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TESIS DOCTORAL

Assessment of genetic and agronomic strategies to enhance ecological resilience and preserve fruit quality in tomato landraces

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Abstract

Tomato is one of the most widely grown horticultural crops worldwide because of its socioeconomic and nutritional value. However, the progressive loss of organoleptic quality in the fruit, resulting from selection aimed primarily at maximising yield, has highlighted the value of traditional varieties as a source of genetic diversity. These varieties exhibit remarkable variability in the morphology, flavour, texture, and bioactive composition of the fruit, enabling the identification of accessions with superior organoleptic and functional attributes compared to commercial varieties, while simultaneously addressing their main limitations, lower yield, and susceptibility to pathogens without compromising their distinctive qualities. Furthermore, their ability to adapt to different local agroclimatic conditions makes them a valuable genetic resource for overcoming agronomic and environmental challenges. The objectives of this doctoral thesis were to: i) identify, within a large collection of traditional tomato varieties, those with outstanding organoleptic and functional qualities; ii) develop and evaluate F1 hybrids derived from these varieties as a strategy to increase yield without compromising quality; iii) analyse hybrids generated from Muchamiel-type varieties and improved lines carrying the ToMV, TSWV, and TYLCV resistance genes, to preserve their characteristic morphology, quality, and balanced yield; and iv) study the response of the selected varieties in low-input cropping systems to identify genotypes capable of maintaining fruit productivity and quality under such conditions. This doctoral thesis provides an exhaustive characterisation of the fruit quality of forty-eight traditional tomato varieties from south-eastern Spain, revealing a significant variability in flavour-related and bioactive compounds. Varieties that exhibited both acceptable agronomic yield and fruit quality characteristics were identified for direct valorisation or use in breeding programs. Eighteen hybrids were developed from these accessions to generate new genetic combinations that integrate the distinctive quality attributes of traditional varieties with agronomic advantages of hybrid vigour. Six of these hybrids excelled due to their balanced performance, combining high yields with exceptional fruit quality in terms of both flavour and bioactive compounds, thus increasing their potential competitiveness in the market. To address the susceptibility to viral diseases commonly found in traditional varieties, hybrids were developed by crossing two breeding lines that carry homozygous virus resistance genes, UMH1139 (*Tm-2a* and *Sw-5*) and UMH1200 (*Tm-2a*, *Sw-5*, and *Ty-1*), with two selected Muchamiel varieties. Introducing a single copy of these genes preserved the morphological identity of the Muchamiel type, maintained or improved fruit quality, and increased yield owing to hybrid vigour. The negative effects of homozygosity associated with linkage drag from the *Ty-1* gene on fruit diameter and organic acid concentration were largely mitigated in the hybrids, which exhibited diameters comparable to those of traditional varieties and intermediate organic acid levels between the UMH1200 breeding

line and traditional varieties. Finally, the evaluation of the potential of the selected varieties in low-input cropping systems revealed that they responded differently depending on the variety. Pera Pinatar demonstrated the greatest resilience, maintaining yield in all treatments and preserving or enhancing certain metabolites. Flor de Baladre and Muchamiel rizado maintained yield in low-input treatments and maintained or improved fruit quality, except in the treatment involving the most concentrated nutrient solution (100F+75I). Negro de Nerpio, which had a lower yield than the other varieties, was particularly sensitive to the most diluted nutrient solution (50F+100I). Hybrids H1 and H2 were more productive than their traditional parent variety, Negro de Nerpio, owing to hybrid vigour. Furthermore, H1 maintained the yield when irrigation was reduced, improving quality by increasing certain metabolic compounds. In contrast, H2 was the most sensitive variety, with a reduced yield and no consistent improvements in metabolites under low-input conditions.

Resumen

El tomate es uno de los cultivos hortícolas más extendidos a nivel mundial debido a su importancia socioeconómica y nutricional. La pérdida progresiva de calidad organoléptica del fruto, como consecuencia de la selección orientada principalmente a maximizar el rendimiento, ha puesto de manifiesto el valor de las variedades tradicionales como fuente de diversidad genética. Estas variedades presentan una notable variabilidad en la morfología, el sabor, la textura y la composición bioactiva del fruto, lo que permite identificar accesiones con atributos organolépticos y funcionales superiores a los de las variedades comerciales, abordando simultáneamente sus principales limitaciones, menor rendimiento y susceptibilidad a patógenos, sin comprometer sus cualidades distintivas. Además, su adaptación a diferentes condiciones agroclimáticas locales las convierte en un recurso genético de gran interés para afrontar los desafíos agronómicos y ambientales actuales. Los objetivos de esta tesis doctoral fueron: i) identificar, dentro de una amplia colección de variedades tradicionales de tomate, aquellas con cualidades organolépticas y funcionales sobresalientes; ii) desarrollar y evaluar híbridos F_1 derivados de dichas variedades como estrategia para aumentar el rendimiento sin comprometer la calidad; iii) analizar los híbridos generados a partir de variedades tipo Muchamiel y líneas mejoradas portadoras de los genes de resistencia ToMV, TSWV y TYLCV, buscando conservar la morfología y calidad características junto con un rendimiento equilibrado; y iv) estudiar la respuesta de las variedades seleccionadas en sistemas de cultivo de bajos insumos, con el fin de identificar genotipos capaces de mantener la productividad y la calidad del fruto bajo dichas condiciones. En la presente tesis doctoral se caracterizó de forma exhaustiva la calidad de los frutos de cuarenta y ocho variedades tradicionales de tomate del sureste de España, observándose una amplia variabilidad en compuestos relacionados con el sabor y bioactivos. Se identificaron variedades con un rendimiento agronómico aceptable y características de calidad de fruto, útiles para su valorización directa o su uso en programas de mejora. A partir de estas accesiones se desarrollaron dieciocho híbridos, lo que permitió generar nuevas combinaciones genéticas que integraron los atributos de calidad distintivos de las variedades tradicionales con las ventajas agronómicas derivadas del vigor híbrido, alcanzando en algunos casos rendimientos comparables a los de las variedades comerciales de referencia. Seis híbridos destacaron por su comportamiento equilibrado, combinando buenos rendimientos con una calidad de fruto destacada, tanto en compuestos de sabor como bioactivos, lo que incrementa su posible competitividad en el mercado. Para superar la alta susceptibilidad a enfermedades víricas que presentan habitualmente las variedades tradicionales, se generaron híbridos mediante el cruce de dos líneas de mejora portadoras de los genes de resistencia a virus en homocigosis, UMH1139 (*Tm-2a* y *Sw-5) y* UMH1200 *(Tm-2a, Sw-5* y *Ty-1*) con dos variedades Muchamiel seleccionadas. La introducción

de una sola copia de estos genes preservó la identidad morfológica del tipo Muchamiel, mantuvo o incluso mejoró la calidad del fruto y aumentó el rendimiento gracias al vigor híbrido. Los efectos negativos en homocigosis, asociados al arrastre del ligamiento del gen *Ty-1,* sobre el diámetro del fruto y la concentración de ácidos orgánicos, se mitigaron en gran medida en los híbridos, que mostraron diámetros similares a los de las variedades tradicionales y niveles intermedios de ácidos orgánicos entre la línea de mejora UMH1200 y las variedades tradicionales. Finalmente, la evaluación del potencial de variedades seleccionadas en sistemas de cultivo de bajos insumos mostró respuestas diferenciales según la variedad. Pera Pinatar mostró la mayor resiliencia, manteniendo el rendimiento en todos los tratamientos y conservando o mejorando algunos metabolitos. Flor de Baladre y Muchamiel rizado mantuvieron el rendimiento en tratamientos de bajos insumos y mantuvieron o mejoraron la calidad de la fruta, excepto en el tratamiento con solución nutritiva más concentrada (100F+75I). Negro de Nerpio, que mostró un rendimiento bajo en comparación con las otras variedades, fue particularmente sensible a la solución nutritiva más diluida (50F+100I). Los híbridos H1 y H2 lograron una mayor productividad que su parental tradicional común, Negro de Nerpio, asociado al vigor híbrido. Además, H1 mantuvo el rendimiento al disminuir el riego, mejorando la calidad mediante el aumento de ciertos compuestos metabólicos. Por el contrario, H2 fue la más sensible, con un rendimiento reducido y sin mejoras consistentes en los metabolitos en condiciones de bajos insumos.

Resum

La tomaca és un dels cultius hortícoles més estesos a nivell mundial a causa de la seua importància socioeconòmica i nutricional. La pèrdua progressiva de qualitat organolèptica del fruit, a conseqüència de la selecció orientada principalment a maximitzar el rendiment, ha posat de manifest el valor de les varietats tradicionals com a font de diversitat genètica. Estes varietats presenten una notable variabilitat en la morfologia, el sabor, la textura i la composició bioactiva del fruit, la qual cosa permet identificar accessions amb atributs organolèptics i funcionals superiors als de les varietats comercials, abordant simultàniament les seues principals limitacions, menor rendiment i susceptibilitat a patògens, sense comprometre les seues qualitats distintives. A més, la seua adaptació a diferents condicions agroclimàtiques locals les convertix en un recurs genètic de gran interés per a afrontar els desafiaments agronòmics i ambientals actuals. Els objectius d'esta tesi doctoral van ser: i) identificar, dins d'una àmplia collecció de varietats tradicionals de tomaca, aquelles amb qualitats organolèptiques i funcionals excellents; ii) desenrotllar i avaluar híbrids F_1 derivats d'estes varietats com a estratègia per a augmentar el rendiment sense comprometre la qualitat; iii) analitzar els híbrids generats a partir de varietats tipus Mutxamel i línies millorades portadores dels gens de resistència ToMV, TSWV i TYLCV, buscant conservar la morfologia i qualitat característiques juntament amb un rendiment equilibrat; iv) estudiar la resposta de les varietats seleccionades en sistemes de cultiu de baixos inputs, amb la finalitat d'identificar genotips capaços de mantindre la productivitat i la qualitat del fruit sota estes condicions. En la present tesi doctoral es va caracteritzar de manera exhaustiva la qualitat dels fruits de quaranta-huit varietats tradicionals de tomaca del sud-est d'Espanya, observantse una àmplia variabilitat en compostos relacionats amb el sabor i bioactivos. Es van identificar varietats amb un rendiment agronòmic acceptable i característiques de qualitat de fruit, útils per a la seua valorització directa o el seu ús en programes de millora. A partir d'estes accessions es van desenrotllar díhuit híbrids, la qual cosa va permetre generar noves combinacions genètiques que van integrar els atributs de qualitat distintius de les varietats tradicionals amb els avantatges agronòmics derivats del vigor híbrid, aconseguint en alguns casos rendiments comparables als de les varietats comercials de referència. Sis híbrids van destacar pel seu comportament equilibrat, combinant bons rendiments amb una qualitat de fruit destacada, tant en compostos de sabor com bioactivos, la qual cosa incrementa la seua possible competitivitat en el mercat. Per a superar l'alta susceptibilitat a malalties víriques que presenten habitualment les varietats tradicionals, es van generar híbrids mitjançant l'encreuament de dos línies de millora portadores dels gens de resistència a virus en homocigosis, UMH1139 (*Tm-2a* i *Sw-5*) i UMH1200 (*Tm-2a, Sw-5* i *Ty-1*) amb dos varietats Mutxamel seleccionades. La introducció d'una sola còpia d'estos gens va preservar la identitat morfològica del tipus Mutxamel, va mantindre o fins i tot va millorar la

qualitat del fruit i va augmentar el rendiment gràcies al vigor híbrid. Els efectes negatius en homocigosis, associats a l'arrossegament del ligamiento del gen *Ty-1*, sobre el diàmetre del fruit i la concentració d'àcids orgànics, es van mitigar en gran manera en els híbrids, que van mostrar diàmetres similars als de les varietats tradicionals i nivells intermedis d'àcids orgànics entre la línia de millora UMH1200 i les varietats tradicionals. Finalment, l'avaluació del potencial de varietats seleccionades en sistemes de cultiu de baixos inputs va mostrar respostes diferencials segons la varietat. Pera Pinatar va mostrar la major resiliència, mantenint el rendiment en tots els tractaments i conservant o millorant alguns metabòlits. Flor de Baladre i Mutxamel arrissat van mantindre el rendiment en tractaments de baixos inputs i van mantindre o van millorar la qualitat de la fruita, excepte en el tractament amb solució nutritiva més concentrada (100F+75I). Negre de Nerpio, que va mostrar un rendiment baix en comparació amb les altres varietats, va anar particularment sensible a la solució nutritiva més diluïda (50F+100I). Els híbrids H1 i H2 van aconseguir una major productivitat que el seu parental tradicional comú, Negre de Nerpio, associat al vigor híbrid. A més, H1 va mantindre el rendiment en disminuir el reg, millorant la qualitat mitjançant l'augment d'uns certs compostos metabòlics. Per contra, H2 va ser la més sensible, amb un rendiment reduït i sense millores consistents en els metabòlits en condicions de baixos inputs.

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1. Introduction

1.1. Socio-economic importance of tomato

The versatility of tomato consumption has driven a significant increase in its production, placing it twelfth in the global ranking and consolidating its position as the most important vegetable worldwide (FAO, 2023a). From 2000 to 2023, the global production of this fruit increased from approximately 109 to over 192 million tonnes (Mt), representing an increase of 57%. This trend has been accompanied by a more moderate expansion in cultivated area, from 3.9 to 5.4 million hectares (Mha), reflecting an improvement in productivity and the intensification of cropping systems. In 2023, China remained the largest producer, contributing 36% of world production, followed by India (11%), Turkey (7%) and the United States (6%) (Figure 1). Spain occupied the tenth position, with 2.1% of global production, equivalent to nearly 4 Mt (FAO, 2023a).

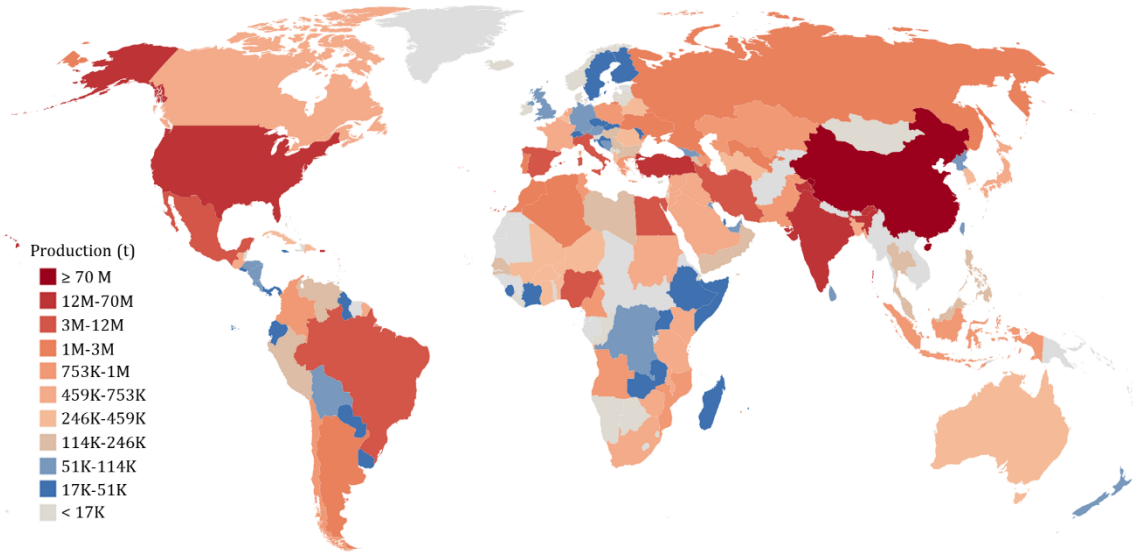

Figure 1. Distribution of global tomato production in 2023 (FAO, 2023).

