22±0.11*/-
	(Z)-3-Nonen-1-ol	Carrizales	n.d.	n.d.	n.d.	n.d.
		La Punta	0.08±0.08	n.d.	n.d.	n.d.
	Benzyl Alcohol	Carrizales	0.29±0.17	3.06±0.71*/*	2.94±0.53*/-	3.74±0.45*/-
		La Punta	0.73±0.15	1.54±0.15 -/*	3.64±0.91*/-	1.55±0.37*/-
	Phenol	Carrizales	0.45±0.02	0.7±0.12*/-	0.77±0.03*/-	0.72±0.02*/-
		La Punta	0.35±0.07	0.36±0.12	0.71±0.05*/-	0.66±0.09
	2-phenylethanol	Carrizales	16.25±0.73	35.73±4.29*/*	35.61±0.49*/-	40.19±0.05*/-
		La Punta	17.07±0.62	19.68±1.39 -/*	39.3±3.38*/-	32.82±3.96*/-
	1-Hexanol	Carrizales	0.06±0.06 **-/***	0.96±0.2*/*	0.7±0.24	0.79±0
		La Punta	0.3±0.02 **-/***	0.48±0.07 -/*	1.04±0.09*/-	0.77±0.06*/-
Aldehydes (ng g^{-1})	Hexanal	Carrizales	59.26±7.24	143.36±2.71 -/*	307.7±49.37*/*	181.88±11.95 */*
		La Punta	48.8±3.19	64.24±7.13 -/*	144.41±15.55*/*	86.62±11.01 */*
	Heptanal	Carrizales	n.d.	n.d.	n.d.	n.d.
		La Punta	n.d.	0.25±0.25	0.39±0.39	n.d.
	(E)-2-Heptenal	Carrizales	4.08±0.23	5.45±0.68	4.53±0.48	9.72±0.19*/-
		La Punta	4.25±0.32	4.9±0.28	7.19±0.99*/-	7.93±1.48*/-
	(E,E)-2,4-Heptadienal	Carrizales	5.14±0.24 **-/***	8.77±1.57	7.01±0.78	12.41±1.6*/-
		La Punta	7.64±0.75 **-/***	10.97±0.63	10.37±1.95	14.12±2.73
	(E)-2-Octenal	Carrizales	2.06±0.25	3.04±0.08	2.18±0.73	4.18±0.08
		La Punta	1.92±0.39	1.15±0.67	3.2±0.4	3.79±0.77
	Nonanal	Carrizales	13.41±2.01 **-/***	20.69±2.1	29.46±8.33	33.25±5.79*/-
		La Punta	9.18±0.74 **-/***	15.53±4.44	34.33±7.29*/-	34.45±10.93*/-
	(Z)-6-Nonenal	Carrizales	1.32±0.76 **-/***	7.86±0.56	8.41±2.09*/*	8.35±1.88
		La Punta	4.19±0.22 **-/***	7.41±1.97	19.53±3.25*/*	10.39±3.46
	(E)-2-Nonenal	Carrizales	161.04±18.39	280.49±33.7*/-	256.82±23.26*/-	378.16±31.09*/-
		La Punta	118.61±11.85	161.24±26.36	378.47±49.02*/-	347.3±34.55*/-
	(E,E)-2,4-Nonadienal	Carrizales	0.64±0.04	1.42±0.22*/-	0.35±0.2	1.15±0.17
		La Punta	0.78±0.04	1.05±0.13	0.94±0.19	1.46±0.37
	(E,Z)-2,6-Nonadienal	Carrizales	449.04±29.3	587.54±85.99	638.66±60.88*/-	1049.28±7.64*/-
		La Punta	356.12±40.47	495±95.57	1053.79±110.05*/-	1059.9±73.41*/-
	(E,E)-2,4-Decadienal	Carrizales	0.5±0.17	0.73±0.12 -/*	0.22±0.22	1.24±0.66
		La Punta	1.07±0.28	2.12±0.24 -/*	1.39±0.63	2.74±1.08
	Benzaldehyde	Carrizales	24.95±1.88	77.57±7.59*/-	67.14±14.86*/-	157.26±6.95*/-
		La Punta	48.07±10.45	46.69±12.01	54.78±8.29	59.07±2.37
	Phenylacetaldehyde	Carrizales	0.74±0.47	15.95±4.17*/*	3.83±0.29	11.76±0.58*/*
		La Punta	2.23±0.45	4.74±0.73 -/*	7±1.54*/-	4.34±1.89 -/*
Esters (ng g^{-1})	2-Methyl propyl acetate	Carrizales	n.d.	0.58±0.58	1.25±0.08	0.93±0.08
		La Punta	n.d.	n.d.	1.02±0.21*/-	1.11±0.24*/-
	(E,E)-2,4-Hexadienoic acid, ethyl ester	Carrizales	1.34±0.15	2.03±0.45*/-	1.76±0.13 -/*	2.98±0.15*/-
		La Punta	1.22±0.1	1.66±0.36	3.53±0.32*/*	3.06±0.22*/-
	butyl butyrate	Carrizales	0.38±0.22	1.23±0.17*/*	1.03±0.09*/-	1.07±0.02
		La Punta	n.d.	n.d. -/*	1.08±0.09*/-	0.92±0.05*/-
	Ethyl butanoate	Carrizales	4.11±0.23	7.24±1.09*/*	8.18±0.36*/-	8.05±0.44*/-
		La Punta	3.67±0.09	4.21±0.27 -/*	8.29±0.61*/-	7.07±1.08*/-
Apocarotenoids (ng g^{-1})	6-methyl-5-Hepten-2-one	Carrizales	n.d.	n.d.	0.19±0.19	n.d.
		La Punta	n.d.	n.d.	n.d.	n.d.
	Geranylacetone	Carrizales	n.d.	n.d.	n.d.	0.58±0.58
		La Punta	0.14±0.14	n.d.	n.d.	n.d.
	Beta-Ionone	Carrizales	1.59±0.12	2.05±0.29	0.85±0.5	3.26±0.46
		La Punta	1.56±0.11	2.14±0.19	1±0.64	2.38±0.55
	Beta-cyclocitral	Carrizales	n.d.	n.d.	n.d.	0.76±0.76
		La Punta	n.d.	n.d.	n.d.	n.d.

A significant impact of grafting on the total VOCs was observed in both fields (Figure 9), with the *Cucumis* rootstocks being those that produced fruits with higher VOC content. The fruits produced in plants grafted onto these two rootstocks had VOC profiles with more aldehydes, more alcohols, and more esters. The *Cucurbita* rootstock also increased

volatile content, especially alcohols and aldehydes, although this increase was not so important as that of *Cucumis* rootstocks and was only significant in Carrizales.

Principal component analyses showed that the different fruit samples were grouped according to their VOC profiles in both fields (Figure 10). Consistently, the samples from NG plants were grouped apart from those obtained from grafted plants, especially those grafted onto Cucumis. They were separated according to the first component that explained more than the 50% of the observed variation. This effect was observed in both fields, and fruits grafted onto *Cucumis* rootstocks, both the melon F_1Pat81 and the wild Fian, resulted in fruits richer in most volatile compounds. This was also true for fruits produced by plants grafted onto the *Cucurbita* rootstock, but the effect of this rootstock was dependent on the field assay, having a similar effect to that of the F_1Pat81 in Carrizales, but with less impact on plants grown in La Punta. The second component, which explained 15% of the observed variation, separated fruits produced in F_1Pat81 grafted plants from those produced on Fian grafted plants, with the latter having higher contents in some aldehydes and apocarotenoids (Figure 10).

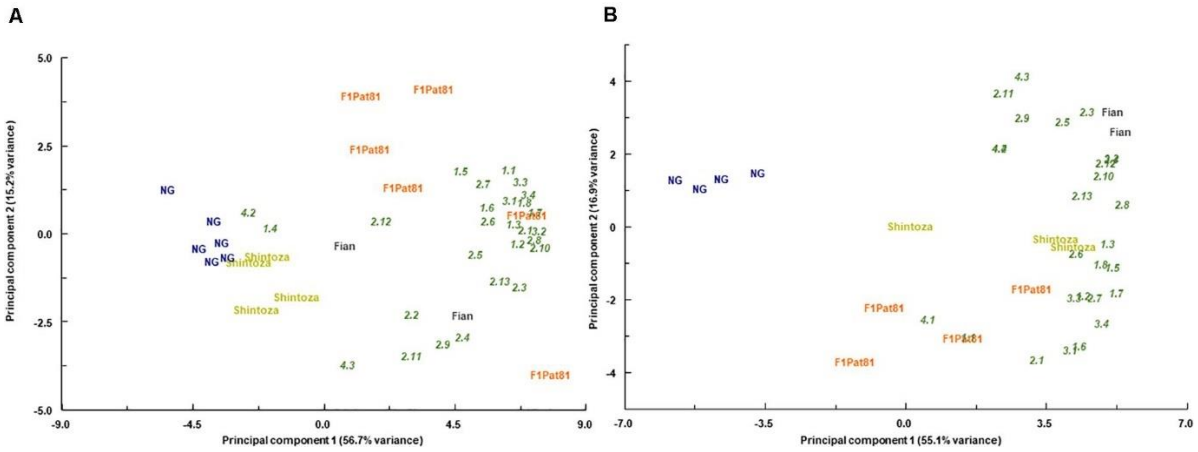


Figure 10. Biplots of scores (bold) and loadings (italics) obtained in the principal component analyses performed with the contents of volatile organic compounds in fruits from non-grafted (NG) and grafted (Shintoza, F_1Pat81, and Fian) grown at La Punta (A) and Carrizales (B). Samples obtained from the sensory analyses performed in 2019 as described in legend of Figure 8. 1_1, 1-pentanol; 1_2, (Z)-3-hexen-1-ol; 1_3, 1-nonanol; 1_4, (Z)-3-nonen-1-ol; 1_5, benzyl alcohol; 1_6, phenol; 1_7, phenylethanol; 1_8, 1-hexanol; 2_1, hexanal; 2_2, heptanal; 2_3, (E)-2-heptenal; 2_4, (E,E)-2,4-heptadienal; 2_5, (E)-2-octenal; 2_6, nonanal; 2_7, (Z)-6-nonenal; 2_8, (E)-2-nonenal; 2_9, (E,E)-2,4-nonadienal; 2_10, (E,Z)-2,6-nonadienal; 2_11, (E,E)-2,4-decadienal; 2_12, benzaldehyde; 2_13, phenylacetaldehyde; 3_1, 2-methyl propyl acetate; 3_2, (E,E)-2,4-headienoic acid, ethyl ester; 3_3, butyl butyrate; 3_4, ethyl butanoate; 4_1, 6-methyl-5-hepten-2-one; 4_2, geranylacetone; 4_3, beta-ionone; 4_4, beta-cyclocitral.

When analyzed in detail (Table 4), we observed in both fields that the F_1Pat81 rootstock increased the content of some of the most abundant aldehydes, (E,Z)-2-6-nonadienal, E-2-nonenal, hexanal, and benzaldehyde (the latter more in Carrizales than in La Punta), of

most alcohols, with the higher increase occurring in 2-phenylethanol, but also in (Z)-3-hexen-1-ol, 1-nonanol, and benzyl alcohol, and also affected ester content, doubling the amount of ethyl butanoate, compared to the NG melons. Most of these effects were also observed in fruits produced by plants grafted onto the wild melon rootstock (Fian), with some differences, the latter with a higher increase in most aldehydes, except from hexanal.

The analysis of specific products also showed the effect of the *Cucurbita* rootstock, Shintoza. Conversely to the consistent effect found in F_1Pat81 and Fian, the *Cucurbita* effect was affected by the field, with the impact on VOCs being higher in Carrizales. The impact on aldehydes was less important, with a significant increase in some compounds regarding NG, but lower than that observed in the *Cucumis* rootstocks, for example, for main aldehydes such as (E,Z)-2-6-nonadienal and hexanal, which did not significantly increase in any of the fields. Benzaldehyde and phenylacetaldehyde, however, significantly increased in the saline conditions of Carrizales, even more than in the F_1Pat81. A similar situation was found for the increase in alcohols and ethyl butanoate, which were only significant in Carrizales.

Correlation analysis performed with sensory scores and metabolite contents is shown in Figure 11. We found moderately significant correlations between flavor and aroma ($R = 0.42$) and between flavor and texture ($R = 0.53$). Flavor acceptability was positively correlated to the sugars ($R = 0.56$ to $R = 0.63$) and malic acid contents ($R = 0.47$), with a higher correlation being detected between flavor and glucose ($R = 0.63$). Significant negative correlations were found between flavor acceptability and contents of (Z)-3-hexen-1-ol ($R = -0.59$), benzyl alcohol ($R = -0.43$), 1-hexanol ($R = -0.40$), benzaldehyde ($R = -0.50$), and phenylacetaldehyde ($R = -0.64$).

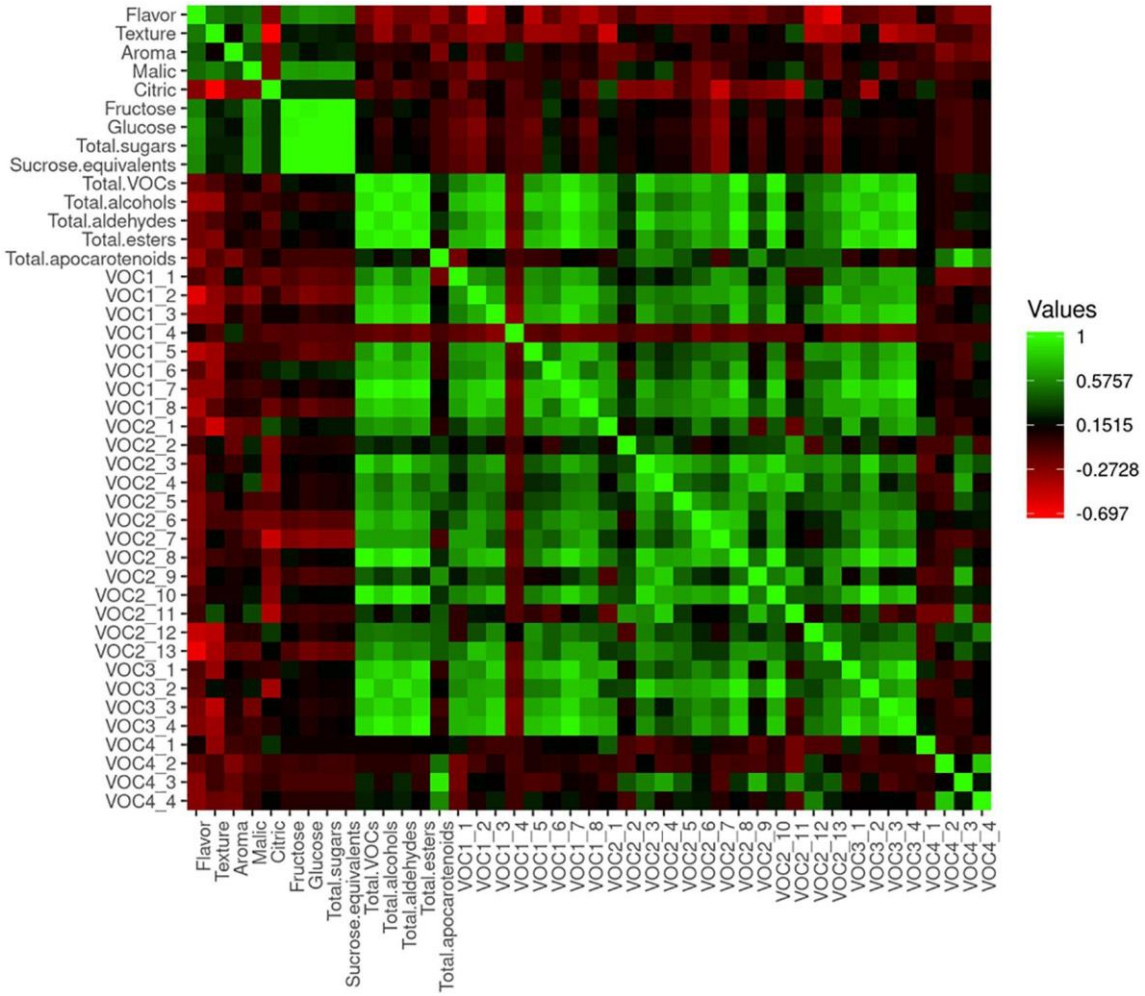


Figure 11. Heatmap of correlation analyses performed with data from the sensory evaluation and metabolite contents of the samples of 2019. 1_1, 1-pentanol; 1_2, (Z)-3-hexen-1-ol; 1_3, 1-nonanol; 1_4, (Z)-3-nonen-1-ol; 1_5, benzyl alcohol; 1_6, phenol; 1_7, 2-phenylethanol; 1_8, 1-hexanol; 2_1, hexanal; 2_2, heptanal; 2_3, (E)-2-heptenal; 2_4, (E,E)-2,4-heptadienal; 2_5, (E)-2-octenal; 2_6, nonanal; 2_7, (Z)-6-nonenal; 2_8, (E)-2-nonenal; 2_9, (E,E)-2,4-nonadienal; 2_10, (E,Z)-2,6-nonadienal; 2_11, (E,E)-2,4-decadienal; 2_12, benzaldehyde; 2_13, phenylacetaldehyde; 3_1, 2-methyl propyl acetate; 3_2, (E,E)-2,4-hexadienoic acid, ethyl ester; 3_3, butyl butyrate; 3_4, ethyl butanoate; 4_1, 6-methyl-5-hepten-2-one; 4_2, geranylacetone; 4_3, beta-ionone; 4_4, beta-cyclocitral.

3.5. Discussion

Melon production is limited by viral and fungal diseases (García-Jiménez et al., 2008; Lecoq and Katis, 2014; González et al., 2020a). Resistances to the main viruses affecting melons have been reported and introgressed into commercial cultivars. However, traditional varieties, such as snake melons, have been neglected in breeding programs, and most of them are susceptible to most viruses (Yousif et al., 2007; Solmaz et al., 2016b). In the current study, we detected several viruses in different years and fields affecting snake melon plants. The aphid-transmitted Potyvirus WMV was the most prevalent, but another potyvirus, ZYMV, and the Begomovirus ToLCNDV were also found with a lower incidence. Resistances to all these viruses are available (Sáez et al., 2017; Pérez-De-Castro et al., 2019; Martín-Hernández and Picó, 2020), but have not been introgressed into the snake melon background. Therefore, introgression programs are needed to develop snake melon cultivars with resistance to these viruses.

Regarding the incidence of fungi, global warming is favoring the increasing incidence of highly damaging fungi to melon (Cohen et al., 2012; de Sousa Linhares et al., 2020; Timmusk et al., 2020). *M. phaseolina* and *N. falciformis* were identified as major pathogens in snake melon NG plants. Our inoculation studies confirm the worse response of snake melon, compared to Piel de Sapo sweet melon, to both pathogens, being highly susceptible to *M. phaseolina* and MR to *N. falciformis*. The former has been described as a main pathogen of melon worldwide, with recently available resistant sources (Ambrósio et al., 2015; Cohen et al., 2016; de Sousa Linhares et al., 2020) and the determination of the genetic control under study. The latter has been very recently reported for the first time as a melon pathogen in Spain (González et al., 2020c, 2020b). The high susceptibility of snake melon to Brazilian isolates of *M. phaseolina* compared to sweet melons was already reported by Ambrósio et al., (2015). The response of this melon to *N. falciformis* is reported here for the first time.

Despite the fact that plant mortality caused by these pathogens was moderate, the impact on the production was very important. The dramatic impact of diseases on snake melon production under organic farming management, almost halving yield, was confirmed in the experiment of 2018, when the production of snake melon in infested soils with previous melon cultivation (La Punta) was compared to that obtained in a soil with no previous cultivation of melon (Moncada). Despite production losses, there was not an

important effect on fruit traits, other than reduction in FF. Compared to the impact of fungal attack, salts stress of Carrizales in 2018 was less damaging for snake melon plants, and productions similar to those of the unstressed control field were obtained. Therefore, snake melons seem to be much more susceptible to fungal stress than to salinity. This is consistent with the general idea that melons, like other Cucurbits, are only moderately sensitive to salinity, compared to other vegetables (Villalobos et al., 2016; Wang et al., 2016). As occurred with fungal stress, FF was slightly reduced under salt stress, which also increases SSC. These effects have been previously reported in sweet melons with higher SSC when grown at high salinity levels (Colla et al., 2006), but our results also show a similar effect even in this non-sweet melon. Also, an effect of salinity on fruit firmness has been reported (Trajkova et al., 2006; Yarsi et al., 2012).

Grafting can be used to reduce the impact of stressful conditions on snake melon production and quality. In fact, the use of *Cucumis* rootstocks, both melon, F_1Pat81, and wild *Cucumis*, Fian and Fimy, reduced plant mortality. *M. phaseolina*, *M. cannonballus*, and different species of *Fusarium* were detected in plants cultivated in both fields. Previous studies showed that F_1Pat81, Fian, and Fimy are quite tolerant to *M. cannonballus* and *M. phaseolina* (Ambrósio et al., 2015; Cáceres et al., 2017; Castro et al., 2020; Gisbert et al., 2020), although the tolerance derived from Pat 81 seems to be temperature dependent (Castro et al., 2020; de Sousa Linhares et al., 2020). The wild *Cucumis* are also resistant to *F. oxysporum* (Matsumoto et al., 2011; Gisbert et al., 2020). These tolerances may account for the reduced mortality found in snake melon plants grafted onto these rootstocks. *Cucurbita* rootstocks are known to have resistance to *F. oxysporum* and tolerance to *M. cannonballus* and *M. phaseolina*. However, in our study, plant mortality similar to that found on NG snake melon occurred in *Cucurbita* grafted plants. Regarding the lower performance of Cucurbita rootstocks, it should be considered that these are usually employed to control *F. oxysporum*. This fungus, along with *M. phaseolina* and *M. cannonballus*, were detected in *Cucurbita* roots. However, in this case, the high levels of mortality could be due to the presence of other fungi as *N. falciformis* and *N. keratoplastica* of the FSSC (O'Donnell et al., 2008; González et al., 2020a), as *F. solani* has been found to very seriously affect the *Cucurbita* hybrid rootstocks in watermelon (Armengol et al., 2000). It is then necessary to include these new pathogens in the selection process of new rootstocks for melon to overcome future pathogenic

problems in these *Cucurbita* species, due to the possible spread of these soilborne fungi in our producing areas.

As stated before, grafting onto the melon F_1Pat81 and wild *Cucumis* rootstocks reduced consistently plant mortality in different agroecological conditions, but the impact on the production per plant was variable between years and fields, and similar to that caused by the *Cucurbita* rootstocks. Despite the variability, in most cases, grafted plants displayed lower production losses than NG, except those grafted onto the Fimy rootstock, which were less productive. Under salt stress, grafting significantly increased production per plant, as occurred in 2018 in Carrizales, with similar effect of melon and *Cucurbita* rootstocks. The combined effect of salinity and fungal stress, and the environmental conditions that favored the stressful scenario, caused the highest impact on production in Carrizales in 2019. Increased susceptibility to soil borne diseases has been reported in tomato under high salinity (Bai et al., 2018). Also, in melons, enhanced fungal damage has been reported under saline conditions (Roustaee et al., 2011; Mirtalebi and Banihashemi, 2019). Grafting contributed to alleviate the impact of these extreme conditions. The lower performance of F_1Pat81 in these conditions could be related to the higher temperatures, as the level of resistance against *M. phaseolina* and *M. cannonballus* of materials derived from F_1Pat81 drop at higher temperatures (Castro et al., 2020; de Sousa Linhares et al., 2020). Therefore, grafting is a good strategy to reduce plant mortality due to fungal stress and can alleviate yield losses, depending on the resistance of the rootstocks and the fungal profile of the soil, and increase production under saline conditions.

The use of grafting in melon is prevented by the impact that the different rootstocks can have on fruit quality (Fita et al., 2007; Fallik and Ilic, 2014; Fredes et al., 2017; Leonardi et al., 2017). In the case of snake melons, the effect of the different rootstocks on fruit traits, such as shape, firmness, pH, or Brix degree, was minimum for both *Cucumis* and *Cucurbita* rootstocks, and only limited effects in certain combinations and environments were detected. Most of these mild effects were associated to the flesh and rind color. Variable effects on fruit color have also been reported for different types of rootstocks in sweet melons (Cáceres et al., 2017). The increase in Brix degree observed in NG snake melons cultivated under salt pressure in Carrizales was also consistently observed both years in grafted plants, which is more intense in plants grafted onto Cucumis rootstocks.

Even when no effect of rootstocks on basic fruit traits is detected, grafting can alter metabolite profiles, which may affect consumers' preferences. Trained sensory panels have been successfully used for the evaluation of sweet melons (Bianchi et al., 2016; Park et al., 2018; Ayres et al., 2019), Spanish sweet melons (traditional landraces) (Escribano and Lázaro, 2012), and even snake melons (Omari et al., 2018). Training such panels is time-consuming and much more difficult to implement in neglected crops such as snake melon. Despite these difficulties, in this study, they consistently showed preferences for snake melon fruits produced in NG plants. In this sense, *Cucurbita* hybrid rootstocks clearly scored below the NG control in most sessions, while the Cucumis rootstock F_1Pat81 showed no significant differences with the NG control. It is also important to note that some tasters reported fibrous or course textures and strange tastes for the Cucurbita grafted plant fruits.

We performed analysis of sugars, acids, and volatiles to evaluate if these consumer preferences might be associated to metabolic profile differences due to grafting. Snake melons do not accumulate sucrose, or present it at very low levels. Burger et al., (2003) related this trait with a high acid invertase activity that prevents the accumulation of sucrose and described the recessive gene *suc* as responsible for high sugar accumulation in sweet melons. The fruits analyzed in this work have shown this profile, with sucrose remaining under the limits of quantification and limited accumulation of hexoses. The main acids detected in our snake melon fruits were citric and malic, with malic being predominant. Burger et al., (2003) described the same profile in the variety "Faqqous" of snake melon, with high acid values being conferred by the dominant gene *So*. Snake melons (along with Indian Momordica and Acidulus melons) are considered sour melons and, in contrast to sweet melons, have malic acid as the main organic acid. The non-sweet and acidic profile found in our snake melon is typical of the *So*/*So*, *Suc*/S*uc* combination, described in other *C. melo* var. *flexuosus* varieties (Burger et al., 2003). (Cohen et al., 2014) identified the gene *CmPH* with a major effect on fruit acidity. They found a high acid profile in the cultivars that lacked a four-amino acid duplication in this gene, among them the flexuosus melons.

Grafting seems to affect the sugars and organic acid profile, with this effect being more accentuated with *Cucurbita* rootstocks that significantly decrease both hexoses, affecting sucrose equivalents and malic acid. Melon rootstocks did not alter malic acid content, and the effect on sugars was only significant for glucose, not affecting sucrose equivalents.

The sugar and organic acid contents can affect consumer acceptability. In fact, flavor scores of sensory evaluations were positively correlated with sugars and malic acid contents.

Snake melons are climacteric melons, aromatic when fully mature (Esteras et al., 2018), but we characterized the aroma profile at commercial maturity, when fruits are physiologically immature. At this ripening stage, VOC profile was more like that of non-climacteric melons, very rich in aldehydes, followed at a considerable distance by alcohols, and low levels of esters and apocarotenoids. The aldehyde profile of the snake melon was characterized by high contents of (E,Z)-2-6-nonadienal, followed by E-2-nonenal, hexanal, and benzaldehyde, which differ from the aldehyde profile usually found on aromatic and non-aromatic sweet melons. These compounds are known to be some of those with the main impact in melon aroma (Gonda et al., 2016). (E,Z)-2-6-nonadienal is reported to contribute with cucumber-like odor, whereas E-2-nonenal and hexanal contribute with green and fresh odor notes. This profile differs from that of sweet climacteric melons, poorer in these aldehydes, but also from other non-climacteric melons, such as the inodorus group, rich in aldehydes, but with a different aldehyde profile, richer in hexanal (Esteras et al., 2018). The alcohol profile was also different to that of aromatic sweet melons, richer in hexanol, stale odor, and fermented notes, and Z-3-hexen–1-ol, grassy-green odor, instead of 2-phenylethanol, floral odor, the main alcohol present in snake melons. The ester compounds, which are the major contributors to the aroma of sweet melons, are nearly absent in this immature fruit, with ethyl butanoate being a major ester contributor to melon aroma and the most abundant, although in very low amounts compared to sweet melons. Also, the apocarotenoid beta-ionone was the only consistently detected, but in very low amounts. This profile was similar to that reported for the “Cai Gua” snake melon by Tang et al., (2015) and Chen et al., (2016), who also reported high levels of phenylethanol among alcohols, and (E-Z)-2-6-nonedienal, (E)-2-nonenal, hexanal, and benzaldehyde among aldehydes.

There was a clear impact of grafting on VOC profile, with a higher effect in *Cucumis* vs *Cucurbita* rootstocks. Grafting onto *Cucumis* rootstocks resulted in fruits with a VOC profile richer in most aldehydes, alcohols, and esters. This effect was lower and more dependent on the field conditions in Cucurbita rootstocks. Flavor acceptability showed significant negative correlations with some of these compounds, (Z)-3-hexen-1-ol, benzyl alcohol, and 1-hexanol, among alcohols, and phenylacetaldehyde and benzaldehyde,

among aldehydes. All grafted plants had significant increases of most of these compounds compared to NG plants. The increase was higher for *Cucurbita* vs *Cucumis* rootstocks in Carrizales for (Z)-3-hexen-1-ol, the compound with the highest negative correlation with flavor acceptability. The increase in the two aldehydes more negatively correlated with flavor acceptability, phenylacetaldehyde and benzaldehyde, was more important in *Cucurbita* in the Carrizales assay, associated with the lower scoring of fruits produced by plants grafted onto these rootstocks in the sensory evaluations.

Our results showed that *Cucurbita* rootstock has a higher impact in sugar and organic acid profile than in VOC profile, resulting in a less favorable consumer perception, and that Cucumis rootstocks affect VOC profile more than sugar and acid profile, which may result in a lower effect on consumer perception, although the increase in specific alcohols and aldehydes could also be related to the less positive perception of consumers of fruits coming from grafted plants.

3.6. Conclusion

Snake melon seems to be moderately susceptible to biotic stress and especially to soilborne diseases. Nonetheless, under high incidence conditions, yield losses can be higher than 50%. This yield loss would be even higher when combined with high salinity, due to a synergic effect. In organic farming, strategies against diseases are limited, and in this context, the use of rootstocks seems to be an efficient alternative. The use of Cucumis rootstocks seems to be more favorable. F_1Pat81 not only reduced yield losses under biotic stress but also increased yield under salt stress. Nonetheless, grafting may have a side effect on consumer acceptability. This effect seems to be related to changes in the profiles of the analyzed metabolites caused by grafting, but it depends on the specific scion–rootstock combination. Cucumis rootstocks had a major effect on the VOC profile, but the incidence on sugar accumulation was limited compared to Cucurbita rootstocks, probably reducing the negative side impact on flavor.

Supplementary Files

All Supplementary files are available for download at: https://www.frontiersin.org/articles/10.3389/fpls.2021.613845/full#supplementary-material

First Report of *Neocosmospora falciformis* Causing Wilt and Root Rot of Muskmelon in Spain

González, V.[1*], García-Martínez, S.[4], Ruiz, J.J.[4], Flores-León, A.[2], B. Picó, B.[2], Garcés-Claver, A.[3],

1. Centro de Investigación y Tecnología Agroalimentaria de Aragón, Unidad de Sanidad Vegetal/Instituto Agroalimentario de Aragón-IA2 (CITA-Universidad de Zaragoza), 50059 Zaragoza, Spain
2. Departamento de Biología Aplicada, Universidad Miguel Hernández de Elche, 03312 Desamparados-Orihuela, Spain
3. Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV-UPV), Universitat Politècnica de València, 46022 Valencia, Spain
4. Centro de Investigación y Tecnología Agroalimentaria de Aragón, Unidad de Hortofruticultura/Instituto Agroalimentario de Aragón-IA2 (CITA-Universidad de Zaragoza), 50059 Zaragoza, Spain

'Cantaloupe' and 'Piel de Sapo' are melon (*Cucumis melo* L.) varieties cultivated in Spain. In 2018, during a pathogens survey in experimental fields of Valencia and Alicante provinces (southeast Spain), wilt and root rot of melon plants were detected in grafted and ungrafted plants. Disease incidence ranged from 10% (Alicante) to 45% (Valencia). Symptoms included yellowing and wilting of leaves, rotting at the stem base and upper root, and collapse of the entire plant. Samplings were conducted from severely decayed and dead plants. Fragments (0.5 to 1 cm) from rotted lower stems and roots were surface disinfected for 1 min in 1.5% NaOCl, washed twice with sterilized distilled water, and plated onto potato dextrose agar (PDA) with streptomycin sulfate (0.5 g/liter). Plates were incubated at 25°C in the dark for 3 to 5 days. Mycelia resembling *Fusarium* were isolated and characterized by morphological and molecular methods. Based on their adpressed beige mycelia, growth in concentric rings, and absence of sporodochia, colonies growing on PDA and Spezieller Nährstoffarmer agar were preliminary identified as belonging to the *Fusarium solani* species complex. On PDA, colonies were white-greyish to pale-cream growing in concentric rings with beige reverse after 6 days. No sporodochia were observed. Macroconidia were slender, falcate, hyaline, three to five septate 43 (38 to 47) × 4.5 (3.8 to 5.2) µm; aerial microconidia were abundant, borne on short, undifferentiated

monophialides, ellipsoidal to reniform, sometimes with a truncate base, and zero to one septate 10 (9.2 to 11.4) × 3.5 (2.5 to 6) µm. Chlamydospores were globose, single or in chains, intercalary and thin- to thick-walled. Sequencing of the internal transcribed spacer (ITS) region, a fragment of translation elongation factor-1α (TEF-1α), and RNA polymerase II (RPB2) partial genes was done using ITS1/ITS4 (White et al., 1990), EF1/EF2 (O'Donnell et al., 1998), and fRPB2-7cF/fRPB2-11aR (Reeb et al., 2004) primers, respectively. After comparisons using BLASTn and the *Fusarium* ID database (http://www.wi.knaw.nl/fusarium/), eight isolates were identified as *Neocosmospora falciformis*. The ITS, EF-1α, and RPB2 sequences of isolate CRR 2-6 showed 99% homology with *N. falciformis* EU329691 (ITS), AB817158 (EF-1α), and EU329650 (RPB2). Sequences were deposited in GenBank with accession numbers MN086327 (ITS), MN509809 (TEF-1α), and MN509810 (RPB2). For pathogenicity tests, isolate CRR 2-6 was grown in 250-ml flasks containing potato sucrose medium for 3 days at 25°C in the dark with constant agitation. Roots of ten 15-day-old Piel de Sapo seedlings grown 6 days in trays with sterilized substrate were submerged into a suspension of 5×10^6 conidia/ml for 2 min and transferred to the plastic containers. Three plants submerged in sterile water served as controls. Plants were incubated in a growth chamber (25°C; 16-h/8-h photoperiod). Scarce development, wilting, and yellowing followed by plant death were observed 15 days post-inoculation. Non-inoculated controls remained asymptomatic. The fungus was reisolated from all the inoculated plants and identified using ITS, TEF-1α, and RPB2. *N. falciformis* belongs to the *Neocosmospora* (*Fusarium*) *solani* species complex (O'Donnell et al., 2008). To our knowledge, this is the first report of *N. falciformis* causing wilt and root rot of melon in Spain. The adoption of molecular-based identification methods should lead to a more precise determination on incidence of the pathogen in this Mediterranean area.

Chapter 4. Evaluating grafted Ibericus Traditional melons under Organic Farming Conditions: effect on agronomic performance and fruit traits.

Chapter 4. Evaluating grafted Ibericus Traditional melons under Organic Farming Conditions: effect on agronomic performance and fruit traits.

This chapter studies the effect that grafting has on a collection of traditional Ibericus melons, with an analysis on their performance under organic farming conditions. The cultivars were evaluated in different localizations, which provided different limiting factors (biotic and abiotic). The main pathogens affecting each melon cultivar were noted, as well as pests. A total of 5 different melon rootstocks (2 *Cucurbita* commercial hybrids and 3 *Cucumis*) were employed and their compatibility, and their effect on agronomic performance and fruit traits were evaluated. This paper is being prepared to be published in Agronomy for Sustainable Development

This work was supported by grants PROMETEO/2017/078 and PROMETEO/2021/072 (to promote excellence groups) funded by Conselleria d'Educació, Investigació, Cultura i Esports (Generalitat Valenciana, Spain) and grants PID2020-116055RB-C21 and AGL2017-85563-C2-1-R-AR funded by AEI 10.13039/501100011033 and by "ERDF A way of making Europe". AFL is a recipient of a predoctoral fellowship from Generalitat Valenciana..

This work was performed by Cucurbits Breeding Group of the COMAV, in collaboration with Dr. Santiago Garcia and Dr. Juan Jose Ruiz from the Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández. Also, collaboration with the Plant Protection Unit and Horticulture Unit of the AgriFood Institute of Aragon (IA2), from the Universidad of Zaragoza, Dr. Vicente González, Dr. Ana Garcés-Claver and Dr. Carmen Julián. Collaborating also with this work was Dr. Maria Ferriol from the Instituto Agroforestal Mediterráneo (IAM-UPV).

Chapter 4. Evaluating grafted Ibericus Traditional melons under Organic Farming Conditions: effect on agronomic performance and fruit traits

Alejandro Flores-León[1], María López-Martín[1], Santiago García-Martínez[2], Vicente González[3], Ana Garcés-Claver[4], Mercedes Valcarcel[1], Carmen Julián[3], Alicia Sifres[1], Ana Pérez-de-Castro[1], María José Díez[1], Carmelo López[1], María Ferriol[5], Carmina Gisbert[1], Juan José Ruiz[2], Belén Picó[1*]

1 Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain,
2 Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández, Orihuela, Spain,
3 Plant Protection Unit/Instituto Agroalimentario de Aragón-IA2, Centro de Investigación y Tecnología Agroalimentaria de Aragón, Universidad de Zaragoza, Zaragoza, Spain,
4 Horticulture Unit/Instituto Agroalimentario de Aragón-IA2, Centro de Investigación y Tecnología Agroalimentaria de Aragón, Universidad de Zaragoza, Zaragoza, Spain,
5 Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Valencia, Spain

Correspondence: mpicosi@btc.upv.es

4.1. Abstract

The performance of several Ibericus traditional cultivars in organic farming conditions was studied under both abiotic (saline) and biotic stress. Soilborne pathogens detected in roots where mainly *Macrophomina phaseolina*, *Fusarium* spp. and *Neocosmospora falciformis*. The main viruses detected where *Watermelon mosaic virus* (WMV), *Cucurbit ahid borne yellow virus* (CABYV), *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Cucumber mosaic virus* (CMV). Infection by *Podosphaera xanthii* affected approximately 20-40% of the plants. Salts stress had a synergic effect, with a loss of yield combined with biotic fungal stress. Saline conditions resulted in an increase of SSC and variation of fruit colour between the fields. Grafting also affected several of the fruit traits such as productivity, fruit size, SSC, seed cavity and fruit flesh colour compared to non-grafted plants. *Cucurbita* rootstocks presenting higher detrimental effect in some of these key fruit characteristics, while plants grafted onto *Cucumis* rootstocks presented similar characteristics to non-grafted plants. The use of *Cucumis* rootstocks appear to be better

than traditional *Cucurbita* hybrids to enable organic production of this high-quality market fruits.

4.2. Introduction

Melon (*Cucumis melo* L.) is an important crop of the Cucurbitaceae family, with its fruits being highly appreciated and mainly consumed as a dessert, although also as a vegetable in some regions (Esteras et al., 2020). Spain is the first producer of melon of the European Union with 660 thousand tonnes, followed by Italy and France. In Spain, this crop is mainly located in the area known as the "Spanish Levant" characterized for both greenhouse and open field production, as well as in the Region of Castilla-La Mancha, where melon is grown in open fields (MAPA, 2021).

The presence of plants of *C. melo* in the Mediterranean basin is really early, with the earliest presence dated to Late Bronze age, although these seeds are closer to modern day non-sweet melons such as those belonging to the horticultural groups Chate, Flexuosus, and Ameri than to modern sweet melons (Sabato et al., 2019). Sweet melons are supposed to have been introduced to Spain during the Middle Ages, with the Islamic conquest of the Iberian Peninsula, when sweet casaba type melons were introduced from Central Asia to Europe (Paris et al., 2009, 2012a). Local farmers cultivated, conserved and exchanged their seed, carrying the best fruits to the most popular markets (Escribano and Lázaro, 2009). Through centuries of selection by the local farmers and consumers, these melons adapted to the diverse agro-climatic conditions of the Iberian Peninsula. Ancient Spanish cultivars, called traditional cultivars, have been maintained for decades by farmers, having been maintained until the genetic erosion started in the XXth century, as a consequence of varietal replacement (Lázaro et al., 2017). A recent classification of melon groups (Pitrat, 2016) recognizes the sweet melons from Spain, classifying them into the group denominated as Ibericus, with sub-groups melons like 'Piel de Sapo', 'Rochet', 'Amarillo', 'Blanco' or 'Tendral'. This group, that includes one of the main worldwide market type 'Piel de Sapo', is characterized for having elliptical or acorn fruit shape, more or less wrinkled, no vein tracts, thick skin, thick light-green or light-orange juicy flesh, high sugar content, low aroma, late maturing and long shelf life (Pitrat, 2016), being quite different from the other main commercial groups: Inodorus (including Honeydew and Earl´s) and Cantalupensis (including Cantaloupes and Charentais). Traditional cultivars from the Ibericus sub-groups are still cultivated nowadays, although

mainly for self-consumption or for local markets with the exception of the 'Piel de Sapo' subgroup, which as stated before is widely cultivated for national and international markets (Esteras et al., 2013).

For many years, Spanish commercial breeders focused on the selection of cultivars from the 'Piel de Sapo' and 'Amarillo' subgroups that share many characteristics (Escribano and Lázaro, 2012). Such is this that about the 50% of cultivated Spanish melons are 'Piel de Sapo', 30 % are those classified as Piel Lisa, mainly 'Amarillo' and 'Blanco' subgroup melons, a low percentage of Ibericus are 'Tendral' (approx. 3%), and only 15% are cultivars of the Cantalupensis group (MAPA, 2021). A study on traditional Spanish melon cultivars (Escribano and Lázaro, 2009) found a wide variability in a very small area with a vast historical importance in melon farming (Madrid Community), which means that by exploring traditional production areas the probability of discovering ancestral genetic resources is higher. Traditional cultivars and their preservation could provide with an excellent opportunity for breeders, as the success of new varieties is mainly due to an increase in its sensorial quality (Lester, 2006). Some studies have already studied the flesh and rind volatile organic compound composition of both commercial and traditional Spanish cultivars and found differences between them (Esteras et al., 2018, 2020). Escribano and Lázaro, (2012) indicate in their study that traditional melon cultivars of Villaconejos (Madrid) are appreciated as much or even more than commercial varieties, but that theses traditional cultivars are no longer available in the market. Hence it is in their great diversity of traits, where they possess their main focus, as they are capable to capture the attention of consumers seeking unique, nutritious, local food sources (Dwivedi et al., 2019). There are more than 1000 different Spanish accessions conserved in several plant germplasm collections (Lázaro et al., 2017). In Valencia, local traditional cultivars can be found in Genebanks such as the Universitat Politècnica de València Genebank (UPV-Genebank available at http://www.upv.es/contenidos/BGCOMAV). One way to make these traditional cultivars more available for the market could be to produce them under organic conditions, as organic foods are widely perceived to be tastier and healthier than conventionally produced products and the production process is less damaging for the environment (De Magistris and Gracia, 2008).

Annunziata and Vecchio (2016) defines organic farming as a production system which sustains the health of soils and ecosystem, relaying on ecological processes, biodiversity and cycles adapted to local conditions, rather than the used of inputs with adverse effect.

In Europe, Spain has the most organic farming surface area with more than 2.35 million hectares, followed by France and Italy (Eurostats, 2021). In the case of organic production, Spain is the third in terms of fresh vegetables (including melons) dedicated area, after France and Italy (Eurostats, 2021). Organic farming faces a series of problems, with the main one being a yield gap with respect to conventional farming systems, with organic crops producing on average 80% of conventional crops (De Ponti et al., 2012; Ponisio et al., 2015).

In order to reduce this yield gap, it is important in organic farming to understand the environment and the factors affecting cultivation, both abiotic and biotic. The main problem many traditional cultivars face is that they lack resistance to important pests and diseases (Dwivedi et al., 2019), leading to a reduction in yields or a less stable production specially under the climate change scenario in which the pressure on yield by biotic and abiotic stressors is expected to increase, challenging global food security (Rouphael et al., 2018a). The use of varieties which are adapted to local pests and pathogens could help in increasing the stability of crop production. In the case of soilborne pathogens, the main way of controlling them is by using resistant rootstocks onto which the desired scions are grafted on. Grafting is a way to secure yield stability and quality in vegetable crops, which is why it is mainly employed for the production of high-value Solanaceae and Cucurbitaceae crops (Kyriacou et al., 2020). The use of grafting on melon is primarily focused to face pathogens such as *Fusarium* wilt, *Monosporascus* wilt and *Meloidogyne* nematodes (Pico et al., 2017; Kyriacou et al., 2018). The combination of scion-rootstock can influence the quality parameters of the fruits depending of the compatibility (Németh et al., 2020). In melon the most popular rootstocks employed are those belonging to the *Cucurbita* genus as they provide protection against many soilborne pathogens and abiotic stresses, but these rootstocks can negatively affect fruit quality parameters such as fruit size, soluble solids and VOCs contents (Pico et al., 2017). Less used are the resistant rootstocks of the *Cucumis* genus, although these rootstocks belonging to the same species could contribute to minimize compatibility problems, they are not sufficiently studied in terms of the advantages they possess to fruit yield and quality (Pico et al., 2017). For this reason, the Valencian Government (Generalitat Valenciana, Conselleria d' Educació, Investigació, Cultura i Esport) financed two projects (PROMETEO 2017/078 and PROMETEO 2021/072) to study Cucurbits under organic farming conditions.

In previous studies performed with the non-sweet, cucumber-like snake melon (Flores-León et al., 2021) in the Comunitat Valenciana under organic farming conditions, the performance and quality of grafted and non-grafted plants was evaluated. The presence of soilborne pathogens was more detrimental to the yield of non-grafted grafted than grafted plants. The *Cucurbita* rootstocks impacted more negatively consumer acceptance and fruit quality than *Cucumis* rootstocks. Fruits from *Cucumis* grafted plants even showed some quality improvement under saline conditions.

In the present paper, we extend the study to a collection of Traditional Spanish sweet melons, also analysing their behaviour under organic farming conditions and identifying the local biotic limiting factors, and the effect of grafting on field performance and fruit quality.

4.3. Materials and Methods

4.3.1. Study Location

A total of 3 different field assays were performed in this study, each representing different agronomic conditions. These fields have already been employed in a previous study (Flores-León et al., 2021). The first field was located near the area of Moncada, in the province of Valencia (39°33'26.8" N, 0°25'06.5" W), with no previous melon cultivation history, having been used for cultivating citrus fruits for the last 20 years. The second field was located in the area of the city of Valencia known as "La Punta" (39°26′41.3″ N, 0°21′'14.9″ W), with a previous history of melon cultivation and high reports of plant disease. The third field was located in the area known as Parque Natural Agrario de Carrizales" (38°08'32.8" N, 0°42'44.7" W) in the province of Alicante, an area famous for melon cultivation and for using saline water irrigation.

Climatic data from each field was obtained from public databases (" Agencia Estatal de Meteorologia", (www.aemet.es) and "Sistema de Información Agroclimática para el Regadío" (http://riegos.ivia.es/red-siar), selecting the stations nearest to the corresponding field. The conductivity of the soil and the water was measured for the 3 different locations. Water conductivity was measured with an electrical conductivity meter (CM35, CRISON, Barcelona, Spain). As for the soil conductivity, it was determined with the method described by Primo and Carrasco (1980). A total of 10 soil

samples were collected and homogenized. Then were dried at room temperature, sieved (2mm) and the conductivity was measured (dS/m).

4.3.2. Plant cycle

The assays were all performed under organic conditions with agricultural practices during the plant cycle previously described in Flores-León et al., (2021). In Carrizales, the water supplied is characterized for its high electric conductivity, resulting in a salt stress condition. In La Punta, the repetitive melon cultivation in the field has led to an infestation of soilborne pathogens. Finally, in Moncada, the lack of melon cultivation in the agronomical history of the field, provides with unstressed conditions. Assays were performed two consecutive years 2018 and 2019. Transplantation of the melon plants (2-3 true leaves stage) occurred between the end of March and the first week of April in the three cultivation sites, in 2018, and the first week of May in 2019.

In 2018, in all fields 17 non-grafted cultivars (NG) were cultivated (5 Piel de Sapo, 4 Amarillo, 4 Blanco, 3 Rochet and 1 Tendral). In the case of La Punta and Carrizales, 8 selected cultivars (2 Piel de Sapo, 2 Amarillo, 2 Blanco and 2 Rochet) representing most of the Spanish types, were additionally cultivated grafted onto two rootstocks, the experimental melon hybrid F1Pat81 and the commercial *Cucurbita* F1 hybrid Cobalt. A randomized complete block design with four plants per treatment and block was used (four blocks).

In 2019, plants were grown only in La Punta and Carrizales. This year 10 of the cultivars were cultivated (2 Piel de Sapo, 1 Amarillo, 2 Blanco, 2 Rochet, 1 Tendral and 2 Commercial Hybrids), with all plants being NG and grafted onto the 5 different rootstocks employed. The rootstocks employed were the previously described F1Pat81 and Commercial *Cucurbita* Cobalt, as well as another Commercial Cucurbita F1 Hybrid Shintoza, and 2 experimental *Cucumis* interspecific rootstocks Fian (*C. ficifolius* x *C. anguria*) and Fimy (*C. ficifolius* x *C. myriocarpus*) (Cáceres et al., 2017). A randomized complete block design with 3 plants per treatment and block was used (3 blocks). Supplementary Table 1 describe cultivars and rootstocks used in each assay.

4.3.3. Pest and Pathogen detection

Pests affecting the plants were identified and any airborne fungal attacks were also recorded. Plants showing viral symptoms were sampled in order to identify the virus responsible for the infection, following the methods described by Pérez-De-Castro et al., (2020).

Plants showing symptoms of soilborne pathogens were also analysed to identify the causal agent or agents, as per the methods employed by (Flores-León et al., 2021). Pieces of necrotic lower steam and upper root tissue were disinfected (1min in 1.5% NaClO solution) and washed with bi-distilled water. The tissue was then placed in a Potato dextrose agar (PDA) medium with streptomycin (0.5g/l). Plates were incubated in dark conditions (25ºC, 3-5 days). The emerging colonies where then transferred individual PDA plates for each isolate subculture, to obtain pure cultures for the characterization. The isolates were identified morphologically by comparing their somatic and/or their sexual/asexual reproductive structures. A molecular characterization was made by PCR amplification of the ribosomal ITS fragment for most of the isolates, and TEF-1α and RPB2 gene fragments for the case of certain Fusarium species, using ITS1/ITS4 (White et al., 1990), EF1 / EF2 (O'Donnell et al., 1998) and fRPB2-7cF / fRPB2-11aR (Reeb et al., 2004) primers respectively. Sequences obtained allowed the identification of isolates by their comparison with homologous sequences deposited in public databases like GenBank (using BLASTn tool) or Fusarium ID Database (http://www.westerdijkinstitute.nl/fusarium/), as well as by performing phylogenetic reconstructions employing Bayesian inference methods from multiloci alignments of combined genomic regions for some of the mentioned Fusarium taxa.

4.3.4. Fruit Characterization

All melon fruit with marketable size were weighted during harvest, in order to calculate the yield per plant. Two Fruit from each plant were characterized for Fruit weight (FW in g, measured with digital scale), fruit length, diameter and cavity (FL, FD and FC, in cm, measured with a ruler), Rind Thickness (in mm, with a Vernier calliper), rind and flesh firmness (RF and FF, measured with a penetrometer in kg/cm2), fruit pH (universal pH indicator paper), soluble solids content (SSC, ºBrix, measured with some drops of juice using a hand-held Pocket refractometer (PAL-α), Atago CO., LTD, Tokyo, Japan).

Finally, both the fruit flesh and rind colours were measured in Hunter L, a and b coordinates (CR-400 colorimeter, Konica Minolta, Inc., Tokyo, Japan).

4.3.5. Statistical analysis

Fruit characterization and agronomic data were analyzed performing MANOVA to evaluate the effect of location, scion and rootstock, as well as their interaction. ANOVA tests, followed by Tukey and Dunnett's test to analyze the performance. StatGraphics Centurion version 17.2.04 for Windows and IBM SPSS Statistics 25 for Windows were used for this purpose.

4.4. Results

4.4.1. Climatic, water and soil results

The rainfall in 2018 was evenly distributed, although high precipitation was recorded for the month of June in both Moncada and La Punta. In the 2019 Carrizales did not present an uniform distribution, with high precipitations at the end of the crop cycle in August, while in La Punta a lower precipitation was observed, but rain was observed at the start and end of the crop cycle (Table 1). Through the whole experiment, temperatures were mostly similar among the three fields, although some exceptions were found. In 2018 a higher temperature, approximately 1ºC, was registered in April in Carrizales and La Punta compared to Moncada, and in 2019 temperatures in La Punta for the month of June and July were lower than in Carrizales. Both years, Carrizales achieved higher values for both water conductivity (4.5-6dS/m) and soil conductivity (3.17dS/m and 1.66dS/m), while La Punta and Moncada presented much lower values of water salinity (≈2dS/m) and low soil salinity (0.3-0.7dS/m).

Table 1. Mean temperature, soil conductivity and Total precipitation for each localization for the 2018 and 2019 assay. Mean temperatures for the month followed by the same letter mean there is no statistical difference between the mean temperature for the month in different localizations according to Tukey's test (P≤0.05) and (*) indicate significant differences for mean temperatures of the month between years (ANOVA, p≤.05). Soil conductivity values followed by the same letter mean there is no statistical difference between the mean soil conductivity according to Tukey's test (P≤0.05).

		CARRIZALES		LA PUNTA		MONCADA	
		Tmean (ºC)	**Precipitation(mm)**	**Tmean (ºC)**	**Precipitation(mm)**	**Tmean(ºC)**	**Precipitation(mm)**
2018	**APRIL**	17.10±0.39b	10.2	16.82±0.36ab	17.6	15.78±0.38a	17
	MAY	19.48±0.33a	4.1	19.42±0.33a	11.2	18.60±0.31a	10
	JUNE	23.63±0.33a	9.6	23.46±0.32a	99.4	23.22±0.35a	38.9
	JULY	26.81±0.17a*	0	26.63±0.19a	1.3	26.56±0.15a	1.2
	AUGUST	27.46±0.24a	13.3	27.17±0.22a	11.8	26.91±0.23a	20.1
	SOIL CE (dS/m)	3.17±0.05 c		0.67±0.08 b		0.36±0.02 a	
2019	**MAY**	19.64±0.3 a	1.1	18.73±0.45a	22.6		
	JUNE	23.62±0.45b	0.5	22.54±0.46a	0		
	JULY	27.58±0.23b*	0.1	26.85±0.23a	7.5		
	AUGUST	26.89±0.28a	45.4	26.66±0.34a	10.3		
	SOIL CE (ds/m)	1.66±0.10 b		0.34±0.02 a			

4.4.2. Pests and Diseases

The main pest detected both years were aphids, which are vectors of different viruses. This pest was mainly detected in both La Punta and Moncada, whereas in Carrizales they were not detected. The main airborne fungus affecting the sweet melons was Powdery Mildew (PM), caused by *Podosphaera xanthii*. In 2018 both La Punta and Moncada suffered severe PM attacks, although in Moncada it was much more severe, with an overall infection of 43.4% of plants compared to 17.1% in La Punta. In La Punta in 2019

the powdery mildew infection was a bit more severe, with approximately 21.2% of plants affected by the fungus. In Carrizales plants did not suffer from this airborne pathogen.

Figure 1 shows the percentage of infected sweet melon plants for each cultivar affected by each virus at each location and year. In 2018 viral infections were detected in the assays performed in Valencia, with *Watermelon mosaic virus* (WMV) being present in La Punta and *Cucumber mosaic virus* (CMV) in Moncada. Rochet 04RC, presented the highest infection of WMV and 02RC the least infection, with the rest having approximately 30% infection. For CMV, only 04RC, 19AM, 16AM, 22AM-GO, 07PS and 03PS were infected (approximately between 10-20%) with the rest presenting low or not being infected. As for the difference between grafted and NG plants, all presented similar infection %, with the lowest being F1Pat81 (≈30%) and the highest NG (≈40%).

In 2019 assay (Fig. 1), viruses were only detected in La Punta, with the most prevalent being *Watermelon mosaic virus* (WMV), followed by *Cucurbit aphid borne yellow virus* (CABYV) and *Tomato leaf curl New Delhi virus* (ToLCNDV), with an approximate infection of 67% for WMV, and ≈30% for both CABYV and TolCNDV. The presence of other viruses such as *Cucumber mosaic virus* (CMV), only appeared in certain instances ((≈8%) while *Zucchini mosaic virus* (ZYMV) was not detected. The infection by WMV affected all cultivars, even the commercial cultivars Cristiano and Finura with these cultivars reaching the highest infection (≈80%) while the lowest observed was in cultivar 04RC (40%), the rest achieved an infection 60-70%. This trend of infection was also observed for CABYV, with the highest being achieved by the commercial melons (40%), and the lowest by cultivar 04RC (21%), with the rest being between 30-38%. As for TolCNDV, commercial Cristiano and cultivar 32BL presented ≈50% infection, while the rest present much lower 20-30%. The presence of CMV was overall the same between cultivars and as for ZYMV, it was not detected at all. Overall, grafting did not affect the infection of viruses, with both grafted and NG presenting similar infection rates.

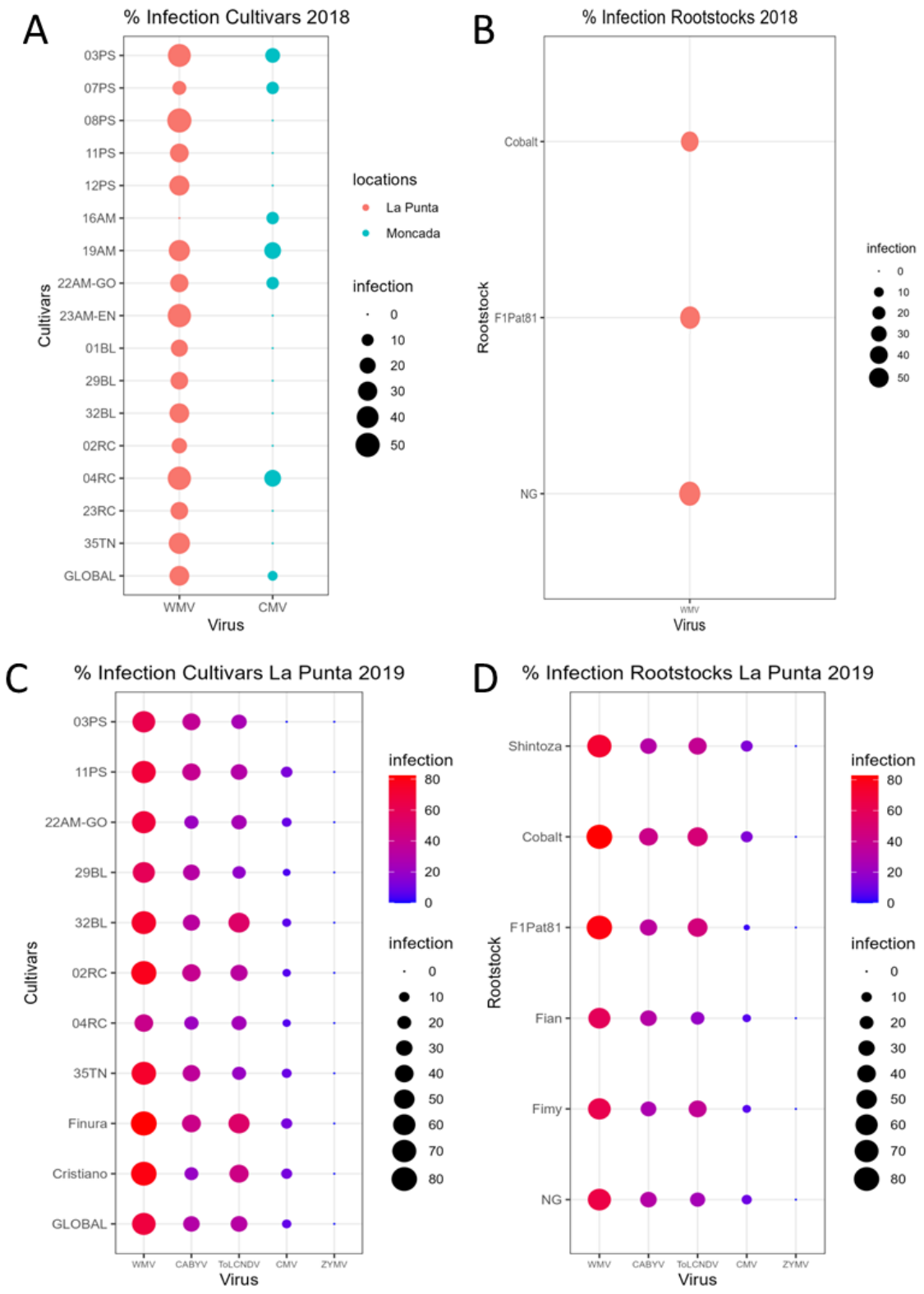


Figure 1. Percentage (%) of infection of different virus detected in sweet melon cultivars in each field and assay. Each year is also accompanied by the (%) of grafted and NG plants infected. The size of the circle indicates de % of plants infected. For the 2018 graphs (A-B) the different colours indicate the prospection location of the detected viruses. In the 2019 graphs (C-D), the colours emphasize the differences in the percentage of infection.

No soilborne pathogens were detected in Moncada in 2018. In both La Punta and Carrizales a number of plants died due to soil borne pathogens. In the case of Carrizales (Fig 2.A.), the mortality for grafted plants was 7% for those grafted onto Cobalt and 14% those on F1Pat81, while non-grafted was 17.1%. As for the main pathogens detected (Fig. 2) the main pathogen affecting F1Pat81 was *Macrophomina phaseolina*, as for Cobalt, again the main pathogen was *M. phaseolina*, while other important pathogens such as *N. falciformis*, *N. keroplastica*, *Fusarium oxysporum* and *Fusarium solani* were also detected. Non-grafted plants presented a higher diversity of soilborne pathogens, although again the main was *M. phaseolina*, followed by *N. falciformis*, *F. solani*, *Monosporascus cannonballus* and *Fusarium sp*.

In La Punta (Fig 2.B.), the mortality was quite higher, 43.8% of non-grafted plants were affected by soilborne pathogens, while Cobalt grafted plants 42% and F1Pat81only 14%. Cobalt grafted plants presented *M. phaseolina* as the main pathogen, followed by *Fusarium* species, including *N. falciformis* of the *F. solani* complex. The pathogens are similar to those detected in Carrizales although with a higher number of *Fusarium* species, although in this field *N. keratoplastica* was not detected. The main pathogens found in F1Pat81 were *Fusarium* species, including *Fusarium oxysporum* f. sp. *melonis*, *Fusarium solani* f. sp. *cucurbitae*, as well as *M. phaseolina* and *M. cannonballus*. Finally, Non-grafted plants, approximately half of the pathogens detected were of the *Fusarium* species, while also *M. phaseolina* being important.

As for the 2019, in Carrizales, only non-grafted and *Cucurbita* grafted plants were affected by soilborne pathogens, with 3.7%, 23.7% and 17.6% (Non-grafted, Shintoza and Cobalt respectively). The main pathogens affecting were *Fusarium equiseti*, *F. oxysporum* f. sp. *melonis*, *M. phaseolina* and *N. falciformis*. The main pathogen affecting Shintoza grafted plants was *M. phaseolina*, followed by *Fusarium* species and *N. falciformis*. Finally, again in Cobalt grafted plants, *M. phaseolina* was the main pathogen, with *F. oxysporum* f. sp. *melonis and F. solani*. In the case of La Punta, both grafted and non-grafted plants were affected with Fimy and Cobalt (18%) presenting the highest, followed by Shintoza (14.3%), Fian (9.8%) and finally F1Pat81 (6%), while non-grafted presented 28% mortality. In terms of soilborne pathogens all presented similar proportions, with the main pathogen being *M. phaseolina*, followed by *Fusarium* species. *N. falciformis* was detected affecting the F1Pat81, Shintoza and non-grafted and Fimy plants.

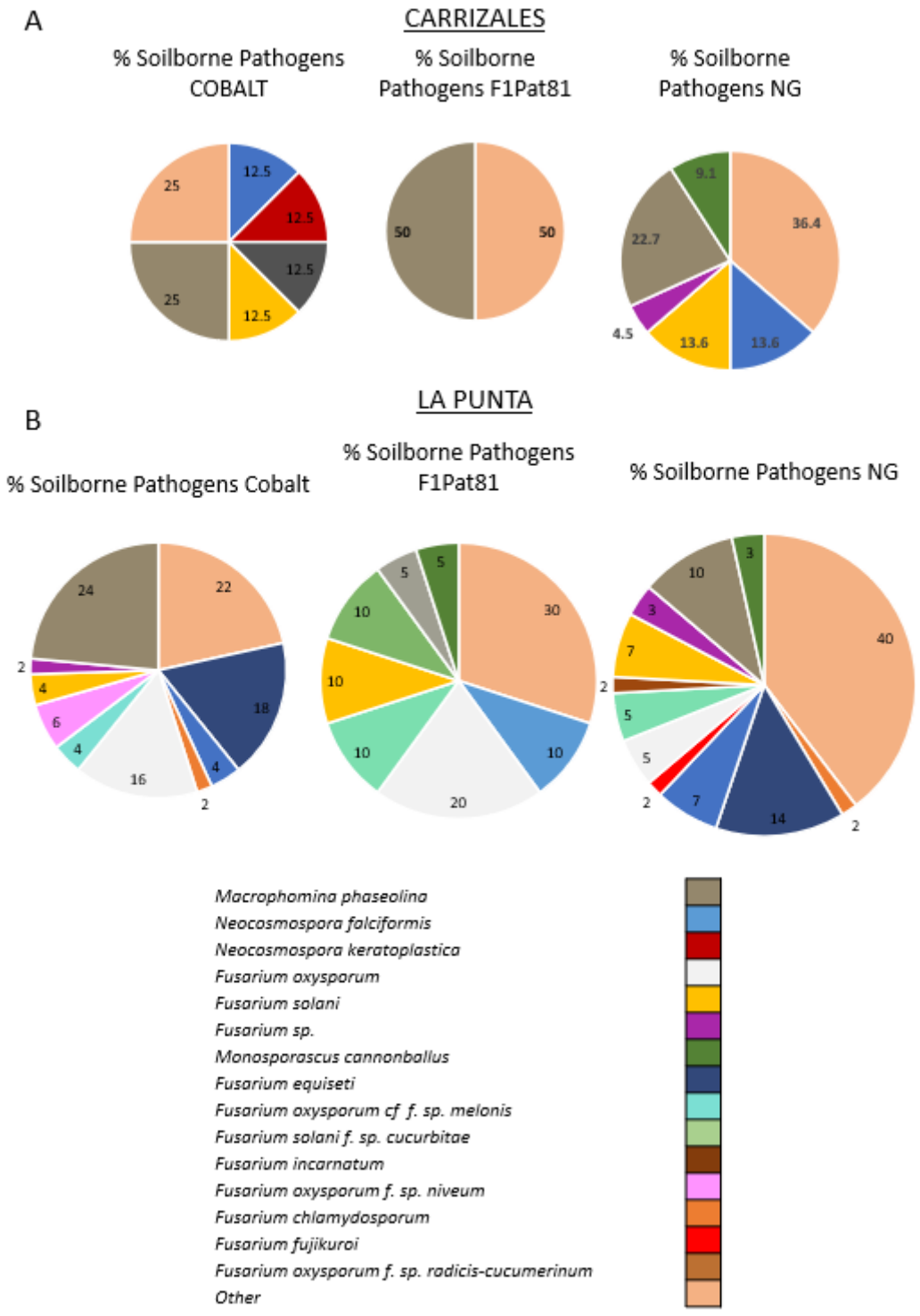


Figure 2. Percentage of soilborne pathogens detected in Carrizales (A) and La Punta (B) for grafted (Cobalt and F1Pat81) and non-grafted (NG) plants for the 2018 assay.

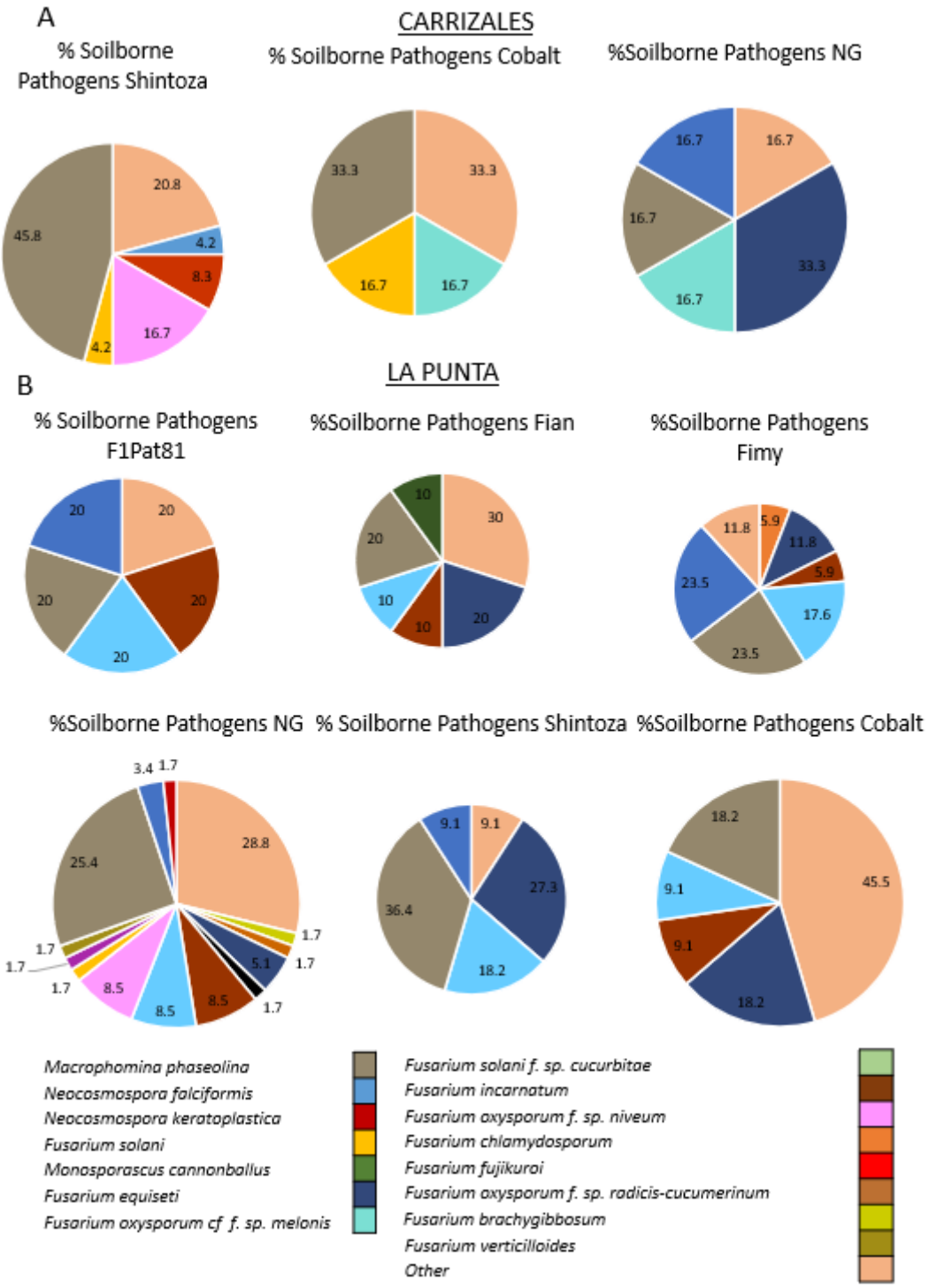


Figure 3. Percentage of soilborne pathogens detected in Carrizales (A) and La Punta (B) for grafted on *Cucurbita* (Cobalt and Shintoza), *Cucumis* (F1Pat81, Fian and Fimy) and non-grafted (NG) plants for the 2019 assay.

4.4.3. Yields and Fruit Characterization

The sweet melons were cultivated in different soil stress with La Punta having fungal and long melon cultivation history, in Carrizales with saline irrigation (4.5dS/m) and Moncada having no known stress. The 2018 accumulated production per plant (Fig. 4) displays the importance of a correct soil sanitation as all subgroups displayed lower values (2.5-4kg/plant). The highest accumulated production was found in Carrizales with values ranging from 4.8-9kg depending in the subgroups. Moncada achieved intermediate results between La Punta and Carrizales. The highest productivity was achieved by Piel de Sapo cultivars, with approximately 6.2kg/plant followed by Rochet (5.4kg/plant), Blanco (5.2kg/plant), Amarillo (4.9kg/plant) and the last Tendral (4.2kg/plant) (Figure 2).

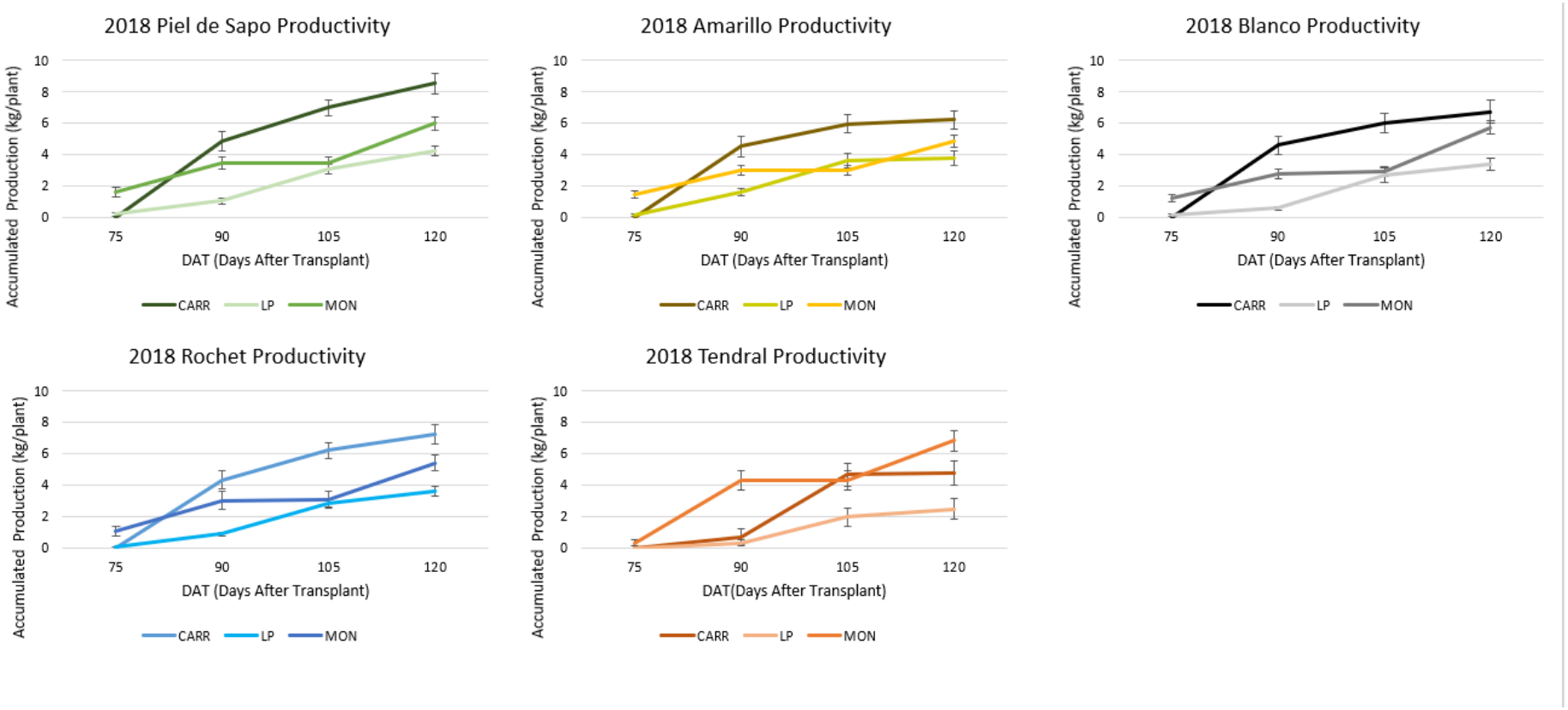


Figure 4. Productivity per plant for the different melon subgroups for non-grafted plants in Moncada (MON), La Punta (LP, light colour) and Carrizales (CARR, darker colour).

In terms of the Fruit characterization in 2018 for the non-grafted plants in the three fields (Fig. 5, Supplementary table 2), the fruit weight ranged between 1-2.1kg, fruit firmness between 1.5-4kgf/cm2 and Soluble Solids content between 10-14ºBrix. As for the effect of the field, fruits from La Punta presented lower fruit weight, lower L/D ratio, indicating smaller fruits. As for Soluble Solid Content, Carrizales Piel de Sapo and Amarillo non-grafted plants presented higher content, on average than in Moncada and La Punta. Fruit Flesh Colour also varied between fields.

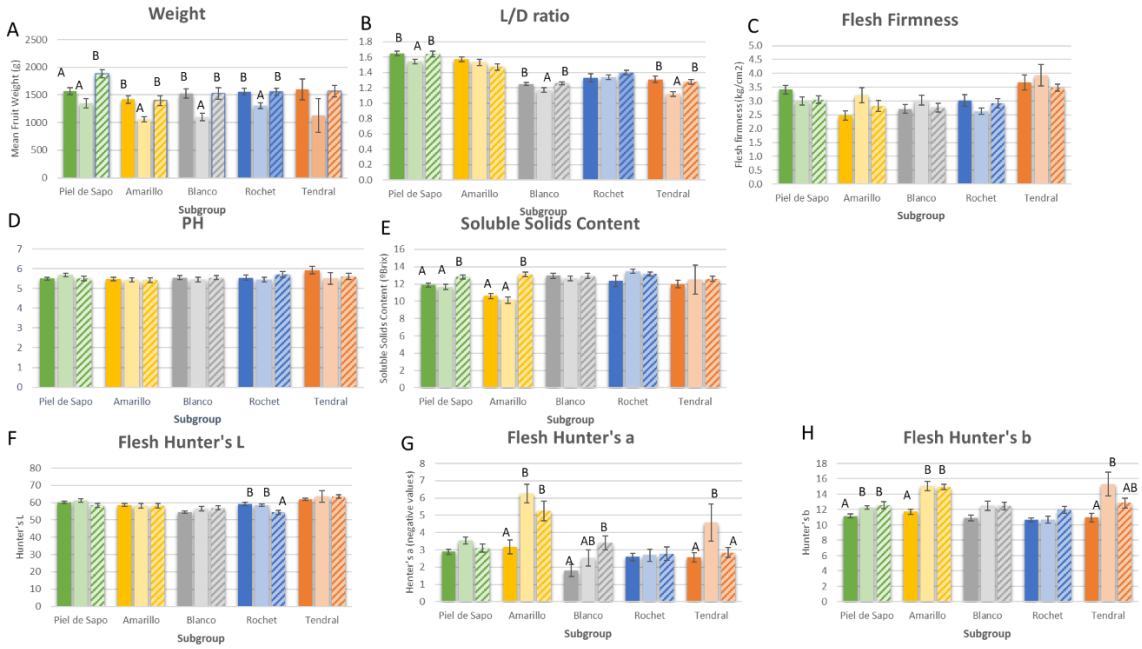


Figure 5. Characteristics of Traditional sweet melon cultivars from each of the 5 subgroups (Piel de Sapo, Amarillo, Blanco, Rochet and Tendral) for Moncada (full colour), La Punta (light colour) and Carrizales (strips). Bars with the same letters indicate no significant differences between each site Tukey's test (P≤0.05).

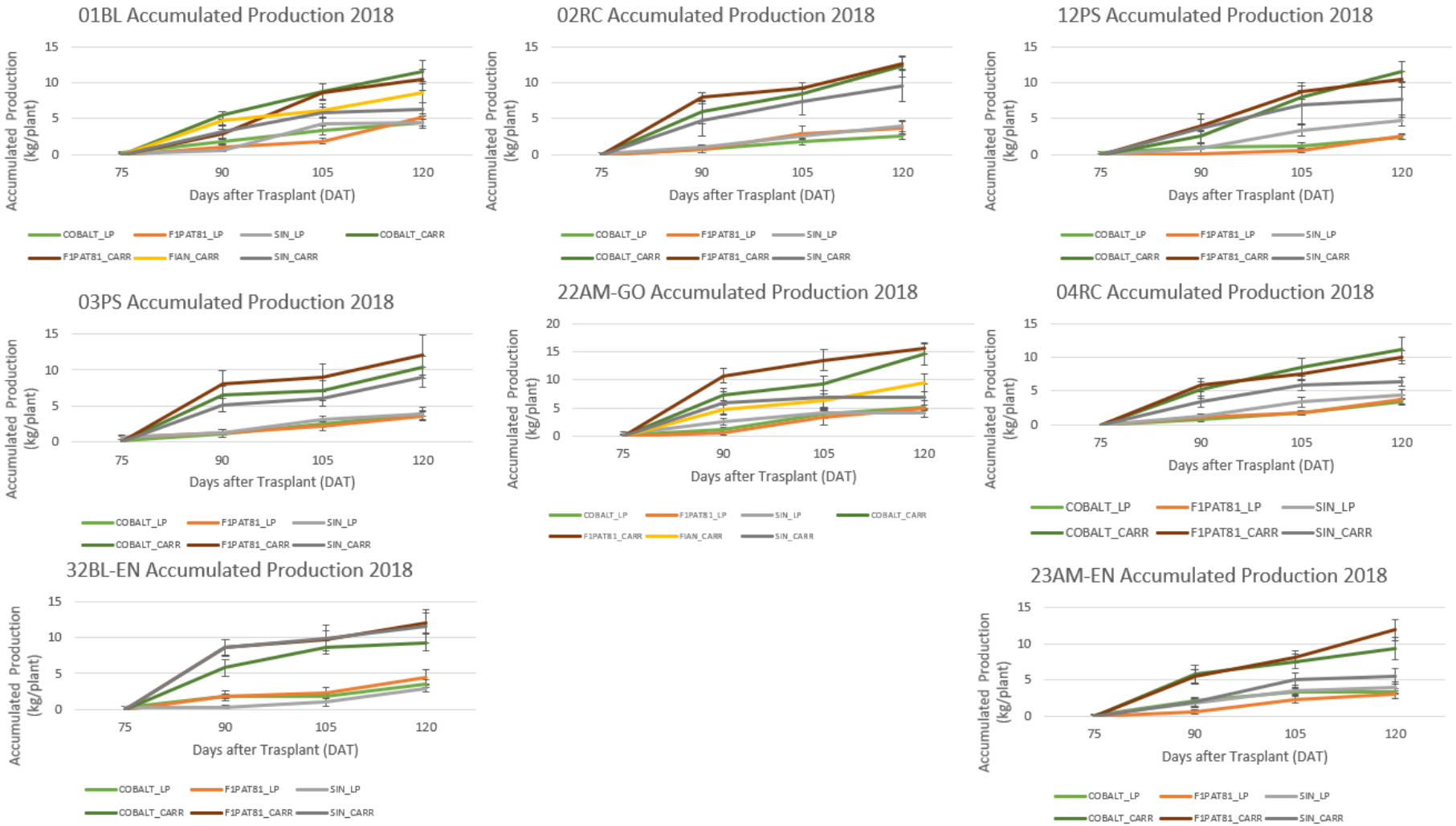


Figure 6. Field accumulated Production for grafted onto COBALT (green), F1PAT81 (Orange) and FIAN (Yellow) and non-grafted (Grey) plant onto from the field of Carrizales (CARR) (dark) and La Punta (LP) (pale) for the 2018 assay.

Table 2. Effect of the location, scion, rootstock and their interaction on the fruit characterization of the traditional melon cultivars for the non-grafted plant fruits (NG) and grafted onto Cucurbita (Cobalt) and Cucumis (F1Pat81) rootstocks for La Punta and Carrizales for the 2018 campaign. The statistical ANOVA relevance is provided. For each effect, different letters indicate significant difference at $p<0.05$ (Tukey test).

		Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Flesh firmness (kg/cm2)	Rind firmness (kg/cm2)	Exocarp Thickness (mm)	Rind Thickness (mm)	pH	Soluble solids content(ºBrix)	Hunter L (F)	Hunter a (F)	Hunter b (F)	Hunter L (R)	Hunter a (R)	Hunter b (R)	Fruit Cavity (cm)
Scion (S)	*P-value*	0.0000	0.0000	0.0000	0.0000	-	0.0000	0.001	0.0006	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	03PS	1557.76±94.5cd	17.73±0.46c	13.21±0.25c	3.44±0.15ab	13±0	5.42±0.23abcd	0.73±0.04bc	5.4±0.08ab	12.74±0.21ab	56.06±0.77ab	-3.51±0.19bc	11.11±0.24abc	32.95±0.82b	-2.85±0.25b	12.3±0.6b	5.63±0.12ab
	12PS	1149.09±55.96a	17.74±0.37cd	11.44±0.23a	3.78±0.1b	13±0	5.88±0.19cd	0.66±0.02abc	5.63±0.09bc	12.88±0.26ab	60.14±0.81bc	-3.11±0.19bc	12.54±0.18bc	29.16±0.41a	-2.98±0.18b	10.19±0.46a	5.35±0.12a
	22AM-GO	1387.36±64.24bc	17.86±0.29cd	12.68±0.25bc	3.58±0.59b	13±0	5.19±0.25abc	0.59±0.03a	5.48±0.09abc	12.49±0.35a	57.87±0.83bc	-5.86±0.54a	14.08±0.6d	70.34±0.58d	0.83±0.65c	38.68±0.35e	5.49±0.15ab
	23AM-EN	1371.18±59.96bc	18.88±0.35d	12.37±0.3b	3.55±0.16b	13±0	5.51±0.2bcd	0.64±0.04abc	5.34±0.07ab	12.59±0.31ab	60.19±0.94bc	-5.35±0.29a	14.24±0.33d	68.33±0.46d	2.51±0.56d	38.34±0.41e	5.51±0.13ab
	01BL	1113.32±35.93a	15.26±0.24a	12.18±0.16ab	3.39±0.16ab	13±0	6.08±0.17d	0.61±0.03abc	5.26±0.08a	12.87±0.2ab	57.71±0.74bc	-3.12±0.28bc	14.05±0.31d	75.5±0.4e	-2.28±0.24b	22.06±0.36d	5.57±0.08ab
	32BL	1709.56±64.75d	15.78±0.32ab	14.65±0.19d	2.43±0.1a	13±0	5.11±0.21abc	0.6±0.05ab	5.35±0.08ab	13.46±0.21ab	53.48±0.89a	-3.86±0.27b	11.6±0.31ab	78.68±0.61f	-1.78±0.17b	20.57±0.4d	5.96±0.13bc
	02RC	1451.08±63.4bc	15.89±0.29ab	13.27±0.22c	2.46±0.11a	13±0	4.82±0.17ab	0.65±0.04abc	5.46±0.1abc	13.24±0.3ab	57.77±0.87bc	-3.62±0.42b	12.85±0.43cd	38.74±1.08c	-5.47±0.22a	17.66±0.78c	6.17±0.13c
	04RC	1279.4±47.12ab	16.43±0.26b	12.36±0.15b	2.75±0.1ab	13±0	4.65±0.21a	0.74±0.04c	5.72±0.1c	13.52±0.16b	55.52±0.67ab	-2.24±0.18c	10.71±0.28a	34.88±0.72b	-5.22±0.23a	14.39±0.53b	5.3±0.11a
Rootstock (R)	*P-value*	0.2798	0.218	0.0204	0.8486	-	0.9613	0.0071	0.0911	0.0002	0.2205	0.0327	0.0001	0.3235	0.0556	0.1412	0.061
	Cobalt	1415.41±44.04	16.81±0.25	13±0.17b	3.23±0.11	13±0	5.4±0.14	0.7±0.03b	5.54±0.05	12.93±0.17ab	57.19±0.51	-3.91±0.22ab	13.08±0.24b	53.24±1.83	-1.76±0.34	21.78±0.99	5.75±0.08
	F1Pat81	1338.94±39.51	18.16±1.21	12.58±0.15a	3.25±0.21	13±0	5.35±0.12	0.61±0.02a	5.39±0.05	13.39±0.12b	57.02±0.49	-3.46±0.2b	12.01±0.22a	54.02±1.88	-2.06±0.34	22.06±0.99	5.52±0.07
	NG	1341.46±38.64	17.14±0.22	12.55±0.15a	3.1±0.09	13±0	5.31±0.13	0.64±0.02ab	5.43±0.05	12.43±0.18a	58.25±0.62	-4.23±0.23a	13.09±0.29b	54.47±2.11	-2.52±0.27	22.02±1.14	5.53±0.08
Localization (L)	*P-value*	0.0000	0.0000	0.0000	0.0023	-	0.0992	0	0.1152	0	0.0016	0.4476	0.4668	0.2389	0.0429	0.0011	0.0000
	Carrizales	1600.83±31.61b	18.16±0.17b	13.47±0.12b	2.91±0.07a	13±0	5.25±0.13	0.74±0.02b	5.41±0.05	13.54±0.11b	56.58±0.41a	-3.73±0.15	12.61±0.17	54.53±1.56	-1.7±0.3b	22.87±0.86b	5.87±0.06b
	La Punta	1137.88±25.69a	16.66±0.89a	11.99±0.12a	3.48±0.16b	13±0	5.46±0.09	0.56±0.01a	5.5±0.04	12.4±0.14a	58.24±0.45b	-3.94±0.21	12.77±0.23	53.22±1.58	-2.46±0.23a	21.05±0.82a	5.35±0.06a
SxR	*P-value*	0.1984	0.301	0.0947	0.7724	-	0.0076	0.0109	0.0068	0.1388	0.9084	0.2215	0.1972	0.2859	0.621	0.0915	0.0138
SxL	*P-value*	0	0.0549	0.0002	0.0139	-	0.0018	0.0126	0.0002	0	0.0149	0.0882	0.0053	0.045	0	0.025	0.21
RxL	*P-value*	0.0001	0.0014	0.0001	0.1071	-	0.9839	0.3169	0.0877	0.0104	0.0023	0.0051	0.2616	0.9458	0.9896	0.9758	0.0224

The effect of grafting on the fruit morphology and productivity (Fig. 6, Table 2, Suppl. Table 3) revealed the effect of localization and rootstocks. Grafted plants in 2018 behaved differently depending of the location (Figure 6). Compared between fields, grafted sweet melons fared better in Carrizales than in La Punta. Grafting did not improve the overall yield per plant in La Punta, with similar yields between non-grafted and grafted and 12PS non-grafted plants even displaying a higher yield per plants than grafted on both F1Pat81 and Cobalt plants. This could be due to the presence of soilborne pathogens and viruses which affected the overall performance of the sweet melon plants. In Carrizales, overall, the use of F1Pat81 and Cobalt increased the yield of the sweet melon plants between 60% and 120%, although Piel de Sapo cultivars 03PS and 12PS, Blanco 32BL-EN and Rochet 02RC was not increased by grafting.