At the national level in Spain, tomato cultivation is the leading horticultural crop in terms of cultivated area, with lettuce and broccoli ranking in second and third places, respectively (MAPA, 2023). Spain produced around 4 Mt of tomatoes on approximately 50,000 hectares in 2023, of which about 40% was destined for the fresh market. Both tomato harvested area and production showed parallel dynamics from 2011 to 2023. They increased steadily until 2016, when production reached a maximum of 5.23 Mt, and subsequently fluctuated, with a marked decline in 2022 followed by a partial recovery in 2023 (Figure 2).

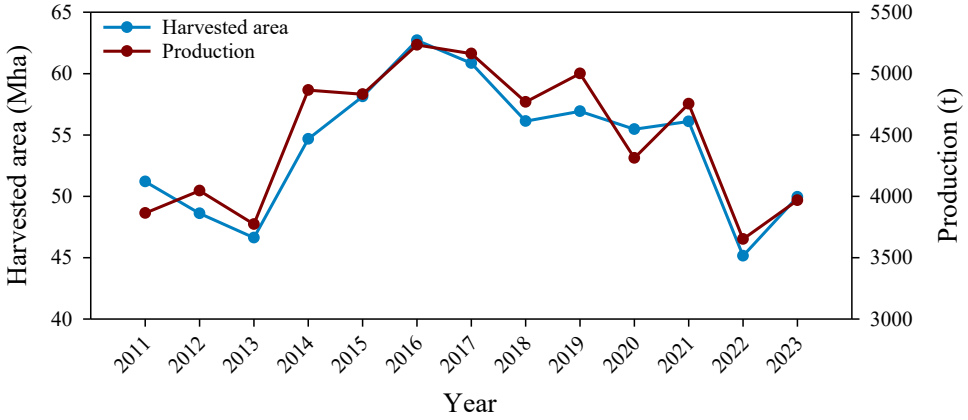

Figure 2. Evolution of harvested area (Mha) and tomato production (t) in Spain between 2011 and 2023 (MAPA, 2023).

Tomato cultivation for fresh consumption in Spain is divided mainly between protected systems, which cover about 80% of the area, and open-field systems, which account for the remaining 20% (MAPA, 2023). Greenhouse tomato production in Spain is concentrated in the south-east of the country, an area with a predominantly semi-arid climate. Within this region, Almería (8,500 ha), Murcia (2,000 ha) and Alicante (187 ha) together represented 57% of the national output in 2023. Other provinces such as Granada, Málaga, and Las Palmas de Gran Canaria, also contribute significantly to the fresh market. In contrast, Extremadura is particularly recognised for its large-scale tomato production destined for processing.

From a socio-economic perspective, tomatoes represent a key pillar of Spanish agriculture. Beyond their annual production volume, they contribute substantially to both direct and indirect employment, thereby acting as an economic driver in many rural regions. In addition, Spain is one of the leading tomato exporters in Europe. In 2023, fresh tomato exports amounted to 493,400 t. Although export volumes have declined in recent seasons, the average export price reached a record 179.6€/100 kg, 33% above the five-year average (MAPA, 2023). Within the EU, Germany continues to be the main destination (25% of total exports), followed by the Netherlands and France.

1.2.Tomato fruit quality

1.2.1. Visual quality

The quality of tomato fruit is determined by a number of attributes, of which external appearance or visual quality is a decisive factor in terms of market acceptance. Visual traits are particularly important at the point of purchase, since characteristics such as flavour and texture can only be assessed after consumption (Oltman et al., 2014).

Fruit colour is one of the most important visual traits, as it directly affects consumer perception of ripeness and market acceptability. In tomato, this attribute exhibits remarkable diversity, with shades ranging from white and yellow to orange, red, pink, purple and even black (Figure 3) (Yang et al., 2023). This variation arises from differences in the concentration and proportion of pigments, primarily carotenoids and chlorophylls, in the pulp and epidermis (Flores et al., 2017). The characteristic red colour of most cultivated varieties is largely due to the accumulation of the carotenoid *all-trans*-lycopene in the pulp and the flavonoid naringenin chalcone in the skin. Variations from this standard pigmentation pattern are often linked to specific genetic mutations that affect pigment biosynthesis or accumulation. The loss-of-function mutation in the *PSY1* gene (locus *r*) promotes phytoene accumulation while blocking downstream carotenoid synthesis, producing yellow fruit (Kang et al., 2017). The orange pigmentation is usually associated with increased *β*-carotene accumulation at the expense of lycopene (Ronen et al., 2000), whereas pink fruit is caused by the absence of naringenin in the epidermis, resulting in colourless skin (Ballester et al., 2010). Finally, black-fruited types are characterised by chlorophyll retention during ripening, which, in combination with lycopene accumulation, confers their distinctive dark appearance (Hu et al., 2011).

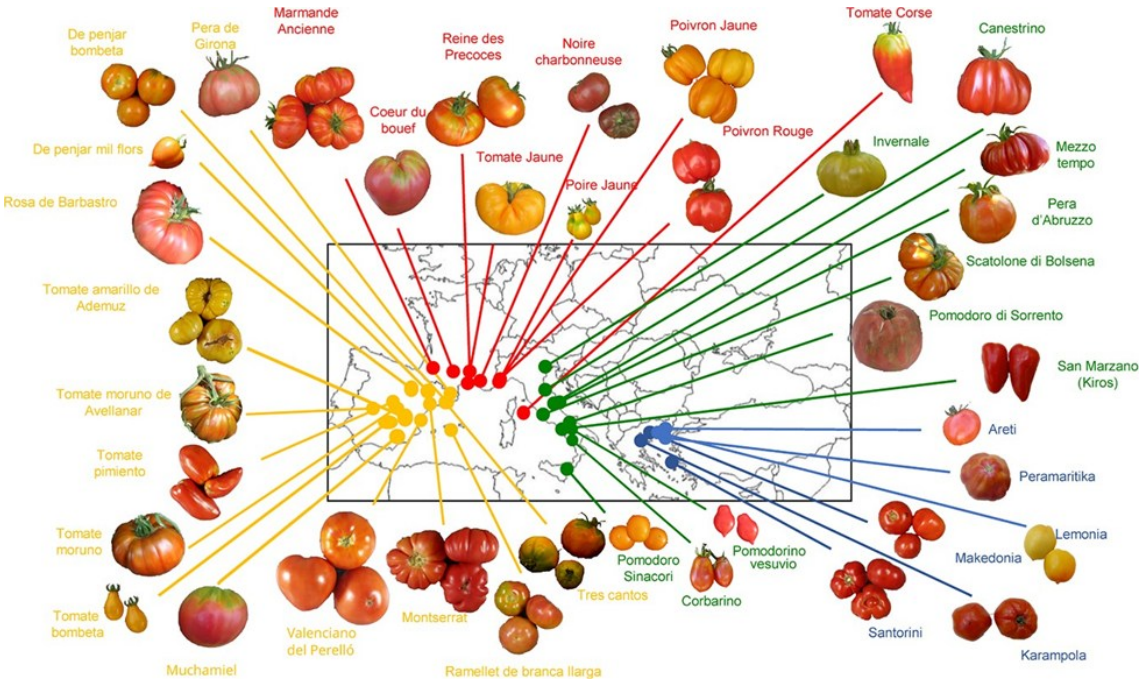

Figure 3. Morphological diversity of tomato fruit varieties in Europe. The map indicates the traditional cultivation area of each variety, with the colour of the lines and names corresponding to the country of origin: Spain (orange), France (red), Italy (green) and Greece (blue). Source: Pons et al. (2022).

Apart from colour, fruit size and shape are key visual attributes, and these are interconnected and shaped by genetic factors as well as culinary selection pressures. Wild tomato relatives typically bear small, round, bilocular fruits, whereas cultivated varieties display

remarkable morphological diversity (Figure 3). According to international descriptors (IPGRI, 1996) and analyses performed using Tomato Analyzer software (Brewer et al., 2006), eight main shape categories have been established: flat, ellipsoid, rectangular, oxheart, heart, elongated, ovoid, and round (Monforte et al., 2014; Rodríguez et al., 2011). Fruit size also varies considerably, ranging from less than 20 g for standard cherry types to over 700 g for large fruited cultivars (Casals et al., 2019). This attribute plays an important role in consumer acceptance, and together with colour, it has been identified as one of the most influential traits in purchase decisions (Wolters and van Gemert, 1990). Subsequent studies have confirmed that approximately half of consumers consider fruit size to be a decisive factor, preferring medium-sized fruits (150–200 g) for salads, as larger tomatoes are often associated with mealy textures (Oltman et al., 2014).

Tomato visual quality can also be severely affected by the occurrence of physiopathies or external defects, which are a frequent cause of commercial rejection. While these disorders are not solely determined by genetic factors, they are strongly influenced by environmental conditions and agronomic practices. One of the most significant disorders is fruit cracking, which results from an imbalance between internal pressure and epidermal resistance. This leads to the formation of radial or concentric fissures which detract from external appearance, shorten post-harvest life, and increase susceptibility to fungal infections (Domínguez et al., 2012). The incidence of cracking is favoured by high humidity, high temperatures, strong irradiance and irregular irrigation regimes. Other common physiopathies include blossom-end rot, which is characterised by a dark, sunken lesion at the distal end of the fruit, and is linked to calcium deficiency; catface, which is a malformation associated with deep scarring and is linked to low temperatures during fruit set; and sunscald, which develops in green fruits that are abruptly exposed to direct solar radiation and produces whitish patches and local tissue collapse (Olle and Williams, 2017; Peet, 2009).

1.2.2. Organoleptic quality

Tomato quality is also defined by sensory attributes such as flavour and texture, which together constitute what is referred to as organoleptic quality (Figàs et al., 2015b). In terms of flavour, tomatoes generally have a sweet–sour profile combined with a complex bouquet of aromas, including fruity, floral, and fresh notes. The intensity of these attributes depends on genetic background, as well as environmental and agronomic factors (Baldwin et al., 2008). Hongsoongnern and Chambers IV (2008) developed a sensory lexicon to describe these perceptions, encompassing descriptors such as ripeness, sweetness, acidity, bitterness, as well as less desirable notes such as herbaceous tones typical of immature fruits, together with wine-like, earthy/musty, and fermented nuances. Fruits that are perceived as particularly flavourful by

trained panels usually display pronounced sweetness and fruity notes, as well as a distinctive tomato flavour, and have a low intensity of pungent or green-herbaceous tones (Tandon et al., 2003). These sensory traits arise from the interaction between volatile compounds and sugars and organic acids, which integrate gustatory perception with retronasal olfaction, resulting in a highly complex sensory phenotype (Klee and Tieman, 2018).

The main soluble sugars found in tomato fruit are fructose and glucose, while sucrose is present at much lower levels (Figure 4A). During ripening, the content of sucrose declines, while the concentrations of glucose and fructose reach their maximum at the stage considered optimal for consumption (Zhu et al., 2022). The fructose-to-glucose ratio is particularly important as fructose is almost twice as sweet as glucose (Biester et al., 1925). An allele originating from *Solanum habrochaites* has been identified that increases this ratio, offering a promising way to enhance perceived sweetness in breeding programmes (Levin et al., 2000). Apart from the concentrations of sugars and organic acids present, their balance is also important in determining the organoleptic profile of tomato fruits (Azodanlou et al., 2003). Malundo et al. (1995) also observed that, for a given level of sugar, there is an optimum acidity threshold above which consumer acceptance tends to decrease.

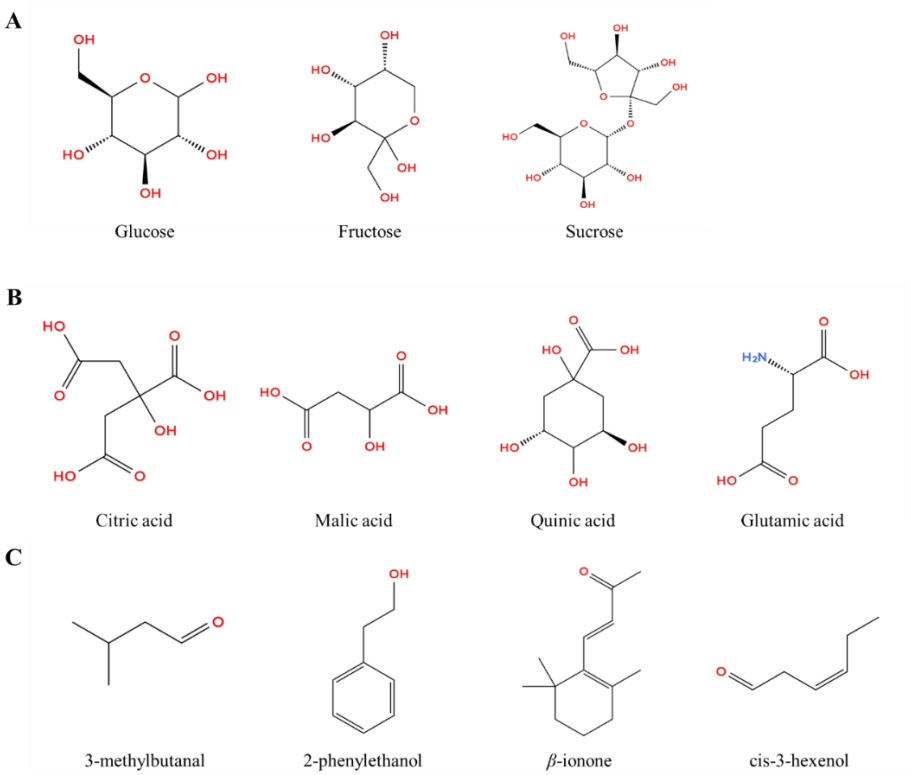

Figure 4. Chemical structures of the main compounds responsible for tomato flavour: A) sugars (glucose, fructose and sucrose); B) organic acids (citric, malic, quinic and glutamic acids); C) representative volatile compounds: 3-methylbutanal (branched-chain amino acid derivative), 2-phenylethanol (phenylalanine derivative), *β*-ionone (carotenoid derivative) and *cis*-3-hexenal (fatty acid derivative).