Several fruit characteristics were affected due to grafting. Cobalt grafted plant fruits presented bigger diameter and rind thickness, while F1Pat81 increased the Soluble Solids Content, and changed the flesh fruit colour (a-value and b-value). Location also impacted fruit morphology with La Punta presenting smaller fruits (longitude, diameter and weight), while the fruits in Carrizales had lower fruit flesh firmness, higher Soluble solids content and fruit cavity. Differences between location were observed for the Flesh luminosity and the rind fruit colour (a-value and b-value). The results showed the rootstock-location interactions, with significance in fruits size, weight, soluble solids content. Cultivar-location interaction was also of great importance as it affected practically all of the fruit morphology components. Individually, 22AM-GO and 12PS did present significant different for fruit pH, mainly between fields, although Cobalt and F1Pat81 grafted plants in La Punta did see an increase in fruit pH compared to the non-grafted fruits.

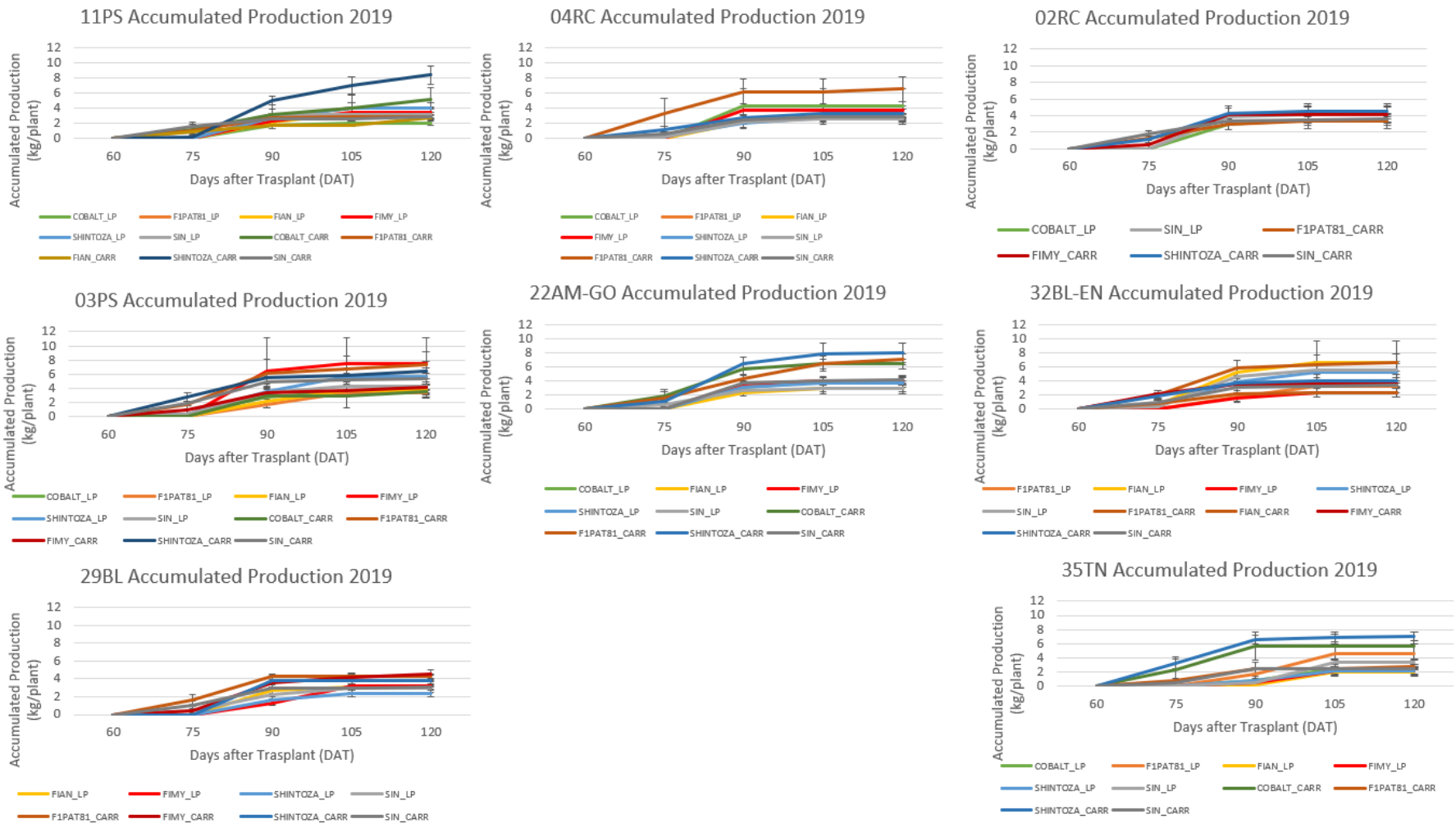


Figure 7. Field accumulated Production for grafted COBALT (green), F1PAT81 (Orange), FIAN (Yellow), FIMY (red) and Shintoza (blue) and non-grafted (grey) plant from the field of Carrizales (CARR) (dark) and La Punta (LP) (pale) for the 2019 assay.

Table 3. Effect of the location, scion, rootstock and their interaction on the fruit characterization of the traditional melon cultivars for the non-grafted plant fruits (NG) and grafted onto *Cucurbita* hybrids (Cobalt and Shintoza) and *Cucumis* (F1Pat81, Fian and Fimy) rootstocks for La Punta and Carrizales for the 2019 campaign. The statistical ANOVA relevance is provided. For each effect, different letters indicate significant difference at $p<0.05$ (Tukey test).

		Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Flesh firmness (kg/cm2)	Rind firmness (kg/cm2)	Exocarp Thickness (mm)	Rind Thickness (mm)	pH	Soluble solids content(°Brix)	Hunter L (F)	Hunter a (F)	Hunter b (F)	Hunter L (R)	Hunter a (R)	Hunter b (R)	Fruit Cavity (cm)
Scion (S)	*P-value*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	03PS	1652.96±61.85 bcd	18.74±0.35c	13.45±0.17bc	3.28±0.06b	13±0	4.44±0.09ab	0.48±0.01d	5.66±0.07bc	12.08±0.17ab	60.16±0.43bc	-3.33±0.17cde	10.4±0.28ab	32.76±0.53c	-3.71±0.16de	11.94±0.33bc	5.35±0.1ab
	11PS	1823.8±53.94de	21.03±0.33d	13.31±0.15bc	3.12±0.05ab	13±0	4.82±0.09bcd	0.41±0.01bc	5.47±0.06abc	12.09±0.24ab	66.29±0.46	-2.87±0.12ef	10.4±0.19ab	35.92±0.86d	-4.93±0.3bc	13.21±0.41c	5.33±0.1ab
	22AM-GO	1683.77±75.21cd	19.12±0.38c	13.67±0.22bcd	3.13±0.05b	13±0	4.92±0.1cde	0.39±0.01ab	5.29±0.06a	12.02±0.17ab	62.41±0.56d	-5.18±0.22a	12.91±0.29d	73.86±0.3f	-4.39±0.33cd	38.5±0.27h	5.65±0.14cd
	29BL	1424.75±75.11ab	15.71±0.32a	13.41±0.25bc	2.99±0.07ab	13±0	4.25±0.08a	0.36±0.01a	5.29±0.07a	12.63±0.23b	61.35±0.54cd	-3.51±0.21cde	11.61±0.29c	78.2±0.48g	-4.07±0.26cde	21.69±0.44g	5.83±0.15cd
	32BL-EN	1719.84±92.18cd	16.27±0.36a	14.22±0.28de	2.56±0.04a	13±0	4.56±0.1abc	0.38±0.01ab	5.69±0.06d	12.53±0.21b	57.94±0.53a	-3.86±0.2bcd	11.56±0.26c	80.3±0.32g	-3.09±0.16ef	19.74±0.43f	5.84±0.14cd
	02RC	1341.73±74.39a	15.73±0.33a	13.01±0.22ab	3.43±0.48bc	13±0	4.18±0.11a	0.44±0.02cd	5.66±0.08bc	11.86±0.17ab	61.29±0.49cd	-4.26±0.22b	12.78±0.31d	41.2±0.75e	-6.64±0.24a	16.94±0.5e	5.68±0.12cd
	04RC	1307.89±42.05a	16.51±0.29ab	12.49±0.13a	2.94±0.05ab	13±0	4.44±0.1ab	0.44±0.01cd	5.97±0.05d	12.36±0.18b	58.16±0.55ab	-2.28±0.13f	9.9±0.21a	36.38±0.71d	-5.81±0.19ab	15.35±0.38d	5.06±0.07a
	35TN	1649.73±64.34bcd	17.54±0.31b	13.96±0.24cde	3.9±0.07cd	13±0	5.14±0.12de	0.42±0.01bc	5.4±0.06ab	12.39±0.22b	65±0.45ef	-3.17±0.15de	11.63±0.23c	21.51±0.33a	-1.99±0.13g	3.38±0.17a	6.66±0.18e
	Cristiano	1526.07±65.58abc	16.52±0.27ab	13.62±0.24bcd	4.1±0.07e	13±0	5.28±0.12e	0.41±0.01abc	5.42±0.06abc	12.37±0.17b	63.46±0.51de	-4.03±0.2bc	11.09±0.27bc	71.93±0.4f	-1.98±0.47g	39.42±0.21h	5.78±0.17cd
	Finuera	2071.06±71.87e	20.32±0.31d	14.57±0.19e	3.5±0.07bc	13±0	5.17±0.08de	0.45±0.01cd	5.35±0.05a	11.48±0.14a	66.31±0.45	-2.98±0.12ef	9.71±0.2a	30.3±0.46b	-2.38±0.12fg	11.13±0.33b	5.88±0.13d
Rootstock (R)	*P-value*	0.0000	0.0041	0.0000	0.0614	0.0000	0.0064	0.9208	0.3316	0.4964	0.4157	0.0047	0.0419	0.1078	0.0000	0.8499	0.0001
	F1Pat81	1558.46±53.07a	17.51±0.31a	13.4±0.16a	3.25±0.05	13±0	4.78±0.09	0.42±0.01	5.47±0.05	12.18±0.16	62.27±0.46	-3.34±0.14ab	11.01±0.2ab	46.49±2.08a	-4.57±0.23a	17.1±1.03a	5.66±0.11ab
	Fian	1609.09±66.42ab	17.77±0.33ab	13.58±0.2ab	3.41±0.07	13±0	4.57±0.08	0.42±0.01	5.58±0.05	12.27±0.2	62.4±0.45	-3.64±0.19ab	10.77±0.25a	52.1±2.37d	-3.29±0.26b	20.66±1.23c	5.71±0.12ab
	Fimy	1630.25±78.89ab	17.71±0.45a	13.52±0.23ab	3.2±0.06	13±0	4.69±0.11	0.42±0.01	5.51±0.06	11.92±0.17	62.55±0.6	-3.85±0.18a	11.64±0.28b	50.28±2.39c	-3.95±0.23ab	17.14±0.97a	5.68±0.12ab
	Cobalt	1762.58±71.47b	18.62±0.44b	13.96±0.21b	3.15±0.08	13±0	4.85±0.12	0.43±0.01	5.54±0.07	12.27±0.17	63.22±0.72	-3.48±0.22ab	11.23±0.31ab	47.44±2.88ab	-4.33±0.27a	19.55±1.69bc	5.88±0.17b
	Shintoza	1765.21±44.8b	18.21±0.24ab	14.02±0.13b	3.26±0.06	13±0	4.85±0.07	0.42±0.01	5.48±0.04	12.26±0.13	61.95±0.43	-3.21±0.12b	10.74±0.16a	48.7±1.78bc	-3.52±0.22b	19.26±0.97b	5.87±0.09b
	NG	1563.35±43.16a	17.95±0.22ab	13.26±0.14a	3.32±0.15	13±0	4.67±0.06	0.42±0.01	5.58±0.04	12.11±0.11	61.98±0.38	-3.62±0.12ab	11.32±0.18ab	50.24±1.72c	-3.84±0.2ab	19.21±0.88b	5.45±0.08a
Localization (L)	*P-value*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.005	0.0446	0.0000	0.0002	0.6518	0.0000	0.2284	0.0309	0.9368	0.0000
	Carrizales	1377.15±25.52a	16.52±0.14a	12.87±0.08a	3.09±0.03a	13±0	4.57±0.04a	0.4±0.01a	5.57±0.03b	12.56±0.08a	61.46±0.25a	-3.57±0.09	11.58±0.12b	50.65±1.19	-4.03±0.11a	18.82±0.59	5.34±0.05a
	La Punta	1952.36±32.94b	19.62±0.18b	14.45±0.1b	3.5±0.09b	13±0	4.93±0.05b	0.44±0b	5.47±0.03a	11.69±0.09b	63.22±0.3b	-3.43±0.09	10.54±0.13a	47.69±1.23	-3.68±0.16b	18.8±0.66	6.09±0.07b
S x R	*P-value*	0.0006	0.0000	0.0001	0.3532	0.0000	0.0086	0.0005	0.0867	0.0041	0.2225	0.0002	0.0034	0.0005	0.0000	0.0859	0.1563
S x L	*P-value*	0.0282	0.0051	0.0489	0.0000	0.0000	0.0001	0.0105	0.0079	0.0000	0.1923	0.0000	0.0004	0.0001	0.0013	0.0000	0.0005
R x L	*P-value*	0.0176	0.0002	0.0039	0.6578	0.0000	0.1615	0.2465	0.422	0.2579	0.1861	0.0285	0.0331	0.6564	0.0569	0.1168	0.6327

In 2019, the productivity (Figure 7) was compared between Carrizales and La Punta. The results showed that non-grafted plants from all Subgroups displayed similar results in both Carrizales and La Punta, between 2-6kg/plants, although some cultivars achieved up to 7kg/plants. In the case of grafting, overall, in La Punta grafting did not affect yield, although in Finura F1Pat81 had a smaller yield than non-grafted plants. In Carrizales, again as in La Punta, grafting did not improve the overall yield per plant, except in the case of some Shintoza grafted plants such as 22AM-GO and 11PS and 35TN, Cobalt grafted 35TN and F1Pat81 29BL and 32BL-EN.

As for the fruit characterization (Table 3, Suppl. Table 4) again we observe the importance of the cultivar in fruit morphology. In this case, we can also observe the effect both grafting and localization had on the morphology. Both *Cucurbita* commercial hybrid rootstocks increased both the fruit weight, fruit diameter and seed cavity, while *Cucumis* hybrids did not, presenting similar fruit weights and sizes as non-grafted plants. Overall, no differences in pH and Soluble solid content were observed between rootstocks, with all presenting values approximately of 12-12.5ºBrix and pH of 5.5. Both Fruit flesh colour was affected by grafting with colour variations for fruit flesh colour (a and b values) between Shintoza and Fian grafted plants. Fruit flesh colour showed great differences were observed between grafts for luminosity, a-value and b-value. Location also influenced all of the fruit parameters. Carrizales fruits presented lower fruit size, seed cavity, weight, flesh firmness, rind thickness but higher pH and Soluble Solids Content than La Punta. Again, the fruit colour parameters for both rind and flesh were also affected, with differences between the fields.

4.5. Discussion

There are several biotic factors which limit melon cultivation both abiotic, such as water quality (Botía et al., 2005), or, biotic such as viruses or fungal diseases (Li et al., 2017; González et al., 2020a; Schoeny et al., 2020). Powdery mildew is a common, widespread and easily recognizable disease affecting the Cucurbitaceae family (Pérez-García et al., 2009). Our results clearly showed that powdery mildew did affect melon cultivation, especially the melons in Moncada. Alvarez et al., (2005) studied the resistance of 127 to *Sphaeratheca fuliginea* (syn. *P.xanthii*) to race 1 and race 2 Spanish isolates, finding that most of the resistant cultivars were from the South of Spain, origin of both races, indicating that their resistance likely originate from the same origin. Although Alvarez et al., (2005) found resistance in Spanish cultivars, only 10 out of the total 69 presented resistant response to any race of *S. fuliginea*, which could explain our results found, as our sweet melons. McCreight (2006) studied the resistance of 22 cultigens to *P. xanthii* (syn. *S. fuliginea*) and found that 'Amarillo', 'Moscatel Grande', and 'Negro' Spanish cultigens were resistant to race 1 of *P. xanthii* but susceptible to race 2. Amarillo was susceptible in one of the race 1 field experiment. McCreight (2006) also indicated the importance of temperature, as high temperatures were able to cause a breakdown of resistance enabling sporulation. High temperatures could explain the results obtained, as the high temperatures in Spain could help overcome the resistance found in any of these traditional melon cultivars.

In terms of viruses, several of them have been detected during the present studies, mainly the Cucumovirus CMV and the Potyvirus WMV, both transmitted by aphids, but also the Polerovirus CABYV and the Begomovirus ToLCNDV transmitted by aphids and white flies respectively (Lecoq and Desbiez, 2012; Kassem et al., 2013; Ruiz et al., 2015). Resistance to viruses have already been found been (Kassem et al., 2015; López et al., 2015; Schoeny et al., 2017; Pérez-De-Castro et al., 2020), as well as virus resistance have been introgressed successfully into a "Ibericus" melon (Palomares-Rius et al., 2018). Therefore, the introduction of resistance into these traditional cultivars is a feasible and important objective as to avoid future damages to sweet melon crops, as the effect of climate change will lead to more severe viral infections (Velasco et al., 2020). Alternative methods to control the viral infections could be done by protecting against the viral vectors, such as anti-insect screens which have proven to be effective in controlling pests such as *Aphis gossyppii* in cucumbers in walk-in tunnels (Antignus et al., 1998) or the use

of parasitoids, which display high specificity towards their prey, leading to less impact to the environment (Lopes et al., 2009). But these alternative methods are not as reliable and the use of resistant cultivar is the best in terms of efficiency, economy and ecology (Gómez et al., 2009; Messelink et al., 2020).

Fungal attacks were the main factor behind the lower productions in La Punta in 2018 and in both fields during the 2019 assay. Spain is one of the countries most exposed to climate change (CEDEX, 2017). This means that there will be an increase in the difficulty of disease management in the future (Garrett et al., 2006). The increase of global temperature will introduce the quick evolution of fungal pathogens due to longer season, such as *M. cannonballus* (Hunjan and Lore, 2020). The high mortality in some cases, was specially observed in the non-grafted plants, as well as in *Cucubita* grafted plants. The main pathogens which affected them were *Fusarium* spp. or *M. phaseolina*, both of which are causal agent of important melon diseases (González et al., 2020a). Resistance to these pathogens have already been found in sweet melon (Alvarez et al., 2005; de Sousa Linhares et al., 2020). Another important factor to consider is that *M. cannonballus* only appeared affecting *Cucumis* grafted or NG plants, with *Cucurbita* rootstocks not being affected by it. Although some resistance to this pathogen have been found in *Cucumis*, it is severely dampened by the effect of temperature and season (Júnior et al., 2019; Castro et al., 2020) with the use of *Cucurbita* hybrid rootstock to control this pathogen being favoured (Al-Daghari et al., 2021). The presence of new pathogens such as *N. falciformis* will be an important factor, in the research and development of new rootstocks to adapt against them. Most importantly NG presented an overall higher mortality than grafted plants, indicating the great importance of using grafted plants but also the correct resistant rootstock to employ.

The presence of these pathogens in the soil lead to drop in productivity in the field. It was particularly dramatic during 2019 in Carrizales, which despite the great performance in the previews year, saw its productions sink to the same levels as La Punta. Grafting is usually employed in order to improve the production, resist soilborne pathogens or even adapt against abiotic stress such as salinity. But in some cases, the use of grafted plants should take into account differences between the rootstocks and the combinations, such as the response of *Cucurbita* root systems to high temperatures, which is a key factor in grafted plants in hot season (Cohen et al., 2017). Our results found that grafted *Cucurbita*

achieved overall the same production as non-grafted plants or even worse, in extreme conditions, although in some cases Shintoza and F1Pat81 grafted plants did achieve higher yields per plant than non-grafted plants. The increase salinity during 2019 in Carrizales could have influenced the higher incidence of fungal attacks in the location. Salinity has been seen to positively influence the incidence of pathogenic fungi in melon (Nischwitz et al., 2002; Roustaee et al., 2011; Mirtalebi and Banihashemi, 2019). Hence it is important to understand the local limiting factors and select the best rootstock-scion combination to alleviate the yield losses.

Grafting is useful tool, but certain rootstock-scion interaction can lead to fruit quality reduction, shorter postharvest time and incompatibility between the rootstock and the scion (Gaion et al., 2018). Our results showed that grafting affected positively in certain factors such as SSC fruit content, specially F1Pat81 grafted plants resulted in higher SSC in some instances leading to higher SSC content in the F1Pat81. Both Flesh and Rind Colour was also affected by grafting both by *Cucurbit* and *Cucumis* rootstocks, as well as in some cases affected the overall flesh firmness of the grafted fruits. Grafting also influenced physical factors such as weight, fruit length, diameter, exocarp and rind thickness. These effects of grafting have been known on melon (Trionfetti Nisini et al., 2002; Verzera et al., 2014; Cáceres et al., 2017). Salinity was another factor which affected the characteristics of the sweet melons. In some case, salinity led to an increase of SSC, change in colour, and negatively affect flesh firmness. In some cases, sweet melon weight was also affected negatively by salinity. These facts have been also been noted before in both grafted melons and non-grafted melons (Edelstein et al., 2005; Akrami and Arzani, 2018, 2019). In the case of loss of weight and flesh firmness, Del Amor et al., (1999) found that the increase of salinity on ‘Galia’ melon led to decrease of fruit weight and loss of flesh firmness. Colla et al., (2006) also found that fruit weight, SSC and flesh firmness of ‘Cyrano’ being affected by both salinity and grafting onto Cucurbita rootstock ‘P360’, increasing the fruit weight, SSC and, contrary to our findings, the flesh firmness of the melon fruits. Colla et al., (2006) also found that rather than salinity, grafting affected the pulp colour. Botía et al., (2005) compared the effect of 2 cultivars ‘Galia’ and the ‘Ibericus’ ‘Amarillo Oro’ to salinity and found that SSC, peel thickness and flesh firmness to be affected by salinity but each cultivar displayed different response to it, with ‘Amarillo Oro’ increasing the firmness and ‘Galia’ decreasing it. Tedeschi et al., (2011) studied the effect of salinity on quality factor of a ‘Ibericus’

‘Tendral’ cultivar found that salinity affected the overall TSS of the Tendral fruits. Our results differ from Tedeschi et al., (2011) as we found that salinity did not affect the Tendral cultivar. This could be explained as Tedeschi et al., (2011) found the differences only between the control and the most saline water conditions of 28.2dS/m, 5 times more than our conditions in Carrizales. Hence again the importance of selecting the best cultivar for agroecological conditions to avoid the loss of fruit quality.

Supplementary files available at:

https://drive.google.com/drive/folders/1jnZUnF_q9yVG6NeZGNnEfc1RiW6oQ6la?usp=share_link

Chapter 5. Sustainable cultivation of melon landraces: effects of grafting on the accumulation of flavour-related compounds.

Chapter 5. Sustainable cultivation of melon landraces: effects of grafting on the accumulation of flavour-related compounds.

This chapter studies the effect that grafting has on flavour-related compounds of a collection of traditional Ibericus melons. The melon cultivars were grown in different locations and a total of 5 different rootstocks were employed, 2 *Cucurbita* commercial hybrids and 3 *Cucumis* hybrids. Acids and sugars of the melons of each location were analysed and compared, as well as the VOCs content, to visualize the effect of both location and grafting on metabolic profiles. Chapter has been submitted to **Food Chemistry** Journal.

This work was supported by grants PROMETEO/2017/078 and PROMETEO/2021/072 (to promote excellence groups) funded by Conselleria d'Educació, Investigació, Cultura i Esports (Generalitat Valenciana, Spain) and grants PID2020-116055RB-C21 and AGL2017-85563-C2-1-R-AR funded by AEI 10.13039/501100011033 and by "ERDF A way of making Europe". AFL is a recipient of a predoctoral fellowship from Generalitat Valenciana. RM is a recipient of a postdoctoral grant (PAID-1020) from Universitat Politècnica de València.

This work was performed by the Cucurbits Breeding Group of the COMAV, in collaboration with Dr. Jaime Cebolla Cornejo, Dr. Raul Martí and Dr. Mercedes Valcarcel of the Joint Research Unit UJI-UPV Improvement of Agri-food Quality. Also collaborating where Dr. Santiago Garcia and Dr. Juan Jose Ruiz from the Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández. Finally, also collaborating are Dr. Salvador Roselló and Dr. Jose Beltran from the Joint Research Unit UJI-UPV Improvement of Agri-food Quality, Universitat Jaume and Instituto Universitario de Plaguicidas y Aguas (IUPA), Universitat Jaume I, respectively.

Chapter 5. Sustainable cultivation of melon landraces: effects of grafting on the accumulation of flavour-related compounds.

Flores-León, A.[1], Martí, R.[2], Valcarcel, M.[2], Roselló, S.[3], Beltrán, J.[4], García-Martínez, S.[5], Ruiz, J.J.[5]; Gisbert, C.[1]; Cebolla-Cornejo J.[2*a]; Picó, B.[1a]

1. Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València. Cno de Vera s.n. 46022. Spain

2. Joint Research Unit UJI-UPV Improvement of Agri-food Quality. COMAV. Universitat Politècnica de València. Cno de Vera s.n. 46022. Spain

3. Joint Research Unit UJI-UPV Improvement of Agri-food Quality. Universitat Jaume I, Avda. Sos Baynat s/n, 12071, Castelló de la Plana, Spain.

4. Instituto Universitario de Plaguicidas y Aguas (IUPA), Universitat Jaume I, Campus de Riu Sec, Avda. Sos Baynat s/n, 12071, Castellón, Spain

5. Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Universidad Miguel Hernández, Ctra. Beniel Km 3,2, 03312, Orihuela, Spain

*Corresponding autor: jaicecor@upv.es

[a]Equal contribution

5.1. Abstract

Melon landraces are highly appreciated by consumers who pay price premiums to compensate for lower yields, enabling on-farm conservation. However, they are highly susceptible to soilborne diseases. This study analyses the impact of Cucurbita and Cucumis rootstocks on the accumulation of flavor-related metabolites in several Spanish landraces of the Ibericus melon group, as a strategy to promote their sustainable cultivation. Scion genotype was the main factor conditioning the accumulation of sugars and acids both under standard and saline organic farming conditions. The effects of grafting on acid accumulation were negligible, while the effects on sugar content and profile were significant. The latter effects were dependent on specific scion-rootstock combinations, though wild *Cucumis* (e.g. Fian) rootstocks represent an alternative that should be further studied. The effect on the accumulation of volatiles was limited, and again depended on specific scion-rootstock combinations. The rootstock effect even differed between populations of the same landrace.

Keywords: *Cucumis melo* L.; sugar; acid; volatile; soilborne diseases; landrace

5.2. Introduction

Melon (*Cucumis melo* L), with an average consumption of 8 g per capita and day (data for 2020), is one of the top ten most consumed fruits in the World (www.fao.org/faostat). It is highly appreciated worldwide, and several countries from Europe, Africa, Asia, and Oceania have consumption levels higher than 15 g per capita and day. In order to satisfy the increasing demand, its production has experienced a steady increase during the last 20 years. In 2020 27.5 million tonnes were produced, more than 50% higher than two decades earlier.

Spain outstands by its particular diversity in this species. Romans already described the cultivation of *C. melo* in this area, although it seems that it was mainly restricted to the Flexuosus group. It would be the Arabs who probably introduced the sweet melon in Spanish agriculture (Lázaro et al., 2017). After centuries of cultivation, a high diversity would develop, specifically within the Ibericus group of the species including the subgroups Piel de Sapo, Amarillo, Tendral, Rochet, and Blanco (Pitrat, 2016). These landraces are still highly appreciated in the area. In fact, they are still more valued than commercial varieties due to their specific sensorial attributes (Escribano and Lázaro, 2012).

The maintenance of this diversity, and the cultivation of melon in general, is jeopardized by the incidence of diseases, especially soilborne diseases. It would be the case of melon wilt and root rot, that affects melon cultivation in arid and semi-arid cucurbit-growing areas worldwide and where it compromises melon cultivation (Castro et al., 2020). Sources of resistance are available, but the main strategy to control these damages relies on the use of grafted plants and the development of new rootstocks (Pico et al., 2017).

In cucurbits, the use of grafting has become commonplace ever since the initial use (circa 1930) of *Lagenaria* rootstocks to provide resistance against *Fusarium* in watermelon (Kawaide, 1985). This practice has been mainly used to provide tolerance against soilborne diseases and abiotic stress (Rouphael et al., 2018a). In fact, cucurbits are increasingly cultivated under unfavorable conditions, including among other soils with high salinity, and fertility problems. In the case of salinity, grafting arises a solution to minimize its impact in yield and quality. In the case of organic farming grafting is an essential approach, considering the limited availability of disease control alternatives and

the high impact of soilborne diseases on *Cucumis* production (Flores-León et al., 2021).

Different types of rootstocks can be employed for melon production. Among them, the interspecific crosses of *Cucurbita maxima* Duch. and *Cucurbita moschata* Duch. lead the use of rootstocks for watermelon and melon production, although other alternatives have been explored (Karaaĝaç and Balkaya, 2013; Cáceres et al., 2017). Nonetheless, the use of intraspecific melon rootstocks is yet to be efficiently exploited and it may interesting alternative influence of the scion, as decreasing the high vigor typical of Cucurbita rootstocks and the negative impact on fruit quality (Pico et al., 2017). Indeed, quality can be affected by grafting. Although this effect is indeed highly dependent on the specific scion-rootstock combination, a majority of studies highlight a negative impact on soluble solids contents, dry matter content and organoleptic perception (as reviewed by (Németh et al., 2020).

Regarding the impact on specific compounds, Kolayli et al., (2010) found that grafted melons reduced total individual sugar contents, and citric acid contents, and increased the fructose to glucose ratio. This, added to a negative impact on aroma perception led to a negative impact on taste perception in sensory evaluations. As regards volatile compounds, a limited amount of literature is available in this species. Nevertheless, pumpkin hybrids have been found to induce a negative effect on the accumulation of key odorant esters. In muskmelon, they reduced the activity of alcohol dehydrogenases and alcohol acyltransferases (Chuan-qiang et al., 2011). This impact can be considerably important, as it was reported in melons of the group reticulatus, in which high reductions in ethyl 2-methylbutanoate and ethyl butanoate contents, 20–55% and 63–95% respectively were induced (Condurso et al., 2012). Within the inodorus group Verzera et al., (2014) found in honeydew melons that the content of key aroma aldehydes, such as (Z)-3-nonen-1-ol and (Z)-6-nonenal were lower in grafted plants, with reductions of 20–60% and 8–45%, respectively. In snake melon (*Cucumis melo* var. *flexuosus*) *Cucumis* and *Cucurbita* rootstocks tended to increase the production of volatiles, especially the former, and the latter reduced the accumulation of hexoses and affected negatively flavour perception (Flores-León et al., 2021).

Despite the progress made, little is known regarding the impact of grafting on melon landraces and the effect of alternative rootstocks. In this context, the purpose of the present work is to analyze the impact of interspecific and intraspecific *Cucurbita* and

Cucumis rootstocks on the accumulation of specific sugars, acids, and volatiles analyzing its effects in melons of the Ibericus group. Melon landraces were selected as materials of study precisely to evaluate the impact on high quality materials and to prospect the use of grafting as an alternative for the production of these resources under constraining conditions such as the impact of soilborne-diseases and high salinity in sustainable organic farming cultivation.

5.3. Materials and Methods

5.3.1. Plant Materials

Eight accessions of five melon landrace types belonging to the C. melo Ibericus group were used as the scion. One accession of the Amarillo landrace type, "Groc d'Ontinyent" 22AM-GO (BGV016451), one accession, 35TN (BGV004298) of the Tendral type, two accessions of Blanco type, 29BL (BGV015753) and 32BL (BGV016453), one accession of Rochet type, 02RC (BGV003718), and two accessions of Piel de Sapo type, 03PS (BGV016356) and 11PS (BGV013188). These accessions are available through the GeneBank of the Universitat Politècnica de Valencia, Spain. One commercial F1 hybrid was included as scion control: Finura RZ F1 (Rijk Zwaan Ibérica S.A.R.L.), representing the Piel de Sapo type.

These scions were grafted onto five rootstocks. F1Pat81, an experimental interspecific cross between a Cucumis melo accession of the *agrestis* subspecies, resistant to *Monosporascus cannonballus* (Flores-León et al., 2021), and another *C. melo* accesion of the melo subespecies, ibericus Piel de Sapo type, two hybrid rootstocks between the wild species *Cucumis ficifolius*, x and *Cucumis anguria* and *Cucumis ficifolius* x *Cucumis myriocarpus* (Fian and Fimy, respectively) with resistance to different soilborne diseases (Cáceres et al., 2017), and one commercial *Cucurbita maxima* x *Cucurbita moschata* hybrid rootstock: Shintoza F1 (Intersemillas S.A.), with resistance to *Fusarium oxysporum* and *Verticillium albo-atrum*.

5.3.2. Experimental design and cultivation

Cultivation was performed during the spring-summer crop cycle (from May to August) in two sites with different agroclimatic characteristics, both open-field, on the East coast of Spain. The field in La Punta (39°26′41.3″ N, 0°21′'14.9″ W, Valencia, province of Valencia), had a long history of melon cultivation with a high incidence of soilborne

diseases. The field of Carrizales (38°08’32.8” N, 0°42’44.7” W, Elche province of Alicante) was selected as representative cultivation of melon under high salinity conditions. The use of saline water irrigation in the area lead to the production of high quality recognized melons. In each field, a randomized complete block design with four blocks and four plants per treatment and block was used. In the case of Fian a lower number of plants was available for some combinations due to the lack of sufficient seed. Not enough samples could be obtained for volatile analysis of 35TN and Finura in Carrizales, and these accessions were excluded from the MANOVA biplot analysis. In the case of 02RC in Carrizales several rootstock combinations could not be sampled due to disease effects and it was exluded from the general ANOVA analysis.

Plants were transplanted onto ridges with black mulch. In La Punta a separation of 2m between ridges and 0.6 m between plants was used. In Carrizales the distance between plants was slightly higher (0.9 m). Flood irrigation every two weeks was used in the case of La Punta and drip irrigation in Carrizales. In both cases, organic farming management was followed.

5.3.3. Analysis of sugars and acids

One fruit was analyzed per plant. A 5 cm wide cross-section of the equatorial area was obtained from each fruit, homogenized, and frozen at -80 ºC until analysis. An aliquot was used to determine individual sugars (sucrose, glucose, and fructose) and organic acids (citric, malic, and glutamic) using an Agilent 7100 capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany) following the procedure described by Cebolla-Cornejo et al., (2012).

Thawed samples were centrifuged at 13200 rpm (F45-24-11 fixed angle rotor, Eppendorf, Hamburg, Germany) for 5 min. The resulting supernatant was diluted (1:20) with ultrapure water (Elix 3, Millipore, Billerica, MA, USA) and filtered using 0.22 µm centrifuge tube filters (Costar® Spin-X®, Corning, Amsterdam, The Netherlands). Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50 µm id, 375 µm od, 67 cm total length, and 60 cm effective length were used for the separation. Before their first use, capillaries were prepared flushing NaOH 1 mol L-1 at 50 °C for 5 min, NaOH 0.1 mol L-1 for 5 min at 20 °C, and water for 10 min. At the beginning of each sequence, the capillary was flushed at 20 °C with the running buffer for 30 min. The

running buffer consisted of 20 mmol L-1 2,6-pyridine dicarboxylic acid and 0.1% w:v hexadimethrine bromide solution at pH 12.1. Between runs, the capillary was flushed with 58 mmol L-1 sodium dodecyl sulphate (2 min) and running buffer (5 min). Samples were hydrodynamically injected at 3400 Pa for 10 s, the separation was performed applying a voltage of −25 kV at 20 °C, and the absorbance was measured at 214 nm. Results were expressed in g kg-1 fresh weight (fw). Total sugars, the ratio fructose to glucose and hexoses to sucrose were determined, as well as sucrose equivalents (SEq), which was calculated by multiplying sucrose, glucose, and fructose contents by their relative sweetening power, 1, 0.74, and 1.73, respectively, and adding them up (Koehler and Kays, 1991).

5.3.4. Analysis of Volatile Compounds

The purge and trap followed by gas chromatography-mass spectrometry (GC-MS) analysis method described by Fredes et al., (2017) were used for the analysis of volatile compounds. Only the samples from Carrizales were analyzed, selecting one random sample per block. Solid Phase Extraction (SPE) cartridges were conditioned with 5 mL of diethyl ether, 5 mL of n-hexane, and air-dried for 10 min. For extraction, 30 g of thawed sample was weighed into a 150 mL stoppered Erlenmeyer flask. A 1.6 mL min-1 nitrogen gas flow was used for the inlet tube of the purge and trap headspace system, and the SPE cartridge for the outlet tube. The samples were extracted for 49 min at 40 °C using magnetic agitation. Then, the cartridges were eluted using 5 mL of diethyl ether/n-hexane 1:1 (v:v) solution, and 5 mL of diethyl ether. Finally, the collected elution solvents were evaporated to 0.5 mL at 35 °C under a nitrogen gas flow. The resulting extracts were divided into two aliquots and frozen at -40 °C in sealed vials until analysis.

The quantification of volatile compounds was performed using a TQ-GC gas chromatography system from Waters (Milford, MA, USA). A Supelcowax 10 column of 30 m x 0.25 mm x 0.25 µm (Sigma-Aldrich, San Luis, MO, USA) and a 1 mL min-1 helium gas flow were used. The samples were injected in splitless mode (1 µL) at 280 °C. The temperature program started at 40 °C during 5 min after the injection followed by a rise to 160 °C (40 °C min-1), and finally, a rise to 250 °C (30 °C min-1) which was maintained for 2 min. Electron ionization in positive mode was used at 250 °C and 230 °C for the interphase and the ion source respectively. The mass spectra were acquired in Selected Ion Monitoring (SIM) mode using the m/z relation for each compound.

5.3.5. Statistical analysis

MANOVA tests were performed with the SPSS 22.0 software (NYSE:IBM, Armonk, NY, USA) to evaluate the effects of the site of cultivation, scion, and rootstock and their interactions. P-value was calculated using the Pillai trace test. ANOVA tests, Tukey and Dunnett's tests were performed to delve into the effect on individual variables. StatGraphics Centurion version 17.2.04 for Windows and IBM SPSS Statistics 25 for Windows were used for this purpose.

The effect of main effects and interaction in the accumulation of volatiles was studied with a graphical MANOVA Biplot representation (freeware licensed software by Vicente-Villardon, 2015). Bonferroni circles were plotted to represent the confidence intervals (α=0.05). Non-overlapping projections of a couple of treatments on each variable indicate significant differences. In the MANOVA Biplot, dashed lines were used to indicate non-significant effects.

5.4. Results

The assays took place under organic farming conditions in order to verify the performance of the rootstocks in actual infestation contexts. In both fields, soilborne pathogens were detected during cultivation, as described in a previous study dealing with snake melon in the same fields (Flores-León et al., 2021), but La Punta presented a higher mortality and pathogen presence than Carrizales. In the case of La Punta *Macrophomina phaseolina*, *Fusarium* species and *Neocosmospora falciformis* represented the main pathogens, while in Carrizales predominated *Macrophomina phaseolina* and *Fusarium* species

Both cultivation sites differed in climatic conditions, management, and even disease incidence, but probably the main difference was related to salinity levels. In Carrizales, salinity of irrigation water (4.5 dS m^{-1}) doubled that of La Punta. Soil salinity was also considerably higher in Carrizales (3.2 dS m^{-1} vs. 0.67 dS m^{-1}). Accordingly, location influenced the accumulation of soluble metabolites leading to significant effects in the levels of glutamic acid, fructose, glucose, sucrose, the ratio fructose to glucose, and hexoses to sucrose, as well as SSC (Table 1). The contents of hexoses were higher in La Punta, while sucrose content and SSC were higher in Carrizales. Regarding acids, higher contents of glutamic acid were found in La Punta. In the case of citric acid, the higher contents of La Punta were not significant but close to the threshold (p=0.07).

The scion had a significant effect on all the variables related to soluble solids (Table 1). Accession 03PS outstood for malic acid accumulation with levels that more than doubled the rest of accessions, even 11PS that also belonged to the same landrace of the Piel de Sapo type. In contrast, 03PS presented the lowest levels of citric acid. Glutamic acid was detected at very low levels, with 35TN having the highest accumulation. As sugars are concerned, Finura, 32BL, and 35TN had the highest values of fructose and 11PS the lowest. A similar trend was found for glucose accumulation. Sucrose levels were similar in all the accessions, but Finura offered lower levels. The differences in total sugars were limited, with significant differences between the 101.20 mg kg^{-1} of Finura and 110.21 mg kg^{-1} of 32BL. A similar trend was observed in the case of sucrose equivalents, which is weighed by the sweetening power of each sugar, and SSC. On the contrary, the profile in sugar accumulation was more variable. In this sense, the fructose to glucose ratio, ranged from 0.82 in 11PS to 1.04 in 32BL. Even higher variation was found for hexoses to sucrose ratio that varied from 0.53 in 11PS to 0.95 in Finura.

The accumulation of acids, SSC and fructose was not significantly affected by the rootstock (Table 1). In fact, the rootstock effect was only significant for the accumulation of glucose, sucrose, the ratio fructose to glucose, total sugars, and sucrose equivalents. The non-grafted control had higher values of glucose and lower levels of sucrose compared to grafted plants. Among the rootstocks, the use of Fian (F1 *C. ficifolius* x *C. anguria*) led to higher sucrose contents. As opposed to the scion effect, the variation in the fructose to glucose ratio was low, with the lowest levels found in Shintoza and Fimy. Finally, the high accumulation of sucrose in Fian led to higher levels of total sugars and sucrose equivalents observed with this rootstock.

Important interactions between factors were detected (Table 1). The interaction location x scion was highly significant for almost all the variables. In the case of location x rootstock it was only significant for glutamic acid contents and the ratio fructose to glucose. The scion x rootstock interaction was significant for all the variables except those regarding acid accumulation.

Considering the existence of such interactions a more thorough evaluation, extended to the volatile profile, was performed in Carrizales, a cultivation site with saline water, that maximizes melon quality, and milder soilborne pathogen stress. This time the focus was placed in the evaluation of a higher range of landraces.

Two independent MANOVAs confirmed highly significant effects of the scion genotype, rootstock, and their interaction in the global accumulation of sugars and acids and volatiles (Pillai trace p-values<0.001). It was confirmed then, the necessity to evaluate the impact of rootstocks on each specific scion genotype.

Table 1. Effect of the location, scion, rootstock and their interaction on the accumulation of sugars and acids in sweet melon fruits from non-grafted (NG) plants and those grafted onto Cucurbita comercial rootstock Shintoza and experimental *Cucumis* rootstocks (F1Pat81, Fian, and Fimy) grown at La Punta and Carrizales. ANOVA p-values are indicated and for each effect, different letters indicate significant difference at p<0.05 (Tukey test).

		Malic acid g kg^{-1}	**Citric acid g kg^{-1}**	**Glutamic acid g kg^{-1}**	**Fructose g kg^{-1}**	**Glucose g kg^{-1}**	**Sucrose g kg^{-1}**	**Fructose/Glucose ratio**	**Hexoses/Sucrose ratio**	**Total sugars g kg^{-1}**	**Sucrose equivalents g kg^{-1}**	**Soluble solids content (ºBrix)**
Location (L)	Carrizales	0.185	4.679	0.019	18.142	19.884	67.248	0.892	0.643	105.275	113.348	12.918
	La Punta	0.222	4.875	0.040	22.102	22.743	60.849	0.977	0.800	105.694	115.915	11.522
	p-value	*0.41*	*0.07*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*0.82*	*0.16*	*<0.01*
Scion (S)	03PS	0.55^{b}	4.08^{a}	0.03^{ab}	17.70^{b}	19.04^{a}	66.27^{b}	0.93^{bc}	0.58^{a}	103.01^{ab}	110.98^{a}	12.4^{b}
	11PS	0.23^{a}	4.86^{b}	0.03^{ab}	14.70^{a}	17.55^{a}	71.82^{b}	0.81^{a}	0.53^{a}	104.06^{ab}	110.22^{a}	12.7^{b}
	32BL	0.06^{a}	4.76^{b}	0.02^{a}	22.84^{c}	21.86^{b}	65.52^{b}	1.04^{d}	0.73^{b}	110.21^{b}	121.20^{b}	12.5^{b}
	35TN	0.12^{a}	5.15^{b}	0.04^{b}	21.68^{c}	23.76^{bc}	63.51^{b}	0.91^{b}	0.82^{bc}	108.94^{ab}	118.59^{ab}	12.2^{ab}
	Finura	0.06^{a}	5.04^{b}	0.03^{ab}	23.70^{c}	24.36^{c}	53.14^{a}	0.97^{c}	0.95^{c}	101.20^{a}	112.17^{a}	11.4^{a}
	p-value	*<0.01*	*<0.01*	*0.03*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*0.01*	*<0.01*	*<0.01*
Rootstock (R)	NG	0.20	4.81	0.03	20.55	22.02^{b}	59.29^{a}	0.93^{ab}	0.79	101.86^{a}	111.14^{a}	12.0
	F1Pat81	0.25	4.89	0.02	19.41	19.80^{a}	65.00^{ab}	0.97^{b}	0.68	104.21^{ab}	113.23^{ab}	12.0
	Shintoza	0.16	4.63	0.03	20.01	21.49^{ab}	63.42^{ab}	0.92^{a}	0.73	104.92^{ab}	113.94^{ab}	12.2
	Fian	0.25	4.69	0.03	20.46	21.67^{ab}	68.73^{b}	0.93^{ab}	0.68	110.86^{b}	120.16^{b}	12.5
	Fimy	0.16	4.87	0.03	20.18	21.59^{ab}	63.80^{ab}	0.92^{a}	0.74	105.57^{ab}	114.69^{ab}	12.4
	p-value	*0.54*	*0.47*	*0.63*	*0.54*	*0.01*	*0.05*	*0.02*	*0.20*	*0.05*	*0.04*	*0.22*
LxS	*p-value*	*0.05*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*0.02*	*0.13*	*<0.01*
LxR	*p-value*	*0.2635*	*0.18*	*0.02*	*0.54*	*0.33*	*0.23*	*0.05*	*0.09*	*0.34*	*0.31*	*0.10*
SxR	*p-value*	*0.75*	*0.23*	*0.23*	*0.05*	*0.05*	*0.04*	*<0.01*	*0.50*	*<0.01*	*<0.01*	*<0.01*

Indeed, in the case of the sugar and acid profile, the response of the plants to grafting depended on the specific scion-rootstock combination. Even more, within the same landrace type, the response varied depending on the precise accession being considered. In the Amarillo landrace accession 22AM-GO, the sugar and acid profile of F1Pat81 was quite similar to that of the non-grafted control (Fig. 1). In this case the Fian rootstock combination was not available. With this landrace, the use of the commercial *Cucurbita* hybrid Shintoza mainly had a small effect increasing the amounts of glutamic acid over the quantification limit, while Fimy tended to offer higher sugar accumulation, though this difference was not significant (Supp. Table 1).