Organic acids are key intermediates in carbon metabolism in plant cells and contribute to the distinctive flavour of fruits by modulating their acidity. In tomato, citric acid predominates, followed by glutamic, malic, and quinic acids (Figure 4B) (Flores et al., 2016). Their relative abundance depends on both the genotype and ripening stage, which directly influence perceived acidity (Anđelini et al., 2023; Batista-Silva et al., 2018). Although malic acid is present at lower concentrations than citric acid, it produces a stronger perception of acidity (De Bruyn et al., 1971). In contrast, glutamic acid contributes little to acidity but plays a key role in enhancing flavour by being responsible for the fifth basic taste, *umami* (savoury in Japanese). Tomatoes are notable among vegetables for their comparatively high glutamic acid content (Tommonaro et al., 2021; Casals et al., 2019).

In addition to sugars and organic acids, volatile compounds play a crucial role in shaping tomato aroma and sweetness perception. Although more than 400 volatiles have been identified in tomatoes, only a fraction occurs at concentrations sufficient to influence sensory perception (Klee and Tieman, 2013). These molecules are typically present at picomolar or nanomolar levels, making them challenging to quantify and select, which has constrained their effective use in breeding programs (Tieman et al., 2017; Rambla et al., 2014). Most tomato volatiles arise from the degradation of primary metabolites and can be grouped according to their biochemical origin into derivatives of branched-chain amino acids (BCAAs), phenylalanine, carotenoids (apocarotenoids), and fatty acids. (Figure 4C) (Kaur et al., 2023; Wang and Seymour, 2017). Their distribution within the fruit is heterogeneous, which can affect sensory perception depending on the part consumed (Li et al., 2019). Certain compounds, particularly apocarotenoids, can enhance sweetness perception even in the presence of low sugar content (Tieman et al., 2017). Colantonio et al. (2022) estimated that volatiles account for 62% of perceived tomato sweetness, compared to the 29% attributed to sugars.

Texture is another fundamental component of the organoleptic quality of tomatoes (Aurand et al., 2012). As tomatoes are composed of approximately 95% water and 5% total solids, texture-related traits are largely determined by turgor pressure, cell wall composition, and the cohesion provided by the middle lamella (Wang and Seymour, 2017). Key sensory descriptors include pulp firmness, mealiness, mellowness, crispness, and juiciness (Hongsoongnern and Chambers IV, 2008). These attributes vary with the ripening stage, temperature, storage conditions, and genotype, reflecting their multifactorial and dynamic nature (Lázaro and Ruiz-Aceituno, 2021). Consumer preferences for texture are equally diverse, necessitating the adaptation of this attribute for different fruit types, morphologies, and end uses (Causse et al., 2003). However, historically, breeding has prioritised firmness to facilitate transport and extend

shelf life, particularly for produce intended for distant markets (Hongsoongnern and Chambers IV, 2008).

1.2.3. Nutritional and functional quality

Tomatoes are notable for their low caloric value (22 kcal/100 g) and high-water content (95%), which, when combined with their richness in essential nutrients and bioactive compounds, grants them with a high nutritional value (Table 1). Their macronutrient profile comprises carbohydrates, proteins, fibre, and lipids (Wang et al., 2023). Although minerals represent only a small fraction of the fresh weight of tomatoes, they are nutritionally significant, particularly potassium, phosphorus, magnesium, and sodium (Davies et al., 1981). The high content of bioactive compounds in tomato is mainly due to secondary metabolites with functional properties, among which carotenoids, phenolic compounds, and vitamins, are particularly prominent (Quinet et al., 2019).

Table 1. Nutritional and functional composition of tomato.

Category	Compound	Range concentration
Nutritional quality[1]	Carbohydrates	3.92-8.05 g/100 g FW
	Proteins	10.50-25.03 g/100 g FW
	Fiber	1.32-19.36 g/100 g FW
	Lipids	3.62-5.39 g/100 g FW
Functional quality[2]	Lycopene	1.86-14.62 mg/100 g FW
	β-carotene	0.11-1.07 mg/100 g FW
	Phytoene	0.47-1.34 mg/100 g FW
	Phytofluene	0.23-1.16 mg/100 g FW
	Lutein	0.08-0.34 mg/100 g FW
	Phenolic acids	2.75-4.68 mg/100 g FW
	Flavonoids	1.15-8.16 mg/100 g FW
	Vitamin C	2.20-21 mg/100 g FW
	Vitamin E	0.11-1.84 mg/100 g FW

Source: [1]Ali et al. 2021; [2]Frusciante et al. 2007

Carotenoids are fat-soluble pigments derived from 40-carbon isoprenoids, classified either as hydrocarbon carotenoids or carotenes (*α*-carotene, *β*-carotene and lycopene) or as oxygenated carotenoids, known as xanthophylls (lutein, zeaxanthin) (Figure 5A) (Saini et al., 2015). Their biosynthesis originates from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which condense to form geranylgeranyl diphosphate (GGPP). From this precursor, phytoene, lycopene, and other carotenoids are synthesised through a sequence of enzymatic reactions and isomerisations (Cazzonelli and Pogson, 2010). In red-fruited cultivars, lycopene is the predominant carotenoid, followed by *β*-carotene (Table 1) (Flores et al., 2017). The carotenoid profile is influenced by genotype, ripening stage, cultivation practices, and environmental conditions (Fibiani et al., 2022; Zhu et al., 2022). During ripening, carotenoid accumulation intensifies and coincides with chlorophyll degradation, which drives the visible

colour transition of the fruit. Mutations in key biosynthetic enzymes can markedly alter carotenoid proportions, ultimately determining fruit pigmentation (Yang et al., 2023). The colour provided by each carotenoid depends on its structure, ranging from pale yellow (ζ-carotene), orange (*β*-carotene), red-orange (*γ*-carotene) to red (lycopene), and the final colour of the fruit is determined by the type, concentration and physical state of the carotenoids present (Naeem et al., 2023). In plants, carotenoids primarily protect photosynthetic tissues against oxidative stress and serve as precursors for phytohormones such as abscisic acid (Sun et al., 2022). Lycopene is particularly noteworthy due to its strong antioxidant capacity, which exceeds that of *β*-carotene, and its intake in humans has been linked to a reduced risk of cardiovascular disease, certain cancers, and cellular ageing (Saini et al., 2020). Meanwhile, *β*-carotene is an important provitamin A compound that contributes to visual health. The precursors phytoene and phytofluene, though present at lower concentrations, have also been associated with potential health benefits (Friedman, 2013).

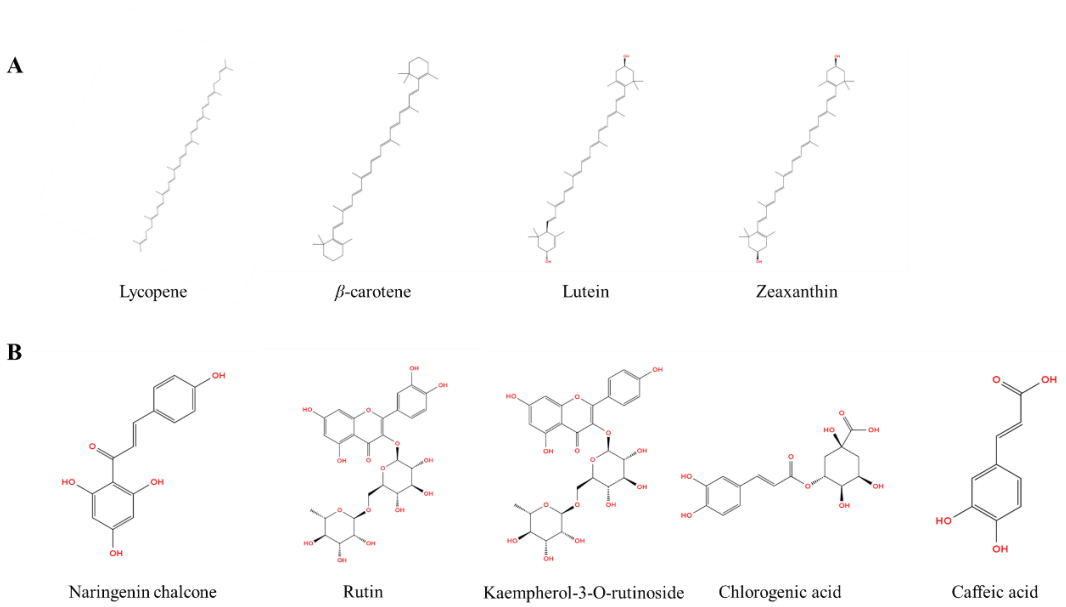

Figure 5. Chemical structures of secondary metabolites with functional relevance in tomato: A) carotenoids (lycopene, *β*-carotene, lutein, and zeaxanthin); B) phenolic compounds: flavonoids (naringenin chalcone, rutin, kaempferol-3-*O*-rutinoside) and non-flavonoids (chlorogenic acid and caffeic acid).

Phenolic compounds are a diverse group of secondary metabolites characterised by the presence of one or more phenolic groups. They are structurally divided into flavonoids and non-flavonoids. Flavonoids share a common backbone of two aromatic rings (A and B) linked by an oxygenated heterocyclic ring (C), and according to the degree of oxidation and the nature of their substituents, they are further classified into flavonols, flavones, flavanones, isoflavones, anthocyanins, and flavonols (Slimestada and Verheulb, 2009). In plants, these compounds are typically present as glycosides. In tomato, common examples of flavonoids include naringenin

chalcone as well as quercetin (rutin) and kaempferol conjugates (Table 1; Figure 5B) (Tamasi et al., 2019). Non-flavonoids comprise phenolic acids, hydrolysable tannins, and stilbenes. Phenolic acids, the most abundant subgroup in plants, are divided into hydroxybenzoic and hydroxycinnamic acid derivatives. Among these, *p*-coumaric, caffeic, ferulic, and synapic acids are particularly relevant, generally occurring in conjugated forms. In tomato, chlorogenic and caffeic acids predominate (Table 1; Figure 5B) (Fibiani et al., 2022).

Phenolic compounds exhibit differential tissue distributions: flavonoids primarily accumulate in the skin, while phenolic acids are present in both the pulp and epidermis (Tamasi et al., 2019). In plants, they play multiple roles in pigmentation, UV protection, and defence responses against biotic and abiotic stresses (Saini et al., 2024). Their aromatic ring structures with hydroxyl substituents confer a high antioxidant capacity, enabling them to donate electrons or hydrogen atoms and neutralise free radicals and reactive oxygen species (ROS) (Quinet et al., 2019). In humans, this activity is associated with protective effects against inflammatory processes, as well as cardiovascular, neurodegenerative, metabolic, and certain cancer disorders. Flavonoids also exhibit antiviral and antihistamine properties, and may also modulate key cell signalling pathways (Ballester et al., 2016; Martí et al., 2016; Soto-Vaca et al., 2012).

Tomatoes are an important dietary source of essential vitamins, including vitamin C and vitamin E (tocopherols), as well as the provitamin A (*β*-carotene) (Figure 6) (Davies et al., 1981). Vitamin C is water-soluble, whereas provitamin A and vitamin E are fat-soluble (Martí et al., 2016). Ascorbic acid (AA), the active form of vitamin C, is a carbohydrate-derived ketolactone that acts as an enzymatic cofactor and an antioxidant in plants. Under oxidative conditions, AA can be reversibly oxidised to form dehydroascorbic acid (DHA), and both forms are considered biologically active and contribute to the neutralisation of free radicals (Mellidou et al., 2021). In humans, vitamin C is associated with preventing chronic and degenerative diseases and plays a role in collagen biosynthesis, steroid hormone production, and neurotransmitter metabolism (Locato et al., 2013). Vitamin E, the main fat-soluble antioxidant, includes eight compounds with vitamin activity: four tocopherols (*α*-, *β*-, *γ*- and *δ*) and four tocotrienols (*α*-, *β*-, *γ*- and *δ*), differentiated by the saturation of their side chain, which is saturated in tocopherols and unsaturated with three double bonds in tocotrienols (Egea et al., 2022). While all forms exhibit biological activity, *α*-tocopherol is the most active and nutritionally significant (Almeida et al., 2011). In plants, vitamin E plays a crucial role in protecting the photosynthetic apparatus by quenching singlet oxygen ($^1O_2$) and limiting lipid peroxidation (Miret and Munné-Bosch, 2015). In humans, its antioxidant properties have been linked to a reduced risk of chronic disease (Bermúdez et al., 2018).

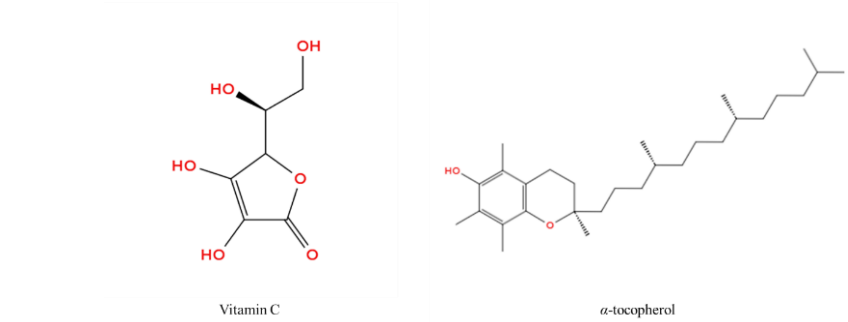

Figure 6. Chemical structures of vitamins with functional relevance in tomato: vitamin C and *α*-tocopherol (vitamin E).

1.3. Challenges for tomato cultivation in arid and semi-arid areas

Arid and semi-arid regions account for over 40% of the Earth's land surface and sustain a considerable share of the global population, whose livelihoods and food security rely heavily on agriculture (Mirzabaev et al., 2022). These regions are of strategic importance for global food security, since countries such as Spain, Italy, and Morocco act as net food exporters to other regions worldwide (Ali et al., 2022). However, their agricultural systems face major challenges arising from both climate change and the prevailing intensive production model.

The Mediterranean region, which lies in the transitional zone between the arid climate of North Africa and the temperate climate of Central Europe, is particularly vulnerable to these pressures and has been identified as a climate change hotspot, due to a sustained temperature rise and a pronounced decline in precipitation. The cumulative environmental impacts of decades of intensive agriculture exacerbate the effects of climate change (Gao and Giorgi, 2008). The Green Revolution promoted the widespread use of synthetic agrochemicals to maximize yields. However, this model has generated adverse impacts on agroecosystems, including soil acidification, compaction, and loss of organic matter, as well as contamination of surface and groundwater through nutrient leaching (Kim et al., 2020; Tripathi et al., 2020; Fess et al., 2011) . Addressing these environmental and climatic challenges is particularly critical for tomato cultivation, a key crop in Mediterranean agriculture that makes a major contribution to both the regional economy and global food supply.