Limited differences were found when the Rochet landrace accession 02RC was used as scion. Although grafted plants tended to reach lower citric acid contents than NG, this difference was not significant.

In the Blanco landrace two accessions were studied, and a different response was observed in each one (Fig. 1). The differences between grafted and non-grafted plants were higher in 29BL. In this accession, NG plants tended to show higher hexoses and lower sucrose accumulation leading to higher hexoses to sucrose ratio. Although grafted plants tended to offer higher sucrose contents, total sugars and SSC, especially in the case of Shintoza, but a high level of variability restricted the significance of this effect on most rootstocks. In the accession 32BL the differences were attenuated, and only Shintoza tender to offer a less acidic profile (Supp table 1).

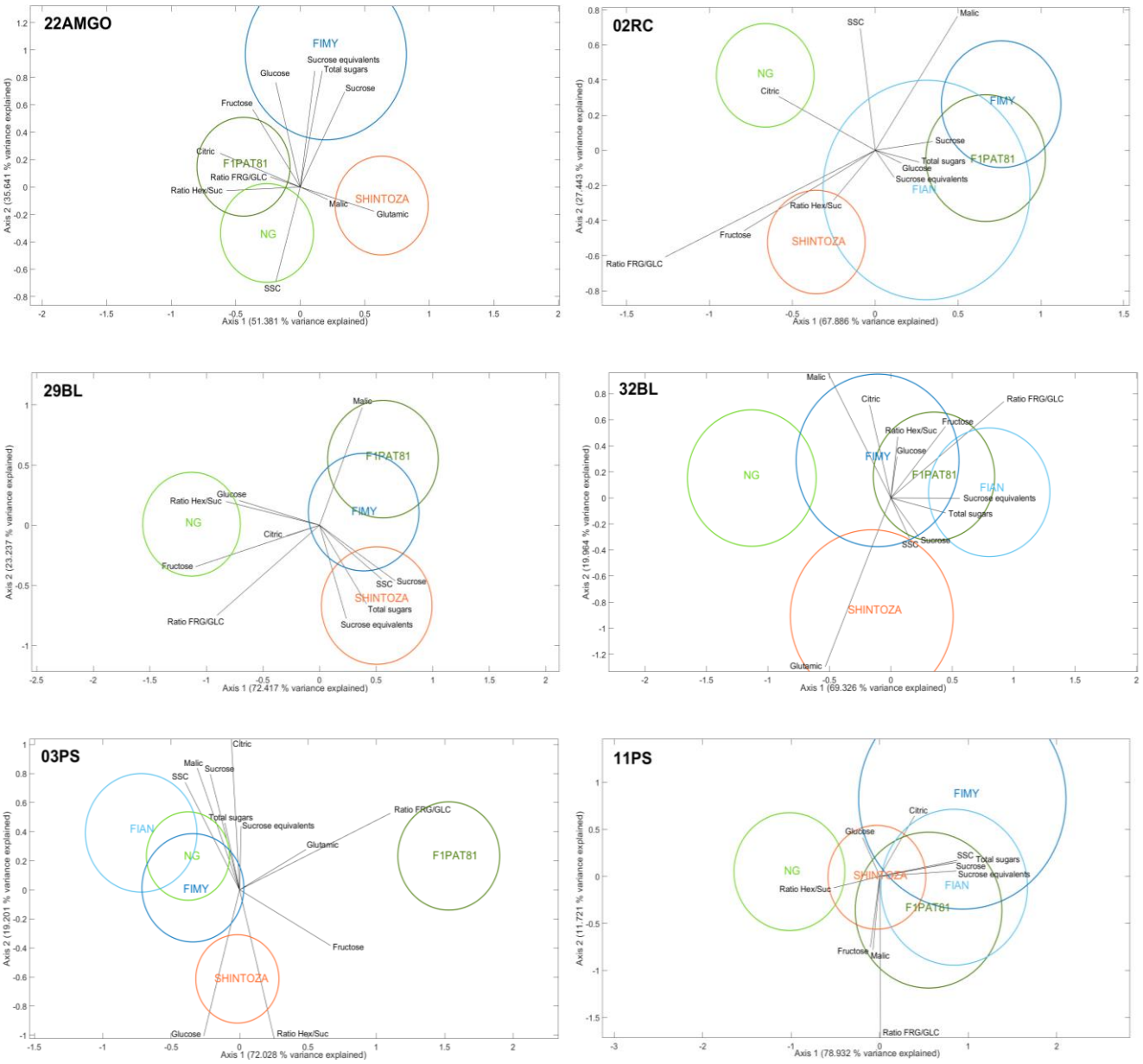


Figure 1. MANOVA Biplot for the Sugars and Acids analyzed in different melon landrace and rootstock combinations grown in Carrizales. Circles represent Bonferroni confidence intervals. NG: non-grafted control, F1PAT81 (*C. melo* subsp *melo* group ibericus, x *C. melo* subsp *agrestis* group chinensis, Pat 81), SHINTOZA (*C. maxima x C. moschata*), FIMY (*C. ficifolius* x *C. myriocarpus*), FIAN (*C. ficifolius* x *C. anguria*)

A difference in the response was also found between the two accessions evaluated of Piel de Sapo. In the 11PS accession, the differences between the NG control and grafted plants were limited, though grafted plants tended to reach higher sugar accumulation and SSC (Fig. 1). But this effect was only significant for Fian and Fimy (Supp table 1). This also applied in the case of 03PS accession with Fian. For this accession Shintoza tended to show a less acidic profile, as it happens with the Blanco accession, but this effect was not significant (Supp Table 1). Finally, F1Pat81 presented a high fructose to glucose ratio.

Table 2. Accumulation of volatiles in fruits grown in Carrizales with different landrace and rootstock combinations. ANOVA p-values are included. Differences with the non-grafted control NG) are indicated (*, Dunnett's test, P=0.05).

Scion	Rootstock	Total volatiles ($ng\ g^{-1}$)	Total esters ($ng\ g^{-1}$)	Total aldehydes ($ng\ g^{-1}$)	Total alcohols ($ng\ g^{-1}$)	Total apocarotenoids ($ng\ g^{-1}$)
22AM-GO	F1Pat81	319.92±36.17	2.17±0.32	207.37±30.87	108.53±11.28	1.85±0.22
	Fimy	223.19±11.89*	2.83±0.86	128.58±36.64	90.41±24.08	1.39±0.19
	Shintoza	265.29±28.7	7.92±2.29*	123.54±36.2*	132.22±8.19	1.61±0.14
	NG	387.68±34.17	2.09±0.2	277.24±28.6	106.91±16.78	1.44±0.15
	p-value	0.0450	0.0404	0.0383	0.3426	0.3269
02RC	F1Pat81	282.02±31.45	6.47±2.02	103.84±21.51	170.05±12.29	1.66±0.08
	Fimy	234.89±37.69	10.35±5.99	35.97±6.62	186.94±38.21	1.63±0.11
	Shintoza	218.86±28.55	2.93±0.15	77.53±7.44	136.39±29.64	2.01±0.29
	NG	201.28±33.91	4.47±0.93	100.11±20.52	95.21±17.92	1.49±0.25
	p-value	0.3688	0.2599	0.1237	0.1214	0.4161
29BL	F1Pat81	328.31±44.31*	2.84±1.09	216.48±51.8*	107.19±8.62	1.8±0.4
	Fimy	452.18±54.59	46.78±44.43	264.2±67.7*	139.91±15.4	1.28±0.23
	Shintoza	460.52±91.35	13.72±7.85	287±42.65*	158.32±45.56	1.48±0.13
	NG	678.92±71.99	3.89±0.33	568.4±44.35	105.02±28.19	1.6±0.49
	p-value	0.0375	0.5131	0.0058	0.5058	0.7513
32BL	F1Pat81	601.02±63.31	7.75±1.52	422.06±85.58	168.53±37.02	2.69±0.07
	Fian	473.22±26.24	10.51±2.34*	318.64±23.24	142.27±34.5	1.8±0.21
	Fimy	1094.38±350.4	7.99±0.76	587.08±69.24	148.29±21.44	1.74±0.16
	Shintoza	571.51±131.18	6.68±0.28	420.3±117.23	142.56±23.33	1.97±0.06
	NG	441.74±63.88	4.73±1.26	329.46±44.93	106.08±19.41	1.47±0.4
	p-value	0.1245	0.1361	0.1491	0.6392	0.1610
11PS	F1Pat81	407.12±66.57	3.45±0.42	276.62±53.28	104.98±56.45	2.26±0.08
	Fian	439.46±126.3	3.46±0.35	164.79±52.35	270.01±179.5	1.2±0.51
	Fimy	217.55±45.47	3.65±0.48	95.11±31.34	116.84±13.71	1.94±0.9
	Shintoza	351.58±65.81	3.11±0.15	222.14±54.14	124.34±25.93	2±0.15
	NG	404.36±126.39	2.13±0.68	292.53±95.55	108.17±30.1	1.53±0.2
	p-value	0.6116	0.5224	0.5136	0.6197	0.4800
03PS	F1Pat81	289.53±57.42	7.74±4.72	133.92±14.08	146.32±61.71	1.54±0.13
	Fian	246.22±18.7	3.79±0.08	118.55±6.95	122.04±25.14	1.85±0.44
	Fimy	216.41±57.92	3.25±0.62	90.51±81.77	121.78±26.13	0.86±0.1
	Shintoza	98.45±31.43	1.53±0.04	27.68±4.46	68.26±27.93	0.98±0.23
	NG	176.73±2.78	2.29±0.29	96.37±5.67	76.81±7.89	1.26±0.13
	p-value	0.0804	0.3927	0.4412	0.5142	0.0458

Important differences were found among accessions in the volatile profile of non-grafted controls, even between those belonging to the same landrace (Table 2). Accession 02RC of Rochet and 03PS from Piel de Sapo showed the lowest total volatiles contents, mainly due to a low accumulation of aldehydes. On the other hand, 29BL from Blanco reached the highest total volatiles and total aldehydes contents, while 02RC and 32BL had high total esters content. The effect of grafting on fruits volatile profile regarding total volatiles

and groups of volatiles was limited. Nonetheless, a more detailed analysis of this effect was studied with independent MANOVA biplots for each accession.

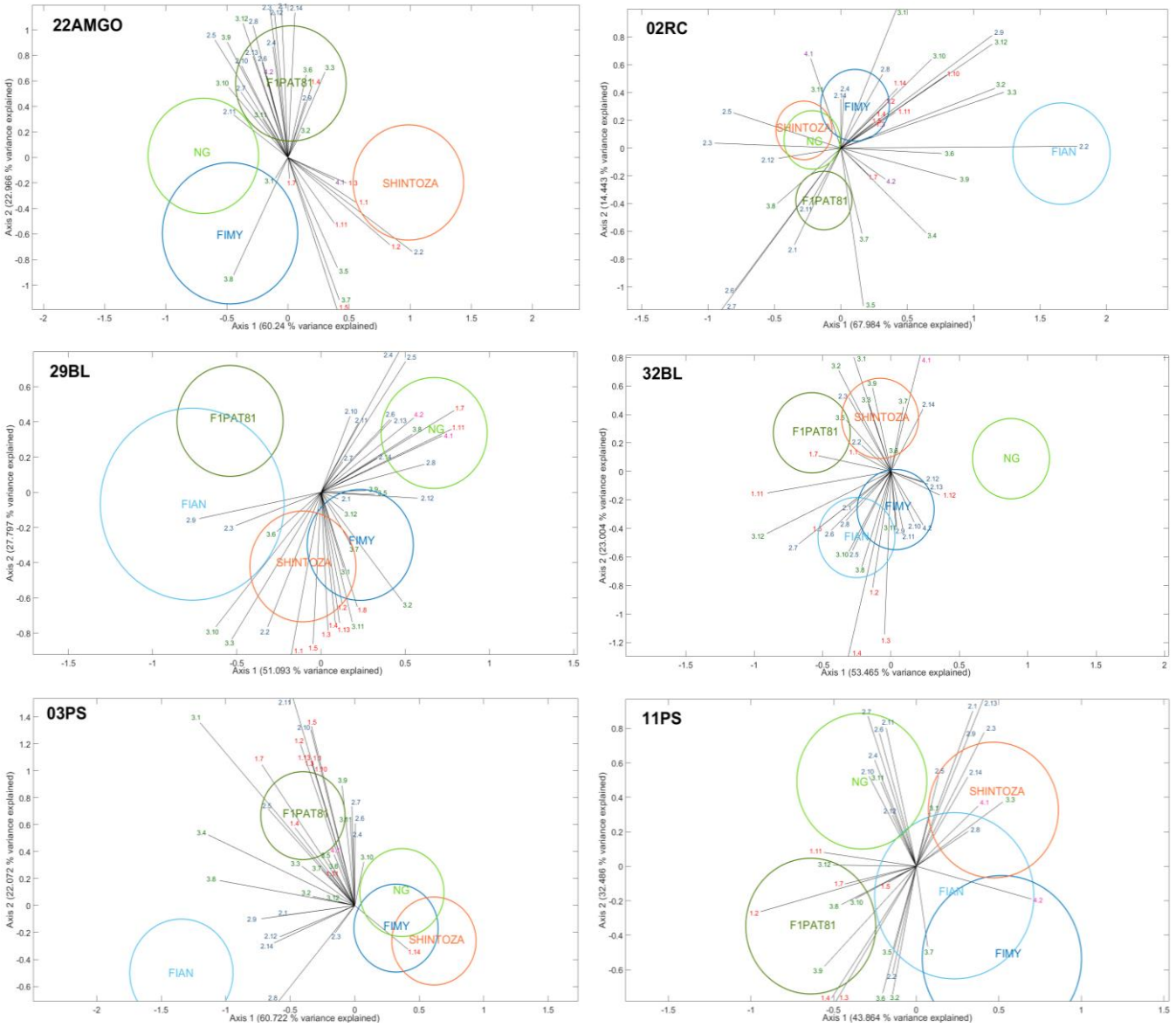


Figure. 3. MANOVA Biplot of organic volatile compounds analyzed in different melon landrace and rootstock combinations grown in Carrizales. Circles represent Bonferroni confidence intervals. NG: non-grafted control, F1PAT81 (*C. melo* subsp *melo* group ibericus, x *C. melo* subsp *agrestis* group chinensis, Pat 81), SHINTOZA (*C. maxima* x *C. moschata*), FIMY (*C. ficifolius* x *C. myriocarpus*), FIAN (*C. ficifolius* x *C. anguria*). Volatile compounds: 1.1 = Methyl-2-methyl butyrate, 1.2 = 2-Methyl propyl acetate, 1.3 = Ethyl butanoate, 1.4 = Ethyl-2-methyl butyrate, 1.5 = Butyl acetate, 1.6 = Diethyl carbonate, 1.7 = Butyl butyrate, 1.8 = Ethyl hexanoate, 1.9 = Hexyl acetate, 1.10 = (Z)-3-Hexen-1-ol, acetate, 1.11 = Heptyl acetate, 1.12 = (E,E)-2,4-Hexadienoic acid, ethyl ester, 1.13 = Ethyl-3-(Methylthio)propanoate, 1.14 = Benzyl acetate, 2.1 = Hexanal, 2.2 = (E)-2-methyl-2-butenal, 2.3 = Heptanal, 2.4 = Octanal, 2.5 = (E)-2-Heptenal, 2.6 = Nonanal, 2.7 = (Z)-6-Nonenal, 2.8 = (E,E)-2,4-Heptadienal, 2.9 = Benzaldehyde, 2.10 = (E)-2-Nonenal, 2.11 = (E,Z)-2,6-Nonadienal, 2.12 = Phenylacetaldehyde, 2.13 = (E,E)-2,4-Nonadienal, 2.14 = (E,E)-2,4-Decadienal, 3.1 = 1-Pentanol, 3.2 = 1-Hexanol. 3.3 = (Z)-3-Hexen-1-ol, 3.4 = 1-Octanol, 3.5 = 1-Nonanol, 3.6 = (Z)-3-Nonen-1-ol, 3.7 = (Z)-6-Nonen-1-ol, 3.8 = 1-Decanol; 3.9 = (E,Z)-2,6-Nonadien-1-ol, 3.10 = Benzyl Alcohol, 3.11 = 2-Phenylethanol, 3.12 = Phenol, 4.1 = Geranylacetone, 4.2 = Beta-Ionone.

Indeed, the specific scion-rootstock combination also seemed determinant in the case of the volatile profile and it was further reviewed in a case-by-case basis. In the Amarillo accession 22AM-GO the use of *Cucumis* rootstocks had a reduced effect on the volatile

profile compared to the non-grafted control (Fig. 2, Supp. Table 2). The highest differences were found between the non-grafted control and Shintoza, the *Cucurbita* F1 Hybrid, which tended to show higher accumulation levels of certain esters (e.g. ethyl butanoate, 1.3 and methyl-2-methyl butyrate, 1.1), and a much lower aldehyde content (e.g. nonanal, 2.6, and (Z)-6-nonenal, 2.7).

In the case of the Rochet accession 02RC the differences between rootstock combinations and the NG control were, in general, negligible. Nonetheless, *Cucumis* rootstocks increased the content of (Z)-3-Nonen-1-ol, 3.6 in the figure (Fig. 2, Supp. Table 2). The use of the Fian rootstock led to a different volatile profile, plotting in MANOVA biplot far from the NG. This roostock tended to increase the levels of certain esters (ethyl butanoate, 1.3 and ethyl-2-methyl butyrate, 1.4) and alcohols, though these changes were not significant when compared in case-by-case basis (Supp. Table 2).

The two accessions of Blanco had certain resemblances in their response (Fig. 2, Supp. Table 2). In both cases, the differences in the volatile profile between grafted combinations were limited, but they seemed to differ from the NG control. Indeed, in both cases the control plotted at some distance from the rest of the treatments and the projections of the Bonferroni confidence circles did not overlap for several volatile vectors. The response of both accessions was different, though in some aspects. Fruits from 29BL grafted plants presented lower aldehyde content (e.g. (E,Z)-2,6-nonadienal, 2.11 and nonanal, 2.6), while those from 32BL grafted plants had higher content of specific compounds in certain combinations. It was the case of 2-methyl propyl acetate (1.2) in Fian, (Z)-6-nonenal (2.7) in Fimy, or geranylacetone (4.1) in F1Pat81.

Differences between accessions of the same landrace were also observed in the case of Piel de Sapo (Fig. 2). In the accession 03PS, melons from plants grafted onto Fian, and F1Pat81 clearly differed from the rest of the treatments and the NG control. They displayed higher content of esters (e.g. heptyl acetate and butyl butyrate in Fian) and alcohols (e.g. 1-pentanol, 3.1, 1 decanol, 3.8 and phenol, 3.12, in Pat81). On the other hand, melons from plants grafted on Shintoza and Fimy had a volatile profile very similar to the NG control. This time those grafted on F1Pat81 tended to show higher levels of esters and certain aldehydes and alcohols (e.g. (E)-2-nonenal, 2.10, (E,Z)-2,6-nonadienal, 2.11), and 1-pentanol, 3.1). In the case of 11PS the differences between the different rootstock/scion combinations and the NG control were rather limited. A higher level of

variation was observed though, which led to higher values for the Bonferroni circles of confidence in the MANOVA biplot. Nonetheless, some common responses were identified. In this sense F1Pat81 again tended to show higher levels of certain alcohols, and lower levels of certain aldehydes, though these differences in a case-by-case analysis were not significant (Supp. Table 2).

5.5. Discussion

Organic farming is becoming increasingly important in areas such as Europe, where an important effort has been made to increase its adoption as a means to contribute to sustainable development (Brzezina et al., 2017). It is true that under organic management crops tend to offer lower yields, but they can also be more profitable, environmentally friendly, and deliver equally or more nutritious foods that contain fewer pesticide traces, compared with conventional farming (Reganold and Wachter, 2016). Consequently, it represents the perfect scenario to promote the active cultivation of melon landraces, contributing to their *in situ* on-farm conservation. Nonetheless, for that purpose it is necessary to assure a price premium that compensates for lower productivity. This is usually achieved targeting the production to high quality markets that value organoleptic and functional quality, as it is the case for example in tomato landraces or high pigment varieties (Cebolla-Cornejo et al., 2007).

Spain represents the perfect scenario for this approach. This area represents a secondary center of melon diversity, characterized by a wide variety of landraces of the Ibericus group, which have been retained in the domestic markets thanks to taste attributes (López-Sesé et al., 2003). But its cultivation is highly jeopardized by the incidence of soilborne diseases. Accordingly, the use of grafting may represent a reliable alternative to promote their cultivation in these conditions, as long as it does not affect their typical quality standards.

The use of grafting also offers an alternative strategy to promote cultivation under saline conditions. Indeed, salinity is a growing concern worldwide, especially in arid and semi-arid areas, and the development of new cultivars tolerant to salinity stress is becoming increasingly important (Akrami and Arzani, 2019). In the case of melon, it has been proved that the use of certain *Cucurbita* rootstocks can help to minimize salinity negative effects (Colla et al., 2006). From the point of view of fruit quality, growing melons in saline conditions usually results in increased SSC. The effect, though, is dependent on

local conditions and the varieties being considered. For example, Huang et al., (2012) reported that increasing water conductivity from 1 to 2.665 and 7.03 dS m^{-1} led to increases in SSC from 9.03° to 11.03° and 11.61°Brix in Northwest China using Huanghemi melon. On the other hand, Tedeschi et al., (2011) observed a more limited increase with Tendral melon grown in Italy. In these conditions salinity (8.7 dS m^{-1}) resulted in 10.5°Brix SSC, only slightly higher than the control (10,1°Brix at 0.9 dS m^{-1}) and a higher rise (11.3°Brix) was only achieved with extreme levels (28.2 dS m^{-1}). Colla et al., (2006) also found an increase in SSC under saline conditions with Cyrano melons in Italy (e.g. 0.6 to 0.7°Brix increase between 2.0 and 4.0 dS m^{-1}), but this effect was reduced in the case of grafted plants.

In our case, the saline waters used in Carrizales probably explained the 1,4°Brix increase in SSC compared to La Punta. Interestingly, this increase would be mainly motivated by a high increase in sucrose content, being hexoses content higher in La Punta. This result agrees with those of (Burger et al., 2000), which also found that differences in SSC were rather conditioned by sucrose contents than hexoses. Nevertheless, it could not be ruled out an advance in the ripening process in Carrizales, as the hexoses to sucrose ratio was clearly lower in this location. In this sense, sucrose contents increase in melon during the last steps of ripening, while hexoses keep constant or tend to decrease (Burger et al., 2006; Perpiñá et al., 2017).

Fewer information is available on the impact of salinity on acid content, as it has received less attention. Del Amor et al., (1999) observed that not only SSC but also acidity increased with increasing salinity levels in Galia melons grown in Spain. Nonetheless, the increase was limited to a 12% in 2dS m^{-1} increases. A similar response was also observed by Colla et al., (2006), again with limited differences. In our case, though, the differences were not significant for malic and citric acids. Even more, lower contents of glutamic acid were found in Carrizales, although the contribution of this amino acid to acidity would be negligible.

Spanish landraces represent a rather particular subgroup in the wide range of global melon variability characterized by big fruits with high sugar content and usually non-climacteric behaviour (Leida et al., 2015). The results obtained in this study confirmed this trend, as SSC were on average higher than 11°Brix. The variation in SSC and sucrose, the main sugar in melon fruits was negligible, with differences only detected with the commercial

control Finura of the Piel de Sapo type. Nonetheless, variability was observed in the ratios fructose to glucose and hexoses to sucrose, evidencing different profiles of sugar accumulation. Wider variability, though, was detected in the accumulation of acids. In Piel de Sapo landraces the accumulation of malic acid in 03PS doubled that of 11PS and was higher than the rest of the landraces. In the case of 32BL of Blanco malic contents were really low, though significant differences with other landraces were limited. The accumulation of citric acid followed an opposite trend, with the lowest contents being found in 03PS. The accumulation of glutamic acid was insignificant. In fact in most samples it remained under the quantification limits. Only 35TN of Tendral outstood for its contents of glutamic acid, which doubled those found in other landraces. The contents of acids and the acidic profile were in accordance with those observed generally in melons, as citric acid tended to predominate (Burger et al., 2010). Flores-León et al., (2022) also obtained similar results for these landraces, although 03PS did achieve a higher content of citric acid. Flores-León et al., (2022) reported lower sugar content for Tendral melons, which was not observed in the accession included in present study. In any case, the accumulation of malic acid was for example considerably lower than that observed in the Cantalupensis Charentaiss group, as in that the accumulation levels were higher than 1 g kg^{-1} (Perpiñá et al., 2017).

The effect of grafting on melon quality has been thoroughly studied in the last decades, spurred by the publication of inconsistent results. Most of these studies have been focused on the effect on SSC or acidity while only a few analysed specific effects on individual sugars and acids. It seems clear, though, that this inconsistency is due to the dependence of the response upon the specific rootstock-scion combinations being considered (Rouphael et al., 2018a). For example, Colla et al., (2006) found that Cyrano Charentais melons grafted on *Cucurbita* hybrid tended to reduce SSC under saline conditions. This effect might not be exclusive of *Cucurbita* rootstocks, as (Fita et al., 2007) found a decrease of SSC of Piel de Sapo melons grafted on Pat81 *C. melo agrestis* rootstock. On the other hand, other authors have found negligible effects on basic quality parameters. In this sense, Crinò et al., (2007) in South Italy tested different *Cucurbita* hybrids and *C. melo* rootstocks on the quality of the winter melon Incas (inodorus group) and found no significant differences in CSS with the non-grafted control. Similarly, Park et al., (2013) in Korea did not find differences between the muskmelon Earls' elite (*reticulatus* type melon) grafted on selected *C. melo* rootstocks and the non-grafted control on SSC.

Verzera et al., (2014) also found that most combinations did not affect SSC, but it was increased in one of the 5 rootstocks evaluated

As stated before, few studies have analysed the impact of grafting on the accumulation of specific sugars. Soteriou et al., (2016) found differences in the profile of sugar accumulation in Galia and Ananas melons grafted on different *Cucurbita* rootstocks and grown in Cyprus. In that work, the authors studied sugar accumulation in scion rootstock combinations with different levels of incompatibility. It became clear that, at least in these cases total and individual sugar content, as well as sweetness index considering each sugar sweetening power would be dependent on specific scion rootstock combinations. In one of the cultivars tested (Elario) grafting tended to increase sucrose and decrease hexoses contents with some of the rootstocks, but without effects on total sugars. Other rootstocks did not affect the sugar profile while in one of them total sugars and sweetening index was higher due to higher sucrose accumulation.

In our case, sugar contents and sugar profile was affected by grafting. In general, the non-grafted control had higher values of glucose and lower levels of sucrose compared to grafted plants, an effect that might be related with differences in the ripening process. Among the rootstocks Fian could be explored as an alternative to increase sugar levels, though this possibility should be further explored as fewer plants were available in this combination. Nonetheless, the effect of rootstock varied with the specific scion/rootstock combination being considered, even between populations of the same landrace.

In watermelon, lower accumulation of hexoses at the onset of fruit development and a reduction in sucrose accumulation during ripening has been involved in the moderate reduction if fruit SSC from plants grafted on *Cucurbita* and *Lagenaria* rootstocks, though this effect is not consistent (Kyriacou et al., 2017). It seems possible that grafting could be affecting flowering and ripening timing. It also seems that these rootstocks would tend to increase acidity.

The effect of grafting on melon fruit acidity is not clear, as few studies are available. Colla et al., (2006) found a reduction while Crinò et al. (2007) failed to find significant effects of the rootstock in acidity, and a recent review pointed out that the rootstock effect on acidity would be much lower than that exerted on sugar accumulation (Kyriacou et al., 2017). Our results also confirm the negligible effect of rootstocks on the accumulation of organic acids in general. In some cases, such as all grafted Rochet combinations or 32BL

grafted on Shintoza tended to show a less acidic profile, but the variation was high and, in most cases, there was no statistical significance. Other cucurbits, such as watermelon, are more prone to changes in acidity, as reviewed by Kyriacou et al., (2017), with a trend to increase acid levels and specifically malic acid contents (Fredes et al., 2017).

The volatile profile of Ibericus melons has recently been reviewed (Flores-León et al., 2022). Our results confirm that in these landraces the main volatile compounds are aldehydes followed by alcohols and esters. In general, the volatile profile of both studies is similar, though differences are found for certain compounds, which would be explained by enviromental factors, as the importance of this effect in the volatile profile of non-climateric melons has been previously reported (Zarid et al., 2020).

Few studies are available regarding the effects of grafting on melon aroma. Condurso et al., (2012) evaluated the effect of different *C. maxima* x *C. moschata* an *C. melo* rootstocks on the volatile profile of Proteo melo (var. *reticulatus*). In their study they found mainly an increase of alcohols and aldehydes responsible for green and fresh notes in fruits from grafted plants. Interestingly, Z-3-nonenol levels representative of melon notes were decreased in all grafted combinations. Regarding esters, pumpkin hybrids tended, in general, to decrease key odorant esters while the opposite happened when the rootstock Sting, from *C. melo*, was used. This effect had previously been reported by Chuan-qiang et al., (2011) in muskmelons grafted on pumpkin rootstocks and it was related to lower alcohol dehydrogenases (ADHs) and, especially, alcohol acyltransferases (AATs) activities. Nonetheless, the alteration of the volatile profile is highly depended on the specific rootstock/scion combination and it was possible to identify both *Cucurbita* and *C melo* rootstocks with a minimum impact on volatile profile as compared to non-grafted control. The effect on ester reduction was not found in our case, probably because Ibericus melons, which are non-climateric, do not accumulate high levels of esters as compared to muskmelons, which are climacteric. Low AATs activities are expected *per se* in this group of melons as these enzymes convert aldehydes generate from alcohols via ADHs into esters (Gonda et al., 2016).

In any case, it seems clear that the effect of grafting on the volatile profile is limited. Some specific trends in some specific rootstock/scion combinations seem evident. For example, the *Cucurbita* rootstock Shintoza decreases aldehyde content in the Amarillo accession 22AM-GO, as in the 29BL F1Pat81 combination. Nonetheless, a high level of

variability generated by uncontrolled factors minimize the significance of most of the specific trends detected. It should also be considered that even within the same landrace the effect of rootstock varies depending on the specific accession being considered. It seems clear then, that it would be difficult to select a grafting solution maximizing the volatile profile, but at the same time it seems that most rootstocks would have a minimum impact on it.

5.6. Conclusion

Scion and salinity exert a higher effect than grafting on the accumulation of soluble and volatile compounds affecting flavour of melon landraces. The effect of experimental rootstocks of *Cucumis* seem to represent a valuable alternative to *Cucurbita* classic rootstocks, as the effect on the accumulation of sugars and acids and volatiles is limited. Among them, further studies should analyse the performance of Fian as it seems to improve the accumulation of sugars compared to the non-grafted control. Despite the limited effects being observed, a high influence of scion x rootstock interaction has been observed, implying the necessity to determine their ideal rootstock for each population, as the response may differ between populations of the same landrace.

Supplementary files available at:

https://drive.google.com/drive/folders/1XqD-biQ8VIwPMnuq97l4YpvD031mFw3h?usp=share_link

General Discussion

6. General Discussion

The genetic diversity present in *Cucumis melo* L. has allowed for the study of an ample number of characteristics, both genetic and agronomic. This has led to a better understanding of these traditional landraces, and how to better focus in future breeding programs. The main objective of this thesis has been the evaluation of a collection of traditional melon landraces from Spain, and to select those best adapted for organic farming conditions. To study this, a large collection of these traditional melon cultivars is needed and a better understanding of their genetics, their morphology and metabolic profiles is needed. By better knowing the available biodiversity, a better judgment can be made, allowing for a better selection of the key and singular melon landraces. It is also important to take into consideration field assays. To better understand how this traditional melon landraces will perform, it is necessary to evaluate them under field conditions akin to those they will face when cultivated by the farmers. This method will provide will more information to the real limiting factors facing the cultivation of melon under organic farming conditions. These limiting factors can be both biotic and abiotic, hence, by employing different locations, more of these limiting factors can be surveyed and taken note, to latter address in future breeding programs. Grafting and the use of resistant rootstocks are part of the modern methods employed to resist against most soilborne pathogens. The use of different resistant rootstocks, both commercial *Cucurbita* hybrids and experimental *Cucumis* (both experimental *C. melo* and *Cucumis* spp. interspecific crosses) has permitted a more thorough evaluation of the effect they have of both non-sweet and sweet melons, how they alter the fruit and the metabolic profiles of the fruits. All these factors are key for the future breeding programs, and the search for scion-rootstock combinations which lessen the impact on fruit quality. Fruit quality has been one of the main aspects taken into consideration in recent years, with both sensory and nutritional values also been considered.

The growing interest and demand for organic products over the last years has led the Cucurbit Breeding Group of the COMAV, to study how to employ neglected traditional melon cultivars. By cultivating employing organic farming conditions the hope of revitalizing these cultivars, as consumer often perceive them as higher quality and safer to eat.

Spanish Melon Landraces: Revealing Useful Diversity by Genomic, Morphological, and Metabolomic Analysis

To better understand the available diversity, a large traditional melon collection was characterized, which included both sweet, non-sweet and exotic melon cultivars. Chapter 1 describes the methods employed, for the genetic characterization (with a GBS approach, population structure, phylogeny, SNP effects), the fruit morphology (at a commercial maturity) and the metabolic profile (sugars, acids and VOCs). Since the first GBS of a melon collection by (Pavan et al., 2017), several of them have been performed to better understand the diversity of melon collections (Abu Zaitoun et al., 2018; Gonzalo et al., 2019; Jung et al., 2020; Moing et al., 2020; Hyun et al., 2021; Wang et al., 2021).These works all are performed employing restriction enzyme *ApeKI*, while our work employed enzyme *MsII*. This has permitted for the study of different genomic areas compared to the rests.

The SNP pipelines employed by different work vary with some employing Tassel-GBS discovery pipeline (Glaubitz et al., 2014) such as Wang et al., (2021), Moing et al., (2020) or Pavan et al., (2017), while others like Jung et al., (2020) or (Hyun et al., 2021) employ programs BWA+SAMtools (Li et al., 2009; Li, 2013) and Bowtie2+GATK (McKenna et al., 2010; Langmead and Salzberg, 2012) respectively. As for SNP filtration criteria, different works employ different methods, especially of maf (minor allele frequency) and maximum missing ranging from 0.01-0.05 and 5-50%. Our filtration criteria ensured that even rare SNPs were detected and that genomic data for that SNP is known for the rest of the accessions studied. This permitted us to obtain a 66971 quality SNPs, which in turn, allowed for a better Population Structure Analysis and Phylogeny. Other works have obtained less SNPs, Gur et al., (2017) in a study of 117 accessions, obtained 23931 quality SNPs, while Wang et al., (2021) obtained 27471 quality SNPs out of a 2083 melon accessions, from a raw 89204 SNPs.

Our study of Population Structure revealed that K=2 and K=3 was the best for the accessions studied, and especially K=3 differentiated 3 subpopulations, one formed by the sweet Ibericus melons, another of the Spanish Flexuosus melons and the third one by the Exotic cultivars. Our results are similar to those obtained by other authors (Leida et al., 2015; Hyun et al., 2021; Wang et al., 2021). Esteras et al., (2013) found that in their study with 74 accessions and 768 SNP markers, that K=4 and K=5 where the best results.

Leida et al., (2015) studied both Chate and Flexuosus melon, but they did not form their own subpopulation, they rather contain proportions of different subpopulations, of *ameri-inodorus* and *ameri* subpopulations. It is also probable that the use of different Flexuosus melons from widely different origins by Leida et al., (2015) derived in not creating a Flexuosus subpopulation.

Most of the previous works perform a Neighbour-Joining phylogeny (Nimmakayala et al., 2016; Gur et al., 2017; Pavan et al., 2017; Abu Zaitoun et al., 2018), although Wang et al., (2021) and (Hyun et al., 2021) employed Maximum-Likelihood and Bayesian regression respectively. Our use of the Maximum-Likelihood method is supported by their greater reliability in multiple sequence alignment (Chang et al., 2014; Kumar Gupta et al., 2021). The results obtained separated the Exotic cultivars, leaving Flexuosus and Ibericus melons forming to clear clades. Interestingly observed was that the Ameri accession was close to sweet cultivated melons, something that was already observed by other authors (Nimmakayala et al., 2016; Sabato et al., 2019; Moing et al., 2020). The Ibericus melon clade, showed no clear grouping based upon their Subgroups, as found by other studies (Esteras et al., 2013; Leida et al., 2015; Moing et al., 2020). Hence, the use of the classification performed by Pitrat (2016) responds to more commercial characterization rather than a genetic one.

The study SNPs associated with specific traits, Genome Wide Association Study (GWAS) are performed, and in melon, these have been performed on traits such as fruit firmness, sex expression, climacteric fruit ripening, agronomic traits, resistance to powdery mildew and *Cucurbit yellow stunting disorder virus* (CYSDV) (Natarajan et al., 2016; Nimmakayala et al., 2016; Gur et al., 2017; Pereira et al., 2020; Hyun et al., 2021; Kishor et al., 2021; Wang et al., 2021). Again, by employing enzyme *Ape*KI and different genome versions, it is difficult to compare with them. Kumar et al., (2018) employed the SNPEff to annotate SNPs and Indels detected in 2 cultivars, and one of the SNPs (T/A) detected (in high drought tolerant cultivar BS5) was also located in this work, with the same effect (Moderate) in gene MELO3C018800 (Transferring glycosyl group transferase), mainly present in Exotic and Flexuosus melons. Oren et al., (2022) performed an QTL analysis on a "Dulce" (*reticulatus*, climacteric) and "Tam Dew" (*inodorus*, non-climacteric) RILs population, employing the newest version of the melon genome. One of the SNPs detected in their candidate genes for Flesh Firmness (FF5.1) was in MELO3C004349.2 (Serine/threonine-protein kinase), with climacteric melons a

T/A change. This SNP was detected on the exotic cultivars, not in the Flexuosus or Ibericus melons.

Morphological characterization and classification of melons have been performed in numerous occasions (Stepansky et al., 1999; Escribano and Lázaro, 2009; Szamosi et al., 2010; Ali-Shtayeh et al., 2017). Different methods can be used to differentiate between each landrace, leading to different classifications. Pitrat, (2016) classifies Ibericus melons based upon their external characteristics, both colour and shape. Lázaro et al., (2017) studied 62 Spanish melon landraces and found 6 different groups ("Piel de Sapo", "Mochuelo", "Tendral", "Yellow/White", "Winter" and "Black") based upon agro-morphological characteristics. These morphological characteristics are what mostly help consumers and farmers differentiate between landraces.

The acid profiles of both non-sweet and sweet melons vary, with non-sweet major acid being malic and sweet melon being citric. The acid profile of melons is determined by the *CmPH* (Cohen et al., 2014), a duplication of 4 amino acids between distinguishes between acidic varieties (no duplication) and modern dessert melons (duplication present). Also, important to note that as melons mature their acid profile varies, with malic acid being more prevalent at immature stages, with a reduction in its concentration as the fruit matures, while citric acid increases as the fruit matures. This helps to explain why the profile of the non-sweet Flexuosus-Chate melons, collected at a commercial maturity (immature) differs from that of sweet Ibericus melons (collected when mature). The difference observed among the Ibericus melons of acidity, allowing for a higher diversity for breeding programs. The VOCs analysis revealed the great diversity among traditional landraces. Non-sweet Flexuosus and Chate presented high content of aldehydes, followed by alcohols, with Chate presenting a richer VOC profile than Flexusous, although the main aldehydes being (E,Z)-2-6-nonadienal, E-2-nonenal, hexanal, and benzaldehyde. This profile of is of high aldehydes followed by alcohols was also observed in other Flexuosus melons (Tang et al., 2015; Chen et al., 2016). As for Ibericus melons, previous works have been performed analysing these melons and found differences between landraces of the Ibericus Group (Esteras et al., 2018). Ibericus melon VOCs generally displayed low levels of esters, with higher content of alcohols, followed by aldehydes and varied content of esters. There exists a great level of variation between landraces of the same Subgroup, with some presenting higher content of esters or lower aldehyde content.

Overall, the landraces studied suggest they all present common genetic backgrounds, with subtle differences between them, and although classified into different Subgroups, high variability between them can still be observed.

Effect of grafting and salinity on Traditional Spanish melon landraces under organic farming conditions: effects on agronomic performance and morphological traits.

Chapter 3 and 4 both study how grafting affect both the agronomic performance and morphological traits of snake melon (chapter 2) and Ibericus melons (chapter 3). The study also observes the limiting factors affecting these traditional landraces, both abiotic (salinity) and biotic (pests and diseases). Studies based on how melon cultivars respond to organic farming have been performed in Spain (Gragera-Facundo et al., 2012; Sánchez-Giráldez et al., 2012) and the effect of grafting melon under organic farming conditions (Guan et al., 2014). Fungal and viral diseases as well as salinity play a huge role in limiting vegetable crop productions (Ahuja et al., 2010; Desbiez et al., 2020; El-Baky and Amara, 2021).

The increase and intensification of cucurbit production has led to the emergence of several viral diseases that threaten the sustainability of these crops (Radouane et al., 2021). In Spain the main viruses affecting Cucurbits according to a recent review (Radouane et al., 2021) are *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), *Papaya ring spot virus* (PRSV), *Cucumber mosaic virus* (CMV), *Cucumber aphid-borne yellows virus* (CABYV), *Melon necrotic spot virus* (MNSV), *Tomato leaf curl New Delhi virus* (ToLCNDV), and *Cucumber vein yellowing virus* (CVYV). Our results detected most of these viruses affecting both snake melons and sweet Ibericus melons, especially WMV and ToLCNDV. The main method to control viral infections in organic farming is to control the viral vector, which are mainly aphids or white-flies. These include the use of entomopathogenic fungi or viruses, traps, nets, predators and parasitoids (Mani, 2022), although the use of resistant cultivars alongside these method is preferable (Messelink et al., 2020). Resistance to these viruses have been detected, mainly in the more exotic melon Groups (Martín-Hernández and Picó, 2020), but breeding has already been performed to obtain resistant breeding line from a Ibericus genetic background (Palomares-Rius et al., 2018) but not into the snake melon genetic background.

As temperatures increase due to global warming, so will increase the incidence of potential soilborne pathogens (de Sousa Linhares et al., 2020; Delgado-Baquerizo et al., 2020). Grafting is an important strategy in managing soilborne plant pathogens, with scions grafted onto disease-resistant rootstocks (Panth et al., 2020). The main pathogens where *Fusarium* spp., *M. phaseolina* and *N. falciformis*, and although also affecting grafted plants, non-grafted where particularly affected by them. Resistance to *Fusarium* spp. have been found in Ibericus and snake melons, although this resistance depends of the *Fusarium* race (Alvarez et al., 2005; Solmaz et al., 2016b). As for *M. phaseolina*, Ibericus and Flexusosu are usually considered as susceptible (Ambrósio et al., 2015; Cohen et al., 2022). Increased temperature and salinity have been observed to affect resistance to soilborne pathogens (Roustaee et al., 2011; Mirtalebi and Banihashemi, 2019; Castro et al., 2020), although this should be further studied. Nevertheless, the use of these rootstocks, especially *Cucumis* hybrids, appear to reduce mortality due to soilborne pathogens. It is also important to note the presence of new soilborne pathogens like *N. falciformis* and *N. keroplastica*, for which resistances must be studied among the different resistant rootstock available.

The impact of grafting and salinity on fruit quality and agronomic performance has been thoroughly studied (Del Amor et al., 1999; Trionfetti Nisini et al., 2002; Edelstein et al., 2005; Crinò et al., 2007; Colla et al., 2006; Schultheis et al., 2015; Visconti et al., 2019). Our results revealed that the presence of soilborne pathogens in the soil reduced production to the same level as non-grafted plants, but when they have little to no presence, grafted plant productivity was higher than non-grafted. The effect of grafting on the snake melons was minimal (mainly associated to colour change), with the differences mainly observed between fields, with an increase in SSC, variation in flesh firmness and fruit colour mainly due to salinity. This increase in SSC was observed in non-grafted and grafted onto *Cucumis* plants two years in a row. As for the sweet Ibericus melons each grafting (both *Cucurbita* and *Cucumis*) resulted in a variation of both fruit flesh and colour something that has already been noted in other works (Cáceres et al., 2017). *Cucumis* hybrid rootstock F1Pat8, as with snake melons, had a positive impact on the SSC fruit content, resulting in higher SSC in grafted melons compared to non-grafted plants. Salinity also affected some of the fruit quality aspects, mainly by increasing SSC, reducing flesh firmness and also changing colour. Reduction of fruit size due to salinity has also been noted to happen (Del Amor et al., 1999; Tedeschi et al., 2011; Akrami and

Arzani, 2019). Botía et al., (2005) highlight how different cultivars respond to salinity, with some common responses (higher SSC, peel thickness) but that the effect on flesh firmness varied between "Amarillo Oro" and "Galia". Another important aspect is the intensity of salination, as Tedeschi et al., (2011) found differences for the total yield for the two highest salination levels with respect to the control, SSC was only different between the control and most extreme salination level, but no differences to flesh firmness regardless of salinity.