1.3.1. Abiotic constraints in tomato cultivation

Tomato cultivation is influenced by multiple abiotic stresses that affect both plant physiology and the yield and quality of fruits. In arid and semi-arid environments, water stress represents one of the main constraints to optimal crop development, as scarce rainfall, limited water quality, and the growing pressure of climate change seriously compromise water availability (Buttaro et al., 2015; Trenberth et al., 2014). Global warming intensifies the frequency and severity of droughts by altering the hydrological cycle, resulting in reduced runoff, lower

surface water flows and diminished aquifer recharge (Mirzabaev et al., 2022). Projections indicate that by 2030, water deficits in the southern Mediterranean basin could reach between 28% and 47%, mainly due to the high agricultural demand, which accounts for the largest share of freshwater consumption (Figure 7) (FAO, 2023b). Tomato is moderately sensitive to drought, particularly during critical phenological stages such as flowering and fruit set, resulting in reduced yields (Franco-Navarro et al., 2025; Nangare et al., 2016). Common responses to water stress in tomatoes include reduced leaf area and stomatal closure. While these responses help conserve water, they also limit CO_2 uptake and photosynthesis, thereby promoting oxidative stress (Conti et al., 2023; Kamanga et al., 2018). In addition, a lower soil moisture slows cell expansion in roots and hinders nutrient uptake, further aggravating its negative effects on plant growth and development (Álvarez and Acosta-Motos, 2022; Yang et al., 2021).

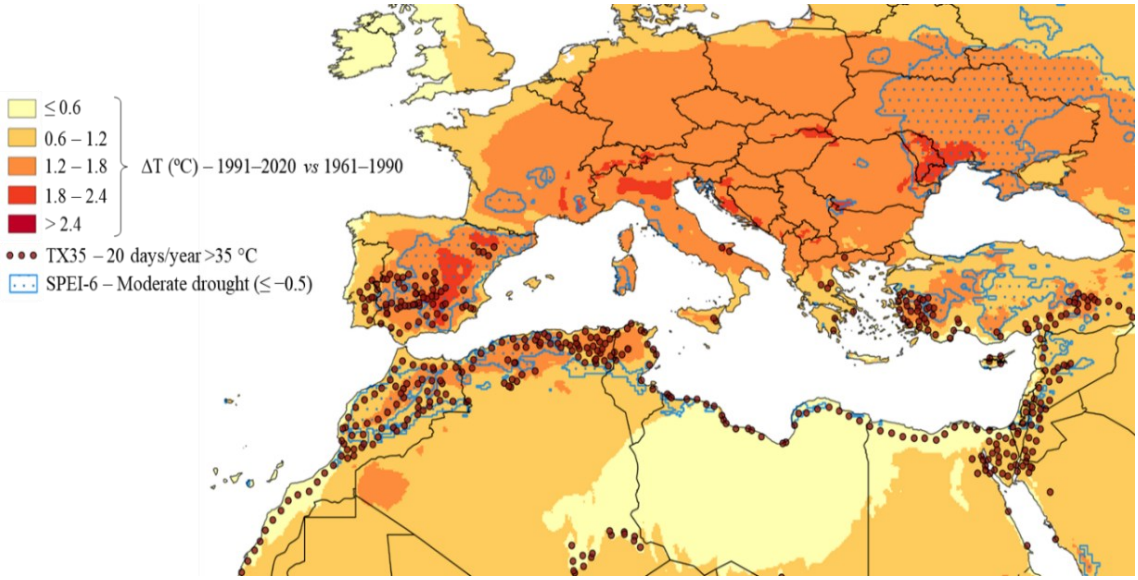

Figure 7. Climate indicators in the Mediterranean basin: increase in mean temperature (ΔT, °C), number of days with maximum temperatures >35 °C (TX35, days/year), and reduction of the water balance (SPEI-6). Source: ERA5-Land, Copernicus Climate Change Service (C3S).

Other relevant abiotic stresses in arid and semi-arid environments include high temperature and salinity. Rising temperatures, particularly in conjunction with water scarcity, pose a significant threat to crops in these regions (Figure 7). In the Mediterranean basin, the current mean temperature already exceeds the pre-industrial levels by 1.5 °C (Ali et al., 2022). Heat stress can manifest chronically as a result of sustained warming or acutely through extreme events, such as heat waves, which are becoming increasingly frequent, intense, and prolonged (Raza et al., 2023). Tomatoes are highly sensitive to high temperatures, particularly during flowering. Temperatures above 35 °C reduce pollen viability, hinder pollen tube growth (Raja et al., 2019), promote floral abscission (Camejo et al., 2005), and decrease photosynthetic efficiency (Thompson et al., 2022), all of which severely compromise yield (Hernández et al., 2022; Scarano

et al., 2020; Paupière et al., 2017). High temperatures can also affect fruit development and ripening, resulting in reduced fruit weight and lower concentrations of bioactive compounds such as carotenoids and phenolics (Hernández et al., 2015). With regard to salinity, the accumulation of salts in soil and water is exacerbated by low rainfall, high evapotranspiration, and overexploitation of aquifers, conditions that also promote seawater intrusion (Mirzabaev et al., 2022). Consequently, salt build-up reduces the osmotic potential of the soil and hinders water uptake by the roots, thereby intensifying the effects of water stress (Naorem et al., 2023). It is estimated that approximately 30% of the arable land in the Mediterranean region is affected by some degree of salinisation (Rasool et al., 2013). Tomato, a crop that is moderately sensitive to salinity stress, exhibits reduced growth and yield under such conditions (Singh et al., 2020). Decreases in plant height, biomass, leaf area, and growth rate have been observed and are associated with ionic toxicity, water deficit, and disruptions in the uptake of K^+, Ca^{2+}, and Mg^{2+} (Roșca et al., 2023; Moles et al., 2016). Cellular damage under high salinity has also been documented, including aggregated chloroplasts and membrane distortion (Najla et al., 2009). However, depending on the cultivar and developmental stage, moderate salt stress may enhance the accumulation of quality-related compounds, such as sugars, organic acids, and carotenoids (Carbonell et al., 2020; Meza et al., 2020).

1.3.2. Biotic constraints in tomato cultivation

In addition to abiotic factors, biotic stresses are another key limitation of agricultural production. It is estimated that up to 40% of global agricultural output is lost annually due to pests and diseases (FAO, 2021). Tomato is susceptible to a wide range of pathogens that can compromise both yield and fruit quality. Specifically, in arid and semi-arid regions such as the Mediterranean basin, the incidence of certain pests and diseases poses a particular threat to tomato crops, resulting in significant yield losses (Panno et al., 2021). Among them, root-knot nematodes of the genus *Meloidogyne* (*M. incognita, M. arenaria, M. javanica)* are of major concern, as they induce root galls that reduce water and nutrient uptake, leading to chlorosis and wilting (Gabriel et al., 2024). Vascular wilt fungi are also significant, including *Fusarium oxysporum* f. sp. *lycopersici*, which causes leaf yellowing, wilting, and plant death (McGovern, 2015), and *Verticillium dahliae*, likewise associated with vascular wilting (Figure 8A) (Fradin and Thomma, 2006). Other common fungi include *Alternaria solani*, the causal agent of early blight, and *Botrytis cinerea*, responsible for rots in flowers, stems, and fruits (Figure 8B) (Panno et al., 2021). Among bacterial diseases, bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis* is the most damaging, producing leaf wilting and characteristic “bird’s eye” lesions on fruits that lead to severe yield losses (Figure 8C) (Sen et al., 2015).

Viral diseases pose a significant threat to tomato cultivation due to the challenges

associated with their management and the substantial yield losses they cause. Cucumber mosaic virus (CMV) causes leaf malformations, dwarfism, and delayed ripening, resulting in non-commercial and necrotic fruits under severe infection (Rivarez et al., 2021). Tomato mosaic virus (ToMV) and Tomato brown rugose fruit virus (ToBRFV), are mechanically transmitted and induce mosaic symptoms, leaf deformation, necrosis of stems, and fruit discolouration (Figure 8D) (Shahriari et al., 2023). ToBRFV is of particular concern, as it is an emerging virus that was first detected in Europe in 2018 (Zhang et al., 2022). Tomato spotted wilt virus (TSWV), transmitted by thrips, causes foliar bronzing, leaf necrosis, and fruit lesions (Figure 8E) (Picó et al., 2002). Finally, Tomato yellow leaf curl virus (TYLCV), transmitted by the whitefly *Bemisia tabaci*, induces leaf yellowing and curling, stunting, and a bushy growth habit, with severe yield losses when infection occurs early (Figure 8F) (Carbonell et al., 2018; Picó et al., 2002).

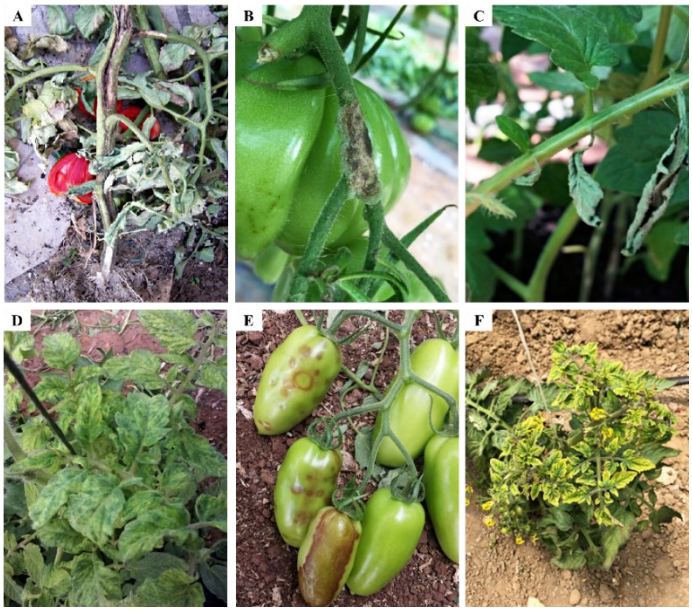

Figure 8. Representative symptoms of biotic diseases in tomato: A) vascular wilt caused by *Verticillium dahliae*; B) stem rot caused by *Botrytis cinerea*; C) leaf lesion caused by *Clavibacter michiganensis*; D) leaf mosaic caused by ToMV; E) fruit bronzing caused by TSWV; F) leaf yellowing and curling caused by TYLCV (adapted from Panno et al. 2021).

Pests negatively affect tomato cultivation both as virus vectors and through direct damage to plants. Among the most relevant, we find the lepidopteran *Tuta absoluta*, one of the most destructive pests in recent years due to the galleries it forms in leaves and fruits, rendering them unmarketable; whiteflies (*Bemisia tabaci, Trialeurodes vaporariorum*) and thrips (*Frankliniella occidentalis*), highly polyphagous insects that damage a wide range of hosts; and mites such as the red spider mite (*Tetranychus urticae*) and the tomato russet mite (*Aculops lycopersici*) (Khan et al., 2020; Santana et al., 2019; Henrique et al., 2018; Perdikis et al., 2008).

Environmental conditions resulting from climate change, such as increased temperature and CO_2 concentration, are altering the life cycles of pests and pathogens, thereby enhancing their virulence, severity, and geographic distribution (Mirzabaev et al., 2022; Olivieri et al., 2021). In tomato, this phenomenon has favoured the expansion of pests such as *Tuta absoluta* and *Bemisia tabaci*, and pathogens such as *Clavibacter michiganensis*, posing an increasing risk in agricultural regions of southern Europe (Hubab et al., 2025; Ramos et al., 2019; Santana et al., 2019). Moreover, plants subjected to abiotic stresses exhibit a greater susceptibility to infections, partly due to the accumulation of reactive oxygen species (ROS), which compromise cellular integrity and facilitate pathogen entry (Kaushik et al., 2023).

1.3.3. Fertilization challenges in tomato cultivation

Intensive tomato cultivation relies on high fertilizer inputs to achieve maximum yields, with nitrogen, potassium, and phosphorus being the essential macronutrients for growth and productivity (Machado et al., 2023). Nitrogen is of particular importance, and in conventional agriculture, excessive fertilization is common to boost yield (Albornoz, 2016). Under nitrogen deficiency, yield reductions are consistently reported, whereas effects on fruit quality remain controversial, with contradictory results depending on the study (Hernández et al., 2020; Bénard et al., 2009). Potassium contributes to vegetative growth, yield, and fruit quality (Li et al., 2021; Liu et al., 2021). Optimal potassium levels have been shown to improve disease resistance, increase resilience to adverse abiotic conditions, and enhance fruit firmness (Wang et al., 2013). Phosphorus plays a central role in several essential biological processes, including photosynthesis, respiration, energy transfer, and nucleic acid synthesis (Sung et al., 2015).

Balanced fertilisation, while avoiding excessive nutrient application, is essential for optimal crop growth and for preventing losses in both yield and quality (Cheng et al., 2021; Elia and Conversa, 2012). Beyond its effects on plant development, over-fertilisation exerts adverse impacts on the environment. Excessive fertiliser use has been shown to contribute to nitrate leaching, eutrophication of aquatic ecosystems, and deterioration of soil structure (Kim et al., 2020; Fess et al., 2011). These impacts are particularly critical in arid and semi-arid regions in the Mediterranean basin, where scarce rainfall, together with intensive irrigated and greenhouse agriculture, exacerbates environmental vulnerability (Ali et al., 2022). Consequently, many intensive production areas have been classified as vulnerable to nitrate pollution under the framework of the European Nitrates Directive (Castro et al., 2019). In addition, fertiliser synthesis and application represent a considerable economic cost and a significant contribution to the agricultural carbon footprint (Machado et al., 2023; Truffault et al., 2019). In response to this situation, the European Commission has promoted the regulation of synthetic input use in agriculture to mitigate its impact on human health and the environment. In 2019, the European

Green Deal set ambitious targets, including a 20% reduction in fertilizer use and a 50% reduction in nutrient losses by 2030, without compromising soil fertility (Cuadros-Casanova et al., 2023). Recent data reflect this trend, with mineral fertilizer use declining after 2021, possibly linked to the implementation of these measures.