Effect of grafting and salinity Traditional Spanish melon landraces under organic farming conditions: effects on metabolic profile and consumer perception

Chapter 3 and 5 both evaluate the effect of grafting on consumer perception, acid, sugars and VOC profile of both snake melon and Ibericus melons. Sensorial evaluations of both snake melons and Ibericus melons have previously been performed (Pardo et al., 2000; Escribano and Lázaro, 2012; Omari et al., 2018) and a tasting method for sweet melons has been established (Escribano et al., 2010). Grafting can result in fruits with fibrous texture, off tastes (Davis et al., 2008a; Rouphael et al., 2010). As for salinity, it too can have an impact on the fruit quality, as it can enhance flavour, aroma and fruit quality parameters of many fruit vegetables (Rouphael et al., 2018b).

Our study evaluated the effect of grafting on the taste of a snake melon landrace from Spain. Omari et al., (2018) performed tastes of 47 traditional snake melon landraces of Israel and Palestine, find that taster preferred crispy texture without any hollows (space between the between pericarp and placenta). Ahlawat et al., (2018) also performed a tasting "Arya" melon (Flexuosus Group), with a 100% consumer acceptability when used as a fresh vegetable for salads. The results of our study showed that panellist prefer non-grafted plants, with *Cucurbita* grafted plants often scoring in the panels, sometimes with reports of strange tastes , while *Cucumis* rootstocks did not display differences with respect to the non-grafted melon. Traka-Mavrona et al., (2000) found that the fruit taste and texture of *Cucurbita* grafted melons was worse than non-grafted, and that this was especially noteworthy under greenhouse conditions.

Sugars, organic acids and volatile organic compounds are the main components responsible for taste (Burger et al., 2003). The results for the snake melon showed that grafting did have an effect on the sugars and organic acid profiles, with *Cucurbita* rootstock decreasing the malic acid and sugar content, with *Cucumis* rootstocks only

decreasing the glucose content, with these only being observed under the saline conditions of Carrizales. The results obtained for the Ibericus grafted melons display that grafting did not alter the acid profile (malic, citric and glutamic acid), with none of the rootstocks affecting its content. Salinity also did not alter the malic or citric acid content, but the fruits from Carrizales displayed lower glutamic acid content. As for the sugar content, non-grafted plants presented lower sucrose content and glucose content than Cucumis grafted onto Fian and F1Pat81 respectively. As for salinity, fruits from Carrizales presented lower fructose and glucose but higher sucrose than La Punta fruits. Colla et al., (2006) found that the acidity (% of citric acid) increased with salinity while grafting reduced it in "Cyrano" melons. More recently, Kaleem et al., (2022) found that grafted "Yuniang" onto *Cucurbita* rootstocks had no effect on the malic acid content, but that it rootstocks "14F17" and "Sizhuang No.12" displayed higher citric acid tan non-grafted plant fruits , while "Tianzhen No.1" had significantly lower citric acid content tan the non-grafted plant fruits. In terms of the sugar content, Kaleem et al., (2022) found that also found that grafting affected the sugar profile of "Yuniang", with "Tianzhen No.1" presenting significantly higher fructose content, while rootstocks "Bijuu", "Yinguang" and "Tianzhen No.1" had ≈50mg/g more sucrose content.

As previously stated, volatile organic compounds are one of the main components responsible for taste. Snake melons are a climacteric fruit, so they are aromatic when they are fully mature (Esteras et al., 2018). In our case, the snake melons studied, where collected at an immature state (commercial), hence their VOC profile is quite different, mainly characterized by high aldehyde, followed by alcohols and low ester content, as has been previously characterized (Tang et al., 2015; Chen et al., 2016). Our results revealed that grafting clearly resulted in a higher content in volatiles in grafted melons than in non-grafted. Particularly, *Cucumis* grafted plants had a higher of aldehydes, alcohols and esters in both fields, but under saline conditions *Cucurbita* grafted plants also presented those higher volatile contents. Cucumis grafted plants had a higher content of certain aldehydes ((E,Z)-2-6-nonadienal, E-2-nonenal and benzaldehyde), alcohols (2-phenylethanol(Z)-3-hexen-1-ol, 1-nonanol) and esters (ethyl butanoate). Our results for the grafted Ibericus melons, revealed that there was no generalized effect of grafting on the VOC profile, but rather specific scion-rootstock combinations had different effects. Both Piel de Sapo did not exhibit overall total volatile content differences, although 03PS grafted onto F1Pat81 and Fian had a higher content of esters (butyl butyrate and Heptyl

acetate), and phenol, with Shintoza and Fimy having similar profile to non-grafted plant fruits. Grafting altered the VOC profile of Blanco fruits, with 29BL grafted plants presenting higher aldehyde content, and 32BL some specific combination effects such as higher ester content with Fian or higher Geranylacetone with F1Pat81. Rochet 02RC had grafted plant fruits and non-grafted presenting similar profiles, although all Cucumis rootstock increased the content of (Z)-3-Nonen-1-ol. Finally, 22AM-GO *Cucurbita* grafted plant fruits, with higher ester content and lower aldehyde content. Cucumis rootstocks have little to no effect on the VOC profile, although Fimy did reduce the content of specific aldehydes (nonanal and (Z)-6-nonenal). Condurso et al., (2012) found that grafting onto *Cucurbita* reduced the content of key aroma esters (ethyl 2-methylbutanoate and ethyl butanoate) of "Proteo" while Sting *C. melo* rootstock did not show this take place. More recently, Lecholocholo et al., (2022) found that grafting cantaloupe "Majestic" and "Hunter" onto *Cucurbita* hybrids rootstocks "Carnivor" and "Kickstart" resulted in higher intensity VOC. Furthermore, "Carnivor" rootstock grated plant fruits presented a much higher VOC profile than "Kickstarter". Lecholocholo et al., (2022) also was able to find that honeydew melons "Honeygoal" and "Honeyval" had different VOC profile than grafted plants fruits. Grafting "Honeygoal" and "Honeyval" onto "Carnivor" resulted in higher content of 4,5 dimethyl-1-hexene, methyl acetate, ethyl hexanoate than "Kickstarter".

Conclusions

7. Conclusions

1) The GBS performed has permitted the study of a new genomic region. The Population Structure Analysis differentiated between the exotic and sweet Ibericus melons, with the Flexuosus, Chate and Ameri in the middle. The phylogeny differentiated Flexuosus and Ibericus clades, with no clear distinction between landraces based upon their subgroups.

2) The morphological and metabolic characterization distinguished between both non-sweet and sweet melons. External colour appeared as the most important trait to differentiate between landraces, although other factor such as SSC, weight, L/D ratio and flesh firmness are also important. Non-sweet melons were characterized for a high malic acid content, little to no citric acid and no sucrose content, while sweet presented high citric acid, low malic and high sugar content. The VOC profile of non-sweet melons presented high aldehyde and alcohols, with low ester content. Ibericus melons presented a range of VOC profiles. This can help cater to specific consumers preferences.

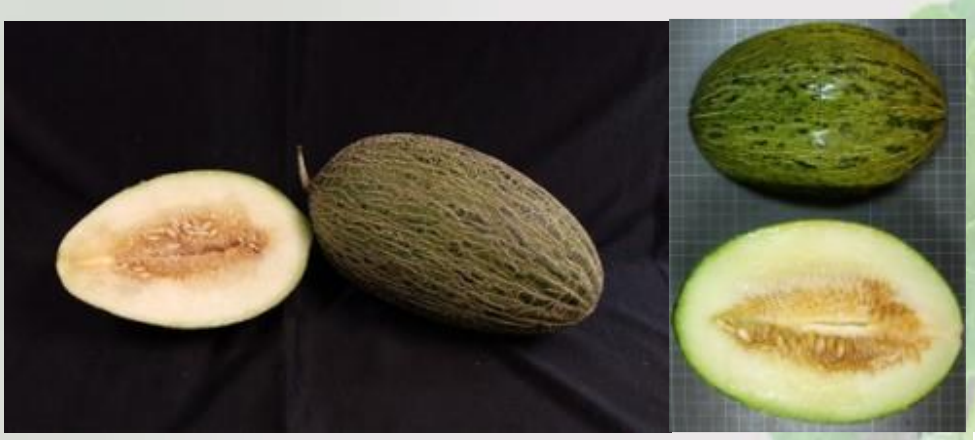
3) In Valencia, the main viral pathogens limiting melon production is WMV, followed by *ToLCNDV*. Salinity has a synergic effect in combination with soilborne pathogens, in limiting yields. These factors must be taken into consideration when selecting future rootstocks, as well as the appearance of new soilborne pathogens such as *N. keratoplastica* and *N. falciformis*.

4) Grafting has little effect on snake melons, although some quality characteristics improved with *Cucumis* rootstocks. Grafting on *Cucurbita* negatively influenced consumer perception of snake melon fruits. Grafting changed the metabolite profile of snake melons, with *Cucurbita* highly influencing acid and sugar profile. *Cucumis* rootstocks altered the VOC profile of snake melons, increasing the content of aldehydes, alcohols and esters. *Cucurbita* rootstocks only had this effect under saline conditions. *Cucumis* rootstocks appear to better fruit quality traits, while minimizing the negative side effects of grafting, compared to commercial *Cucurbita*.

5) Sweet Ibericus melons where highly influenced by salinity, with increased SSC, while grafting affected agronomic and fruit quality parameters. The metabolite profile was not affected by grafting but rather by both salinity and individual landrace. *Cucumis* rootstocks represent an alternative to classic *Cucurbita* rootstock due to their limited effect on the VOCs, acid and sugar profile in sweet melons. High influence of rootstock-scion has been observed, implying the necessity to determine their ideal rootstock for

each population, as the response may differ between difference landraces of the same Subgroup.

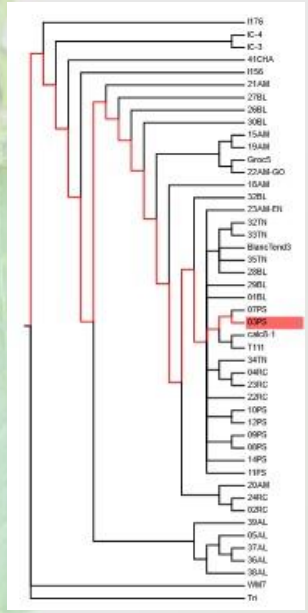
Annex



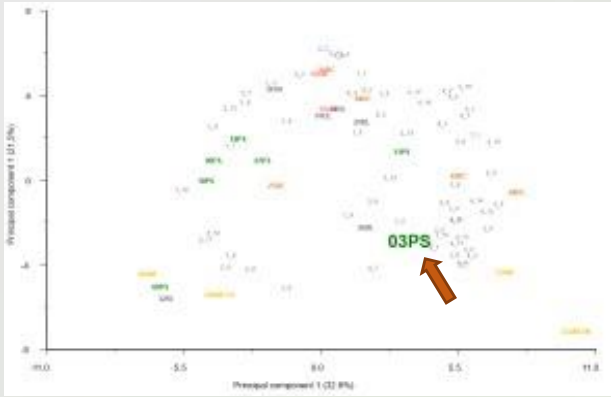
03PS

Localidad de Origen: Moncófar (Castellón)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV 016356

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 2.6 ± 0.23
Anchura (cm) : 15.3 ± 0.4
Longitud (cm) : 23.9 ± 1.18
Firmeza (kg/cm2): 2.2 ± 0.21

Forma de fruto : elipsoidal
Color de la Corteza: Verde
Color de la carne: crema
Escriturado: presente

Características Nutricionales del Fruto

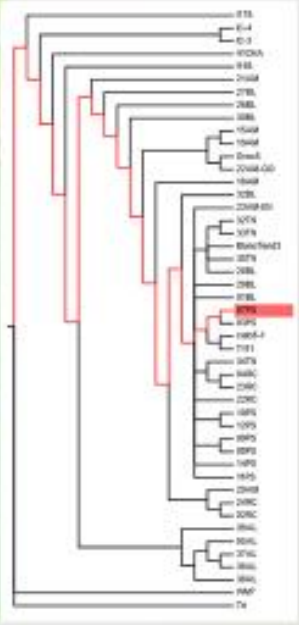
º Brix: 10.8 ± 0.31
pH: 5.6 ± 0.3
Ácido Málico (g/kg): 0.53±0.1
Ácido Cítrico (g/kg): 5.1±0.1

Fructosa (g/kg) : 19.04±0.97
Glucosa (g/kg): 16.29±0.9
Sucrosa (g/kg): 54.71±2.5
Equiv. Sucrosa (g/kg): 99.7±0.92

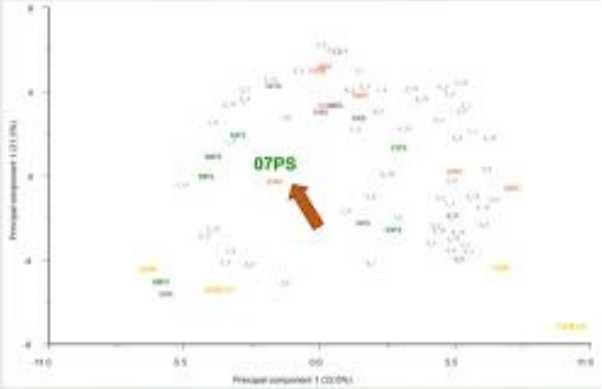
07PS

Localidad de Origen: Villalonga (Valencia)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV016385

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 2.08 ± 0.49
Anchura (cm): 13.35 ± 1.24
Longitud (cm): 24±1.89
Firmeza (kg/cm2): 3±0.2

Forma de fruto: elipsoidal
Color de la Corteza: Verde
Color de la carne: crema-verde
Escriturado: presente

Características Nutricionales del Fruto

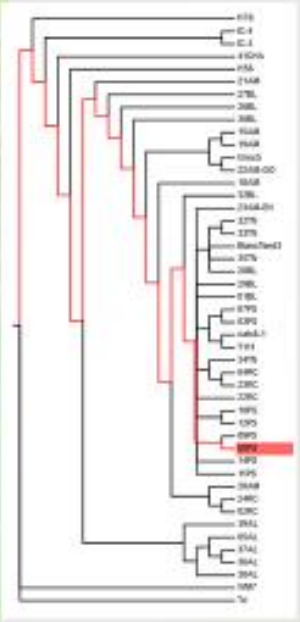
º Brix: 11.88 ± 0.06
pH: 5.5 ± 0
Ácido Málico (g/kg): 0.14 ± 0.03
Ácido Cítrico (g/kg): 4.17 ± 0.59

Fructosa (g/kg): 20.27 ± 2.98
Glucosa (g/kg): 19.83 ± 2.16
Sucrosa (g/kg): 57.48 ± 18.17
Equiv. Sucrosa (g/kg): 107.22 = 12.2

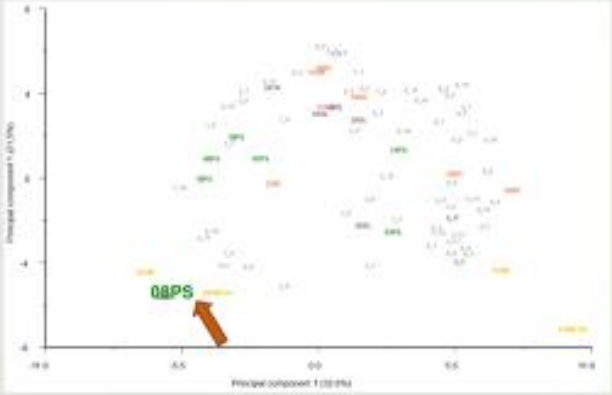
08PS

Localidad de Origen: Vilafranca de Bonany (Baleares)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV001417

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 2.1 ± 0.21	Forma de fruto: elipsoidal
Anchura (cm): 14.2 ± 0.49	Color de la Corteza: Verde
Longitud (cm): 19.62 ± 3.65	Color de la carne : crema
Firmeza (kg/cm2): 2.4 ± 0.17	Escriturado: muy leve

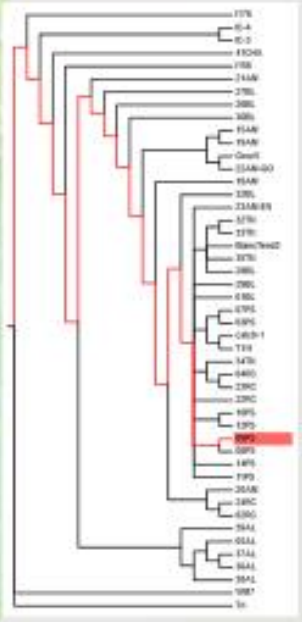
Características Nutricionales del Fruto

º Brix : 10.9 ± 0.15	Fructosa (g/kg) : 17.29 ± 0.7
pH: 5.67 ± 0.11	Glucosa (g/kg): 16.17 ± 0.65
Ácido Málico (g/kg): 0.07 ± 0	Sucrosa (g/kg): 49 ± 3.49
Ácido Cítrico (g/kg): 4.48 ± 0.27	Equiv. Sucrosa (g/kg): 90.89±2.81

09PS

Localidad de Origen: Ademuz (Valencia)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004891

Filogenia



Perfil Aroma

Descripción del Fruto

Peso (kg): 2.22 ± 0.16	Forma de fruto : elipsoidal
Anchura (cm): 14.76 ± 0.52	Color de la Corteza : Verde
Longitud (cm): 21.6 ± 0.67	Color de la carne : crema
Firmeza (kg/cm2): 2.4 ± 0.24	Escriturado: no

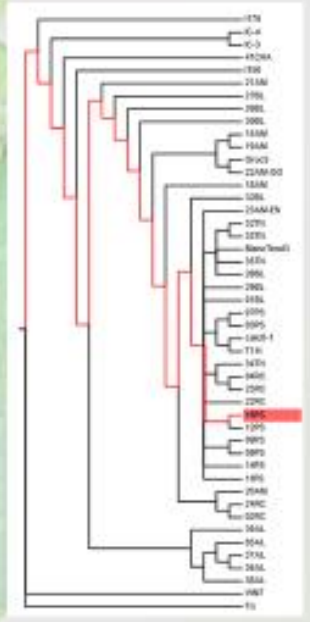
Características Nutricionales del Fruto

º Brix: 12.04 ± 0.57	Fructosa (g/kg) : 16.8 ± 0.69
pH: 5.2 ± 0.12	Glucosa (g/kg): 16.44 ± 1
Ácido Málico (g/kg): 0.09 ± 0.01	Sucrosa (g/kg): 49.99 ± 9
Ácido Cítrico (g/kg): 4.83 ± 0.66	Equiv. Sucrosa (g/kg): 91.23±10.62

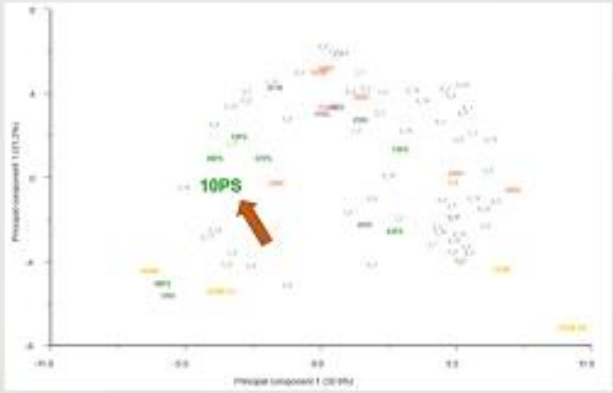
10PS

Localidad de Origen: Argelita (Castellón)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004914

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 1.78 ± 0.13	Forma de fruto: elipsoidal
Anchura (cm): 13.08 ± 0.25	Color de la Corteza: Verde
Longitud (cm): 21.4 ± 0.99	Color de la carne: crema-verde
Firmeza (kg/cm2): 2.65 ± 0.27	Escriturado: no

Características Nutricionales del Fruto

º Brix: 12.14 ± 0.57	Fructosa (g/kg): 15.62±0.51
pH: 5.2 ± 0.12	Glucosa (g/kg): 14.54±0.92
Ácido Málico (g/kg): 0.09 ± 0.01	Sucrosa (g/kg): 64.28±4.16
Ácido Cítrico (g/kg): 6.59 ± 0.36	Equiv. Sucrosa (g/kg): 102.07±4.27

11PS

Localidad de Origen: Membrilla (Ciudad Real)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV013188

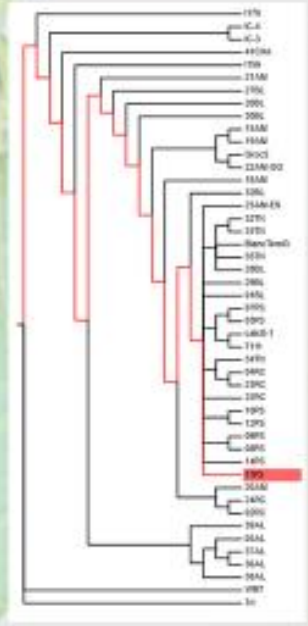
Descripción del Fruto

Peso (kg): 2.79 ± 0.17
Anchura (cm): 14.4 ± 0.27
Longitud (cm): 26.92 ± 1.19
Firmeza (kg/cm2): 1.7 ± 0.22
Forma de fruto: elipsoidal
Color de la Corteza: Verde
Color de la carne: crema-verde
Escriturado: no

Características Nutricionales del Fruto

° Brix: 13.44 ± 0.23
pH: 6 ± 0.22
Ácido Málico (g/kg): 0.3 ± 0.21
Ácido Cítrico (g/kg): 4.61 ± 0.43
Fructosa (g/kg): 17.3±0.66
Glucosa (g/kg): 16.42±0.52
Sucrosa (g/kg): 77.91±8.29
Equiv. Sucrosa (g/kg): 119.99±6.78

Filogenia



Perfil Aroma

12PS

Localidad de Origen: Alborea (Albacete)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV003686

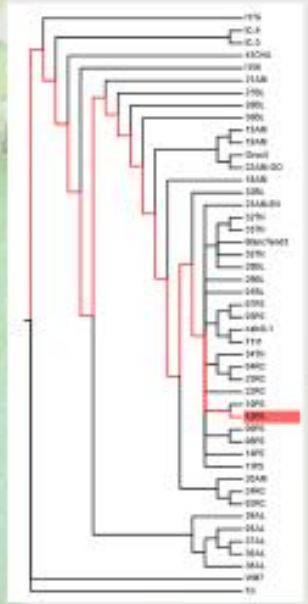
Descripción del Fruto

Peso (kg): 2.04 ±0.196
Anchura (cm): 13.68 ± 0.49
Longitud (cm): 22.38 ± 0.85
Firmeza (kg/cm2): 3.3 ± 0.34
Forma de fruto: elipsoidal
Color de la Corteza: Verde
Color de la carne: crema-verde
Escriturado: no

Características Nutricionales del Fruto

° Brix: 11.98 ± 0.68
pH: 5.3 ± 0.12
Ácido Málico (g/kg): 0.11 ± 0.02
Ácido Cítrico (g/kg): 5.62 ± 0.6
Fructosa (g/kg): 17.12 ± 1.01
Glucosa (g/kg): 15.76 ± 1.22
Sucrosa (g/kg): 62.83 ± 6.78
Equiv. Sucrosa (g/kg): 104.11 ± 4.3

Filogenia

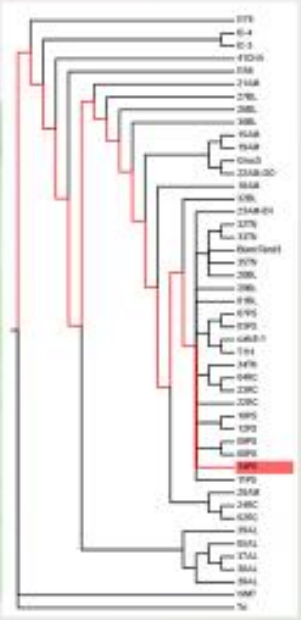


Perfil Aroma

14PS

Localidad de Origen: Cartagena (Murcia)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV012815

Filogenia



Descripción del Fruto

Peso (kg): 2.30 ± 0.47	Forma de fruto: elipsoidal
Anchura (cm): 13.8 ± 0.4	Color de la Corteza: Verde
Longitud (cm): 25.9 ± 3.33	Color de la carne: crema-verde
Firmeza (kg/cm2): 3.08 ± 0.08	Escriturado: no

Características Nutricionales del Fruto

º Brix: 11.4 ± 0.95
pH: 5.17 ± 0.17

15AM

Localidad de Origen: Torredonjimeno (Jaén)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV010747

Descripción del Fruto

Peso (kg): 1.91 ± 0.158
Anchura (cm): 13.72 ± 0.44
Longitud (cm): 23.62 ± 0.43
Firmeza (kg/cm2): 2.67±0.28

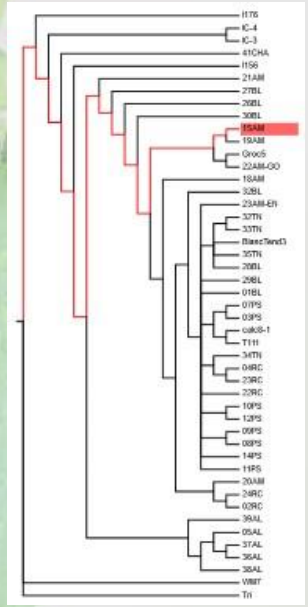
Forma de fruto: elipsoidal
Color de la Corteza: amarillo
Color de la carne: crema
Escriturado: no

Características Nutricionales del Fruto

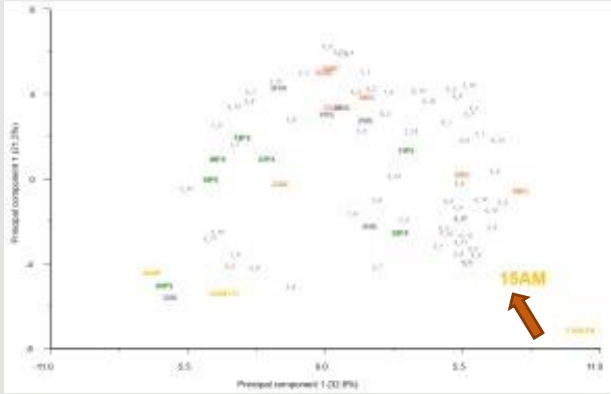
º Brix: 11.23 ± 0.33
pH: 5.17 ± 0.11
Ácido Málico (g/kg): 0.57 ± 0.18
Ácido Cítrico (g/kg): 4.75 ± 0.28

Fructosa (g/kg): 17.2 ± 0.93
Glucosa (g/kg): 14.3±1.11
Sucrosa (g/kg): 51.62±1.61
Equiv. Sucrosa (g/kg): 91.96±0.9

Filogenia



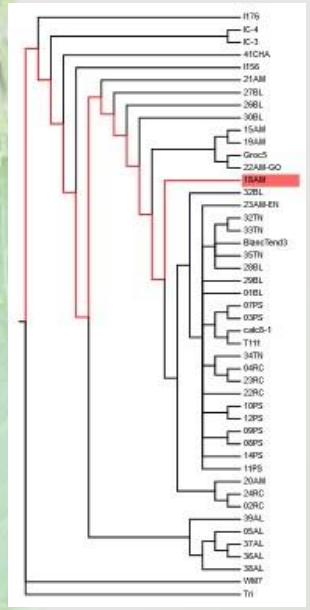
Perfil Aroma



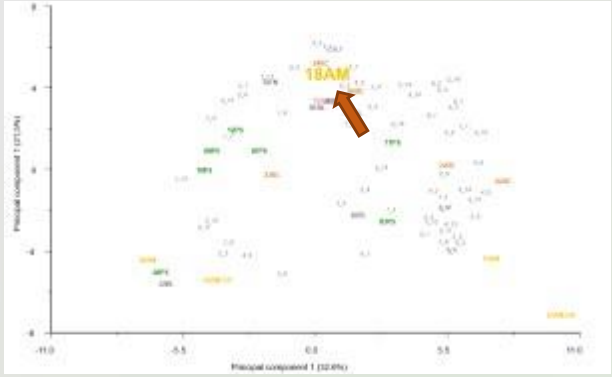
18AM

Localidad de Origen: Benissa (Alicante)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004869

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 2.03 ± 0.05
Anchura (cm): 13.75 ± 0.2
Longitud (cm): 22.92 ± 0.43
Firmeza (kg/cm2): 2.21 ± 0.31
Forma de fruto: elipsoidal
Color de la Corteza: Amarilla
Color de la carne: crema-verde
Escriturado: no

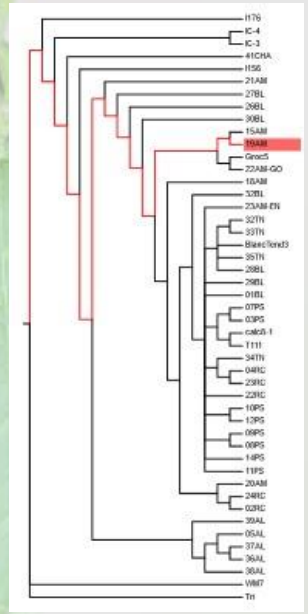
Características Nutricionales del Fruto

º Brix : 11.52±0.57
pH: 5.75 ± 0.28
Ácido Málico (g/kg): 0.17 ± 0.03
Ácido Cítrico (g/kg): 4.35 ± 0.19
Fructosa (g/kg): 16.1 ± 0.19
Glucosa (g/kg): 15.96 ± 0.32
Sucrosa (g/kg): 63.31 ± 11.49
Equiv. Sucrosa (g/kg): 102.98 ± 11.67

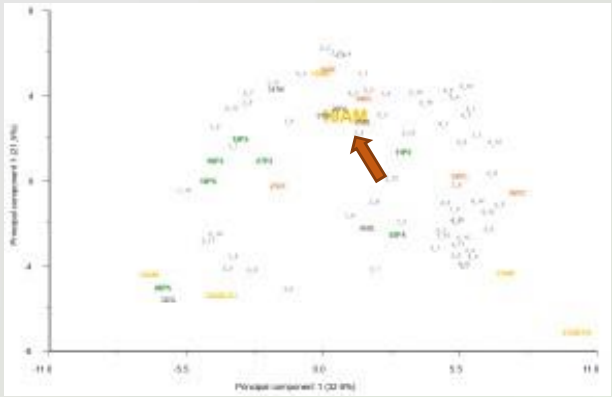
19AM

Localidad de Origen: Torre-Pacheco (Murcia)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004271

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 1.92 ±0.01
Anchura (cm): 13.62 ± 0.26
Longitud (cm): 22.5 ± 0.71
Firmeza (kg/cm2): 2.9 ± 0.2

Forma de fruto: elipsoidal
Color de la Corteza: Amarilla
Color de la carne: crema-verde
Escriturado: no

Características Nutricionales del Fruto

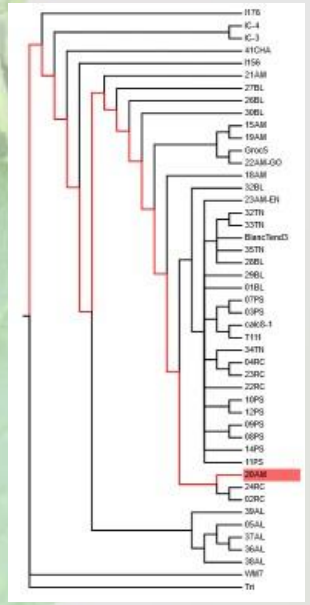
º Brix: 11.3 ± 0.3
pH: 5.4 ± 0.1
Ácido Málico (g/kg): 0.16 ± 0.01
Ácido Cítrico (g/kg): 4.38 ± 0.36

Fructosa (g/kg): 16.62 ± 0.37
Glucosa (g/kg): 14.04 ± 0.62
Sucrosa (g/kg): 49.98 ± 3.63
Equiv. Sucrosa (g/kg): 89.13±3.82

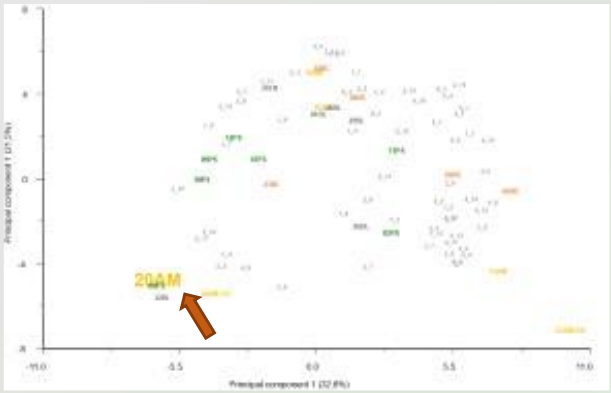
20AM

Localidad de Origen: Massamagrell (Valencia)
Colección : Banco de Germoplasma UPV
Código de Banco: BGV004937

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 1.84 ± 0.24
Anchura (cm) : 14.83 ± 0.63
Longitud (cm) : 16.9 ± 0.68
Firmeza (kg/cm2): 2.71 ± 0.15
Forma de fruto: redondeado
Color de la Corteza : Amarilla
Color de la carne: crema
Escriturado : no

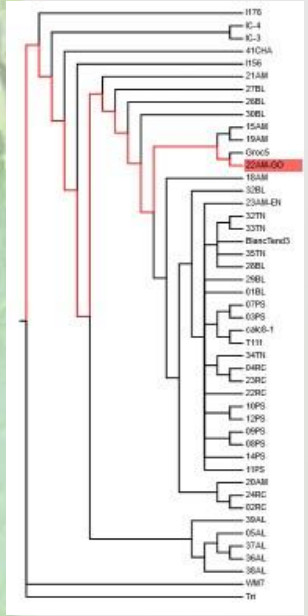
Características Nutricionales del Fruto

º Brix : 11.4 ± 0.36
pH: 5.71 ± 0.1
Ácido Málico (g/kg): 0.11 ± 0.01
Ácido Cítrico (g/kg): 4.17 ± 0.37
Fructosa (g/kg): 18.19 ± 2.16
Glucosa (g/kg): 16.04 ± 1.48
Sucrosa (g/kg): 52.92 ± 4.28
Equiv. Sucrosa (g/kg): 96.27±6.22

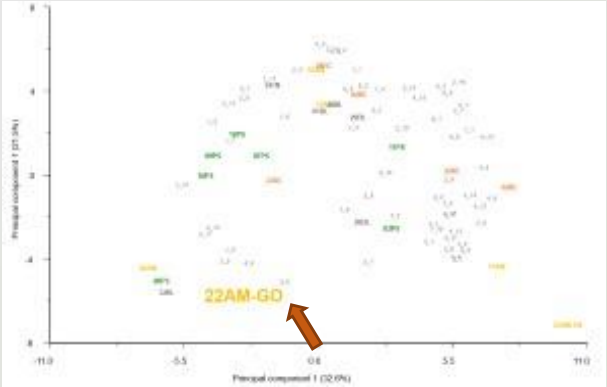
22AM-GO

Localidad de Origen: Ontinyent (Valencia)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV016451

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 2.55 ± 0.44
Anchura (cm): 15.85 ± 0.99
Longitud (cm): 22.48 ± 1.15
Firmeza (kg/cm2): 2.71 ± 0.15
Forma de fruto: ovalado
Color de la Corteza: Amarilla
Color de la carne: crema-verde
Escriturado: no

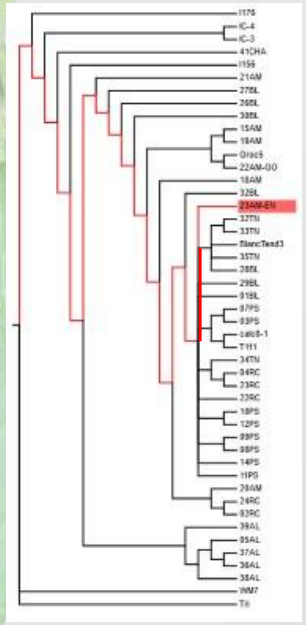
Características Nutricionales del Fruto

º Brix: 12.23 ± 0.32
pH: 5.5 ± 0.2
Ácido Málico (g/kg): 0.36 ± 0.18
Ácido Cítrico (g/kg): 4.56 ± 0.69
Fructosa (g/kg): 19.43 ± 2.33
Glucosa (g/kg): 15.81 ± 2.6
Sucrosa (g/kg): 55.41±4.61
Equiv. Sucrosa (g/kg): 100.72±8.26

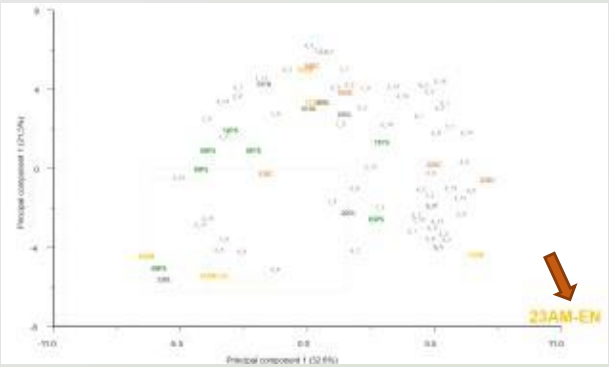
23AM-EN

Localidad de Origen: Alzira (Valencia)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV016452

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 2.77 ± 0.45
Anchura (cm): 16.24 ± 0.66
Longitud (cm): 26.08 ± 1.17
Firmeza (kg/cm2): 1.95 ± 0.25
Forma de fruto: elipsoidal
Color de la Corteza: Amarilla
Color de la carne: crema-verde
Escriturado: no

Características Nutricionales del Fruto

º Brix: 11.46 ± 0.53
pH: 6.4 ± 0.24
Ácido Málico (g/kg): 1.52 ± 0.43
Ácido Cítrico (g/kg): 2.81 ± 0.69
Fructosa (g/kg): 19.03±1.83
Glucosa (g/kg): 17.77±1.99
Sucrosa (g/kg): 53.73±1.24
Equiv. Sucrosa (g/kg): 99.79±4.08

01BL

Localidad de Origen: Montalvos (Albacete)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV003692

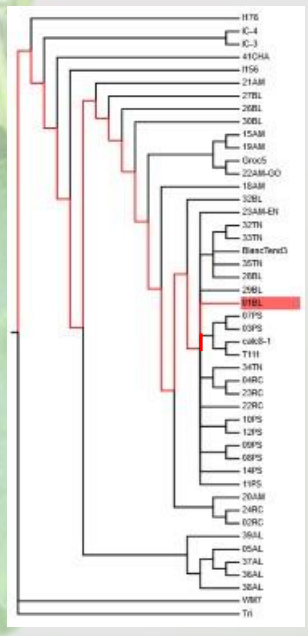
Descripción del Fruto

Peso (kg): 1.67 ± 0.09	Forma de fruto: redondeado
Anchura (cm): 14 ± 0.41	Color de la Corteza: Blanca
Longitud (cm): 16.62 ± 0.18	Color de la carne: crema-verde
Firmeza (kg/cm2): 2.25 ± 0.16	Escriturado: no

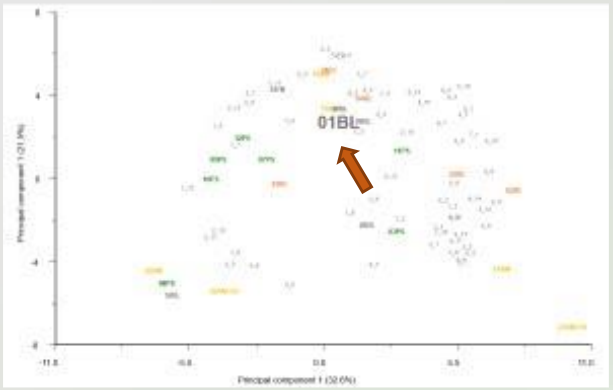
Características Nutricionales del Fruto

º Brix: 12.76 ± 0.64	Fructosa (g/kg): 16.66 ± 0.82
pH: 5.1 ± 0.1	Glucosa (g/kg): 15.76 ± 0.87
Ácido Málico (g/kg): 0.15 ± 0.02	Sucrosa (g/kg): 67.22 ± 4.45
Ácido Cítrico (g/kg): 5.87 ± 0.19	Equiv. Sucrosa (g/kg): 107.7 ± 6.46

Filogenia



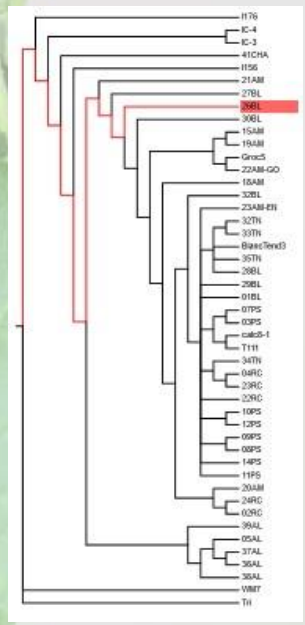
Perfil Aroma



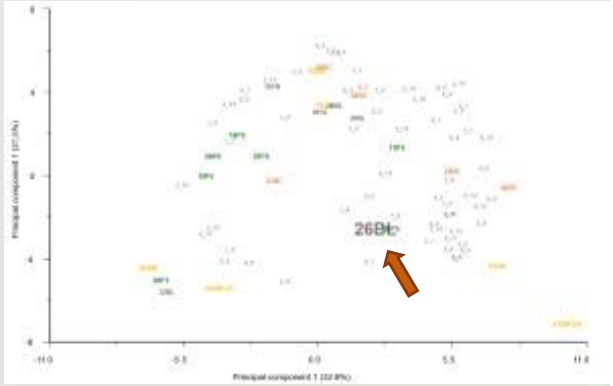
26BL

Localidad de Origen: Canena (Jaén)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV000444

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 1.26 ±0.11
Anchura (cm): 12.05 ± 0.36
Longitud (cm): 17.33 ± 0.75
Firmeza (kg/cm2): 2.25 ± 0.16
Forma de fruto: elipsoidal
Color de la Corteza: Blanca
Color de la carne: crema
Escriturado: no

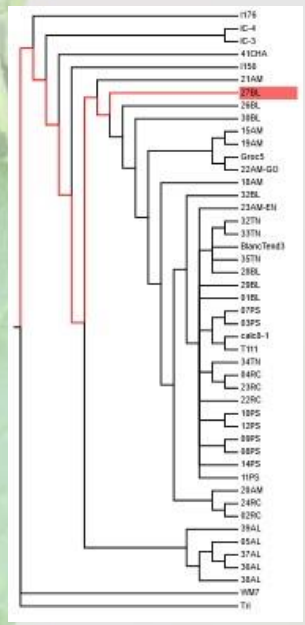
Características Nutricionales del Fruto

º Brix: 11.25 ± 0.62
pH: 5.5 ± 0.2
Ácido Málico (g/kg): 0.26 ± 0.14
Ácido Cítrico (g/kg): 4.38 ± 0.48
Fructosa (g/kg): 21.16 ± 2.45
Glucosa (g/kg): 19.36 ± 3.25
Sucrosa (g/kg): 51.29 ± 13.79
Equiv. Sucrosa (g/kg): 102.22 ± 7.32

27BL

Localidad de Origen: Piedrabuena (Ciudad Real)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV013194

Filogenia



Descripción del Fruto

Peso (kg): 2.67 ± 0.51	Forma de fruto: alargado
Anchura (cm): 15.32 ± 0.87	Color de la Corteza: Blanca
Longitud (cm): 28.54 ± 2.8	Color de la carne: crema
Firmeza (kg/cm2): 3.01 ± 0.18	Escriturado: no

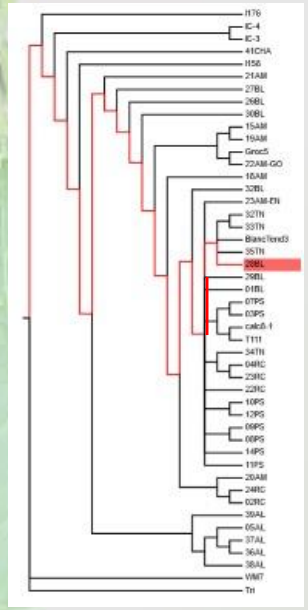
Características Nutricionales del Fruto

º Brix: 10.04 ± 0.12	Fructosa (g/kg): 17.93 ± 1.72
pH: 5.1 ± 0.1	Glucosa (g/kg): 18.64 ± 1.82
Ácido Málico (g/kg): 0.1 ± 0.01	Sucrosa (g/kg): 35.54 ± 4.29
Ácido Cítrico (g/kg): 3.27 ± 0.05	Equiv. Sucrosa (g/kg): 80.36±3.09

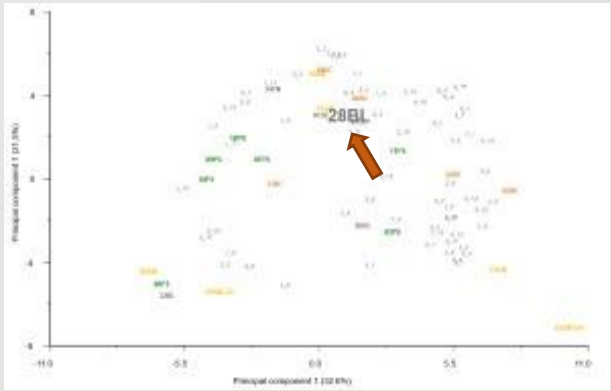
28BL

Localidad de Origen: Benlloch (Castellón)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV014212