1.4. Strategies for enhancing the sustainability of tomato cultivation

In view of the challenges outlined above, related to the incidence of abiotic and biotic stresses as well as fertilisation issues, several strategies have been proposed to enhance the sustainability of tomato cultivation. Implementing fertigation systems and selecting varieties with lower input requirements and better adaptation to limiting conditions are key approaches to optimising the use of water and fertilisers. Additionally, biotechnological tools such as biostimulants (Franzoni et al., 2022; Hernández et al., 2022; Castiglione et al., 2021; Ruzzi and Aroca, 2015), nanofertilisers (Echeverría-Pérez et al., 2025) and rootstocks (Carbonell et al., 2022; Voutsela et al., 2012; He et al., 2009), have emerged as promising alternatives to strengthen tomato tolerance to drought, salinity and high temperatures, while reducing dependence on conventional inputs and promoting beneficial interactions within the soil–plant–atmosphere continuum. In addition, agronomic practices such as the application of organic or inorganic mulches (Mendonça et al., 2021), organic fertilizers (Wu et al., 2020), and cover crops (Massantini et al., 2021) have shown positive effects on soil structure, moisture conservation, and organic matter content, with direct implications for the efficiency and sustainability of tomato cultivation.

Among these alternatives, a fundamental first step in arid and semi-arid regions such as the Mediterranean basin is the efficient management of water and nutrients. These inputs are essential for tomato cultivation, and under the increasing incidence of droughts and the enforcement of stricter environmental regulations on mineral fertilisers, their management requires high precision. Likewise, pest and disease control is crucial in this crop, given the high phytosanitary pressure in intensive systems and the need for sustainable alternatives to reduce chemical inputs (Alrteimei et al., 2022; Panno et al., 2021; Patanè and Cosentino, 2010; Gao and Giorgi, 2008) (Figure 10).

Figure 10. Key strategies to enhance the sustainability of tomato cultivation, including efficient management of water and fertilizers, pest and disease control, and the use of agronomic and biotechnological tools.

1.4.1. Irrigation and fertilisation strategies

Efficient water management is essential for maintaining crop yield, particularly in arid and semi-arid regions exposed to water scarcity. This approach contributes to improving environmental sustainability without compromising production viability, an aspect that is especially relevant given the projected increase in food demand (Mirzabaev et al., 2022). Within this framework, various strategies have been developed to enhance input-use efficiency. Drip irrigation has been consolidated as one of the most effective techniques, as it delivers water locally and directly to the root zone, significantly reducing losses through evaporation and leaching, while replacing other conventional systems such as flood irrigation (Franco-Navarro et al., 2025; Huang et al., 2023; Wang and Xing, 2017). Together with soil moisture sensors, irrigation scheduling tools, and weather stations, this technique allows the efficiency of water resources to be maximised (Kim et al., 2020; Buttaro et al., 2015). Complementarily, deficit irrigation, based on the controlled supply of water volumes below crop requirements, has proven effective in improving water-use efficiency without significantly reducing yield (Fullana-Pericàs et al., 2019; Kamanga et al., 2018; Nangare et al., 2016; Patanè and Cosentino, 2010). Its effectiveness depends on precise knowledge of crop phenology. Its application during critical stages, such as flowering or fruit set, may cause severe yield losses. However, its application during late vegetative phases can improve fruit quality by increasing soluble solids and antioxidant compounds as an adaptive response to stress (Hou et al., 2020; Ripoll et al., 2014).

With regard to fertilisation, the adequate management of mineral nutrition in tomato is essential. This should include balanced fertilisation, adjusted to the actual crop requirements and the nutrient availability in the soil. It also involves correcting the applied doses, selecting the appropriate type of fertiliser, and incorporating strategies and technologies that enhance

nutrient-use efficiency (Mustafa et al., 2022). In this context, fertigation through drip irrigation is particularly effective in semi-arid regions, as it enables the localized and combined application of water and nutrients, thereby minimizing leaching losses (Abdelhady et al., 2017; Wang and Xing, 2017). This strategy, however, requires detailed knowledge of soil fertility status and crop nutritional demand throughout the growth cycle. Split nutrient application according to phenological stage further improves absorption, prevents excess supply, and reduces environmental losses (Zotarelli et al., 2009). In recent years, emerging techniques such as slow- and controlled-release fertilizers or real-time sensors for monitoring nitrogen and other soil nutrients have also been developed, which can help reduce nutrient losses and increase fertilizer-use efficiency (Ameer et al., 2024).

1.4.2. Pest and disease control

Reducing the use of agrochemicals, particularly pesticides, constitutes another fundamental pillar of sustainable agriculture. Integrated pest management involves the strategic combination of multiple control methods, including cultural, biological, and chemical tools, with the aim of maintaining pest populations below economically damaging thresholds while minimizing risks to the environment and public health (Barzman et al., 2015). Biological control, which is based on the use of natural enemies such as parasitoids, predators and pathogens, represents a key component of pest management and an effective approach to reducing reliance on chemical pesticides (Perdikis et al., 2008). However, in regions such as the Mediterranean basin, where there is high phytosanitary pressure, its effectiveness may be limited by the persistence of soil-borne pathogens, the mechanical transmission of diseases, or the complexity of pest and pathogen life cycles (Rezk et al., 2021).

The use of genetic resistance has become established as an effective, sustainable strategy for controlling crop diseases. In the case of tomatoes, wild species of the genus *Solanum* are a valuable source of genes that provide resistance against viruses, fungi, bacteria, and nematodes, and have been widely exploited in breeding programmes (Table 2) (Rezk et al., 2021). Genes such as *Tm-2a*, *Sw-5* and *Ty-1*, which originate from *S. peruvianum*, *S. habrochaites* and *S. chilense* respectively, have been identified as providing resistance to viruses. Resistance genes have also been reported against bacterial pathogens, as well as against fungal pathogens (*Ph-3*, *I-3*) and root-knot nematodes (*Meloidogyne* spp.), including *Mi-1* and its other variants (*Mi-3*, *Mi-9*, *Mi-HT*). However, the effectiveness of genetic resistance can be limited by factors such as pathogen evolution, loss of resistance under high-temperature conditions, or linkage drag effects on yield and fruit quality, which highlights the importance of combining resistance breeding with complementary management strategies (Mundt, 2014; Verlaan et al., 2011).

Table 2. Resistance genes identified in wild and cultivated tomato species against viruses, fungi, bacteria, and nematodes.

Resistance gene	Source of resistance	Pathogen
Viruses		
Ty-1	*Solanum chilense*	TYLCV
Ty-2	*S. habrochaites*	TYLCV
Ty-3	*S. chilense*	TYLCV
Ty-4	*S. chilense*	TYLCV
Ty-5	*S. peruvianum*	TYLCV
Ty-6	*S. chilense*	TYLCV
Tm-2a	*S. peruvianum*	ToMV
Sw-5	*S. peruvianum*	TSWV
Fungi		
Ph-3	*S. pimpinellifolium*	*Phytophtora infestans*
I-1	*S. pimpinellifolium*	*Fusarium oxysporum* f. sp. *lycopersici*
I-2	*S. pimpinellifolium*	*F. oxysporum* f. sp. *lycopersici*
I-3	*S. pennellii*	*F. oxysporum* f. sp. *lycopersici*
Ve-1	*S. lycopersicum*	*Verticillium dahlia* Kleb.
Ve-2	*S. lycopersicum*	*Verticillium alboatrum*
Bacteria		
Bwr-6	*S. lycopersicum* var. *cerasiforme*	*Ralstonia solanacearum*
Pto-1 / Pto-2	*S. pimpinellifolium*	*Pseudomonas syringae* pv. *tomato*
Rx-4	*S. pimpinellifolium*	*Xanthomonas perforans* (Race T3)
RXopJ4	*S. pennellii*	*X. perforans* (Race T4)
Nematodes		
Mi-1, Mi-HT y Mi-9	*S. peruvianum*	*Meliodogyne* spp.
Mi-3 y Mi-5	*S. peruvianum*	*Meliodogyne* spp.

*Table adapted from Rezk et al. (2021).

1.5. The role of traditional tomato varieties in sustainable agriculture

Traditional tomato landraces, which are the result of natural and artificial selection processes in local environments, are a valuable source of genetic diversity and have great potential in addressing the challenges of sustainable agriculture. Although breeding programs have historically relied on related wild species to enhance the tolerance of cultivated tomato to biotic and abiotic stresses (Rezk et al., 2021), these species often present genetic barriers, compatibility issues, and linkage drag, which hinder their direct use (Egea et al., 2022). By contrast, landraces, which are genetically closer to modern cultivars, offer a complementary and more accessible alternative for breeding programs aimed at developing sustainable and resilient production systems (Gascuel et al., 2017). Many of these landraces have proven to be sources of traits associated with tolerance, which are essential for maintaining yield under suboptimal conditions where modern cultivars often show limitations (Ficiciyan et al., 2018; Fess et al., 2011). In this context, traditional varieties have gained renewed attention as an alternative to modern cultivars

and as a source of traits that promote resilience for breeding programmes targeting agricultural sustainability (Caramante et al., 2023; Tian et al., 2021; Fita et al., 2015).

1.5.1. Origin, genetic erosion and revalorization

The cultivated tomato (*Solanum lycopersicum* L.) belongs to the *Solanaceae* family, the *Solanum* genus and the *Lycopersicon* section. It was first described by Linnaeus in 1753 (Peralta et al., 2005). Its origin is located in South America, specifically in areas of Peru, Ecuador, Bolivia, southern Colombia, and northern Chile, where numerous wild species occur (Figure 11) (Zuriaga et al., 2009a). Among these, *S. pimpinellifolium* L. (SP) is considered the closest relative of cultivated tomato; its accessions are grouped into three genetic lineages adapted to different ecological regions along the Ecuadorian and Peruvian coast (Blanca et al., 2012; Zuriaga et al., 2009b). Another relevant taxon is *S. lycopersicum* var. *cerasiforme* (SLC), which has been identified in the humid environments of Ecuador and Peru on the eastern edge of the Amazon basin. Both species lack reproductive barriers but are geographically separated by the Andes Mountain range (Blanca et al., 2015).

The process of tomato domestication has been widely debated and is currently understood to be a two-step process (Figure 11) (Blanca et al., 2015). The first step, pre-domestication, occurred in the Andean region through the selection of primitive SP, which gave rise to the SLC populations of Ecuador and northern Peru. The second step took place in Mesoamerica, where these pre-domesticated SLC were further selected, leading to the modern cultivated form *S. lycopersicum* var. *lycopersicum* (SLL) (Razifard et al., 2020; Blanca et al., 2015).

After it arrived in Europe in the 16th century, tomato experienced a strong genetic bottleneck due to the introduction and selection of a limited number of accessions (Park et al., 2004). Italy and Spain were the first countries to incorporate it into their gastronomy, whereas its adoption in other regions of the world was not consolidated until the 18th century (Blanca et al., 2012; Cebolla-Cornejo et al., 2007). Once established in Europe, particularly in the Mediterranean basin, considered a secondary centre of diversity, the crop diversified through the intuitive genetic improvement carried out by farmers, who preserved and selected seeds from genotypes with desirable traits such as flavour, shape, colour or ripening time. This artificial selection, together with adaptation to diverse agro-climatic conditions, resulted in a wide diversity of varieties, with remarkable morphological and functional differentiation (Cebolla-Cornejo et al., 2013; Blanca et al., 2012; Mazzucato et al., 2008). In the mid-19th century, the establishment of breeding companies and the intensification of agriculture led to the gradual replacement of traditional varieties with more productive commercial cultivars. This phenomenon, known as genetic erosion, intensified during the Green Revolution and was further accelerated by the widespread use of F_1 hybrids, which provided agronomic advantages but entailed genetic homogenization

(Frison et al., 2011; Cebolla-Cornejo et al., 2007). Additionally, globalisation and international trade facilitated the spread of pests and diseases, favouring the preferential use of cultivars carrying resistance genes and thus further reducing genetic diversity (Hajjar and Hodgkin, 2007).

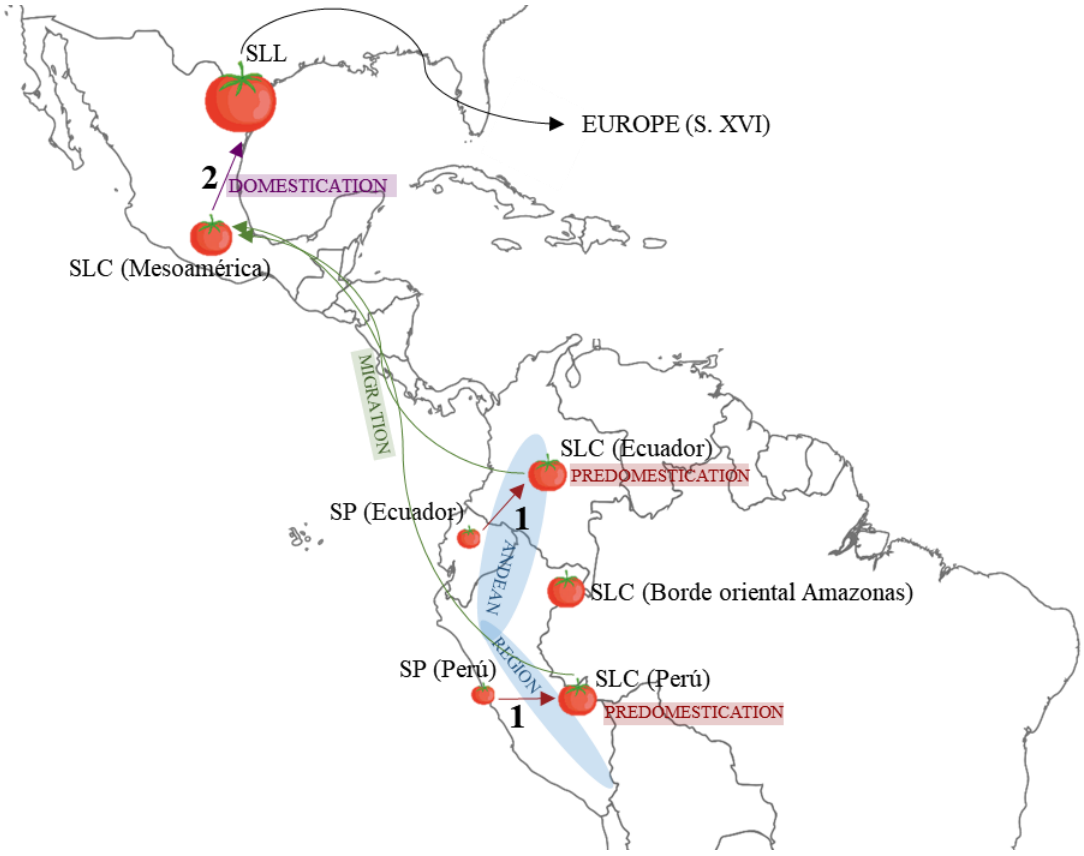

Figure 11. Domestication routes of tomato from wild populations (*Solanum pimpinellifolium*, SP) in the Andes, through intermediate forms (*S. lycopersicum* var. *cerasiforme*, SLC), to the cultivated form (*S. lycopersicum* var. *lycopersicum*, SLL) in Mesoamerica.