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 1.97 ±0.06	Forma de fruto: elipsoidal
Anchura (cm): 14.2 ± 0.21	Color de la Corteza: Blanca
Longitud (cm): 20.81 ± 0.42	Color de la carne: crema
Firmeza (kg/cm2): 2.42 ± 0.28	Escriturado: no

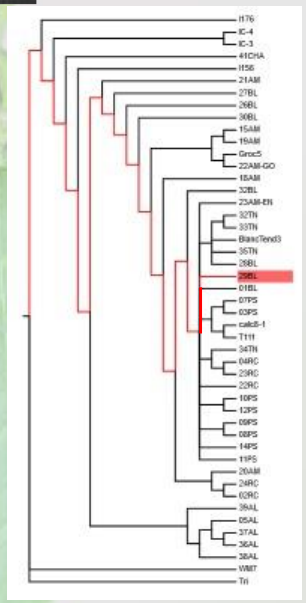
Características Nutricionales del Fruto

º Brix: 12.93 ± 0.7	Fructosa (g/kg): 18.29 ± 1.95
pH: 6 ± 0.19	Glucosa (g/kg): 17.57 ± 2.5
Ácido Málico (g/kg): 0.07 ± 0.01	Sucrosa (g/kg): 46.2 ± 9.96
Ácido Cítrico (g/kg): 4.12 ± 0.06	Equiv. Sucrosa (g/kg): 90.85 ± 7.94

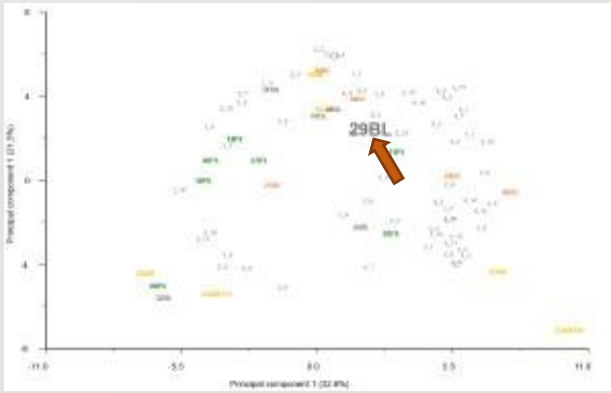
29BL

Localidad de Origen: Carcaixent (Valencia)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV014212

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 2.56 ± 0.22	Forma de fruto : redondo
Anchura (cm): 18.85 ± 0.74	Color de la Corteza : Blanca
Longitud (cm): 16.68 ± 0.55	Color de la carne : crema-verde
Firmeza (kg/cm2): 2.69 ± 0.45	Escriturado: no

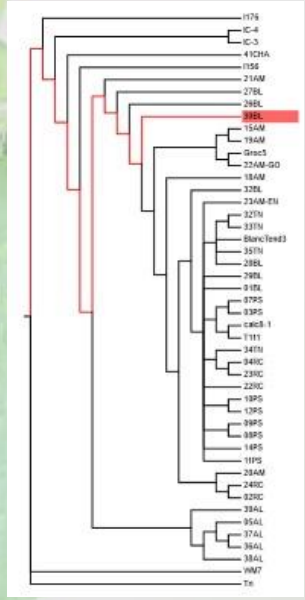
Características Nutricionales del Fruto

º Brix : 12.2 ± 0.86	Fructosa (g/kg) : 16.05 ± 0.58
pH: 5.75 ± 0.32	Glucosa (g/kg): 16.09 ± 0.84
Ácido Málico (g/kg): 0.09 ± 0.02	Sucrosa (g/kg): 67.37 ± 9.71
Ácido Cítrico (g/kg): 4.25 ± 0.19	Equiv. Sucrosa (g/kg): 107.05±11.28

30BL

Localidad de Origen: Rota (Cádiz)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV000424

Filogenia



Descripción del Fruto

Peso (kg): 2.82 ± 0.23	Forma de fruto: elipsoidal
Anchura (cm): 16.15 ± 0.41	Color de la Corteza: Blanca
Longitud (cm): 23.98 ± 1.2	Color de la carne: verde
Firmeza (kg/cm2): 3.75 ± 0.66	Escriturado: abundante

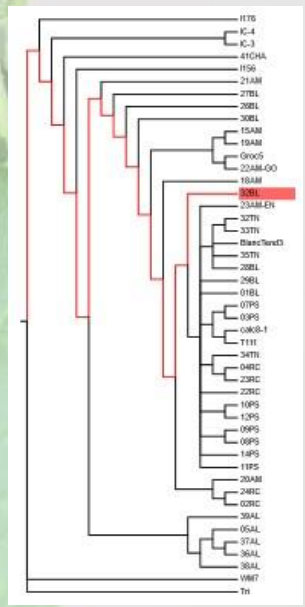
Características Nutricionales del Fruto

º Brix: 9.18 ± 0.37
pH: 5.5 ± 0.2

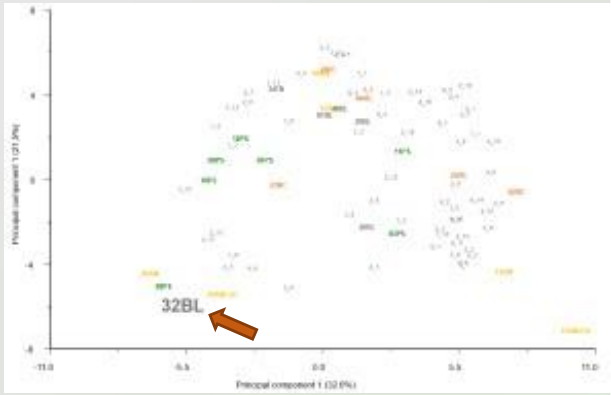
32BL

Localidad de Origen: Sueca (Valencia)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV016453

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 2.38 ±0.14	Forma de fruto: redondo
Anchura (cm): 15.57 ± 0.12	Color de la Corteza: Blanca
Longitud (cm): 21.3 ± 1.88	Color de la carne: crema-verde
Firmeza (kg/cm2): 2.13 ± 0.33	Escriturado: no

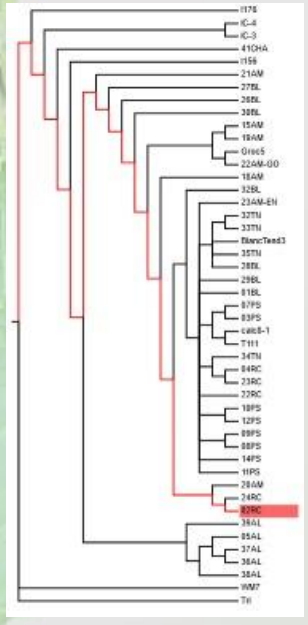
Características Nutricionales del Fruto

º Brix: 12.17 ± 0.8	Fructosa (g/kg): 21.98 ± 2.09
pH: 6 ± 0	Glucosa (g/kg): 19.02 ± 1.36
Ácido Málico (g/kg): 0.2±0.1	Sucrosa (g/kg): 46.7 ± 5.21
Ácido Cítrico (g/kg): 3.74 ± 0.12	Equiv. Sucrosa (g/kg): 98.81±9.07

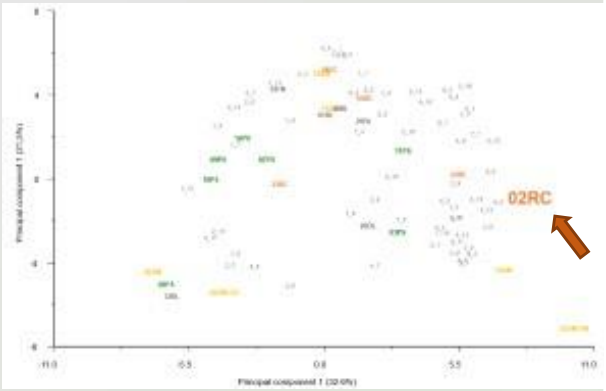
02RC

Localidad de Origen: Rielves (Toledo)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV003718

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 2.12 ±0.15	Forma de fruto: redondo
Anchura (cm): 15.54 ± 0.43	Color de la Corteza: verde
Longitud (cm): 17.76 ± 0.24	Color de la carne: crema-verde
Firmeza (kg/cm2): 1.65 ± 0.22	Escriturado: no

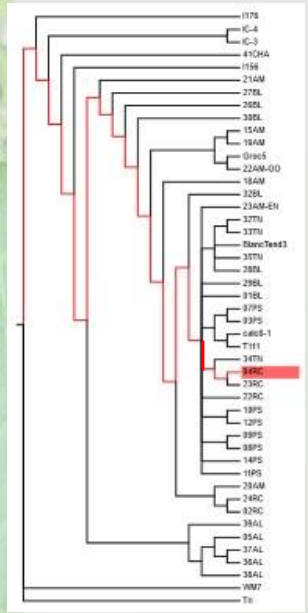
Características Nutricionales del Fruto

º Brix: 11.36 ± 0.41	Fructosa (g/kg): 14.55 ± 0.31
pH: 5.3 ± 0.3	Glucosa (g/kg): 11.72 ± 0.42
Acido Málico (g/kg): 0.21 ± 0	Sucrosa (g/kg): 58.4 ± 3.92
Acido Cítrico (g/kg): 5.65 ± 0.12	Equiv. Sucrosa (g/kg): 92.24±3.5

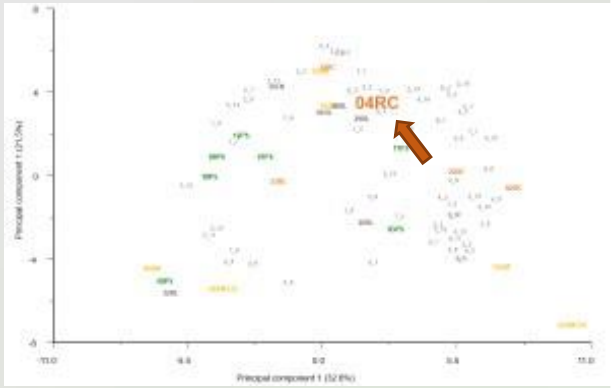
04RC

Localidad de Origen: La Pobla de Benifassa (Castellón)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004884

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 1.83 ±0.07
Anchura (cm): 14.24 ± 0.24
Longitud (cm): 19 ± 0.3
Firmeza (kg/cm2): 2.54 ± 0.14
Forma de fruto: redondo
Color de la Corteza: verde
Color de la carne: crema
Escriturado: muy leve

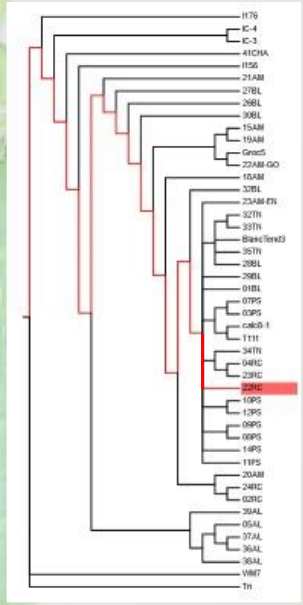
Características Nutricionales del Fruto

º Brix: 12.3 ± 0.49
pH: 5.8 ± 0.12
Ácido Málico (g/kg): 0.07 ± 0.01
Ácido Cítrico (g/kg): 4.01 ± 0.14
Fructosa (g/kg): 18.37 ± 1.23
Glucosa (g/kg): 18.1 ± 1.7
Sucrosa (g/kg): 65.58 ± 3.31
Equiv. Sucrosa (g/kg): 110.76±1.47

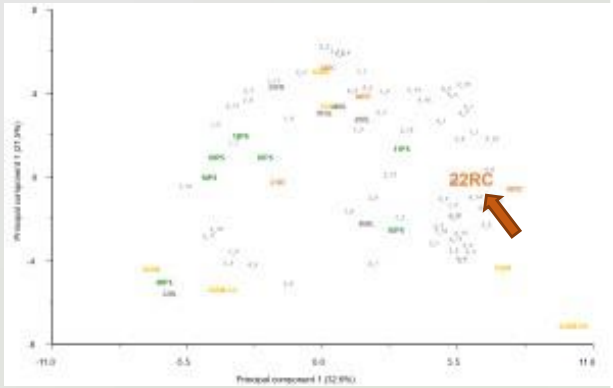
22RC

Localidad de Origen: Jalón (Alicante)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004848

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 2.14 ± 0.51	Forma de fruto: elipsoidal
Anchura (cm): 14.48 ± 1.37	Color de la Corteza: verde
Longitud (cm): 20.23 ± 1.12	Color de la carne: crema-verde
Firmeza (kg/cm2): 2.56 ± 0.5	Escriturado: no

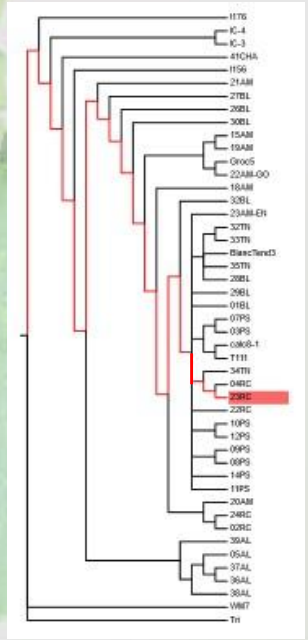
Características Nutricionales del Fruto

º Brix: 11.05 ± 0.62	Fructosa (g/kg) : 18 ± 3.84
pH: 5.25 ± 0.25	Glucosa (g/kg): 17.26 ± 4.17
Ácido Málico (g/kg): 0.69 ± 0.53	Sucrosa (g/kg): 49.93 ± 6.55
Ácido Cítrico (g/kg): 3.9 ± 0.53	Equiv. Sucrosa (g/kg): 93.84±4.9

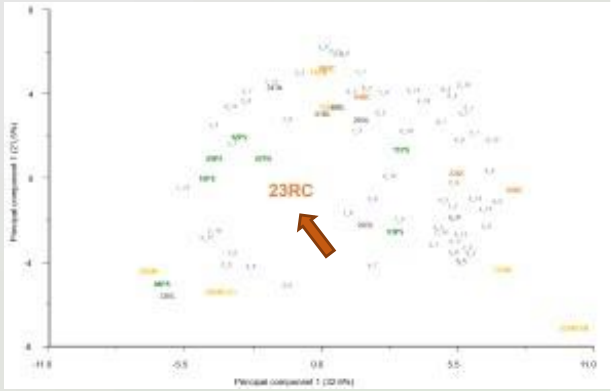
23RC

Localidad de Origen: Massalfassar (Valencia)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV014771

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 1.70 ± 0.33	Forma de fruto: elipsoidal
Anchura (cm): 13.44 ± 1.42	Color de la Corteza: verde
Longitud (cm): 19.2 ± 1.43	Color de la carne: crema-verde
Firmeza (kg/cm2): 2.58 ± 0.47	Escriturado: no

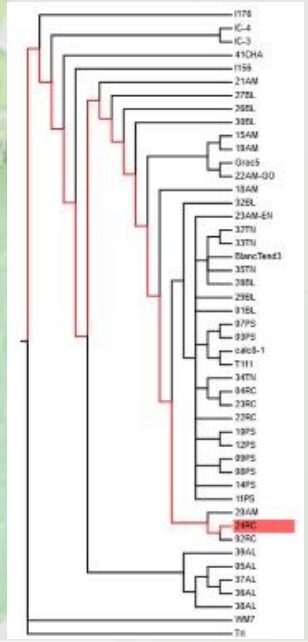
Características Nutricionales del Fruto

º Brix: 12.78 ± 1.94	Fructosa (g/kg): 19.62 ± 1.9
pH: 5.2 ± 0.27	Glucosa (g/kg): 18.77 ± 2.51
Ácido Málico (g/kg): 0.25 ± 0.16	Sucrosa (g/kg): 65.15 ± 3.12
Ácido Cítrico (g/kg): 4.09 ± 0.09	Equiv. Sucrosa (g/kg): 112.98 ± 4.02

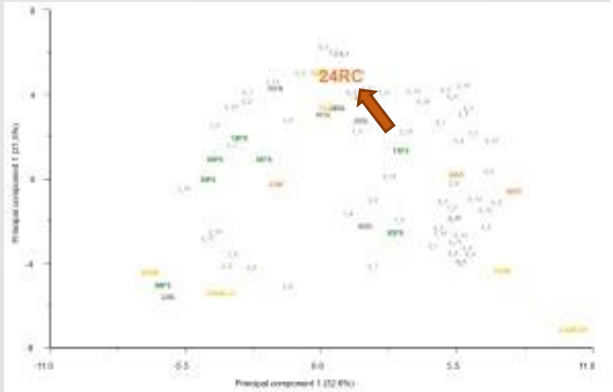
24RC

Localidad de Origen: Miguelturra (Ciudad Real)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV014765

Filogenia



Perfil Aroma



Descripción del Fruto

Peso (kg): 1.69 ±0.11	Forma de fruto: ovalado
Anchura (cm): 13.78 ± 0.52	Color de la Corteza: verde
Longitud (cm): 18.88 ± 0.5	Color de la carne: crema-verde
Firmeza (kg/cm2): 1.91 ± 0.18	Escriturado: no

Características Nutricionales del Fruto

º Brix: 12.56 ± 0.37	Fructosa (g/kg): 16.99 ± 1.34
pH: 5 ± 0	Glucosa (g/kg): 14.84 ± 1.71
Ácido Málico (g/kg): 0.06 ± 0	Sucrosa (g/kg): 73.59 ± 3.93
Ácido Cítrico (g/kg): 5.09 ± 0.49	Equiv. Sucrosa (g/kg): 113.97 ± 0.93

32TN

Localidad de Origen: La Roda (Albacete)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV003722

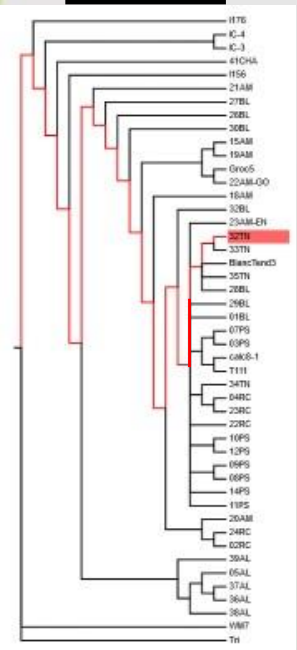
Descripción del Fruto

Peso (kg): 2.04 ± 0.29
Anchura (cm): 15.42 ± 0.74
Longitud (cm): 17.8 ± 0.79
Firmeza (kg/cm2): 2.8 ± 0.28
Forma de fruto: redondo
Color de la Corteza: verde oscuro
Color de la carne: crema-verde
Escriturado: no

Características Nutricionales del Fruto

º Brix: 10.93 ± 0.34
pH: 5.08 ± 0.08
Ácido Málico (g/kg): 0.09 ± 0.02
Ácido Cítrico (g/kg): 3.42 ± 0.36
Fructosa (g/kg): 17.21 ± 1.7
Glucosa (g/kg): 19.07 ± 1.13
Sucrosa (g/kg): 43.42±10.69
Equiv. Sucrosa (g/kg): 87.3±8.48

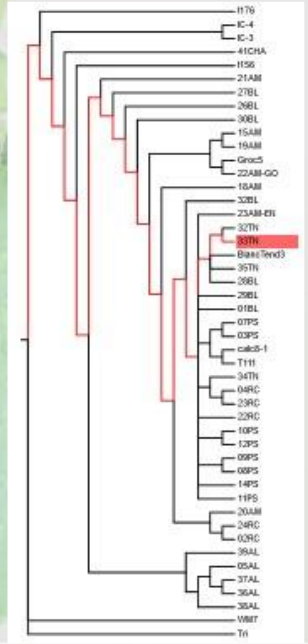
Filogenia



33TN

Localidad de Origen: Santa Fe (Granada)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV000471

Filogenia



Descripción del Fruto

Peso (kg): 2.04 ± 0.24	Forma de fruto : redondo
Anchura (cm) : 15.4 ± 0.76	Color de la Corteza : verde oscuro
Longitud (cm) : 18.25 ± 0.95	Color de la carne : crema-verde
Firmeza (kg/cm2): 3.15 ± 0.65	Escriturado: no

Características Nutricionales del Fruto

º Brix : 11.08 ± 0.57	Fructosa (g/kg): 18.34 ± 4.79
pH: 5 ± 0	Glucosa (g/kg): 18.78 ± 4.97
Acido Málico (g/kg): 0.21 ± 0.1	Sucrosa (g/kg): 48.38 ± 9.69
Acido Cítrico (g/kg): 3.76 ± 0.34	Equiv. Sucrosa (g/kg): 94.01 ± 2.36

34TN

Localidad de Origen: Benissa (Alicante)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004871

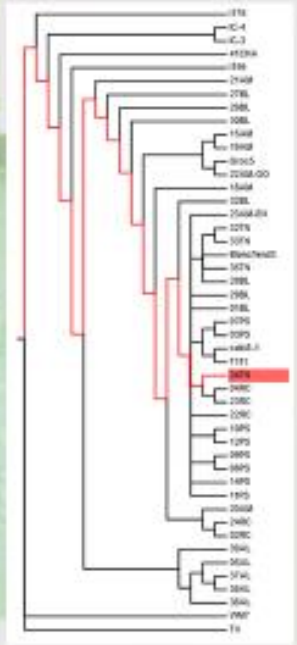
Descripción del Fruto

Peso (kg): 1.63 ± 0.15	Forma de fruto: ovalado
Anchura (cm): 13.64 ± 0.78	Color de la Corteza: verde
Longitud (cm): 18.6±1.62	Color de la carne: crema
Firmeza (kg/cm2): 2.95 ± 0.34	Escriturado: no

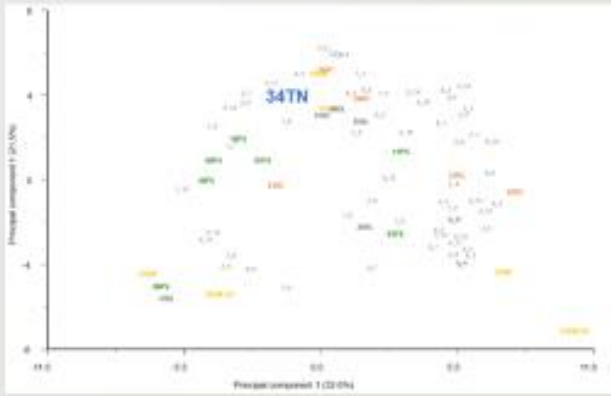
Características Nutricionales del Fruto

° Brix: 11.32 ± 0.49	Fructosa (g/kg): 20.94 ± 1.91
pH: 5.5 ± 0.39	Glucosa (g/kg): 19.39 ± 1.67
Ácido Málico (g/kg): 0.09 ± 0.02	Sucrosa (g/kg): 42.81 ± 5.96
Ácido Cítrico (g/kg): 4.11 ± 0.37	Equiv. Sucrosa (g/kg): 93.39±2.37

Filogenia



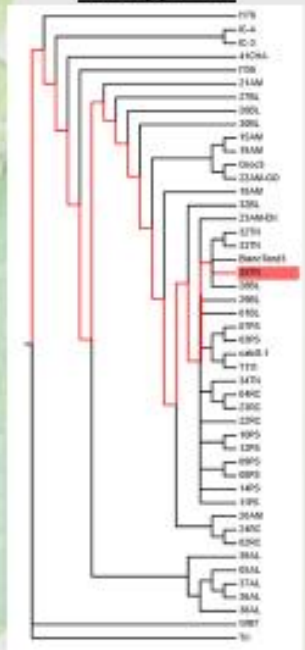
Perfil Aroma



35TN

Localidad de Origen: Puerto Lumbreras (Murcia)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004298

Filogenia



Descripción del Fruto

Peso (kg): 1.57 ± 0.09	Forma de fruto : redondo
Anchura (cm): 14.23 ± 0.31	Color de la Corteza : verde oscuro
Longitud (cm): 16.85 ± 0.37	Color de la carne : crema-verde
Firmeza (kg/cm2): 3.28 ± 0.11	Escriturado: no

Características Nutricionales del Fruto

° Brix : 11.08 ± 0.49	Fructosa (g/kg): 17.18 ± 1
pH: 5 ± 0	Glucosa (g/kg): 19.22 ± 1.01
Ácido Málico (g/kg): 0 ± 0	Sucrosa (g/kg): 47.59 ± 4.31
Ácido Cítrico (g/kg):5.11 ± 0.36	Equiv. Sucrosa (g/kg): 91.53±6.45

05AL

Localidad de Origen: Jalón (Alicante)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004853

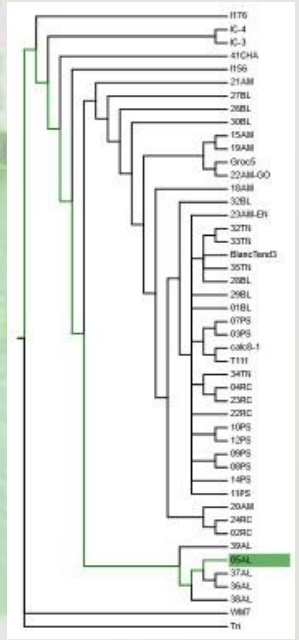
Descripción del Fruto

Peso (g): 226.2 ± 31.47	Forma de fruto: serpentino
Anchura (cm): 2.78 ± 0.17	Color de la Corteza: verde
Longitud (cm): 40.24 ± 1.08	Color de la carne: verde
Firmeza (kg/cm2): 2.94 ± 0.38	Escriturado: no

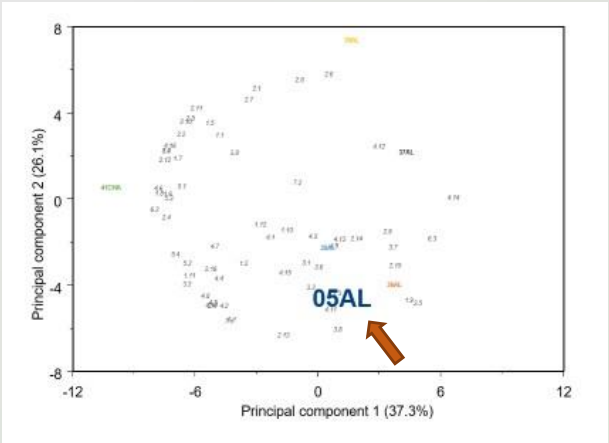
Características Nutricionales del Fruto

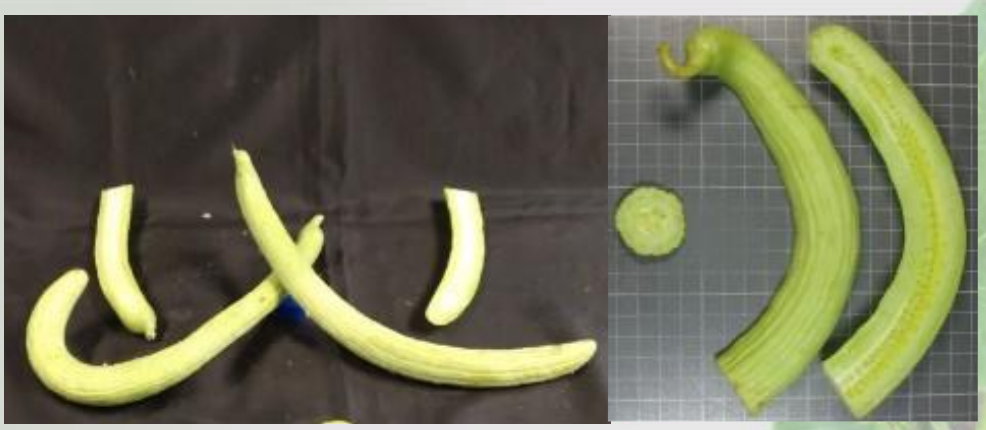
º Brix: 2.34 ± 0.05	Fructosa (g/kg): 10.46 ± 0.77
pH: 4.6 ± 0.1	Glucosa (g/kg): 9.18 ± 0.84
Ácido Málico (g/kg): 1.92 ± 0.11	Sucrosa: -
Ácido Cítrico (g/kg): 0.06 ± 0.01	Equiv. Sucrosa (g/kg): 24.89±1.94

Filogenia



Perfil Aroma





36AL

Localidad de Origen: Elche (Alicante)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004963

Descripción del Fruto

Peso (g): 173.17 ± 18.68
Anchura (cm) : 2.77 ± 0.14
Longitud (cm) : 46.35 ± 3.76
Firmeza (kg/cm2): 2.78 ± 0.16

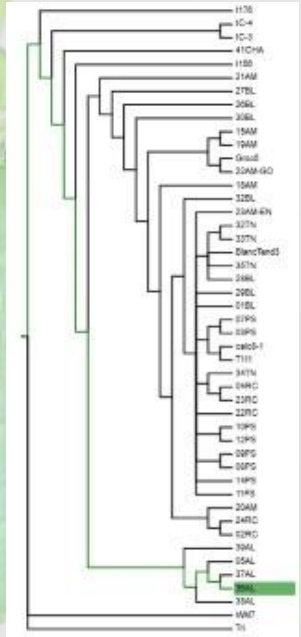
Forma de fruto : serpentino
Color de la Corteza : verde
Color de la carne : verde
Escriturado : no

Características Nutricionales del Fruto

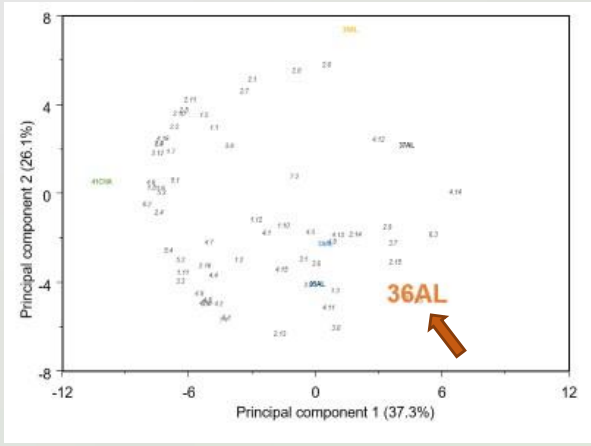
º Brix : 2.72 ± 0.22
pH: 4.33 ± 0.11
Ácido Málico (g/kg): 2.32 ± 0.44
Ácido Cítrico (g/kg): 0.07 ± 0.02

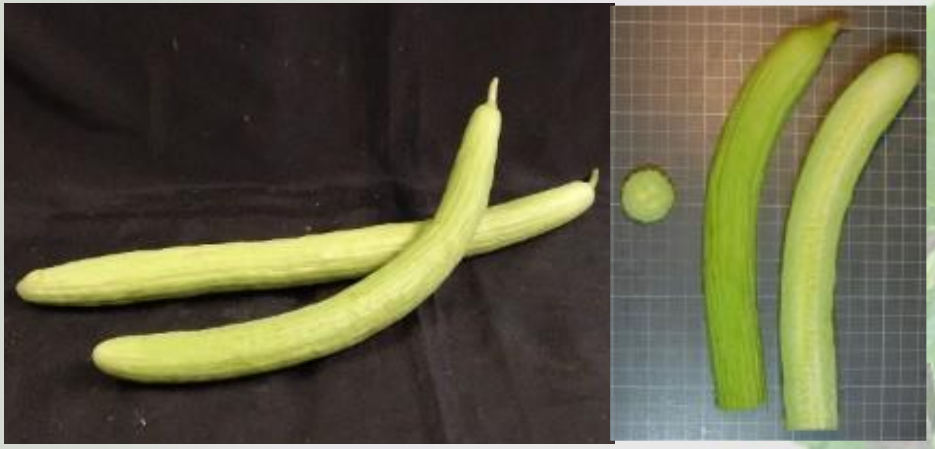
Fructosa (g/kg): 10.57 ± 0.81
Glucosa (g/kg): 9.93±1.04
Sucrosa: -
Equiv. Sucrosa (g/kg): 25.63 ± 2.16

Filogenia



Perfil Aroma





37AL

Localidad de Origen: Totana (Murcia)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004310

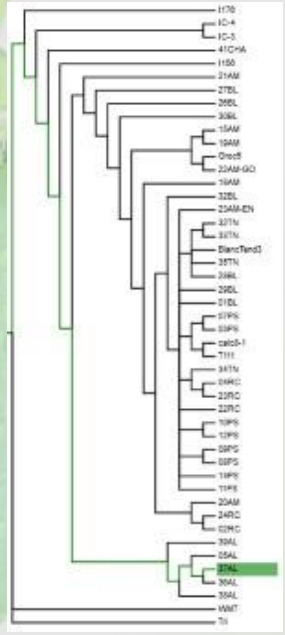
Descripción del Fruto

Peso (g): 236.8 ± 14.12	Forma de fruto: serpentino
Anchura (cm): 3.24 ± 0.09	Color de la Corteza: verde
Longitud (cm): 41.04 ± 2.23	Color de la carne: verde
Firmeza (kg/cm2): 3.18 ± 0.21	Escriturado: no

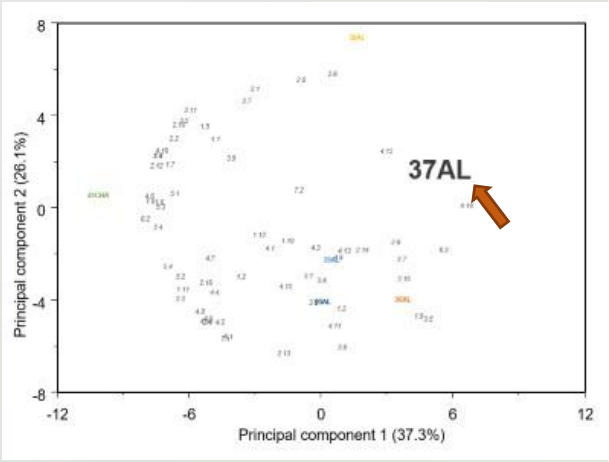
Características Nutricionales del Fruto

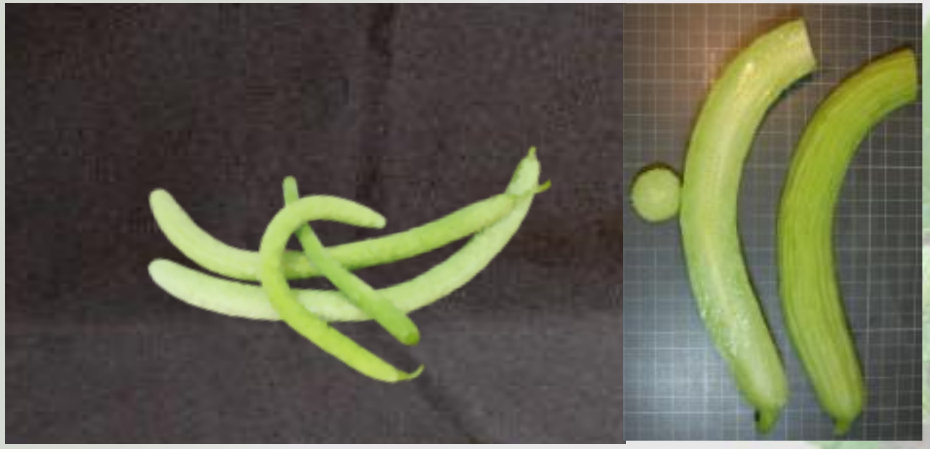
º Brix: 1.78±0.21	Fructosa (g/kg): 10.04 ± 0.71
pH: 4 ± 0	Glucosa (g/kg): 9.49 ± 0.41
Ácido Málico (g/kg): 1.65±0.09	Sucrose: -
Ácido Cítrico (g/kg): 0.03±0	Equiv. Sucrosa (g/kg): 24.39±1.5

Filogenia



Perfil Aroma





38AL

Localidad de Origen: Benissa (Alicante)
Colección: Banco de Germoplasma UPV
Código de Banco: BGV004860

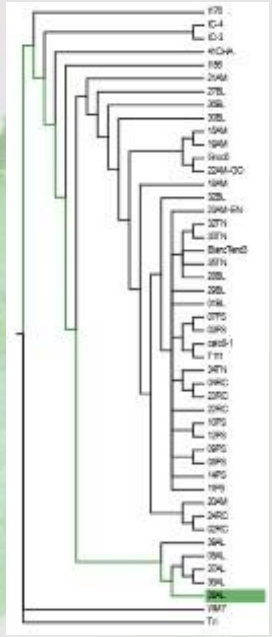
Descripción del Fruto

Peso (g): 243.5 ± 67.35	Forma de fruto: serpentino
Anchura (cm): 3.35 ± 0.15	Color de la Corteza: verde
Longitud (cm): 36.43 ± 3.6	Color de la carne: verde
Firmeza (kg/cm2): 3.38 ± 0.24	Escriturado: no

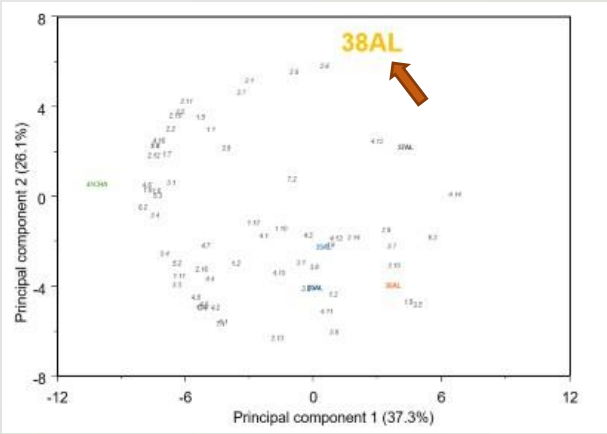
Características Nutricionales del Fruto

º Brix: 1.93 ± 0.17	Fructosa (g/kg): 7.43 ± 0.45
pH: 4.25 ± 0.14	Glucosa (g/kg): 7.25 ± 0.36
Ácido Málico (g/kg): 1.53 ± 0.41	Sucrose: -
Ácido Cítrico (g/kg): 0.06 ± 0.02	Equiv. Sucrosa (g/kg): 18.23±1.04

Filogenia



Perfil Aroma



39AL

Localidad de Origen: Argelia
Colección: Banco de Germoplasma UPV
Código de Banco: BGV015843

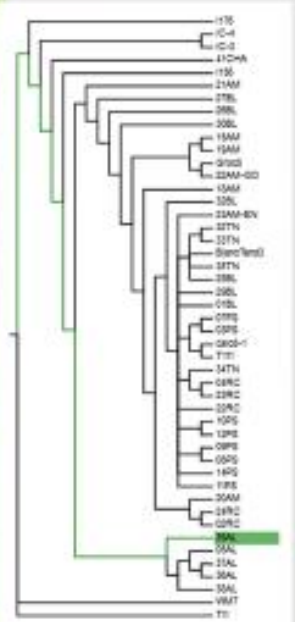
Descripción del Fruto

Peso (g): 461.2 ± 63.01	Forma de fruto: alargado
Anchura (cm): 5.92 ± 0.27	Color de la Corteza: verde
Longitud (cm): 25.58 ± 1.29	Color de la carne: verde
Firmeza (kg/cm2): 3.7 ± 0.41	Escriturado: no

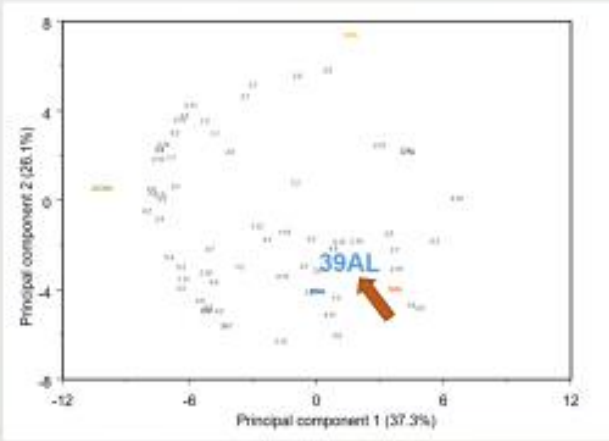
Características Nutricionales del Fruto

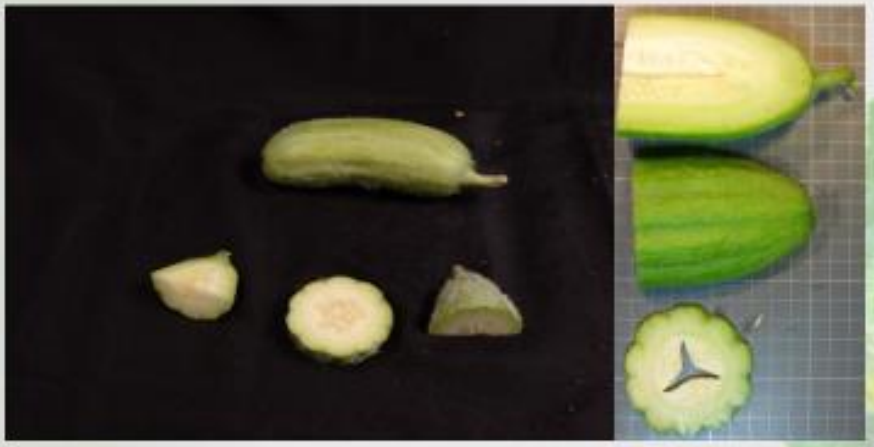
º Brix: 2.26 ± 0.09	Fructosa (g/kg): 8.56 ± 0.31
pH: 4.6 ± 0.24	Glucosa (g/kg): 8.68 ± 0.39
Ácido Málico (g/kg): 1.46 ± 0.08	Sucrosa: -
Ácido Cítrico (g/kg): 0.03 ± 0	Equiv. Sucrosa (g/kg): 21.23±0.8

Filogenia



Perfil Aroma





41CHA

Localidad de Origen: Benissa (Alicante)
Colección: Esteras et al., (2013)
Código de Banco: Chate-Carr

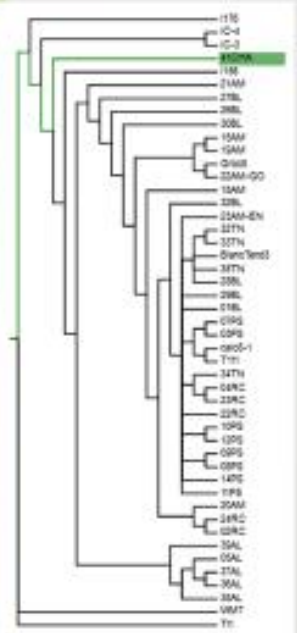
Descripción del Fruto

Peso (g): 309 ± 49.79	Forma de fruto: alargado
Anchura (cm): 6.1 ± 0.39	Color de la Corteza: verde
Longitud (cm): 14.66 ± 1.03	Color de la carne: verde
Firmeza (kg/cm2): 5.3 ± 0.25	Escriturado: no

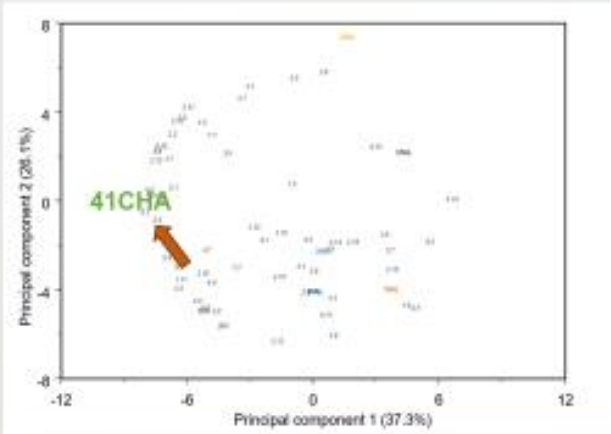
Características Nutricionales del Fruto

º Brix: 3.08 ± 0.47	Fructosa (g/kg): 10.16 ± 0.78
pH: 4.7 ± 0.2	Glucosa (g/kg): 8.5 ± 0.55
Ácido Málico (g/kg): 1.82 ± 0.1	Sucrosa: -
Ácido Cítrico (g/kg): 0.13 ± 0.06	Equiv. Sucrosa (g/kg): 23.86±1.75

Filogenia



Perfil Aroma



Bibliography

Bibliography

Abdelmohsin, M. E., El Jack, A. E., Yousif, M. T., El Naim, A. M., Ahmed, E. A., and Pitrat, M. (2015). Impact of breeding hermaphroditic melon on early production and yield: case of snake melon (*Cucumis melo* var. *flexuosus*) and Tibish (*C. melo* var. *tibish*). *Int. J. Life Sci. Eng* 1, 171–176.

Abu Zaitoun, S. Y., Jamous, R. M., Shtaya, M. J., Mallah, O. B., Eid, I. S., and Ali-Shtayeh, M. S. (2018). Characterizing Palestinian snake melon (*Cucumis melo* var. *flexuosus*) germplasm diversity and structure using SNP and DArTseq markers. *BMC Plant Biol.* 18, 1–12. doi: 10.1186/s12870-018-1475-2.

Aguiar, R. W. S., Alves, G. B., Queiroz, A. P., Nascimento, I. R., and Lima, M. F. (2018). Evaluation of weeds as virus reservoirs in watermelon crops. *Planta Daninha* 36. doi: 10.1590/S0100-83582018360100032.

Ahlawat, S. P., Pandey, A., Malav, P. K., Bhardwaj, R., and Dhariwal, O. P. (2018). A less-known vegetable melon landrace "Arya" (*Cucumis melo* L.) from Rajasthan and Haryana, India: morphological, biochemical and taxonomic study. *Genet. Resour. Crop Evol.* 65, 2037–2047. doi: 10.1007/S10722-018-0668-8/TABLES/2.

Ahuja, I., de Vos, R. C. H., Bones, A. M., and Hall, R. D. (2010). Plant molecular stress responses face climate change. *Trends Plant Sci.* 15, 664–674. doi: 10.1016/J.TPLANTS.2010.08.002.

Akrami, M., and Arzani, A. (2018). Physiological alterations due to field salinity stress in melon (*Cucumis melo* L.). *Acta Physiol. Plant.* 40, 1–14. doi: 10.1007/s11738-018-2657-0.

Akrami, M., and Arzani, A. (2019). Inheritance of fruit yield and quality in melon (*Cucumis melo* L.) grown under field salinity stress. *Sci. Rep.* 9, 1–13. doi: 10.1038/s41598-019-43616-6.

Akrami, M., Arzani, A., and Majnoun, Z. (2019). Leaf ion content, yield and fruit quality of field-grown melon under saline conditions. *Exp. Agric.* 55, 707–722. doi: 10.1017/S0014479718000303.

Al-Daghari, D. S. S., Al-Sadi, A. M., and Rethinasamy, V. (2021). *Monosporascus* root rot and vine decline disease of muskmelon: an overview. *Trop. Agric.* 98, 406. Available at: https://journals.sta.uwi.edu/ojs/index.php/ta/article/view/7958

Albuquerque, B., Lidon, F. C., and Barreiro, M. G. (2006). A case study on the flavor properties of melon (*Cucumis melo* L.) cultivars. *Fruits* 61, 333–339. doi: 10.1051/fruits:2006032.

Alexander, D. H., Novembre, J., and Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 19, 1655–1664. doi: 10.1101/gr.094052.109.

Ali-Shtayeh, M. S., Jamous, R. M., Shtaya, M. J., Mallah, O. B., Eid, I. S., and Zaitoun, S. Y. A. (2017). Morphological characterization of snake melon (*Cucumis melo* var. *flexuosus*) populations from Palestine. *Genet. Resour. Crop Evol.* 64, 7–22. doi: 10.1007/s10722-015-0329-0.

Aloni, B., Karni, L., Deventurero, G., Levin, Z., Cohen, R., Katzir, N., et al. (2008). Physiological and biochemical changes at the rootstock-scion interface in graft combinations between *Cucurbita* rootstocks and a melon scion. *J. Hortic. Sci. Biotechnol.* 83, 777–783. doi: 10.1080/14620316.2008.11512460.

Alvarez, J. M., González-Torres, R., Mallor, C., and Gómez-Guillamón, M. L. (2005). Potential sources of resistance to *fusarium* wilt and powdery mildew in melons. *HortScience* 40, 1657–1660. doi: 10.21273/HORTSCI.40.6.1657.

Ambrósio, M. M. Q., Dantas, A. C. A., Martínez-Perez, E., Medeiros, A. C., Nunes, G. H. S., and Picó, M. B. (2015). Screening a variable germplasm collection of *Cucumis melo* L. for seedling resistance to *Macrophomina phaseolina*. *Euphytica* 206, 287–300. doi: 10.1007/s10681-015-1452-x.

Annunziata, A., and Vecchio, R. (2016). Organic Farming and Sustainability in Food Choices: An Analysis of Consumer Preference in Southern Italy. *Agric. Agric. Sci. Procedia* 8, 193–200. doi: 10.1016/j.aaspro.2016.02.093.

Antignus, Y., Lapidot, M., Hadar, D., Messika, Y., and Cohen, S. (1998). Ultraviolet-Absorbing Screens Serve as Optical Barriers to Protect Crops from Virus and Insect Pests. *J. Econ. Entomol.* 91, 1401–1405. doi: 10.1093/jee/91.6.1401.

Araya, E. M., Adamu, B. A., Periasamy, G., Sintayehu, B., and Gebrelibanos Hiben, M. (2019). In vivo hepatoprotective and In vitro radical scavenging activities of Cucumis ficifolius A. rich root extract. *J. Ethnopharmacol.* 242, 112031. doi: 10.1016/j.jep.2019.112031.

Argyris, J. M., Díaz, A., Ruggieri, V., Fernández, M., Jahrmann, T., Gibon, Y., et al. (2017). QTL Analyses in Multiple Populations Employed for the Fine Mapping and Identification of Candidate Genes at a Locus Affecting Sugar Accumulation in Melon (Cucumis melo L.). *Front. Plant Sci.* 8, 1679. doi: 10.3389/fpls.2017.01679.

Armengol, J., José, C. M., Moya, M. J., Sales, R., Vicent, A., and García-Jiménez, J. (2000). *Fusarium solani* f. sp. *cucurbitae* race 1, a potential pathogen of grafted watermelon production in Spain. *EPPO Bull.* 30, 179–183. doi: 10.1111/j.1365-2338.2000.tb00875.x.

Artés, F., Escriche, A. J., Martinez, J. A., and Marin, J. G. (1993). Quality factors in four varieties of melon (*Cucumis melo*, L.). *J. Food Qual.* 16, 91–100.

Avery, D. T. (2001). Nature's Toxic Tools: The Organic Myth of Pesticide-Free Farming. Hudson Institute.

Baldwin, E. A., Scott, J. W., Einstein, M. A., Malundo, T. M. M., Carr, B. T., Shewfelt, R. L., et al. (1998). Relationship between sensory and instrumental analysis for tomato flavor. *J. Am. Soc. Hortic. Sci.* 123, 906–915. doi: 10.21273/jashs.123.5.906.

Barreiro, M. D. G., Lidon, F. C., and Pinto, M. (2001). Caractérisation physicochimique de la sénescence après récolte du melon d'hiver Tendral. *Fruits* 56, 51–58. doi: 10.1051/fruits:2001112.

Beaulieu, J. C., and Grimm, C. C. (2001). Identification of volatile compounds in cantaloupe at various developmental stages using solid phase microextraction. *J. Agric. Food Chem.* 49, 1345–1352. doi: 10.1021/jf0005768.

Bekhradi, F., Kashi, A., and Delshad, M. (2011). Effect of three cucurbits rootstocks on vegetative and yield of "Charleston Gray" watermelon. *Int. J. Plant Prod.* 5(2), 105-109.

Bianchi, T., Guerrero, L., Gratacós-Cubarsí, M., Claret, A., Argyris, J., Garcia-Mas, J., et al. (2016). Textural properties of different melon (*Cucumis melo* L.) fruit types: Sensory and physical-chemical evaluation. *Sci. Hortic. (Amsterdam).* 201, 46–56. doi: 10.1016/j.scienta.2016.01.028.

Bie, Z., Nawaz, M. A., Huang, Y., Lee, J.-M., Colla, G., and others (2017). Introduction of vegetable grafting. *Veg. grafting, Princ. Pract.*, 1–21.

Blanca, J., Esteras, C., Ziarsolo, P., Pérez, D., FernÃndez-Pedrosa, V., Collado, C., et al. (2012). Transcriptome sequencing for SNP discovery across *Cucumis melo*. *BMC Genomics* 13, 280. doi: 10.1186/1471-2164-13-280.

Botía, P., Navarro, J. M., Cerdá, A., and Martínez, V. (2005). Yield and fruit quality of two melon cultivars irrigated with saline water at different stages of development. *Eur. J. Agron.* 23, 243–253. doi: 10.1016/j.eja.2004.11.003.

Boualem, A., Troadec, C., Camps, C., Lemhemdi, A., Morin, H., Sari, M. A., et al. (2015). A cucurbit androecy gene reveals how unisexual flowers develop and dioecy emerges. *Science (80-.).* 350, 688–691. doi: 10.1126/science.aac8370.

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., and Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23, 2633–2635. doi: 10.1093/bioinformatics/btm308.

Brzezina, N., Biely, K., Helfgott, A., Kopainsky, B., Vervoort, J., and Mathijs, E. (2017). Development of organic farming in Europe at the crossroads: Looking for the way forward through system archetypes lenses. *Sustainability* 9, 821. doi: 10.3390/SU9050821.

Buller, S., Inglis, D., and Miles, C. (2013). Plant growth, fruit yield and quality, and tolerance to verticillium wilt of grafted watermelon and tomato in field production in the Pacific Northwest. *HortScience* 48, 1003–1009. doi: 10.21273/hortsci.48.8.1003.

Burger, Y., Paris, H. S., Cohen, R., Katzir, N., Tadmor, Y., Lewinsohn, E., et al. (2010). 3 Genetic Diversity of Cucumis Melo. *Hortic. Rev. (Am. Soc. Hortic. Sci).* 36, 165–198.

Burger, Y., Sa'ar, U., Distelfeld, A., Katzir, N., Yeselson, Y., Shen, S., et al. (2003). Development of sweet melon (*Cucumis melo*) genotypes combining high sucrose and organic acid content. *J. Am. Soc. Hortic. Sci.* 128, 537–540. doi: 10.21273/jashs.128.4.0537.

Burger, Y., Sa'ar, U., Paris, H. S., Lewinsohn, E., Katzir, N., Tadmor, Y., et al. (2006). Genetic variability for valuable fruit quality traits in *Cucumis melo*. *Isr. J. Plant Sci.* 54, 233–242. doi: 10.1560/IJPS_54_3_233.

Burger, Y., Sa'ar, U., Katzir, N., Paris, H. S., Yeselson, Y., Levin, I., et al. (2002). A single recessive gene for sucrose accumulation m cucumis melo fruit. *J. Am. Soc. Hortic. Sci.* 127, 938–943. doi: 10.21273/jashs.127.6.938.

Burger, Y., Shen, S., Petreikov, M., and Schaffer, A. A. (2000). The contribution of sucrose to total sugar content in melons. in *Acta Horticulturae* (International Society for Horticultural Science), 479–485. doi: 10.17660/actahortic.2000.510.75.

Buttaro, D., Bonasia, A., Minuto, A., Serio, F., and Santamaria, P. (2009). Effect of silicon in the nutrient solution on the incidence of powdery mildew and quality traits in carosello and barattiere (*Cucumis* melo L.) grown in a soilless system. *J. Hortic. Sci. Biotechnol.* 84, 300–304. doi: 10.1080/14620316.2009.11512521.

Cáceres, A., Perpiña, G., Ferriol, M., Picó, B., and Gisbert, C. (2017). New Cucumis rootstocks for melon: 'UPV-FA' and 'UPV-FMY.' *HortScience* 52, 792–797. doi: 10.21273/HORTSCI11791-17.

Calatayud, Á., San Bautista, A., Pascual, B., Maroto, J. V., and López-Galarza, S. (2013). Use of chlorophyll fluorescence imaging as diagnostic technique to predict compatibility in melon graft. *Sci. Hortic. (Amsterdam).* 149, 13–18. doi: 10.1016/j.scienta.2012.04.019.

Casals, J., Pascual, L., Cañizares, J., Cebolla-Cornejo, J., Casañas, F., and Nuez, F. (2011). The risks of success in quality vegetable markets: Possible genetic erosion in Marmande tomatoes (*Solanum lycopersicum* L.) and consumer dissatisfaction. *Sci. Hortic. (Amsterdam).* 130, 78–84. doi: 10.1016/j.scienta.2011.06.013.

Castanera, R., Ruggieri, V., Pujol, M., Garcia-Mas, J., and Casacuberta, J. M. (2020). An Improved Melon Reference Genome With Single-Molecule Sequencing Uncovers a Recent Burst of Transposable Elements With Potential Impact on Genes. *Front. Plant Sci.* 10, 1815. doi: 10.3389/fpls.2019.01815.

Castro, G., Perpiñá, G., Esteras, C., Armengol, J., Picó, B., and Pérez-de-Castro, A. (2020). Resistance in melon to *Monosporascus cannonballus* and *M. eutypoides*: Fungal pathogens associated with Monosporascus root rot and vine decline. *Ann. Appl. Biol.* 177, 101–111. doi: 10.1111/aab.12590

Cebolla-Cornejo, J., Roselló, S., and Nuez, F. (2013). Phenotypic and genetic diversity of Spanish tomato landraces. *Sci. Hortic. (Amsterdam).* 162, 150–164. doi: 10.1016/j.scienta.2013.07.044.

Cebolla-Cornejo, J., Soler, S., and Nuez, F. (2007). Genetic erosion of traditional varieties of vegetable crops in Europe: tomato cultivation in Valencia (Spain) as a case study. *Int. J. Plant Prod.* 1, 113–128.

Cebolla-Cornejo, J., Valcárcel, M., Herrero-Martínez, J. M., Roselló, S., and Nuez, F. (2012). High efficiency joint CZE determination of sugars and acids in vegetables and fruits. *Electrophoresis* 33, 2416–2423. doi: 10.1002/elps.201100640.

CEDEX Evaluación del impacto del cambio climático en los recursos hídricos y sequías en España (2017) [Accessed August 4, 2022].

Chang, J. M., Di Tommaso, P., and Notredame, C. (2014). TCS: A New Multiple Sequence Alignment Reliability Measure to Estimate Alignment Accuracy and Improve Phylogenetic Tree Reconstruction. *Mol. Biol. Evol.* 31, 1625–1637. doi: 10.1093/MOLBEV/MSU117.

Chang, X., Gao, Z., Wang, S., and Chen, H. (2019). Modelling long-term soil salinity dynamics using SaltMod in Hetao Irrigation District, China. *Comput. Electron. Agric.* 156, 447–458. doi: 10.1016/j.compag.2018.12.005.

Chen, H., Cao, S., Jin, Y., Tang, Y., and Qi, H. (2016). The Relationship between CmADHs and the Diversity of Volatile Organic Compounds of Three Aroma Types of Melon (*Cucumis melo*). *Front. Physiol.* 7, 254. doi: 10.3389/fphys.2016.00254.

Chomicki, G., Schaefer, H., and Renner, S. S. (2019). Origin and domestication of Cucurbitaceae crops: insights from phylogenies, genomics and archaeology. *New Phytol.*, nph.16015. doi: 10.1111/nph.16015.

Chuan-qiang, X., Yu-xue, H., and Lin, Y. (2011). Effects of grafting on four characteristic ester aromas contents and related enzyme activities in muskmelon fruit. *North. Hortic* 16, 41–44.

Cicia, G., Del Giudice, T., and Ramunno, I. (2009). Environmental and Health Components in Consumer Perception of Organic Products: Estimation of Willingness to Pay. *J. Food Prod. Mark.* 15, 324–336. doi: 10.1080/10454440902925930.

Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., et al. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly (Austin).* 6, 80–92. doi: 10.4161/fly.19695.

Clayberg, C. D. (1992). Interaction and linkage test of flesh colour genes in *Cucumis melo* L. *Cucurbit Genet. Coop.* 15, 53.

Cohen, R., Dombrovsky, A., Louws, F. J., and others (2017). Grafting as agrotechnology for reducing disease damage. *Veg. Grafting Princ. Pract. Colla, G., Pérez-Alfocea, F., Schwarz. D., Eds*, 155–170.

Cohen, R., Elkabetz, M., and Edelstein, M. (2016). Variation in the responses of melon and watermelon to Macrophomina phaseolina. *Crop Prot.* 85, 46–51. doi: 10.1016/j.cropro.2016.03.015.

Cohen, R., Elkabetz, M., Paris, H. S., Freeman, S., and Gur, A. (2022). Charcoal rot (*Macrophomina phaseolina*) across melon diversity: evaluating the interaction between the pathogen, plant age and environmental conditions as a step towards breeding for resistance. *Eur. J. Plant Pathol.* 163, 601–613. doi: 10.1007/S10658-022-02500-2.

Cohen, R., Pivonia, S., Crosby, K. M., and Martyn, R. D. (2012). Advances in the biology and management of monosporascus vine decline and wilt of melons and other cucurbits. *Hortic. Rev. (Am. Soc. Hortic. Sci).* 39, 77–120. doi: 10.1002/9781118100592.ch2.

Cohen, S., Itkin, M., Yeselson, Y., Tzuri, G., Portnoy, V., Harel-Baja, R., et al. (2014). The PH gene determines fruit acidity and contributes to the evolution of sweet melons. *Nat. Commun.* 5, 1–9. doi: 10.1038/ncomms5026.

Colla, G., Rouphael, Y., Cardarelli, M., Massa, D., Salerno, A., and Rea, E. (2006). Yield, fruit quality and mineral composition of grafted melon plants grown under saline conditions. *J. Hortic. Sci. Biotechnol.* 81, 146–152. doi: 10.1080/14620316.2006.11512041.

Colla, G., Suãrez, C. M. C., Cardarelli, M., and Rouphael, Y. (2010). Improving nitrogen use efficiency in melon by grafting. *HortScience* 45, 559–565. doi: 10.21273/hortsci.45.4.559.

Condurso, C., Verzera, A., Dima, G., Tripodi, G., Crinò, P., Paratore, A., et al. (2012). Effects of different rootstocks on aroma volatile compounds and carotenoid content of melon fruits. *Sci. Hortic. (Amsterdam).* 148, 9–16. doi: 10.1016/j.scienta.2012.09.015.

Cortés-Olmos, C., Valcárcel, J. V., Roselló, J., Díez, M. J., and Cebolla-Cornejo, J. (2015). Traditional Eastern Spanish varieties of tomato. *Sci. Agric.* 72, 420–431. doi: 10.1590/0103-9016-2014-0322.

Cothiere, O., González, V., Sales, E., Montaner-Otín, C., and Garcés-Claver, A. (2016). Optimización de la inoculación artificial de *Fusarium oxysporum* f. sp. niveum en sandía. *Actas Hortic.* 74, 99–100.

Crinò, P., Lo Bianco, C., Rouphael, Y., Colla, G., Saccardo, F., and Paratore, A. (2007). Evaluation of rootstock resistance to fusarium wilt and gummy stem blight and effect on yield and quality of a grafted “inodorus” melon. *HortScience* 42, 521–525. doi: 10.21273/hortsci.42.3.521.

Díaz, A., Martín-Hernández, A. M., Dolcet-Sanjuan, R., Garcés-Claver, A., Álvarez, J. M. M. M., Garcia-Mas, J., et al. (2017). Quantitative trait loci analysis of melon (*Cucumis melo* L.) domestication-related traits. *Theor. Appl. Genet.* 130, 1837–1856. doi: 10.1007/s00122-017-2928-y.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., et al. (2011). The variant call format and VCFtools. *Bioinformatics* 27, 2156–2158. doi: 10.1093/bioinformatics/btr330.

Darnhofer, I., Lindenthal, T., Bartel-Kratochvil, R., and Zollitsch, W. (2010). Conventionalisation of organic farming practices: from structural criteria towards an assessment based on organic principles. A review. *Agron. Sustain. Dev.* 30, 67–81. doi: 10.1051/agro/2009011.

Dasgan, H. Y., Balacheva, E., Yetişir, H., Yarsi, G., Altuntas, O., Akhoundnejad, Y., et al. (2015). The Effectiveness of Grafting to Improve Salt Tolerance of Sensitive Melon when the Tolerant Melon is Use as Rootstock. *Procedia Environ. Sci.* 29, 268. doi: 10.1016/j.proenv.2015.07.203.

Dastranji, N., Shojaeiyan, A., and Falahati-Anbaran, M. (2017). Assessment of genetic diversity on snake melon (*Cucumis melo* var. *flexuosus*) using ISSR markers in Iran. *Acta Hortic.* 1151, 45–50. doi: 10.17660/ActaHortic.2017.1151.8.

Davis, A. R., Perkins-Veazie, P., Hassell, R., Levi, A., King, S. R., and Zhang, X. (2008a). Grafting effects on vegetable quality. in *HortScience* (American Society for Horticultural Science), 1670–1672. doi: 10.21273/hortsci.43.6.1670.

Davis, A. R., Perkins-Veazie, P., Sakata, Y., López-Galarza, S., Maroto, J. V., Lee, S. G., et al. (2008b). Cucurbit grafting. *CRC. Crit. Rev. Plant Sci.* 27, 50–74. doi: 10.1080/07352680802053940.

De Magistris, T., and Gracia, A. (2008). The decision to buy organic food products in Southern Italy. *Br. Food J.* 110, 929–947. doi: 10.1108/00070700810900620.

De Ponti, T., Rijk, B., and Van Ittersum, M. K. (2012). The crop yield gap between organic and conventional agriculture. *Agric. Syst.* 108, 1–9. doi: 10.1016/j.agsy.2011.12.004.

de Sousa Linhares, C. M., Ambrósio, M. M. Q., Castro, G., Torres, S. B., Esteras, C., de Sousa Nunes, G. H., et al. (2020). Effect of temperature on disease severity of charcoal rot of melons caused by *Macrophomina phaseolina*: implications for selection of resistance sources. *Eur. J. Plant Pathol.* 158, 431–441. doi: 10.1007/s10658-020-02083-w.

Del Amor, F. M., Martinez, V., and Cerdá, A. (1999). Salinity duration and concentration affect fruit yield and quality, and growth and mineral composition of melon plants grown in perlite. *HortScience* 34, 1234–1237. doi: 10.21273/hortsci.34.7.1234.

Delgado-Baquerizo, M., Guerra, C. A., Cano-Díaz, C., Egidi, E., Wang, J. T., Eisenhauer, N., et al. (2020). The proportion of soil-borne pathogens increases with warming at the global scale. *Nat. Clim. Chang. 2020 106* 10, 550–554. doi: 10.1038/s41558-020-0759-3.

Denna, D. W. (1962). A simple grafting technique for cucurbits. in *Proc. Amer. Soc. Hort. Sci.*, 369–370.

Desbiez, C., Wipf-Scheibel, C., Millot, P., Berthier, K., Girardot, G., Gognalons, P., et al. (2020). Distribution and evolution of the major viruses infecting cucurbitaceous and solanaceous crops in the French Mediterranean area. *Virus Res.* 286, 198042. doi: 10.1016/j.virusres.2020.198042.

Dhillon, N. P. S., Ranjana, R., Singh, K., Eduardo, I., Monforte, A. J., Pitrat, M., et al. (2007). Diversity among landraces of Indian snapmelon (*Cucumis melo* var. *momordica*). *Genet. Resour. Crop Evol.* 54, 1267–1283. doi: 10.1007/s10722-006-9108-2.

Díaz, A., Zarouri, B., Fergany, M., Eduardo, I., Álvarez, J. M., Picó, B., et al. (2014). Mapping and Introgression of QTL Involved in Fruit Shape Transgressive Segregation into 'Piel de Sapo' Melon (*Cucumis melo* L.). *PLoS One* 9, e104188. doi: 10.1371/journal.pone.0104188.

Díaz, J. A., Mallor, C., Soria, C., Camero, R., Garzo, E., Fereres, A., et al. (2003). Potential sources of resistance for melon to nonpersistently aphid-borne viruses. *Plant Dis.* 87, 960–964. doi: 10.1094/PDIS.2003.87.8.960.

Doyle, J. (1991). "DNA Protocols for Plants," in *Molecular Techniques in Taxonomy* (Springer Berlin Heidelberg), 283–293. doi: 10.1007/978-3-642-83962-7_18.

Dwivedi, Goldman, and Ortiz (2019). Pursuing the Potential of Heirloom Cultivars to Improve Adaptation, Nutritional, and Culinary Features of Food Crops. *Agronomy* 9, 441. doi: 10.3390/agronomy9080441.

Edelstein, M., Ben-Hur, M., Cohen, R., Burger, Y., and Ravina, I. (2005). Boron and salinity effects on grafted and non-grafted melon plants. *Plant and Soil* (Springer), 273–284. doi: 10.1007/s11104-004-0598-4.

Eduardo, I., Arús, P., and Monforte, A. J. (2005). Development of a genomic library of near isogenic lines (NILs) in melon (*Cucumis melo* L.) from the exotic accession PI161375. *Theor. Appl. Genet.* 112, 139–148. doi: 10.1007/s00122-005-0116-y.

El-Baky, N. A., and Amara, A. A. A. F. (2021). Recent Approaches towards Control of Fungal Diseases in Plants: An Updated Review. *J. Fungi 2021, Vol. 7, Page 900* 7, 900. doi: 10.3390/JOF7110900.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., et al. (2011). A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species. *PLoS One* 6, e19379. doi: 10.1371/journal.pone.0019379.

Endl, J., Achigan-Dako, E. G., Pandey, A. K., Monforte, A. J., Pico, B., and Schaefer, H. (2018). Repeated domestication of melon (*Cucumis melo*) in Africa and Asia and a new close relative from India. *Am. J. Bot.* 105, 1662–1671. doi: 10.1002/AJB2.1172.

Escribano, S., and Lázaro, A. (2009). Agro-morphological diversity of Spanish traditional melons (*Cucumis melo* L.) of the Madrid provenance. *Genet. Resour. Crop Evol.* 56, 481–497. doi: 10.1007/s10722-008-9380-4.

Escribano, S., and Lázaro, A. (2012). Sensorial characteristics of Spanish traditional melon genotypes: Has the flavor of melon changed in the last century? *Eur. Food Res. Technol.* 234, 581–592. doi: 10.1007/s00217-012-1661-7.

Escribano, S., Lázaro, A., Cuevas, H. E., López-Sesé, A. I., and Staub, J. E. (2012). Spanish melons (*Cucumis melo* L.) of the Madrid provenance: A unique germplasm reservoir. *Genet. Resour. Crop Evol.* 59, 359–373. doi: 10.1007/s10722-011-9687-4.

Escribano, S., Sánchez, F. J., and Lázaro, A. (2010). Establishment of a sensory characterization protocol for melon (*Cucumis melo* L.) and its correlation with physical-chemical attributes: Indications for future genetic improvements. *Eur. Food Res. Technol.* 231, 611–621. doi: 10.1007/s00217-010-1313-8.

Escribano, S., Sánchez, F. J., Salces, R., and Lázaro, A. (2007). Caracterización morfológica de variedades tradicionales del melón de Villaconejos. in *Actas XI Congreso SECH. Albacete*, 23–27.

Esteras, C., Formisano, G., Roig, C., Díaz, A., Blanca, J., Garcia-Mas, J., et al. (2013). SNP genotyping in melons: Genetic variation, population structure, and linkage disequilibrium. *Theor. Appl. Genet.* 126, 1285–1303. doi: 10.1007/s00122-013-2053-5.

Esteras, C., Nuez, F., and Picó, B. (2012). "Genetics, Genomics and Breeding of Cucurbits," in *Cucurbits: genetics, genomics and breeding of cucurbits*, eds. Y. Wang, T. K. Behera, and C. Kole (New Hampshire: Science Publishers Inc, Enfield).

Esteras, C., Rambla, J. L., Sánchez, G., Granell, A., and Picó, M. B. (2020). Melon Genetic Resources Characterization for Rind Volatile Profile. *Agronomy* 10, 1512. doi: 10.3390/agronomy10101512.

Esteras, C., Rambla, J. L., Sánchez, G., López-Gresa, M. P., González-Mas, M. C., Fernández-Trujillo, J. P., et al. (2018). Fruit flesh volatile and carotenoid profile analysis within the *Cucumis melo* L. species reveals unexploited variability for future genetic breeding. *J. Sci. Food Agric.* 98, 3915–3925. doi: 10.1002/jsfa.8909.

Eurostats (2021). Available online at: https://ec.europa.eu/eurostat/data/database (accessed June 2022).

Fallik, E., and Ilic, Z. (2014). Grafted vegetables – the influence of rootstock and scion on postharvest quality. *Folia Hortic.* 26, 79–90. doi: 10.2478/fhort-2014-0008.

Fallik, E., and Ziv, C. (2020). How rootstock/scion combinations affect watermelon fruit quality after harvest? *J. Sci. Food Agric.* 100, 3275–3282. doi: 10.1002/jsfa.10325.

FAO (2020). Available online at: http://www.fao.org/faostat/es/#data (accessed June 2022).

FEPEX (2021). Available online at: https://www.fepex.es (accessed June 2022)

Finckh, M. R., Schulte-Geldermann, E., and Bruns, C. (2006). Challenges to organic potato farming: Disease and nutrient management. *Potato Res.* 49, 27–42. doi: 10.1007/s11540-006-9004-3.

Fita, A., Picó, B., Roig, C., and Nuez, F. (2007). Performance of *Cucumis melo* ssp. agrestis as a rootstock for melon. 82, 184–190. doi: 10.1080/14620316.2007.11512218.

Flexas, J., Bota, J., Loreto, F., Cornic, G., and Sharkey, T. D. (2004). Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biol.* 6, 269–279. doi: 10.1055/s-2004-820867.

Flores-León, A., García-Martínez, S., González, V., Garcés-Claver, A., Martí, R., Julián, C., et al. (2021). Grafting Snake Melon [*Cucumis melo* L. subsp. *melo* var. *flexuosus* (L.) Naudin] in Organic Farming: Effects on Agronomic Performance; Resistance to Pathogens; Sugar, Acid, and VOC Profiles; and Consumer Acceptance. *Front. Plant Sci.* 12, 113. doi: 10.3389/fpls.2021.613845.

Flores-León, A., Peréz Moro, C., Martí, R., Beltran, J., Roselló, S., Cebolla-Cornejo, J., et al. (2022). Spanish Melon Landraces: Revealing Useful Diversity by Genomic, Morphological, and Metabolomic Analysis. *Int. J. Mol. Sci.* 23, 7162. doi: 10.3390/ijms23137162.

Forster, D., Andres, C., Verma, R., Zundel, C., Messmer, M. M., and Mäder, P. (2013). Yield and economic performance of organic and conventional cotton-based farming systems - Results from a field trial in India. *PLoS One* 8. doi: 10.1371/journal.pone.0081039.

Fredes, A., Roselló, S., Beltrán, J., Cebolla-Cornejo, J., Pérez-de-Castro, A., Gisbert, C., et al. (2017). Fruit quality assessment of watermelons grafted onto citron melon rootstock. *J. Sci. Food Agric.* 97, 1646–1655. doi: 10.1002/jsfa.7915.

Freilich, S., Lev, S., Gonda, I., Reuveni, E., Portnoy, V., Oren, E., et al. (2015). Systems approach for exploring the intricate associations between sweetness, color and aroma in melon fruits. *BMC Plant Biol.* 15, 1–16. doi: 10.1186/s12870-015-0449-x.

Fu, Q., Zhang, X., Kong, Q., Bie, Z., Wang, H., and others (2018). Grafting onto pumpkin rootstock is an efficient alternative to improve melon tolerance to nacl stress. *Eur. J. Hortic. Sci.* 83, 337–344.

Furno, M., Del Giudice, T., and Cicia, G. (2021). Organic consumers' profile beyond the mean. *Org. Agric.* 11, 337–349. doi: 10.1007/s13165-020-00333-z.

Gaion, L. A., Braz, L. T., and Carvalho, R. F. (2018). Grafting in vegetable crops: A great technique for agriculture. *Int. J. Veg. Sci.* 24, 85–102.

Galpaz, N., Gonda, I., Shem-Tov, D., Barad, O., Tzuri, G., Lev, S., et al. (2018). Deciphering genetic factors that determine melon fruit-quality traits using RNA-Seq-based high-resolution QTL and eQTL mapping. *Plant J.* 94, 169–191. doi: 10.1111/tpj.13838.

García-Jiménez, J., Armengol, J., Sales, R., Jordá, C., and Bruton, B. D. (2008). Fungal pathogens associated with melon collapse in Spain. *EPPO Bull.* 30, 169–173. doi: 10.1111/j.1365-2338.2000.tb00873.x.

Garrett, K. A., Dendy, S. P., Frank, E. E., Rouse, M. N., and Travers, S. E. (2006). Climate change effects on plant disease: genomes to ecosystems.

Garrison, E., and Marth, G. (2012). Haplotype-based variant detection from short-read sequencing. Available at: http://arxiv.org/abs/1207.3907 [Accessed June 3, 2022].

Genesys PGR Available at: https://www.genesys-pgr.org/ [Accessed August 18, 2022].

Giordano, A., Santo Domingo, M., Quadrana, L., Pujol, M., Martín-Hernández, A. M., and Garcia-Mas, J. (2022). CRISPR/Cas9 gene editing uncovers the roles of CONSTITUTIVE TRIPLE RESPONSE 1 and REPRESSOR OF SILENCING 1 in melon fruit ripening and epigenetic regulation. *J. Exp. Bot.* 73, 4022–4033. doi: 10.1093/jxb/erac148.

Gisbert, C., Cáceres, A., Perpiñà, G., Garcés-Claver, A., González, V., Gómez-Guillamón, M. L., et al. (2020). Interspecific hybrids of wild *Cucumis* species ("Fian" and 'Fimy'): New rootstocks for melon highly resistant to biotic soil stress. *Acta Hortic.* 1294, 169–172. doi: 10.17660/ActaHortic.2020.1294.22.

Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., Sun, Q., et al. (2014). TASSEL-GBS: A High Capacity Genotyping by Sequencing Analysis Pipeline. *PLoS One* 9, e90346. doi: 10.1371/JOURNAL.PONE.0090346.

Gómez, P., Rodríguez-Hernández, A. M., Moury, B., and Aranda, M. (2009). Genetic resistance for the sustainable control of plant virus diseases: Breeding, mechanisms and durability. *Eur. J. Plant Pathol.* 125, 1–22. doi: 10.1007/s10658-009-9468-5.

Gonda, I., Burger, Y., Schaffer, A. A., Ibdah, M., Tadmor, Y., Katzir, N., et al. (2016). Biosynthesis and perception of melon aroma. *Biotechnol. flavor Prod.*, 281–305.

González, V., Armijos, E., and Garcés-Claver, A. (2020a). Fungal endophytes as biocontrol agents against the main soil-borne diseases of melon and watermelon in Spain. *Agronomy* 10. doi: 10.3390/agronomy10060820.

González, V., García-Martínez, S., Flores-León, A., Ruiz, J. J., Picó, B., and Garcés-Claver, A. (2020b). *Neocosmospora keratoplastica*, a relevant human fusarial pathogen is found to be associated with wilt and root rot of Muskmelon and Watermelon crops in Spain: epidemiological and molecular evidences. *Eur. J. Plant Pathol.* 156, 1189–1196. doi: 10.1007/s10658-020-01931-z.

González, V., García-Martínez, S., Ruiz, J. J., Flores-León, A., Picó, B., and Garcés-Claver, A. (2020c). First report of *Neocosmospora falciformis* Causing Wilt and Root Rot of Muskmelon in Spain. *Plant Dis.* 104. doi: 10.1094/PDIS-09-19-2013-PDN.

Gonzalo, M. J., Díaz, A., Dhillon, N. P. S., Reddy, U. K., Picó, B., and Monforte, A. J. (2019). Re-evaluation of the role of Indian germplasm as center of melon diversification based on genotyping-by-sequencing analysis. *BMC Genomics* 20, 1–13. doi: 10.1186/s12864-019-5784-0.

Gosme, M., de Villemandy, M., Bazot, M., and Jeuffroy, M. H. (2012). Local and neighbourhood effects of organic and conventional wheat management on aphids, weeds, and foliar diseases. *Agric. Ecosyst. Environ.* 161, 121–129. doi: 10.1016/j.agee.2012.07.009.

Gragera-Facundo, J., Gil-Torralvo, C. G., Gutiérrez-Perera, J. M., Cano-Suárez, J. M., Ávila-Lozano, C., and Esteban-Perdigón, A. (2012). Agronomic and quality evaluation of spanish melon landraces grown under organic farming in extremadura. *Acta Hortic.* 933, 61–68. doi: 10.17660/ACTAHORTIC.2012.933.5.

Grebenščikov, I. (1986). Cucurbitaceae. 914–951 In: *Schultze-Motel, J. (ed.), Rudolf Mansfelds Verzeichnis landwirtschaftlicher und g¨artnerischer Kulturpflanzen, Akademie-Verlag,* Berlin, 914–951.

Grumet, R., McCreight, J. D., McGregor, C., Weng, Y., Mazourek, M., Reitsma, K., et al. (2021). Genetic resources and vulnerabilities of major cucurbit crops. *Genes (Basel).* 12. doi: 10.3390/genes12081222.

Guan, W., Haseman, D., and Nowaskie, D. (2020). Rootstock evaluation for grafted cucumbers grown in high tunnels: Yield and plant growth. *HortScience* 55, 914–919. doi: 10.21273/HORTSCI14867-20.

Guan, W., and Zhao, X. (2014). Techniques for melon grafting. *IFAS Extension. Univ. Florida. Doc. No. HS1257*, 1–5.

Guan, W., Zhao, X., Dickson, D. W., Mendes, M. L., and Thies, J. (2014). Root-knot Nematode Resistance, Yield, and Fruit Quality of Specialty Melons Grafted onto Cucumis metulifer. *HortScience* 49, 1046–1051. doi: 10.21273/HORTSCI.49.8.1046.

Guilherme, M. M. D., Willame, dos S. C., Edgard, H. C. S., Marcus, V. M., Carolina, A. F., Leila, T. B., et al. (2016). Screening melon genotypes for resistance to Meloidogyne enterolobii. *African J. Agric. Res.* 11, 2271–2276. doi: 10.5897/ajar2015.11175.

Guler, Z., Candir, E., Yetisir, H., Karaca, F., and Solmaz, I. (2014). Volatile organic compounds in watermelon (*Citrullus lanatus*) grafted onto 21 local and two commercial bottle gourd (*Lagenaria siceraria*) rootstocks. *J. Hortic. Sci. Biotechnol.* 89, 448–452. doi: 10.1080/14620316.2014.11513105.

Gur, A., Tzuri, G., Meir, A., Sa'Ar, U., Portnoy, V., Katzir, N., et al. (2017). Genome-Wide Linkage-Disequilibrium Mapping to the Candidate Gene Level in Melon (Cucumis melo). *Sci. Rep.* 7, 1–13. doi: 10.1038/s41598-017-09987-4.

Hammer, K., and Gladis, T. (2014). Notes on infraspecific nomenclature and classifications of cultivated plants in Compositae, Cruciferae, Cucurbitaceae, Gramineae (with a remark on *Triticum dicoccon* Schrank) and Leguminosae. *Genet. Resour. Crop Evol.* 61, 1455–1467. doi: 10.1007/s10722-014-0148-8.

He, Y., Zhu, Z., Yang, J., Ni, X., and Zhu, B. (2009). Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity. *Environ. Exp. Bot.* 66, 270–278. doi: 10.1016/j.envexpbot.2009.02.007.

Hoberg, E., Ulrich, D., Schulz, H., Tuvia-Alkali, S., and Fallik, E. (2003). Sensory and quality analysis of different melon cultivars after prolonged storage. 47, 320–324. doi: 10.1002/food.200390074.

Huang, C. H., Zong, L., Buonanno, M., Xue, X., Wang, T., and Tedeschi, A. (2012). Impact of saline water irrigation on yield and quality of melon (*Cucumis melo* cv. Huanghemi) in northwest China. *Eur. J. Agron.* 43, 68–76. doi: 10.1016/j.eja.2012.05.008.

Huang, Y., Bie, Z., He, S., Hua, B., Zhen, A., and Liu, Z. (2010). Improving cucumber tolerance to major nutrients induced salinity by grafting onto *Cucurbita ficifolia*. *Environ. Exp. Bot.* 69, 32–38. doi: 10.1016/j.envexpbot.2010.02.002.

Hunjan, M. S., and Lore, J. S. (2020). "Climate Change: Impact on Plant Pathogens, Diseases, and Their Management," in *Crop Protection Under Changing Climate* (Springer International Publishing), 85–100. doi: 10.1007/978-3-030-46111-9_4.

Huxham, I. M., Jarvis, M. C., Shakespeare, L., Dover, C. J., Johnson, D., Knox, J. P., et al. (1999). Electron-energy-loss spectroscopic imaging of calcium and nitrogen in the cell walls of apple fruits. *Planta* 208, 438–443. doi: 10.1007/s004250050580.

Hyun, D. Y., Sebastin, R., Lee, G.-A., Lee, K. J., Kim, S.-H., Yoo, E., et al. (2021). Genome-Wide SNP Markers for Genotypic and Phenotypic Differentiation of Melon (*Cucumis melo* L.) Varieties Using Genotyping-by-Sequencing. *Int. J. Mol. Sci.* 22, 6722. doi: 10.3390/ijms22136722.

Ibrahim, D. S. (2017). Neuroprotective effect of *Cucumis melo* var. *flexuosus* leaf extract on the brains of rats with streptozotocin-induced diabetes. *Metab. Brain Dis.* 32, 69–75. doi: 10.1007/s11011-016-9886-y.

IFOAM - Organics International | Home Available at: https://www.ifoam.bio/ (Accessed June 2, 2022).

Iman, M. K., Abo-Bakr, M. A., and Hanna, H. Y. (1972). Inheritance of some economic characters in crosses between sweet melon and snake cucumber. I. *Inherit. Qual. characters. Assiut J Ag Sco* 3, 363–380.

Ito, L. A., Gaion, L. A., Galatti, F. S., Braz, L. T., and Santos, J. M. (2014). Resistance of cucurbita rootstocks to nematodes and compatibility of grafting with net melon. *Hortic. Bras.* 32, 297–302.

IUCN (2021).International Union for Conservation of Nature's Red List of Threatened Species. Available at : https://www.iucnredlist.org/ (Accessed June 2022).

Jang, Y., Huh, Y. C., Park, D. K., Mun, B., Lee, S., and Um, Y. (2014). Greenhouse evaluation of melon rootstock resistance to monosporascus root rot and vine decline as well as of yield and fruit quality in grafted 'Inodorus' melons. *Korean J. Hortic. Sci. Technol.* 32, 614–622. doi: 10.7235/hort.2014.14065.

Janick, J., Paris, H. S., and Parrish, D. C. (2007). The Cucurbits of Mediterranean Antiquity: Identification of Taxa from Ancient Images and Descriptions. *Ann. Bot.* 100, 1441–1457. doi: 10.1093/aob/mcm242.

Jeffrey, C. (1980). A review of the Cucurbitaceae. *Bot. J. Linn. Soc.* 81, 233–247. doi: 10.1111/j.1095-8339.1980.tb01676.x.

Johnson, S. J., Kreider, P., and Miles, C. A. (2011). Vegetable Grafting: Eggplants and Tomatoes. Washington State University Extension Pullman, WA, USA.

Jung, J. K. J., Park, G., Oh, J., Jung, J. K. J., Shim, E. J., Chung, S. M., et al. (2020). Assessment of the current infraspecific classification scheme in melon (*Cucumis melo* L.) based on genome-wide single nucleotide polymorphisms. *Hortic. Environ. Biotechnol.* 61, 537–547. doi: 10.1007/s13580-020-00230-0

Júnior, R. S., Senhor, R. F., Michereff, S. J., and Negreiros, A. M. P. (2019). Reaction of melon genotypes to the roots' rot caused by *Monosporascus*. *Rev. Caatinga* 32, 288–294.

Kader, A. A. (2008). Flavor quality of fruits and vegetables. *J. Sci. Food Agric.* 88, 1863–1868. doi: 10.1002/jsfa.3293.

Kaleem, M. M., Nawaz, M. A., Ding, X., Wen, S., Shireen, F., Cheng, J., et al. (2022). Comparative analysis of pumpkin rootstocks mediated impact on melon sensory fruit quality through integration of non-targeted metabolomics and sensory evaluation. *Plant Physiol. Biochem.* 192, 320–330. doi: 10.1016/J.PLAPHY.2022.10.010.

Karaaĝaç, O., and Balkaya, A. (2013). Interspecific hybridization and hybrid seed yield of winter squash *(Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.) lines for rootstock breeding. *Sci. Hortic. (Amsterdam).* 149, 9–12. doi: 10.1016/J.SCIENTA.2012.10.021.

Kassem, M. A., Gosalvez, B., Garzo, E., Fereres, A., Gómez-Guillamon, M. L., and Aranda, M. A. (2015). Resistance to cucurbit aphid-borne yellows virus in melon accession TGR-1551. *Phytopathology* 105, 1389–1396. doi: 10.1094/PHYTO-02-15-0041-R.

Kassem, M. A., Juarez, M., Gómez, P., Mengual, C. M., Sempere, R. N., Plaza, M., et al. (2013). Genetic diversity and potential vectors and reservoirs of Cucurbit aphid-borne yellows virus in southeastern Spain. *Phytopathology* 103, 1188–1197.

Kawaide, T. (1985). Utilization of rootstocks in cucurbits production in Japan. *Japanese Agric. Res. Quaterly* 18, 285–288.

Kishor, D. S., Noh, Y., Song, W. H., Lee, G. P., Park, Y., Jung, J. K., et al. (2021). SNP marker assay and candidate gene identification for sex expression via genotyping-by-sequencing-based genome-wide associations (GWAS) analyses in Oriental melon (*Cucumis melo* L.var.*makuwa*). *Sci. Hortic. (Amsterdam).* 276, 109711. doi: 10.1016/j.scienta.2020.109711.

Kleiman, B., Primoli, A., Koptur, S., and Jayachandran, K. (2020). Weeds, pollinators, and parasitoids-Using weeds for insect manipulation in agriculture. *J. Res. Weed Sci.* 3, 382–390.

Koehler, P. E., and Kays, S. J. (1991). Sweet potato flavor: quantitative and qualitative assessment of optimum sweetness. *J. Food Qual.* 14, 241–249.

Kolayli, S., Kara, M., Tezcan, F., Erim, F. B., Sahin, H., Ulusoy, E., et al. (2010). Comparative study of chemical and biochemical properties of different melon cultivars: Standard, hybrid, and grafted melons. *J. Agric. Food Chem.* 58, 9764–9769. doi: 10.1021/ jf102408y

Koutsika-Sotiriou, M., and Traka-Mavrona, E. (2002). The cultivation of grafted melons in Greece, current status and prospects. in *Acta Horticulturae* (International Society for Horticultural Science), 325–330. doi: 10.17660/ActaHortic.2002.579.54.

Kubota, C., McClure, M. A., Kokalis-Burelle, N., Bausher, M. G., and Rosskopf, E. N. (2008). Vegetable grafting: History, use, and current technology status in North America. in *HortScience* (American Society for Horticultural Science), 1664–1669. doi: 10.21273/hortsci.43.6.1664.

Kumar Gupta, M., Gouda, G., Sabarinathan, S., Donde, R., Rajesh, N., Pati, P., et al. (2021). Phylogenetic Analysis. *Bioinforma. Rice Res.*, 179–207. doi: 10.1007/978-981-16-3993-7_9.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018a). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–1549. doi: 10.1093/molbev/msy096.