In response to this increase in genetic erosion, a renewed interest in traditional varieties, also referred to as landraces or heirlooms, was observed in recent decades (Pons et al., 2022, 2023). This change responds both to their value as genetic reservoirs and to their link with sustainability, local food, and territorial identity (Pérez-Caselles et al., 2020; Petrescu et al., 2020; Casañas et al., 2017). The concept of landrace has evolved from its original use, referring to cultivars developed without formal breeding, towards broader and more integrative definitions. Casañas et al. (2017) proposed a definition that acknowledges their continuous evolution, whether through traditional or modern techniques, within a specific ecogeographical environment and shaped by local culture. Throughout the 20th century, germplasm banks were established worldwide to safeguard plant diversity. In the case of tomato, Spain hosts important collections (Figure 12), including the COMAV-UPV Germplasm Bank in Valencia, the Horticultural Germplasm Bank of Zaragoza at the CITA of Aragón, and the IMIDA Germplasm Bank in Murcia. At the international level, key institutions include the Germplasm Resources Information

Network of the United States Department of Agriculture, the Leibniz Institute of Plant Genetics and Crop Plant Research in Germany, and the INIA Germplasm Bank in Peru, as well as the Svalbard Global Seed Vault in Norway, which preserves accessions from around the world. These resources are fundamental for conserving horticultural biodiversity, particularly local and traditional tomato varieties, through the collection, multiplication, and long-term maintenance of seeds and genetic material.

Figure 12. Example of the morphological diversity of fruit from the collection of traditional tomato varieties from southeastern Spain, conserved in the Murcia Germplasm Bank (BAGERIM).

Germplasm banks, in addition to their role in collection and conservation, have enabled extensive genetic, morphological, and metabolomic characterization of traditional varieties, supporting their revalorization, cultivation, and incorporation into breeding programmes as valuable sources of variation (Table 3). As a result, in Spain, varieties such as Muchamiel, Moruno, Pera de Girona, Penjar, and Ramellet have regained prominence due to their organoleptic qualities and their close link with regional gastronomic identity (Villena et al., 2023b; Carbonell et al., 2018; Figàs et al., 2015a; Casals et al., 2012). Similarly, in Italy, varieties such as Pomodoro di Sorrento, del Piennolo, and San Marzano, the latter even holding a Protected Designation of Origin, have become consolidated (Parisi et al., 2016; Sinesio et al., 2007). This trend is also observed in other European countries, such as France, where Coeur de Boeuf and Marmande retain their commercial value (Pons et al., 2022; Casals et al., 2011), or Greece, with Tomataki Santorinis (Koutsika-Sotiriou et al., 2016).

Table 3. Morphological, genetic, and biochemical characterization studies of traditional tomato varieties.

Reference	Country/Region	Accessions	Type of characterization
(Terzopoulos and Bebeli, 2010)	Greece	34	Phenotypic (36 descriptors)
(Sim et al., 2011)	USA, Canada, Costa Rica, Panama, Mexico & Peru	23	Genetic (173 markers: SNPs, SSRs, indels)
(Casals et al., 2012)	Spain (Catalonia)	27	Phenotypic + sensory + genetic (AFLP)
(García-Martínez et al., 2013)	Italy & Spain	26	Genetic ($(GATA)_4$ fingerprinting)
(Corrado et al., 2013)	Italy	76	Genetic (SNP-panel Illumina, 175 loci)
(Figàs et al., 2015a)	Spain	69	Phenotypic (64 descriptors + 38 Tomato Analyzer)
(Cortés-Olmos et al., 2015)	Spain	63	Phenotypic + agronomic + genetic (32 SNPs)
(Sacco et al., 2015)	Europe, Africa, Asia & America	102	Phenotypic (18 descriptors) + genetic (7,720 SNPs, SolCAP array) + GWAS
(Baldina et al., 2016)	Italy & France	15	Phenotypic + biochemical + SNP association
(Parisi et al., 2016)	Italy (Sorrento)	10	Phenotypic (13 descriptors) + genetic (Illumina SNP array, 1450 SNPs)
(Flores et al., 2017)	Spain	53	Biochemical
(Esposito et al., 2020)	Italy & Spain	200	Genomic (ddRAD-seq, 32,799 SNPs)
(Nankar et al., 2020)	Europe, America & Asia	43	Phenotypic (28 descriptors + 47 Tomato Analyzer descriptors)
(Farinon et al., 2022)	Italy (Lazio)	51	Phenotypic (25 descriptors) + genetic (7,720 SNPs, SolCAP array)
(Blanca et al., 2022)	Spain, France, Italy, Greece, Israel, Ecuador & Peru	1254	Genomic (GBS, GWAS)
(Pons et al., 2022)	Spain, Italy, Greece, France, Ukraine & Israel	1499	Phenotypic (70 descriptors) + genomic (GWAS, meta-analysis)
(Pons et al., 2023)	Spain, Italy, Greece, France, South America & Russia	226	Phenotypic (33 agro-morphological descriptors) + genomic (GWAS, G×E)
(Tripodi et al., 2023)	Italy	60	Phenotypic + biochemical + genetic (7,591 SNPs, SolCAP array)

1.5.2. Potential of traditional tomato varieties

When factors beyond yield maximization are considered in the choice of crop varieties, traditional varieties or landraces emerge as a valuable alternative. Due to their origin through local adaptation processes and their closer genetic proximity to modern cultivars than to wild relatives, they are a vital source of genetic diversity (Gonzalo et al., 2021; Gascuel et al., 2017; Cebolla-Cornejo et al., 2007). This diversity helps to mitigate genetic erosion, which threatens food

security and increases the vulnerability of the future food supply (Lázaro, 2018). These varieties can be used either through direct cultivation or by incorporating them into breeding programmes aimed at addressing future agricultural challenges (Farinon et al., 2022).

1.5.2.1. Organoleptic and functional quality

In terms of fruit quality, landraces are notable for their remarkable variability in terms of morphology, flavour, texture, and functional composition (Villena et al., 2023a; Renna et al., 2019; Baldina et al., 2016). This variability is the result of long-term selection carried out by farmers, who preferentially conserved genotypes with superior flavour (Farinon et al., 2022). Consequently, many landraces have retained alleles associated with higher contents of sugars, volatile compounds, and other metabolites linked to organoleptic quality, which have been reduced in commercial cultivars subjected to intensive selection based on criteria such as fruit size, colour, or firmness (Villena et al., 2023a; Tieman et al., 2017). Consistent with this, recent surveys indicate that consumers particularly value traits such as intense flavour, fleshy texture, red colouring, and firmness. Notably, this intense flavour is associated with a local origin rather than intensive cultivation (Pérez-Caselles et al., 2020). Changes in the metabolome affected not only primary metabolites but also secondary metabolites, many of which include compounds with potential health benefits, as illustrated by Zhu et al. (2018). The identification of these alleles through modern genomic tools allows for their reintroduction into breeding programmes, thereby highlighting the genetic value of landraces. This approach also aligns with the growing demand for healthier and better-tasting foods (Casañas et al., 2017; Baldina et al., 2016).

1.5.2.2. Adaptation to limiting cultivation conditions

In addition to their value for improving organoleptic and nutritional quality, the genetic diversity present in landraces also underpins their ability to adapt to a range of environmental stresses (Casals et al., 2021; Fullana-Pericàs et al., 2019; Fita et al., 2015). This resilience is largely attributed to their long history of selection in specific local environments, carried out over generations by farmers. As a result, many landraces have developed a notable buffering capacity against changing environmental conditions, expressed in a broad variability of adaptive responses to stress and restrictive cultivation environments (Ficiciyan et al., 2018; Fita et al., 2015).

Several studies have documented the performance of landraces under drought. In Spain and Italy, some varieties have been linked to a higher tolerance to water limitation, attributed to their long tradition of cultivation without supplemental irrigation during the summer (Fullana-Pericàs et al., 2022; Guida et al., 2017; Conesa et al., 2014; Siracusa et al., 2012; Galmés et al., 2011). These studies have also identified specific adaptive mechanisms, such as an increased water-use efficiency. Complementary evidence from germplasm collections in Iran and Bulgaria confirmed that genotypes originating from arid regions exhibited superior tolerance to water

deficit (Grozeva et al., 2024; Sousaraei et al., 2021). Another major challenge in arid and semi-arid regions is salinity stress. Several Mediterranean landraces have shown tolerance to moderate and even severe levels of this stress. These include varieties from south-eastern Spain, such as Muchamiel, De la Pera, Verdal, Negro de Yeste and Tomate Pimiento, as well as the south Italian cultivar, Ciettaicale. In some cases, tolerance was associated not only with crop stability but also with improvements in fruit quality and increased metabolite accumulation (Ntanasi et al., 2023; Carbonell et al., 2020; Meza et al., 2020; Moles et al., 2016, 2019; Massaretto et al., 2018). It has been suggested that such tolerance may be related to their long-term cultivation in saline soils, and in many cases, to the use of water from salinized aquifers, conditions typical of these regions. Regarding heat stress, studies involving landraces of diverse origins have confirmed that several varieties can maintain superior quality and nutritional value, and sometimes even stable yields, at high temperatures. This reinforces their importance for breeding programmes (Gonzalo et al., 2021; Olivieri et al., 2021; Scarano et al., 2020). Finally, under nutrient limitations, some landraces such as De la Pera maintained stable yields in the absence of fertilization, while others demonstrated resilience under low nitrogen or organic farming conditions, leading to the identification of promising ideotypes for breeding (Cirillo et al., 2025; Tagiakas et al., 2022; Rosa-Martínez et al., 2021; Carbonell et al., 2020).

1.5.2.3. Sociocultural value

At the sociocultural and economic level, traditional varieties are closely linked to local agricultural practices and are often preferred by small-scale farmers for reasons of family tradition, ancestral knowledge, or specific culinary uses (Pons et al., 2022; Lázaro, 2018). This dual symbolic and economic value enhances their positioning in differentiated markets, where consumers are willing to pay significantly higher prices, up to 4–6 times more for varieties such as Muchamiel and De la Pera or up to five times more in the case of San Marzano tomatoes with PDO status (García-Martínez et al., 2013; Cebolla-Cornejo et al., 2007). Their promotion also supports sustainable production models based on short supply chains, lower carbon footprints, and stronger local economies (Lázaro, 2018). Surveys of European farmers suggest that the primary reasons for preserving these varieties are flavour and nutritional value (37.5%), tradition and food security (25%), and ideological motivations (16.7%) (Calvet-Mir et al., 2011). As well as being relevant in the market, they therefore play a key role in safeguarding culinary heritage, the knowledge of farmers, and territorial identity (Ficiciyan et al., 2018).

1.5.3. Limitations of traditional tomato varieties

The use of traditional varieties in modern agriculture presents several limitations that hinder their integration into current production systems. While these varieties offer benefits such as increased genetic diversity and desirable sensory properties, their adoption is limited by various

factors. In the current context, where profitability is a key determinant for farmers, production decisions are usually oriented towards cultivars that ensure high yields, uniformity, and extended postharvest shelf life (Pérez-Caselles et al., 2020). By contrast, traditional varieties exhibit greater variability in fruit shape and size and shorter shelf life, which limits both their profitability and their incorporation into large-scale supply chains (Asensio et al., 2019). Moreover, under optimal cultivation conditions, landraces generally achieve lower yields than commercial cultivars (Ronga et al., 2021; Ficiciyan et al., 2018). As a result, they are cultivated almost exclusively by small- and medium-scale farmers, reducing their economic viability in production systems oriented towards wholesale markets (Pérez-Caselles et al., 2020; Casañas et al., 2017).

Another major limitation is their vulnerability to foreign pathogens. Viral diseases represent the most serious threat in intensive systems, owing to their high transmissibility and limited options for control, often leading to severe economic losses (Carbonell et al., 2018; Picó et al., 2002). This susceptibility is partly explained by the significant loss of genetic diversity during domestication, particularly in defence-related genes, which reduced the presence of resistance alleles compared with wild relatives (Blanca et al., 2015). In addition, landraces established in Europe were selected in local contexts prior to the emergence of many of today's pathogens. While genetic improvement through the introgression of resistance genes is considered a sustainable strategy to mitigate the impact of disease, negative effects on fruit quality have been reported in the case of virus resistance (Rubio et al., 2016; Verlaan et al., 2011). Modern methods like genome editing, especially CRISPR/Cas systems, are strong tools for introducing virus resistance with high precision. However, using these methods in agricultural production systems, especially in Europe, is difficult due to technical, legal, and social challenges (Chen et al., 2019). This necessitates the meticulous evaluation of landraces, whose primary value lies in their superior fruit quality.

Finally, their strong local adaptation can also constitute a significant limitation, as it may negatively affect agronomic performance and consumer acceptance outside their region of origin. These varieties have been selected historically under specific soil, climate, and management conditions, which can restrict their adaptability to other agricultural environments (Lázaro, 2018). This territorial linkage also influences consumer perception: a comparative study of traditional varieties from Italy, Spain, and France showed that sensory preferences varied by country, and in some cases modern cultivars were favoured over traditional foreign ones (Sinesio et al., 2021). These findings suggest that landraces do not always meet organoleptic expectations in broader markets, thereby reinforcing their positioning within local and niche circuits.

2. Thesis objectives

Tomato is one of the most important horticultural crops worldwide, both in terms of production and consumption. In recent years, the evident decline in tomato quality and flavour has renewed interest in traditional varieties, particularly in Mediterranean areas, where they constitute an important reservoir of cultivated biodiversity and a key component of the regional diet. In addition to mitigating genetic erosion, many of these varieties are highly valued for their flavour and functional composition attributes. The recovery of these varieties for cultivation in modern agriculture requires an integrated approach that combines the identification of materials with added value, improvement of their main limitations, and evaluation of their potential under cropping systems adapted to current challenges.

The main objective of this doctoral thesis was to broaden the genetic and phenotypic diversity of cultivated tomatoes through the development of a collection of traditional varieties and their derived hybrids. This work aimed to preserve the distinctive qualitative attributes of these materials, while addressing the main limitations that constrain their commercial potential, particularly low yield and susceptibility to viral diseases, and to further explore their suitability for sustainable agricultural systems, especially those based on low-input conditions.

Based on this general aim, the specific objectives of the present doctoral thesis are to:

1. Identify, from a diverse collection of traditional indeterminate tomato varieties from south-eastern Spain (IMIDA Germplasm Bank in Murcia), those with acceptable agronomic performance and outstanding fruit quality, including metabolites related to flavour and bioactive compounds (Chapter I).

2. Develop and characterise F_1 hybrids from targeted crosses of selected traditional varieties as a strategy to enhance agronomic performance while maintaining fruit quality (Chapter II).