Kumar, S., Vats, S., Barman, P., Tyagi, N., Kumari, R., Bangar, P., et al. (2018b). Whole genome SNP identification and validation in *Cucumis melo* L. cultivars using genome resequencing approach. *Indian J. Genet. Plant Breed.* 78, 478–486.

Kusvuran, S., Yasar, F., Ellialtioglu, S., and Abak, K. (2007). Utilizing some of screening methods in order to determine of tolerance of salt stress in the melon (*Cucumis melo* L.). *Res. J. Agric. Biol. Sci.* 3, 40–45.

Kyriacou, M. C., Colla, G., and Rouphael, Y. (2020). Grafting as a Sustainable Means for Securing Yield Stability and Quality in Vegetable Crops. *Agronomy* 10, 1945. doi: 10.3390/agronomy10121945.

Kyriacou, M. C., Leskovar, D. I., Colla, G., and Rouphael, Y. (2018). Watermelon and melon fruit quality: The genotypic and agro-environmental factors implicated. *Sci. Hortic. (Amsterdam).* 234, 393–408. doi: 10.1016/J.SCIENTA.2018.01.032.

Kyriacou, M. C., Rouphael, Y., Colla, G., Zrenner, R., and Schwarz, D. (2017). Vegetable Grafting: The Implications of a Growing Agronomic Imperative for Vegetable Fruit Quality and Nutritive Value. *Front. Plant Sci.* 8, 741. doi: 10.3389/fpls.2017.00741.

Lammerts Van Bueren, E. T., Jones, S. S., Tamm, L., Murphy, K. M., Myers, J. R., Leifert, C., et al. (2011). The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: A review. *NJAS - Wageningen J. Life Sci.* 58, 193–205. doi: 10.1016/j.njas.2010.04.001.

Langmead, B., and Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359. doi: 10.1038/nmeth.1923.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., et al. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* 23, 2947–2948. doi: 10.1093/bioinformatics/btm404.

Lázaro, A., Fernández, I. C., Borrero, M. J., Cabello, F., López-Sesé, A. I., Gómez-Guillamón, M. L., et al. (2017). Agromorphological genetic diversity of Spanish traditional melons. *Genet. Resour. Crop Evol.* 64, 1687–1706. doi: 10.1007/s10722-016-0466-0.

Lecholocholo, N., Shoko, T., Manhivi, V. E., Maboko, M. M., Akinola, S. A., and Sivakumar, D. (2022). Influence of different rootstocks on quality and volatile constituents of cantaloupe and honeydew melons (*Cucumis melo*. L) grown in high tunnels. *Food Chem.* 393, 133388. doi: 10.1016/J.FOODCHEM.2022.133388.

Lecoq, H., and Desbiez, C. (2012). "Viruses of Cucurbit Crops in the Mediterranean Region. An Ever-Changing Picture," in *Advances in Virus Research* (Academic Press Inc.), 67–126. doi: 10.1016/B978-0-12-394314-9.00003-8.

Lecoq, H., and Katis, N. (2014). "Control of Cucurbit Viruses," in *Advances in Virus Research* (Academic Press Inc.), 255–296. doi: 10.1016/B978-0-12-801246-8.00005-6.

Lee, J.-M. (1994). Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. *HortScience* 29, 235–239.

Lee, J.-M. M., and Oda, M. (2003). "Grafting of Herbaceous Vegetable and Ornamental Crops," in *Horticultural Reviews*, 61–124. doi: 10.1002/9780470650851.ch2.

Lee, J. M., Kubota, C., Tsao, S. J., Bie, Z., Echevarria, P. H., Morra, L., et al. (2010). Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Sci. Hortic. (Amsterdam).* 127, 93–105. doi: 10.1016/j.scienta.2010.08.003.

Leida, C., Moser, C., Esteras, C., Sulpice, R., Lunn, J. E., de Langen, F., et al. (2015). Variability of candidate genes, genetic structure and association with sugar accumulation and climacteric behavior in a broad germplasm collection of melon (*Cucumis melo* L.). *BMC Genet.* 16, 1–17. doi: 10.1186/s12863-015-0183-2.

Leonardi, C. (2016). Vegetable grafting tour introduction. *Univ. Catania, Sicily, Italy* 23.

Leonardi, C., Kyriacou, M. C., Gisbert, C., Oztekin, G. B., Mourão, I., Rouphael, Y., et al. (2017). Quality of grafted vegetables. *Veg. grafting Princ. Pract.*, 216–244.

Lester, G. (2006). Consumer preference quality attributes of melon fruits. in *Acta Horticulturae* (International Society for Horticultural Science), 175–181. doi: 10.17660/actahortic.2006.712.17.

Li, B., Zhao, Y., Zhu, Q., Zhang, Z., Fan, C., Amanullah, S., et al. (2017). Mapping of powdery mildew resistance genes in melon (*Cucumis melo* L.) by bulked segregant analysis. *Sci. Hortic. (Amsterdam).* 220, 160–167. doi: 10.1016/j.scienta.2017.04.001.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. doi: 10.48550/arxiv.1303.3997.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25, 2078–2079. doi: 10.1093/bioinformatics/btp352.

Liu, B., Santo Domingo, M., Mayobre, C., Martín-Hernández, A. M., Pujol, M., and Garcia-Mas, J. (2022). Knock-Out of CmNAC-NOR Affects Melon Climacteric Fruit Ripening. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.878037.

López-Sesé, A. I., Staub, J. E., and Gómez-Guillamón, M. L. (2003). Genetic analysis of Spanish melon (*Cucumis melo* L.) germplasm using a standardized molecular-marker array and geographically diverse reference accessions. *Theor. Appl. Genet.* 108, 41–52. doi: 10.1007/s00122-003-1404-z.

López, C., Ferriol, M., and Picó, M. B. (2015). Mechanical transmission of Tomato leaf curl New Delhi virus to cucurbit germplasm: selection of tolerance sources in Cucumis melo. *Euphytica* 204, 679–691. doi: 10.1007/s10681-015-1371-x.

Mani, M. (2022). Organic Pest Management in Horticultural Crops. *Trends Hortic. Entomol.*, 211–241. doi: 10.1007/978-981-19-0343-4_7.

MAPA (2021). Available online at https://www.mapa.gob.es/es/ (Accessed June 2022).

Maroto, J. V., Pascual, B., Miguel, A., San Bautista, A., López-Galarza, S., Baixauli, C., et al. (2003). Influence of different rootstocks and cycles on serpent melon (*Cucumis melo* var. *flexuosus*) production in soilless cultivation. in *Acta Horticulturae* (International Society for Horticultural Science), 319–322. doi: 10.17660/ActaHortic.2003.614.48.

Martí, R., Sánchez, G., Valcárcel, M., Roselló, S., and Cebolla-Cornejo, J. (2019). High throughput FT-MIR indirect analysis of sugars and acids in watermelon. *Food Chem.* 300, 125227. doi: 10.1016/j.foodchem.2019.125227.

Martín-Hernández, A. M., and Picó, B. (2020). Natural Resistances to Viruses in Cucurbits. *Agronomy* 11, 23. doi: 10.3390/agronomy11010023.

Martínez-Martínez, C., Gonzalo, M. J., Sipowicz, P., Campos, M., Martínez-Fernández, I., Leida, C., et al. (2022). A cryptic variation in a member of the Ovate Family Proteins is underlying the melon fruit shape QTL fsqs8.1. *Theor. Appl. Genet.* 135, 785–801. doi: 10.1007/s00122-021-03998-6.

Massey, M., O'Cass, A., and Otahal, P. (2018). A meta-analytic study of the factors driving the purchase of organic food. *Appetite* 125, 418–427. doi: 10.1016/j.appet.2018.02.029.

Matsumoto, Y., Ogawara, T., Miyagi, M., Watanabe, N., and Kuboyama, T. (2011). Response of wild *Cucumis* species to inoculation with fusarium oxysporum f. sp. melonis race 1,2y. *J. Japanese Soc. Hortic. Sci.* 80, 414–419. doi: 10.2503/jjshs1.80.414.

Maurya, D., Pandey, A. K., Kumar, V., Dubey, S., and Prakash, V. (2019). Grafting techniques in vegetable crops: A review. *Int. J. Chem. Stud.* 7, 1664–1672.

McCreight, J. D. (2006). Melon-powdery mildew interactions reveal variation in melon cultigens and *Podosphaera xanthii* races 1 and 2. *J. Am. Soc. Hortic. Sci.* 131, 59–65. doi: 10.21273/jashs.131.1.59.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., et al. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 20, 1297–1303. doi: 10.1101/GR.107524.110.

Mendlinger, S., and Pasternak, D. (1992). Effect of time of salinization on flowering, yield and fruit quality factors in melon, Cucumis melo L. . *J. Hortic. Sci.* 67, 529–534. doi: 10.1080/00221589.1992.11516279.

Menezes Ayres, E. M., Lee, S. M., Boyden, L., and Guinard, J. X. (2019). Sensory Properties and Consumer Acceptance of Cantaloupe Melon Cultivars. *J. Food Sci.* 84, 2278–2288. doi: 10.1111/1750-3841.14724.

Merheb, J., Pawełkowicz, M., Branca, F., Bolibok-Brągoszewska, H., Skarzyńska, A., Pląder, W., et al. (2020). Characterization of lebanese germplasm of snake melon (*Cucumis melo* subsp. *melo* var. *flexuosus*) using morphological traits and ssr markers. *Agronomy* 10. doi: 10.3390/agronomy10091293.

Messelink, G. J., Calvo, F. J., Marín, F., Janssen, D., Messelink, G. J., Calvo, F. J., et al. (2020). Cucurbits. *Integr. Pest Dis. Manag. Greenh. Crop.*, 537–566. doi: 10.1007/978-3-030-22304-5_19.

Miguel, A. and Camacho Ferre, F. (2014). Grafting horticultural plants as technique for the control of soil pathogens. Org. Control Pathog. Prot. Crop. Cult. Pract. Sustain. Agric., 467–502.

Mirtalebi, M., and Banihashemi, Z. (2019). Effect of salinity on root rot of Cucumis melo L. caused by phytophthora melonis. *J. Agric. Sci. Technol.* 21, 209–220.

Moing, A., Allwood, J. W., Aharoni, A., Baker, J., Beale, M. H., Ben-Dor, S., et al. (2020). Comparative Metabolomics and Molecular Phylogenetics of Melon (*Cucumis melo*, Cucurbitaceae) Biodiversity. *Metabolites* 10, 121. doi: 10.3390/metabo10030121.

Monforte, A. J., and Alvarez, J. M. (2006). Mejora de la calidad en melón. *Mejor. genética la Calid. en plantas. SECH, SEG, Univ. Politécnica Val. Val.*, 395–413.

Monforte, A. J., Oliver, M., Gonzalo, M. J., Alvarez, J. M., Dolcet-Sanjuan, R., and Arús, P. (2004). Identification of quantitative trait loci involved in fruit quality traits in melon (*Cucumis melo* L.). *Theor. Appl. Genet.* 108, 750–758. doi: 10.1007/s00122-003-1483-x.

Mudge, K., Janick, J., Scofield, S., and Goldschmidt, E. E. (2009). A history of grafting. in *Horticultural Reviews*, John Wiley & Sons, Ltd, 2009, doi: https://doi.org/10.1002/9780470593776.ch9

Munns, R., Schachtman, D. P., and Condon, A. G. (1995). The significance of a two-phase growth response to salinity in wheat and barley. *Aust. J. Plant Physiol.* 22, 561–569. doi: 10.1071/PP9950561.

Munns, R., and Tester, M. (2008). Mechanisms of Salinity Tolerance. *Annu. Rev. Plant Biol.* 59, 651–681. doi: 10.1146/annurev.arplant.59.032607.092911.

Murphy, K. M., Campbell, K. G., Lyon, S. R., and Jones, S. S. (2007). Evidence of varietal adaptation to organic farming systems. *F. Crop. Res.* 102, 172–177. doi: 10.1016/j.fcr.2007.03.011.

Natarajan, S., Kim, H.-T., Thamilarasan, S. K., Veerappan, K., Park, J.-I., and Nou, I.-S. (2016). Whole Genome Re-Sequencing and Characterization of Powdery Mildew Disease-Associated Allelic Variation in Melon. *PLoS One* 11, e0157524. doi: 10.1371/journal.pone.0157524.

Naudin, C. (1859). Essais d'une monographie des especès et des variétés du genre *Cucumis*. *Ann. des Sci. Nat.* 4, 5–87.

Németh, D., Balázs, G., Bodor, Z., Zaukuu, J. L. Z., Kovács, Z., and Kappel, N. (2020). Food quality attributes of melon (*Cucumis melo* L.) influenced by grafting. in *Progress in Agricultural Engineering Sciences* (Akademiai Kiado ZRt.), 53–66. doi: 10.1556/446.2020.10006.

Nimmakayala, P., Tomason, Y. R., Abburi, V. L., Alvarado, A., Saminathan, T., Vajja, V. G., et al. (2016). Genome-Wide Differentiation of Various Melon Horticultural Groups for Use in GWAS for Fruit Firmness and Construction of a High Resolution Genetic Map. *Front. Plant Sci.* 7, 1437. doi: 10.3389/fpls.2016.01437.

Nischwitz, C., Olsen, M., and Rasmussen, S. (2002). Influence of salinity and root-knot nematode as stress factors in charcoal rot of melon. *Univ. Arizona, Veg. Rep. Artic.*

O'Donnell, K., Kistlerr, H. C., Cigelnik, E., and Ploetz, R. C. (1998). Multiple evolutionary origins of the fungus causing panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proc. Natl. Acad. Sci. U. S. A.* 95, 2044–2049. doi: 10.1073/pnas.95.5.2044.

O'Donnell, K., Sutton, D. A., Fothergill, A., McCarthy, D., Rinaldi, M. G., Brandt, M. E., et al. (2008). Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* Species complex. *J. Clin. Microbiol.* 46, 2477–2490. doi: 10.1128/JCM.02371-07.

Obando-Ulloa, J. M., Ruiz, J., Monforte, A. J., and Fernández-Trujillo, J. P. (2010). Aroma profile of a collection of near-isogenic lines of melon (*Cucumis melo* L.). *Food Chem.* 118, 815–822. doi: 10.1016/j.foodchem.2009.05.068.

Obando, J., Fernández-Trujilio, J. P., Martínez, J. A., Alarcón, A. L., Eduardo, I., Arús, P., et al. (2008). Identification of melon fruit quality quantitative trait loci using near-isogenic lines. *J. Am. Soc. Hortic. Sci.* 133, 139–151. doi: 10.21273/jashs.133.1.139.

Omari, S., Kamenir, Y., Benichou, J. I. C., Pariente, S., Sela, H., and Perl-Treves, R. (2018). Landraces of snake melon, an ancient Middle Eastern crop, reveal extensive morphological and DNA diversity for potential genetic improvement. *BMC Genet.* 19, 34. doi: 10.1186/s12863-018-0619-6.

Oren, E., Tzuri, G., Dafna, A., Rees, E. R., Song, B., Freilich, S., et al. (2022). QTL mapping and genomic analyses of earliness and fruit ripening traits in a melon recombinant inbred lines population supported by de novo assembly of their parental genomes. *Hortic. Res.* 9. doi: 10.1093/HR/UHAB081.

Orsini, F., Sanoubar, R., Oztekin, G. B., Kappel, N., Tepecik, M., Quacquarelli, C., et al. (2013). Improved stomatal regulation and ion partitioning boosts salt tolerance in grafted melon. *Funct. Plant Biol.* 40, 628. doi: 10.1071/FP12350.

Östman, Ö., Ekbom, B., and Bengtsson, J. (2003). Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. *Ecol. Econ.* 45, 149–158. doi: 10.1016/S0921-8009(03)00007-7.

Oumouloud, A., Arnedo-Andrés, M. S., González-Torres, R., and Álvarez, J. M. (2009). Morphological and molecular characterization of melon accessions resistant to *Fusarium* wilts. *Euphytica* 169, 69–79. doi: 10.1007/s10681-009-9942-3.

Ozbahce, A., Kosker, Y., Gultekin, R., Gorgisen, C., Avag, K., Demir, Y., et al. (2021). Impact of different rootstocks and limited water on yield and fruit quality of melon grown in a field naturally infested with Fusarium wilt. *Sci. Hortic. (Amsterdam).* 289, 110482. doi: 10.1016/j.scienta.2021.110482.

Palmer, M. W., Cooper, J., Tétard-Jones, C., Średnicka-Tober, D., Barański, M., Eyre, M., et al. (2013). The influence of organic and conventional fertilisation and crop protection practices, preceding crop, harvest year and weather conditions on yield and quality of potato (Solanum tuberosum) in a long-term management trial. *Eur. J. Agron.* 49, 83–92. doi: 10.1016/j.eja.2013.03.004.

Palomares-Rius, F. J., Garcés-Claver, A., Picó, M. B., Esteras, C., Yuste-Lisbona, F. J., and Gómez-Guillamón, M. L. (2018). 'Carmen', a yellow canary melon breeding line resistant to *Podosphaera xanthii*, *Aphis gossypii*, and *Cucurbit yellow stunting disorder virus*. *HortScience* 53, 1072–1075. doi: 10.21273/HORTSCI13013-18.

Pangalo, K. (1958). Melons [Dyni]. State publisher [Gosudarstvennoe izdatel'stvo]

Panth, M., Hassler, S. C., and Baysal-Gurel, F. (2020). Methods for Management of Soilborne Diseases in Crop Production. *Agric. 2020, Vol. 10, Page 16* 10, 16. doi: 10.3390/AGRICULTURE10010016.

Pardo, J. E., Alvarruiz, A., Varón, R., and Gómez, R. (2000). Quality evaluation of melon cultivars. Correlation among physical-chemical and sensory parameters. *J. Food Qual.* 23, 161–170.

Paris, H. S. (2012). Semitic-language records of snake melons (*Cucumis melo*, Cucurbitaceae) in the medieval period and the "piqqus" of the "faqqous." *Genet. Resour. Crop Evol.* 59, 31–38. doi: 10.1007/s10722-011-9664-y.

Paris, H. S., Amar, Z., and Lev, E. (2012a). Medieval emergence of sweet melons, *Cucumis melo* (Cucurbitaceae). *Ann. Bot.* 110, 23–33. doi: 10.1093/aob/mcs098.

Paris, H. S., Daunay, M.-C., and Janick, J. (2009). The Cucurbitaceae and Solanaceae illustrated in medieval manuscripts known as the Tacuinum Sanitatis. *Ann. Bot.* 103, 1187–1205. doi: 10.1093/aob/mcp055.

Paris, H. S., Daunay, M. C., and Janick, J. (2012b). Occidental diffusion of cucumber (*Cucumis sativus*) 500-1300 CE: Two routes to Europe. *Ann. Bot.* 109, 117–126. doi: 10.1093/aob/mcr281.

Park, D. K., Son, S.-H., Kim, S., Lee, W. M., Lee, H. J., Choi, H. S., et al. (2013). Selection of Melon Genotypes with Resistance to *Fusarium* Wilt and *Monosporascus* Root Rot for Rootstocks. *Plant Breed. Biotechnol.* 1, 277–282. doi: 10.9787/pbb.2013.1.3.277.

Park, E., Luo, Y., Marine, S. C., Everts, K. L., Micallef, S. A., Bolten, S., et al. (2018). Consumer preference and physicochemical evaluation of organically grown melons. *Postharvest Biol. Technol.* 141, 77–85. doi: 10.1016/j.postharvbio.2018.03.001.

Pavan, S., Marcotrigiano, A. R., Ciani, E., Mazzeo, R., Zonno, V., Ruggieri, V., et al. (2017). Genotyping-by-sequencing of a melon (*Cucumis melo* L.) germplasm collection from a secondary center of diversity highlights patterns of genetic variation and genomic features of different gene pools. *BMC Genomics* 18, 59. doi: 10.1186/s12864-016-3429-0.

Pech, J.-C., Purgatto, E., Bouzayen, M., and Latché, A. (2012). "Ethylene and Fruit Ripening," in *Annual Plant Reviews Volume 44* (Oxford, UK: Wiley-Blackwell), 275–304. doi: 10.1002/9781118223086.ch11.

Pech, J. C., Bouzayen, M., and Latché, A. (2008). Climacteric fruit ripening: Ethylene-dependent and independent regulation of ripening pathways in melon fruit. *Plant Sci.* 175, 114–120. doi: 10.1016/j.plantsci.2008.01.003.

Perchepied, L., and Pitrat, M. (2004). Polygenic inheritance of partial resistance to Fusarium oxysporum f. sp. melonis race 1.2 in melon. *Phytopathology* 94, 1331–1336. doi: 10.1094/PHYTO.2004.94.12.1331.

Pereira, L., Ruggieri, V., Pérez, S., Alexiou, K. G., Fernández, M., Jahrmann, T., et al. (2018). QTL mapping of melon fruit quality traits using a high-density GBS-based genetic map. *BMC Plant Biol.* 18, 324. doi: 10.1186/s12870-018-1537-5.

Pereira, L., Santo Domingo, M., Ruggieri, V., Argyris, J., Phillips, M. A., Zhao, G., et al. (2020). Genetic dissection of climacteric fruit ripening in a melon population segregating for ripening behavior. *Hortic. Res.* 7, 187. doi: 10.1038/s41438-020-00411-z.

Pérez-De-Castro, A., Martınez de Alba, A. E., Sáez, C., Flores, A., Sifres, A., Gómez-Guillamón, M. L., et al. (2020). Incidence and genetic diversity of cucurbit viruses in Spain. *Acta Hortic.* 1294, 203–210. doi: 10.17660/ActaHortic.2020.1294.25.

Pérez-García, A., Romero, D., Fernández-Ortuño, D., López-Ruiz, F., de Vicente, A., and Torés, J. A. (2009). The powdery mildew fungus *Podosphaera fusca* (synonym *Podosphaera xanthii*), a constant threat to cucurbits. *Mol. Plant Pathol.* 10, 153–160. doi: 10.1111/j.1364-3703.2008.00527.x.

Perpiñá, G., Cebolla-Cornejo, J., Esteras, C., Monforte, A. J., and Picó, B. (2017). 'MAK-10': A Long Shelf-life Charentais Breeding Line Developed by Introgression of a Genomic Region from Makuwa Melon. *HortScience* 52, 1633–1638. doi: 10.21273/HORTSCI12068-17.

Perpiñá, G., Roselló, S., Esteras, C., Beltrán, J., Monforte, A. J., Cebolla-Cornejo, J., et al. (2021). Analysis of aroma-related volatile compounds affected by 'Ginsen Makuwa' genomic regions introgressed in 'Vedrantais' melon background. *Sci. Hortic. (Amsterdam).* 276, 109664. doi: 10.1016/j.scienta.2020.109664.

Pico, B., Thompson, A. J., Gisbert, C., YETISir, H., and Bebeli, P. (2017). Genetic resources for rootstock breeding. *Veg. grafting Princ. Pract.* 1, 22–69.

Pina, A., Cookson, S. J., Calatayud, Á., Trinchera, A., Errea, P., and others (2017). Physiological and molecular mechanisms underlying graft compatibility. *Veg. grafting Princ. Pract.*, 132–154.

Piperno, D. R., Andres, T. C., and Stothert, K. E. (2000). Phytoliths in Cucurbita and other Neotropical Cucurbitaceae and their occurrence in early archaeological sites from the lowland American tropics. *J. Archaeol. Sci.* 27, 193–208. doi: 10.1006/jasc.1999.0443.

Pitrat, M. (2008). "Melon," in *Vegetables I* (New York, NY: Springer New York), 283–315. doi: 10.1007/978-0-387-30443-4_9.

Pitrat, M. (2016). "Melon Genetic Resources: Phenotypic Diversity and Horticultural Taxonomy," in (Springer, Cham), 25–60. doi: 10.1007/7397_2016_10.

Pitrat, M., Hanelt, P., and Hammer, K. (2000). Some comments on infraspecific classification of cultivars of melon. in *Acta Horticulturae* (International Society for Horticultural Science), 29–36. doi: 10.17660/ActaHortic.2000.510.4.

Ponisio, L. C., M'gonigle, L. K., Mace, K. C., Palomino, J., Valpine, P. De, and Kremen, C. (2015). Diversification practices reduce organic to conventional yield gap. *Proc. R. Soc. B Biol. Sci.* 282. doi: 10.1098/rspb.2014.1396.

Primo, E., and Carrasco, J. M. (1980). *Química Agrícola I. Suelos y Fertilizantes*. Madrid: Alhambra S.A

Radouane, N., Ezrari, S., Belabess, Z., Tahiri, A., Tahzima, R., Massart, S., et al. (2021). Viruses of cucurbit crops: current status in the Mediterranean Region. *Phytopathol. Mediterr.* 60, 493–519.

Reeb, V., Lutzoni, F., and Roux, C. (2004). Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. *Mol. Phylogenet. Evol.* 32, 1036–1060. doi: 10.1016/j.ympev.2004.04.012.

Reganold, J. P., and Wachter, J. M. (2016). Organic agriculture in the twenty-first century. *Nat. plants* 2, 15221. doi: 10.1038/nplants.2015.221.

Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007 *https://webarchive.nationalarchives.gov.uk/eu-exit/https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02018R0848-20201114*.

Rengasamy, P. (2006). World salinization with emphasis on Australia. Oxford Academic doi: 10.1093/jxb/erj108.

Ríos, P., Argyris, J., Vegas, J., Leida, C., Kenigswald, M., Tzuri, G., et al. (2017). *ETHQV6.3* is involved in melon climacteric fruit ripening and is encoded by a NAC domain transcription factor. *Plant J.* 91, 671–683. doi: 10.1111/tpj.13596.

Robinson, R., and Decker-Walters, D. (1997). Cucurbits. New York: CAB International.

Roig, C., Fita, A., Ríos, G., Hammond, J. P., Nuez, F., and Picó, B. (2012). Root transcriptional responses of two melon genotypes with contrasting resistance to *Monosporascus cannonballus* (Pollack et Uecker) infection. *BMC Genomics* 13. doi: 10.1186/1471-2164-13-601.

Rouphael, Y., Cardarelli, M., Rea, E., and Colla, G. (2012). Improving melon and cucumber photosynthetic activity, mineral composition, and growth performance under salinity stress by grafting onto Cucurbita hybrid rootstocks. *Photosynthetica* 50, 180–188. doi: 10.1007/s11099-012-0002-1.

Rouphael, Y., Kyriacou, M. C., and Colla, G. (2018a). Vegetable Grafting: A Toolbox for Securing Yield Stability under Multiple Stress Conditions. *Front. Plant Sci.* 8, 2255. doi: 10.3389/fpls.2017.02255.

Rouphael, Y., Petropoulos, S. A., Cardarelli, M., and Colla, G. (2018b). Salinity as eustressor for enhancing quality of vegetables. *Sci. Hortic. (Amsterdam).* 234, 361–369. doi: 10.1016/J.SCIENTA.2018.02.048.

Rouphael, Y., Schwarz, D., Krumbein, A., and Colla, G. (2010). Impact of grafting on product quality of fruit vegetables. *Sci. Hortic. (Amsterdam)* 127,172-179. doi: 10.1016/j.scienta.2010.09.001

Roustaee, A., Karimpour Reyhan, M., and Jafari, M. (2011). Study of interaction between salinity and charcoal rot diseases of melon (*Macrophomina phaseolina*) in Semnan and Garmsar. *Desert* 16, 175–180. doi: 10.22059/JDESERT.2012.24749.

Roy, A., Bal, S. S., Fergany, M., Kaur, S., Singh, H., Malik, A. A., et al. (2012). Wild melon diversity in India (Punjab State). *Genet. Resour. Crop Evol.* 59, 755–767. doi: 10.1007/s10722-011-9716-3.

Ruiz, M. L., Simón, A., Velasco, L., García, M. C., and Janssen, D. (2015). First report of Tomato leaf curl New Delhi virus infecting tomato in Spain. *Plant Dis.* 99, 894. doi: 10.1094/PDIS-10-14-1072-PDN.

Sabato, D., Esteras, C., Grillo, O., Peña-Chocarro, L., Leida, C., Ucchesu, M., et al. (2019). Molecular and morphological characterisation of the oldest *Cucumis melo* L. seeds found in the Western Mediterranean Basin. *Archaeol. Anthropol. Sci.* 11, 789–810. doi: 10.1007/s12520-017-0560-z.

Sáez, C., Esteras, C., Martínez, C., Ferriol, M., Dhillon, N. P. S., López, C., et al. (2017). Resistance to tomato leaf curl New Delhi virus in melon is controlled by a major QTL located in chromosome 11. *Plant Cell Rep.* 36, 1571–1584. doi: 10.1007/s00299-017-2175-3.

Sáez, C., Flores-León, A., Montero-Pau, J., Sifres, A., Dhillon, N. P. S., López, C., et al. (2022). RNA-Seq Transcriptome Analysis Provides Candidate Genes for Resistance to Tomato Leaf Curl New Delhi Virus in Melon. *Front. Plant Sci.* 12, 3039. doi: 10.3389/fpls.2021.798858.

Safdar, H., Amin, A., Shafiq, Y., Ali, A., Yasin, R., Shoukat, A., et al. (2019). A review: Impact of salinity on plant growth. *Nat. Sci* 17, 34–40.

Saftner, R., Abbott, J. A., Lester, G., and Vinyard, B. (2006). Sensory and analytical comparison of orange-fleshed honeydew to cantaloupe and green-fleshed honeydew for fresh-cut chunks. *Postharvest Biol. Technol.* 42, 150–160. doi: 10.1016/j.postharvbio.2006.02.011.

Saladié, M., Matas, A. J., Isaacson, T., Jenks, M. A., Goodwin, S. M., Niklas, K. J., et al. (2007). A Reevaluation of the Key Factors That Influence Tomato Fruit Softening and Integrity. *Plant Physiol.* 144, 1012–1028. doi: 10.1104/pp.107.097477.

San Bautista, A., Calatayud, A., Nebauer, S. G., Pascual, B., Maroto, J. V., and López-Galarza, S. (2011). Effects of simple and double grafting melon plants on mineral absorption, photosynthesis, biomass and yield. *Sci. Hortic. (Amsterdam).* 130, 575–580. doi: 10.1016/j.scienta.2011.08.009.

Sánchez-Giráldez, H., Ramos, M., and De La Cuadra, C. (2012). Melon cultivars evaluation for organic horticultural production in extremadura. *Acta Hortic.* 933, 69–74. doi: 10.17660/ACTAHORTIC.2012.933.6.

Sari, N., Tan, A., Yanmaz, R., Yetisir, H. I., Balkaya, A., Solmaz, I., et al. (2008). General status of cucurbit genetic resources in Turkey. Proceeding of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae (Pitrat M, ed), INRA, Avignon (France), 21-32

Schoeny, A., Desbiez, C., Millot, P., Wipf-Scheibel, C., Nozeran, K., Gognalons, P., et al. (2017). Impact of Vat resistance in melon on viral epidemics and genetic structure of virus populations. *Virus Res.* 241, 105–115. doi: 10.1016/j.virusres.2017.05.024.

Schoeny, A., Rimbaud, L., Gognalons, P., Girardot, G., Millot, P., Nozeran, K., et al. (2020). Can Winged Aphid Abundance Be a Predictor of Cucurbit Aphid-Borne Yellows Virus Epidemics in Melon Crop? *Viruses* 12, 911. doi: 10.3390/v12090911.

Schrama, M., de Haan, J. J. J., Kroonen, M., Verstegen, H., and Van der Putten, W. H. H. (2018). Crop yield gap and stability in organic and conventional farming systems. *Agric. Ecosyst. Environ.* 256, 123–130. doi: 10.1016/j.agee.2017.12.023.

Schultheis, J., Thompson, W., and Hassell, R. (2015). Specialty melon yield and quality response to grafting in trials conducted in the southeastern United States. *Acta Hortic.* 1086, 269–278. doi: 10.17660/ActaHortic.2015.1086.34.

Sebastian, P., Schaefer, H., Telford, I. R. H., and Renner, S. S. (2010). Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. *Proc. Natl. Acad. Sci. U. S. A.* 107, 14269–14273. doi: 10.1073/pnas.1005338107.

Seufert, V. (2019). "Comparing Yields: Organic Versus Conventional Agriculture," in *Encyclopedia of Food Security and Sustainability*, eds. P. Ferranti, E. M. Berry, and J. R. Anderson (Oxford: Elsevier), 196–208. doi: https://doi.org/10.1016/B978-0-08-100596-5.22027-1.

Seufert, V., Ramankutty, N., and Foley, J. A. (2012). Comparing the yields of organic and conventional agriculture. *Nature* 485, 229–232. doi: 10.1038/nature11069.

Shaik, R. S., Burrows, G. E., Urwin, N. A. R., Gopurenko, D., Lepschi, B. J., and Weston, L. A. (2017). The biology and management of prickly paddy melon (*Cucumis myriocarpus* L.), an important summer annual weed in Australia. *Crop Prot.* 92, 29–40. doi: 10.1016/j.cropro.2016.10.005.

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., et al. (2003). Cytoscape: A software Environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504. doi: 10.1101/gr.1239303.

Sivritepe, N., Sivritepe, H. O., and Eris, A. (2003). The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. *Sci. Hortic. (Amsterdam).* 97, 229–237. doi: 10.1016/S0304-4238(02)00198-X.

Solmaz, I., Kacar, Y. A., Simsek, O., and Sari, N. (2016a). Genetic Characterization of Turkish Snake Melon (*Cucumis melo* L. subsp. *melo flexuosus* Group) Accessions Revealed by SSR Markers. *Biochem. Genet.* 54, 534–543. doi: 10.1007/s10528-016-9739-8.

Solmaz, I., Sari, N., Dogimont, C., and Pitrat, M. (2016b). Evaluation of Turkish melon ccessions for resistance to *Fusarium* wilt, downy mildew, powdery mildew, *Cucumber mosaic* virus and *Zucchini yellow mosaic virus*. *Acta Horticulturae*, 133–139. doi: 10.17660/ActaHortic.2016.1127.22.

Soltani, F., Akashi, Y., Kashi, A., Zamani, Z., Mostofi, Y., and Kato, K. (2010). Characterization of Iranian melon landraces of *Cucumis melo* L. Groups Flexuosus and Dudaim by analysis of morphological characters and random amplified polymorphic DNA. *Breed. Sci.* 60, 34–45. doi: 10.1270/jsbbs.60.34.

Soteriou, G. A., Papayiannis, L. C., and Kyriacou, M. C. (2016). Indexing melon physiological decline to fruit quality and vine morphometric parameters. *Sci. Hortic. (Amsterdam).* 203, 207–215. doi: 10.1016/j.scienta.2016.03.032.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313. doi: 10.1093/bioinformatics/btu033.

Stepansky, A., Kovalski, I., and Perl-Treves, R. (1999). Intraspecific classification of melons (*Cucumis melo* L.) in view of their phenotypic and molecular variation. *Plant Syst. Evol. 1999 2173* 217, 313–332. doi: 10.1007/BF00984373.

Miguel, A., Camacho Ferre, F., and others (2014). Grafting horticultural plants as technique for the control of soil pathogens. Org. Control Pathog. Prot. Crop. Cult. Pract. Sustain. Agric., 467–502.

Suansia, A., and Samal, K. C. (2021). Vegetable grafting: A sustainable and eco-friendly strategy for soil-borne pest and disease management. J. Pharmacogn. Phytochem. 10, 1634–1642.

Subramanian, B., Gao, S., Lercher, M. J., Hu, S., and Chen, W. H. (2019). Evolview v3: A webserver for visualization, annotation, and management of phylogenetic trees. *Nucleic Acids Res.* 47, W270–W275. doi: 10.1093/nar/gkz357.

Swofford, D. L. (2002). Version 4. Sinauer Associates. *PAUP^*. Phylogenetic Anal. Using Parsimony (^* Other Methods)*.

Szamosi, C., Solmaz, I., Sari, N., and Bársony, C. (2010). Morphological evaluation and comparison of Hungarian and Turkish melon (*Cucumis melo* L.) germplasm. *Sci. Hortic. (Amsterdam).* 124, 170–182.

Tang, M., Bie, Z. long, Wu, M. zhu, Yi, H. ping, and Feng, J. Xin (2010). Changes in organic acids and acid metabolism enzymes in melon fruit during development. *Sci. Hortic. (Amsterdam).* 123, 360–365. doi: 10.1016/j.scienta.2009.11.001.

Tang, Y., Zhang, C., Cao, S., Wang, X., and Qi, H. (2015). The Effect of *CmLOX*s on the Production of Volatile Organic Compounds in Four Aroma Types of Melon (*Cucumis melo*). *PLoS One* 10, e0143567. doi: 10.1371/journal.pone.0143567.

Tedeschi, A., Lavini, A., Riccardi, M., Pulvento, C., and d'Andria, R. (2011). Melon crops (*Cucumis melo* L., cv. Tendral) grown in a mediterranean environment under saline-sodic conditions: Part I. Yield and quality. *Agric. Water Manag.* 98, 1329–1338. doi: 10.1016/j.agwat.2011.04.007.

Thangamani, C., Pugalendhi, L., and Punithaveni, V. (2018). Screening wild and cultivated cucurbits against root knot nematode to exploit as rootstocks for grafting in cucumber. *J. Hortic. Sci.* 13, 32–41. doi: 10.24154/jhs.2018.v13i01.003.

Timmusk, S., Nevo, E., Ayele, F., Noe, S., and Niinemets, Y. (2020). Fighting fusarium pathogens in the era of climate change: A conceptual approach. *Pathogens* 9. doi: 10.3390/pathogens9060419.

Trajkova, F., Papadantonakis, N., and Savvas, D. (2006). Comparative effects of NaCl and CaCl2 salinity on cucumber grown in a closed hydroponic system. *HortScience* 41, 437–441. doi: 10.21273/hortsci.41.2.437.

Traka-Mavrona, E., Koutsika-Sotiriou, M., and Pritsa, T. (2000). Response of squash (*Cucurbita* spp.) as rootstock for melon (*Cucumis melo* L.). *Sci. Hortic. (Amsterdam).* 83, 353–362. doi: 10.1016/S0304-4238(99)00088-6.

Trionfetti Nisini, P., Colla, G., Granati, E., Temperini, O., Crinò, P., and Saccardo, F. (2002). Rootstock resistance to fusarium wilt and effect on fruit yield and quality of two muskmelon cultivars. *Sci. Hortic. (Amsterdam).* 93, 281–288. doi: 10.1016/S0304-4238(01)00335-1.

Tripodi, G., Condurso, C., Cincotta, F., Merlino, M., and Verzera, A. (2020). Aroma compounds in mini-watermelon fruits from different grafting combinations. *J. Sci. Food Agric.* 100, 1328–1335. doi: 10.1002/jsfa.10149.

Tzuri, G., Zhou, X., Chayut, N., Yuan, H., Portnoy, V., Meir, A., et al. (2015). A 'golden' SNP in *CmOr* governs the fruit flesh color of melon (*Cucumis melo*). *Plant J.* 82, 267–279. doi: 10.1111/tpj.12814.

Ulas, A., Aydin, A., Ulas, F., Yetisir, H., and Miano, T. F. (2020). *Cucurbita* Rootstocks Improve Salt Tolerance of Melon Scions by Inducing Physiological, Biochemical and Nutritional Responses. *Horticulturae* 6, 66. doi: 10.3390/horticulturae6040066.

Velasco, L., Ruiz, L., Galipienso, L., Rubio, L., and Janssen, D. (2020). A Historical Account of Viruses in Intensive Horticultural Crops in the Spanish Mediterranean Arc: New Challenges for a Sustainable Agriculture. *Agronomy* 10, 860. doi: 10.3390/agronomy10060860.

Verzera, A., Dima, G., Tripodi, G., Condurso, C., Crinò, P., Romano, D., et al. (2014). Aroma and sensory quality of honeydew melon fruits (*Cucumis melo* L. subsp. melo var. *inodorus* H. Jacq.) in relation to different rootstocks. *Sci. Hortic. (Amsterdam).* 169, 118–124. doi: 10.1016/j.scienta.2014.02.008.

Villalobos, F. J., Mateos, L., Quemada, M., Delgado, A., and Fereres, E. (2016). "Control of salinity," in *Principles of Agronomy for Sustainable Agriculture* (Springer), 295–320.

Visconti, F., Salvador, A., Navarro, P., and de Paz, J. M. (2019). Effects of three irrigation systems on 'Piel de sapo' melon yield and quality under salinity conditions. *Agric. Water Manag.* 226, 105829. doi: 10.1016/j.agwat.2019.105829.

Wafi Al-Taae, H. H., and Al-Taae, A. K. (2019). First molecular identification of *Fusarium oxysporum* causing *Fusarium* wilt of Armenian cucumber in Iraq. in *IOP Conference Series: Earth and Environmental Science* (Institute of Physics Publishing), 012005. doi: 10.1088/1755-1315/388/1/012005.

Wang, L. M., Zhang, L. D., Chen, J. B., Huang, D. F., Zhang, Y. D., and others (2016). Physiological analysis and transcriptome comparison of two muskmelon (*Cucumis melo* L.) cultivars in response to salt stress. *Genet. Mol. Res* 15, 1–18.

Wang, X., Ando, K., Wu, S., Reddy, U. K., Tamang, P., Bao, K., et al. (2021). Genetic characterization of melon accessions in the U.S. National Plant Germplasm System and construction of a melon core collection. *Mol. Hortic.* 1, 11. doi: 10.1186/s43897-021-00014-9.

Wasylikowa, K., and Van Der Veen, M. (2004). An archaeobotanical contribution to the history of watermelon, *Citrullus lanatus* (Thunb.) Matsum. & Nakai (syn. C. vulgaris Schrad.). *Veg. Hist. Archaeobot.* 13, 213–217. doi: 10.1007/s00334-004-0039-6.

Watson, C. A. A., Bengtsson, H., Ebbesvik, M., Løes, A.-K., Myrbeck, A., Salomon, E., et al. (2002). A review of farm-scale nutrient budgets for organic farms as a tool for management of soil fertility. *Soil Use Manag.* 18, 264–273. doi: 10.1111/j.1475-2743.2002.tb00268.x.

White, T. J., Bruns, T., Lee, S., Taylor, J., and others (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protoc. a Guid. to methods Appl.* 18, 315–322.

Wickham, H. (2016). *ggplot2: elegant graphics for data analysis*. Springer.

Wild, A. (2003). Soils, land and food: managing the land during the twenty-first century. Cambridge University Press.

Wimer, J., Inglis, D., and Miles, C. (2015). Field and greenhouse evaluations of cucurbit rootstocks to improve *Verticillium* resistance for grafted watermelon. *HortScience* 50, 1625–1630. doi: 10.21273/hortsci.50.11.1625.

Yarsi, G., Sari, N., and Yetisir, H. (2012). Effect of different rootstocks on the yield and quality of grafted melon plants. in *Acta Horticulturae* (International Society for Horticultural Science), 411–416. doi: 10.17660/ActaHortic.2012.936.54.

Yousif, M. T., Kheyr-Pour, A., Gronenborn, B., Pitrat, M., and Dogimont, C. (2007). Sources of resistance to Watermelon Chlorotic Stunt Virus in melon. *Plant Breed.* 126, 422–427. doi: 10.1111/j.1439-0523.2007.01366.x.

Zarid, M., Bueso, M. C., and Fernández-Trujillo, J. P. (2020). Seasonal effects on flesh volatile concentrations and texture at harvest in a near-isogenic line of melon with introgression in LG X. *Sci. Hortic. (Amsterdam).* 266, 109244. doi: 10.1016/J.SCIENTA.2020.109244.

Zhang, C., Dong, S.-S., Xu, J.-Y., He, W.-M., and Yang, T.-L. (2019). PopLDdecay: a fast and effective tool for linkage disequilibrium decay analysis based on variant call format files. *Bioinformatics* 35, 1786–1788. doi: 10.1093/bioinformatics/bty875.

Zhang, H., Dong, H., Li, W., Sun, Y., Chen, S., and Kong, X. (2009). Increased glycine betaine synthesis and salinity tolerance in AhCMO transgenic cotton lines. *Mol. Breed.* 23, 289–298. doi: 10.1007/s11032-008-9233-z.

Zhang, N., Huang, X., Bao, Y., Wang, B., Zeng, H., Cheng, W., et al. (2017). Genome-wide identification of SAUR genes in watermelon (*Citrullus lanatus*). *Physiol. Mol. Biol. Plants* 23, 619–628. doi: 10.1007/s12298-017-0442-y.

Zhao, G., Lian, Q., Zhang, Z., Fu, Q., He, Y., Ma, S., et al. (2019). A comprehensive genome variation map of melon identifies multiple domestication events and loci influencing agronomic traits. *Nat. Genet.* 51, 1607–1615. doi: 10.1038/s41588-019-0522-8.

Zhao, X., Guo, Y., Huber, D. J., and Lee, J. (2011). Grafting effects on postharvest ripening and quality of 1-methylcyclopropene-treated muskmelon fruit. *Sci. Hortic. (Amsterdam).* 130, 581–587. doi: 10.1016/j.scienta.2011.08.010.

Zheng, J., Zhang, Z., Gong, Z., Liang, Y., Sang, Z., Xu, Y., et al. (2021). Genome-Wide Association Analysis of Salt-Tolerant Traits in Terrestrial Cotton at Seedling Stage. *Plants* 11, 97. doi: 10.3390/plants11010097.

Zhou, X., Wu, Y., Chen, S., Chen, Y., Zhang, W., Sun, X., et al. (2014). Using *Cucurbita* rootstocks to reduce fusarium wilt incidence and increase fruit yield and carotenoid content in oriental melons. *HortScience* 49, 1365–1369. doi: 10.21273/hortsci.49.11.1365.

Zikeli, S., Deil, L., and Möller, K. (2017). The challenge of imbalanced nutrient flows in organic farming systems: A study of organic greenhouses in Southern Germany. *Agric. Ecosyst. Environ.* 244, 1–13. doi: 10.1016/j.agee.2017.04.017.

Zohary, D. (2000). Domestication of plants in the Old World: the origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. 3rd ed. Oxford : Oxford University Press,.