3. Identify, among the hybrids derived from selected Muchamiel-type varieties and breeding lines incorporating resistance genes to viruses ToMV, TSWV, and TYLCV (developed by the Breeding Group of the CIAGRO-UMH), those that preserve the distinctive fruit morphology, outstanding quality, and favourable agronomic performance (Chapter III).

4. Evaluate the performance of selected tomato accessions under low-input cultivation conditions as a means to identify well-adapted varieties capable of maintaining fruit yield and quality across different environments, taking advantage of their broad adaptive diversity to local growing conditions (Chapter IV).

3. Chapter I

Characterization and phenotypic evaluation of fruit quality traits related to functional and organoleptic quality of Spanish tomato landraces

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Abstract

Among the different vegetable crops, tomato is one of the most popular and widely cultivated in the world due to its socio-economic and nutritional importance. There are more than 25,000 tomato varieties, although only 20% are marketed, which is causing a loss of valuable genetic diversity through the loss of cultivars and the disappearance of traditional and local varieties. In this sense, landraces cover a wide genetic diversity that can help mitigate the current genetic erosion within agricultural diversity, improving nutritional and organoleptic traits compared to commercial F_1. The objective of this study was to determine the diversity of fruit quality parameters related to nutritional and bioactive compounds such as glucose and fructose, organic acids as glutamic, malic and citric acid, vitamin C and carotenoids of forty-eight tomato landraces belonging to nine different cultivar groups: 'Flor de Baladre', 'Corazón', 'de la Sierra', 'Muchamiel', 'de la Pera', 'Pimiento', 'Kumato', 'Mesa Murciano' and 'Cherry'. Fruits with different shapes (squared, slightly flattened, rounded, heart-shaped, long oblong and pyriform), colours (yellow, P and R) and sizes (from very small to very large) were included in this study. The highest values in compounds related to the organoleptic quality of the fruits were found in the orange and yellow 'Cherry' varieties. While the varieties belonging to the 'Cherry' and 'Pimiento' types showed a high content of compounds related to the functional quality (vitamin C and carotenoids). The results obtained show an important intravarietal and intervarietal variability, which would allow the selection of varieties to be used in breeding lines focused on obtaining fruits of high organoleptic and nutritional quality.

3.1. Introduction

In addition to its economic importance, the agricultural sector has a direct impact on the preservation of the rural way of life and the environment and therefore plays a key role in land management and has a great responsibility in the preservation of natural resources. In the European area, almost 50% of the territory is covered by agricultural land, so monitoring the conditions for sustainable agriculture is very important in order to achieve the desired balance between agriculture and the environment. While the agricultural sector continues to grow, global agrobiodiversity is decreasing due to the loss of genetic diversity in food crop varieties (Messmer et al., 2015). This has led to a notable loss in the nutritional quality, flavour and taste of vegetables (Ribes-Moya et al., 2018; Figàs et al., 2015).

Unfortunately, modern agriculture does not make the right choices, as it focuses its efforts on the development of economically important plant varieties, prioritizing traits such as high yield, longevity and visual impact, but neglecting organoleptic and nutritional quality, leading to consumer dissatisfaction (Oltman et al., 2014; Casals et al., 2011). In this context, traditional varieties play a fundamental social, environmental and agronomic role in ecosystems. These local varieties (or landraces) represent a genetic heritage and a source of biodiversity and are the result of a selection process carried out by farmers over generations. The main selection criterion has been organoleptic properties, of which flavour is one of the most important. In this sense, several studies show the superiority of traditional vegetable varieties over commercial ones, taking into account aspects of flavour (Klee and Tieman, 2018), aroma (Cheng et al., 2020), content of compounds beneficial to health (Martínez-Ispizua et al., 2021) and consumer appreciation (Sinesio et al., 2021). Another interesting aspect of traditional varieties is that their selection/breeding process has been based on their ability to adapt to local environmental conditions with specific edaphic-climatic characteristics, mainly in the context of traditional low-input farming systems, so that in many cases they are expected to show satisfactory performance under stress conditions (Fita et al., 2015). In particular, the cultivation of horticultural products in areas of eastern Spain is conditioned by various factors that limit production and affect crop quality, such as scarcity of water resources, high temperatures and poor soil quality (erosion and salinisation) (Alrteimei et al., 2022; Bonachela et al., 2022; Polade et al., 2017). In this scenario, traditional varieties are usually excellent starting materials for breeding programmes, since farmers have selected these landraces or heirlooms for their great behaviour in low input systems and under stress conditions (Fullana-Pericàs et al., 2022; Ribes-Moya et al., 2018; Gonzalez-Cebrino et al., 2011). In addition, promoting their cultivation contributes to the enhancement of agrobiodiversity in the agrifood sector, thus stabilising food production (Renard and Tilman, 2019).

Tomato (*Solanum lycopersicum*) crops are of great economic, social and cultural importance, as they are consumed all over the world. This crop occupies a large area in the eastern part of Spain (about 30,000 ha) and is the most important vegetable produced in Spain, with a total production of 200,000 T. From a nutritional point of view, tomato is considered a high quality food in terms of its content of vitamins, phenolics, carotenoids and antioxidant capacity (Guijarro-Real et al., 2023; Ribes-Moya et al., 2020; Flores et al., 2016, 2017, 2018; Kavitha et al., 2014; Morales-Soto et al., 2014) and traditional genotypes have been found to have very high levels of these compounds and 'taste-of-the-past' flavours, making them more attractive and healthier for consumers (Moreno-Peris et al., 2020; Ribes-Moya et al., 2018; Figàs et al., 2015).

The aim of this study was to determine the diversity of fruit quality parameters related to nutritional and bioactive compounds such as glucose and fructose, organic acids such as glutamic, malic and citric acids, vitamin C and carotenoids of forty-eight tomato landraces belonging to nine different cultivar groups: 'Flor de Baladre', 'Corazón', 'de la Sierra', 'Muchamiel', 'de la Pera', 'Pimiento', 'Kumato', 'Mesa Murciano' and 'Cherry'. Fruits of different shapes (square, slightly flattened, rounded, heart-shaped, oblong and pyriform), colours (yellow, pink, red and orange) and sizes (from very small to very large) were included in this study.

3.2. Materials and methods

3.2.1. Materials

Forty-eight traditional genotypes of tomato (*Solanum lycopersicum* L., Figure 1) were provided by the genebank of the Instituto Murciano de Investigación y Desarrollo Agrario y Medioambiental (BAGERIM-IMIDA) and grown in a greenhouse at an experimental farm (La Pilica) located in Águilas (Murcia, SE, Spain, -1.483, 37.433). This area has a desert-arid climate with mild temperatures and was irrigated with a mixture of desalinated water and well water (1.8-2 dS m^{-1}). The crop design was the same for all varieties, in single rows with 40 cm plant spacing within the row and 1 m row spacing. The plots were managed according to a low-input tomato production management system, using integrated pest management with the support of flower margins to promote the presence of auxiliary fauna, mainly *Macrolophus* sp. to control fruit worms as *Tuta absoluta* and whiteflies. The plants were maintained until the end of the production phase (150-240 days after transplanting, depending on the genotype). Throughout production, fruits were collected and weighed individually to determine total number and average weight. For the analysis of quality parameters, fully red and ripe fruits were selected from trusses 2-4, discarding those that were not homogeneous in colour or had any defect. The ripeness of the fruit was measured daily to avoid over-ripening.

3.2.2. Methods

To determine the mean weight, colour and equatorial and longitudinal diameter of the fruits, four sub-samples of at least ten ripe fruits were randomly collected and processed individually. The weight of each fruit was determined using a Mettler Toledo PG 6002-S (Uznach, Switzerland) balance, colour was measured with a MinoltaCR-200 colourimeter (Ramsey, NJ, USA) and the diameter was determined using a Mitutoyo Digimatic caliper (Japan). Tomatoes belonging to the same tank were cut into small pieces, mixed, frozen in liquid N_2, and homogenized in a food processor (Thermomix, Vorwerk, Switzerland). The homogenate of each sample was frozen at -80 °C for further analysis.

Total soluble solids (TSS) content was determined with a calibrated hand-held refractometer (Atago, USA) and expressed as °Brix, and titratable acidity (TA) were determined by titration with 0.1 N NaOH with an endpoint titrator (Mettler Toledo, USA) and expressed as grams of citric acid per litre of juice.

Figure 1. Pictures of the accessions studied according to the key in table 1. From one to forty-eight, from right to left and from top to bottom.

Soluble sugars and organic acids were extracted with water and subsequently purified using C18 Sep-Pak cartridges (Flores et al., 2016). Soluble sugars were analysed in a liquid chromatograph (Agilent 1100 Waldbronn, Germany) equipped with a refraction index detector and a CARBOSep CHO-682 LEAD column using ultrapure water as mobile phase at a flow rate of 0.4 mL min^{-1}, and organic acids were determined by liquid chromatography using a G6410A triple quadrupole mass spectrometer detector (Agilent Technologies, Santa Clara, CA, USA) according to (Flores et al., 2012).

Vitamin C (ascorbic and dehydroascorbic acids) was determined according to the method developed by Fenoll et al. (2011), briefly, tomato samples were homogenised with EDTA, 0.05% (w/v) and dithiothreitol and analysed by LC-MS-MS (Agilent Technologies, Santa Clara, CA, USA).

Total carotenoids and chlorophyll were extracted with ethyl acetate and determined by measuring the optical density at 450, 663 and 645 nm in a Shimadzu UV-2401PC spectrophotometer (Kyoto, Japan) according to Nagata and Yamashita, (1992), carotenoids were quantified by comparison with a standard curve of lycopene and *β*-carotene (Merck KGaA, Damstadt, Germany).

The results were statistically analysed using IBM SPSS Statistic 25 with analysis of variance (ANOVA) and Duncan's multiple range test for differences between means.

3.3. Results

Based on previous results (Flores et al., 2017), a selection of 48 *Solanum lycopersicum* accessions (Figure 1) from the IMIDA germplasm bank was made to be evaluated as sources of variability for the valorisation and breeding of high quality horticultural cultivars.

The selected landraces belong to nine cultivars such as 'Flor de Baladre', 'Corazón', 'de la Sierra', 'Muchamiel', 'de la Pera', 'Pimiento', 'Kumato', 'Mesa Murciano' and 'Cherry'. Within each variety there are fruits of different colour, size and shape (Table 1). In terms of colour, the selection includes four yellow (Y), one orange (O), nine pink (P) and thirty-four red (34) varieties. They include carriers (7) and non-carriers (41) of the STAY-GREEN (*SGR*) protein mutation. This mutation causes the inhibition of chlorophyll degradation during the ripening process and therefore the presence of chlorophyll in the ripe fruit. As a result of this mutation, the fruits are pink-black (P-B), as in 'de la Sierra', or reddish-black (R-B), as in 'Negro de Nerpio', 'Bola Negra', 'Tomate Redondo', 'Pera Negro', 'Tomate Negro' and 'Tomate Redondico', which have a dark colour of varying intensity. A high variability in size and weight was also observed, including varieties with flattened (15), squared (8), rounded (11), heart-shaped (3), pepper-shaped (2), pyriform (6), spherical (1) and truncated (2) fruits, and with different sizes and weights, from very small to very large. Thus, fruits were classified as very small (<15 g), small (≥15 and <70 g), medium (≥70 and <150 g), large (≥150 and <300 g) and very large (≥300 g).

Table 1. Fruit morphological characteristics in fully ripened fruits of the 48 tomato genotypes studied.

Type	Local Name (Number Identification)	Colour*	Size**	Shape
Corazón Toro	Tomate Corazón (1)	P	XL	Heart-shaped
	Corazón de Toro (2)	R	XL	Heart-shaped
de la Sierra	Amarillo Chaparral (3)	Y	L	Flattened
	Tomate Amarillo (4)	Y	XL	Flattened
	Tomate de Agramón (5)	P	XL	Flattened
	Tomate de la Sierra (6)	P-B	XL	Squared
	Negro de Nerpio (7)	R-B	XL	Flattened
Flor de Baladre	Tomate Rosa (8)	P	XL	Flattened
	Flor de Baladre 1 (9)	P	XL	Flattened
	Flor de Baladre 2 (10)	P	XL	Squared
	Flor de Baladre 3 (11)	P	XL	Flattened
Kumato	Bola Negra (12)	R-B	M	Rounded
	Tomate Redondo (13)	R-B	M	Rounded
	Tomate Pera Negro (14)	R-B	M	Pruned
Mesa Murciano	Rosa de Barranda (15)	P	XL	Flattened
	Rosa de la Arboleja (16)	P	XL	Flattened
	Tomate Acorazonado (17)	R	XL	Heart-shaped
	Tomate Cuarenteno (18)	R	L	Flattened
	Tomate Murciano (19)	R	L	Rounded
	Tomate del País 1 (20)	R	L	Rounded
	Tomate del País 2 (21)	R	XL	Flattened
	Tomate Mesa 1 (22)	R	XL	Squared
	Tomate Mesa 2 (23)	R	L	Rounded
	Tomate Gordo (24)	R	L	Globose
Muchamiel	Muchamiel (25)	R	XL	Pruned
	Pera Muchamiel (26)	R	L	Flattened
	Tomate Aplastado (27)	R	L	Flattened
	Tomate de Molina (28)	R	XL	Squared
	Tomate Muchamiel 1 (29)	R	XL	Squared
	Tomate Rizado (30)	R	XL	Flattened
	Muchamiel Corazón (31)	R	XL	Pyriform
	Muchamiel Gordo (32)	R	XL	Squared
	Tomate Muchamiel 2 (33)	R	XL	Flattened
Pera	Pera Pinatar (34)	R	L	Pyriform
	Tomate Alargado (35)	R	M	Squared
	Tomate Aperado (36)	R	M	Pyriform
	Tomate de Mesa (37)	R	L	Squared
	Tomate Pera (38)	R	L	Pyriform
Pimiento	Tomate Pimiento 1 (39)	R	L	Pepper
	Tomate Pimiento 2 (40)	R	L	Pepper
Cherry	Bombilla Amarillo (41)	Y	S	Pyriform
	Cherry Amarillo (42)	Y	S	Rounded
	Bolica Naranja (43)	O	S	Rounded l
	Bolica Roja (44	R	XS	Rounded
	Cherry (45)	R	XS	Rounded
	Tomate Bombilla (46)	R	S	Pyriform
	Tomate Negro (47)	R-B	S	Rounded
	Tomate Redondico (48)	R-B	S	Rounded

*P: Pink, R: Red, Y: Yellow, R-B: Red-Black, P-B: Pink-Black, O: Orange. **XL: Very Large, L: Large, M: Medium, S: Small, XS: Very Small.

This variability was observed both between types and between varieties. As expected, the smallest varieties were of the 'cherry' type (41-48), with weights ranging from about 8 to 50 grams (Table 2), the smallest being the so-called 'cherry' variety with an average weight of 8 grams. On the other hand, the largest varieties were 'Corazon' and 'Flor de Baladre', with fruits weighing over 400 grams. For the 'Pera' and 'Pimiento' varieties, the average weight and size of the fruit was between 130 and 190 grams per fruit.

With regard to titratable acidity (TA) and total soluble solids (TSS), the values obtained showed a high variability within types and varieties (Table 2), ranging from 2 to 7.8 g citric acid L^{-1} and 3.3 to 8.7 °Brix. In general, the highest values of both parameters corresponded to the 'Cherry' type, among which the varieties 'Bolica Roja (44)' and 'Cherry (45)' stood out. On the other hand, the lowest values, between 2 and 3 for total acidity and 3 and 4 for TSS, were found in the varieties 'Pimiento', 'Muchamiel' and 'Mesa Murciano' and in 'de la Sierra', 'Flor de Baladre', 'Mesa Murciano' and 'Muchamiel'. Within these varieties, 'Tomate Gordo (24)' and 'Tomate Rosa (8)' stood out for their low TSS values, 3.3 and 3.6 °Brix, respectively, and 'Tomate Gordo 2 (32)' and 'Tomate Pimiento 2 (40)' for their low acidity values, 2 and 2.4 g L^{-1}, respectively.

As with the previous parameters, a large variability was observed between both types and varieties in the equatorial and longitudinal diameters and in the colour, parameters determined, which were consistently related to the shape and colour defined by the IPGRI (International Plant Genetic Resources Institute) descriptors and were in line with what was expected within the characteristics of each variety. The variability observed in the size, colour and shape of the selected varieties was also observed in the nutritional and organoleptic composition of the fruits (Figures 2, 3 and 4). In general, the cherry genotypes were superior in terms of organoleptic and nutritional compounds, with high concentrations of sugars, organic acids, vitamin C and carotenoids.

With regard to sugars, the main compounds found were glucose and fructose (Figure 2), whose values ranged between 6-25 mg g^{-1} and 6-30 mg g^{-1}, respectively. For both sugars, the highest values were found in genotypes 42, 43 and 45 (cherry type). Among the remaining genotypes, 6, 13 and 18 showed the highest values for both sugars.

Table 2. Fruit weight (g), equatorial diameter (Eq. Diameter, mm), longitudinal diameter (Lg. Diameter, mm), colour parameters (L, a*, b*), total soluble solids (SST, °Brix) and acidity (AT, g L^{-1}) of landrace tomatoes at harvest.

Landrace	Fruit Weight	Eq. Diameter	Lg. Diameter	L	a*	b*	SST	AT
1	406.8	92.4	94.3	35.0	19.4	11.1	4.7	3.1
2	441.4	96.8	89.7	35.5	18.1	10.5	4.4	3.1
3	207.0	86.3	50.7	48.9	-4.5	21.6	5.0	3.5
4	507.4	112.5	77.0	51.9	-5.2	23.0	4.0	3.7
5	524.1	111.6	68.4	33.9	11.4	12.3	4.4	3.2
6	387.0	100.0	70.0	34.7	11.0	10.0	5.3	4.7
7	535.7	113.8	70.6	32.6	10.3	11.9	4.0	3.1
8	586.2	116.0	64.9	36.2	15.8	10.4	3.6	2.9
9	474.9	172.5	132.9	38.0	17.4	12.1	4.7	3.4
10	529.0	120.6	70.4	37.3	18.7	11.6	4.7	3.0
11	535.9	119.6	72.7	39.8	18.1	12.5	4.6	3.1
12	120.0	62.8	54.8	31.3	10.4	11.0	5.8	3.3
13	123.1	61.5	55.7	30.8	7.9	10.0	5.6	3.1
14	54.2	42.3	52.3	31.9	10.0	11.0	5.7	5.0
15	494.9	111.2	70.6	42.9	14.5	12.3	5.7	2.6
16	451.8	105.9	70.2	41.4	19.2	13.8	5.5	4.2
17	325.8	91.2	71.1	48.7	10.0	24.5	5.0	3.6
18	235.7	83.6	62.0	35.3	19.4	14.7	5.8	3.0
19	259.0	81.8	67.6	37.0	20.8	15.9	4.5	4.0
20	288.1	90.4	69.6	36.0	18.5	14.9	4.7	4.5
21	462.3	110.8	70.7	31.5	15.2	12.1	5.7	3.8
22	313.7	88.6	62.9	35.2	16.1	12.8	4.8	3.5
23	237.1	79.7	75.2	37.7	16.4	15.1	4.3	3.5
24	269.9	84.7	65.8	37.0	18.8	16.0	3.3	2.9
25	477.7	113.6	68.7	38.3	15.3	15.2	3.9	2.6
26	274.7	94.3	65.8	36.6	22.3	14.5	4.5	3.6
27	417.2	108.8	65.6	36.6	17.9	13.5	4.4	3.2
28	371.9	98.2	66.1	37.5	19.2	14.6	4.3	3.1
29	378.3	106.1	70.9	35.2	20.5	13.7	4.4	2.7
30	284.5	91.5	74.9	38.2	21.5	15.8	5.0	2.6
31	284.2	92.4	77.8	36.5	20.2	15.0	4.9	2.7
32	420.7	113.2	69.8	35.2	22.9	14.9	4.1	2.0
33	391.1	99.9	68.3	38.6	17.7	11.7	3.9	2.6
34	159.2	72.5	73.4	37.6	19.1	13.9	4.9	3.2
35	135.6	68.4	58.3	35.5	19.0	13.8	5.3	3.8
36	135.5	63.9	64.7	36.0	19.0	14.1	4.8	4.0
37	171.6	72.2	72.1	36.2	22.0	15.1	5.0	2.7
38	140.9	62.2	66.9	37.5	21.1	16.2	5.0	3.6
39	195.5	57.8	117.3	33.9	20.2	14.6	5.2	3.1
40	190.9	62.8	103.2	35.8	21.2	15.3	5.0	2.4
41	15.3	28.1	43.7	48.2	-3.7	23.3	6.3	4.6
42	16.1	29.5	30.8	40.6	-0.9	18.9	8.7	5.2
43	19.6	28.0	39.8	39.5	11.4	19.4	8.2	5.5
44	11.9	26.9	26.4	30.7	14.9	11.1	8.3	7.8
45	7.9	23.6	22.1	33.4	17.9	13.3	8.7	5.0
46	46.9	46.3	43.4	34.2	18.1	13.1	5.6	4.8
47	41.5	41.7	40.7	29.2	3.3	9.3	7.7	4.6
48	51.8	42.1	52.4	30.7	10.8	10.3	5.6	3.4

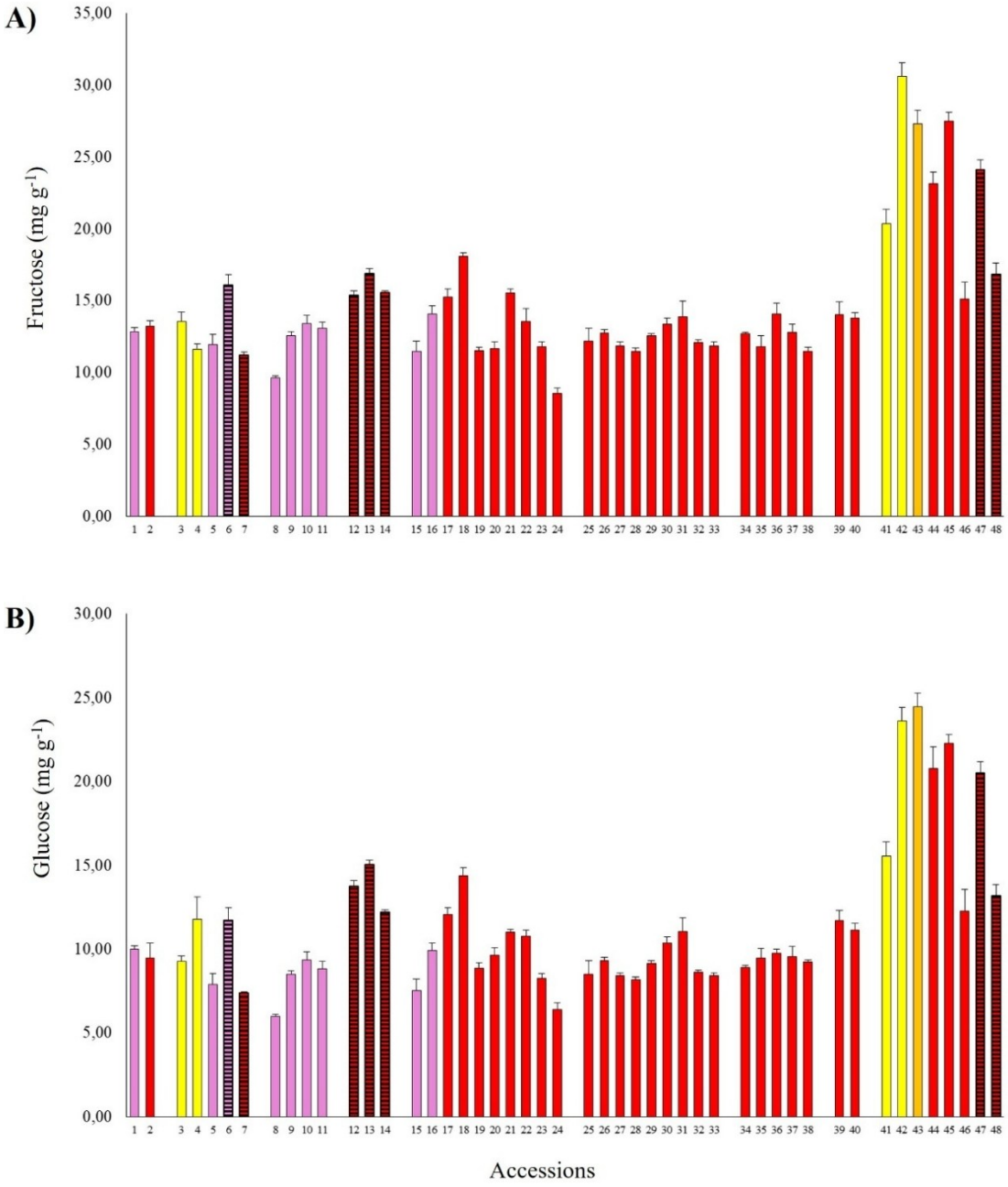

Figure 2. Concentration (mg g^{-1} FW) of fructose (A) and glucose (B) in tomato accessions classified from 1 to 48 (according to the key in Table 1) and coloured according to the external colour of the fruit. Bars represent mean ± SE (n=4).

With regard to the organic acids (Figure 3), thirteen compounds were determined, three major acids, citric, malic and glutamic, which ranged between 4.5-13.6, 0.6-1.7 and 4.4-10.4 mg g^{-1} respectively, and ten minor acids (quinic, tartaric, pyruvic, isocitric, shikimic, malonic, ketoglutaric, succinic, fumaric and glutaric), which were present in all the genotypes studied. In general, the citric acid content (Figure 3a) of the cherry-type fruits was higher than that of the other varieties, with the 'Bolica Roja' genotype standing out (13.6 mg g^{-1}). On the other hand, genotype 3 (de la Sierra type, Amarillo Chaparral) stood out for its malic acid content (1.7 mg

g^{-1}). Cherry tomatoes also stood out for their minority acid content (Figure 3d). The pepper genotypes also showed the lowest values of organic acids. The glutamic acid content ranged from 4.4 (44, Bolica Roja) to 10.4 mg g^{-1} (26, Pera Muchamiel).

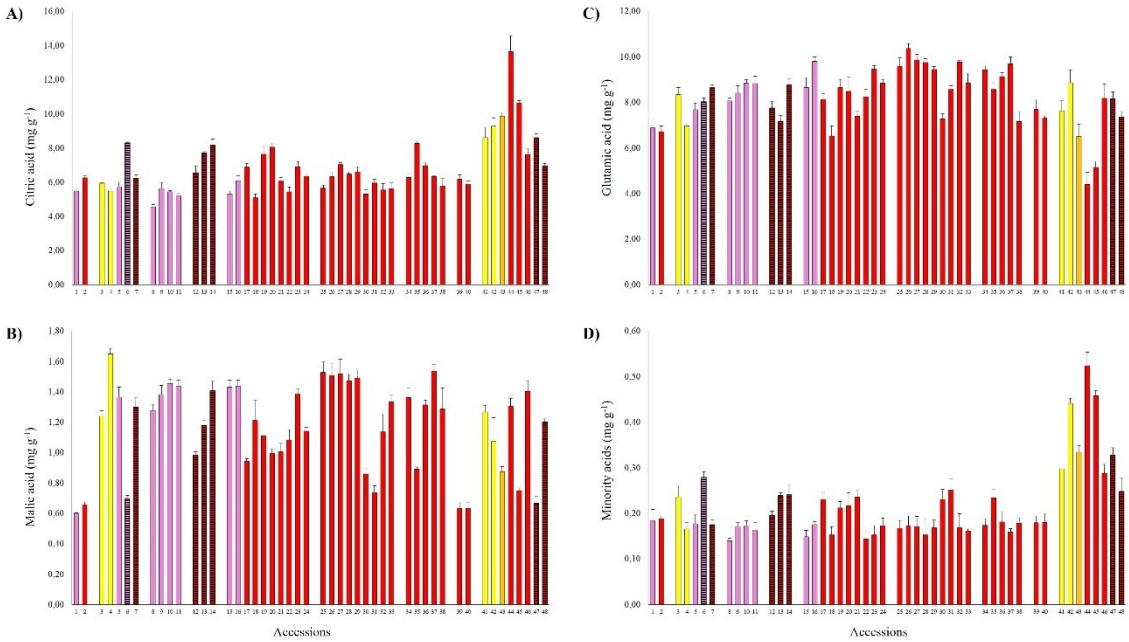

Figure 3. Concentration (mg g^{-1} FW) of citric acid (a), malic acid (b), glutamic acid (c) and sum of minority acids (d), in tomato accessions classified from 1 to 48 (according to the key in Table 1) and coloured according to the external colour of the fruit. Bars represent mean ± SE (n=4).

Similarly to the previous results, the vitamin C content (Figure 4) was related to the type and size of the fruit, with the highest concentrations being obtained in the small or very small cherry genotypes. The minimum value (9 mg 100 g^{-1}) was found in 23 and 24 (Mesa Murciano type) and the maximum value (40 mg 100 g^{-1}) in 45 (Cherry).

Finally, the content of total carotenoids (Figure 4), which ranged from 18 to 165 µg g^{-1}, was directly related to the colour of the fruit, being absent or present in low concentrations in yellow and pink fruits. Red, orange and red-black coloured fruits showed the best results. Again, the highest levels were found in cherry fruits, especially in genotype 47 (Black Tomato), which had a very intense red-black colour.

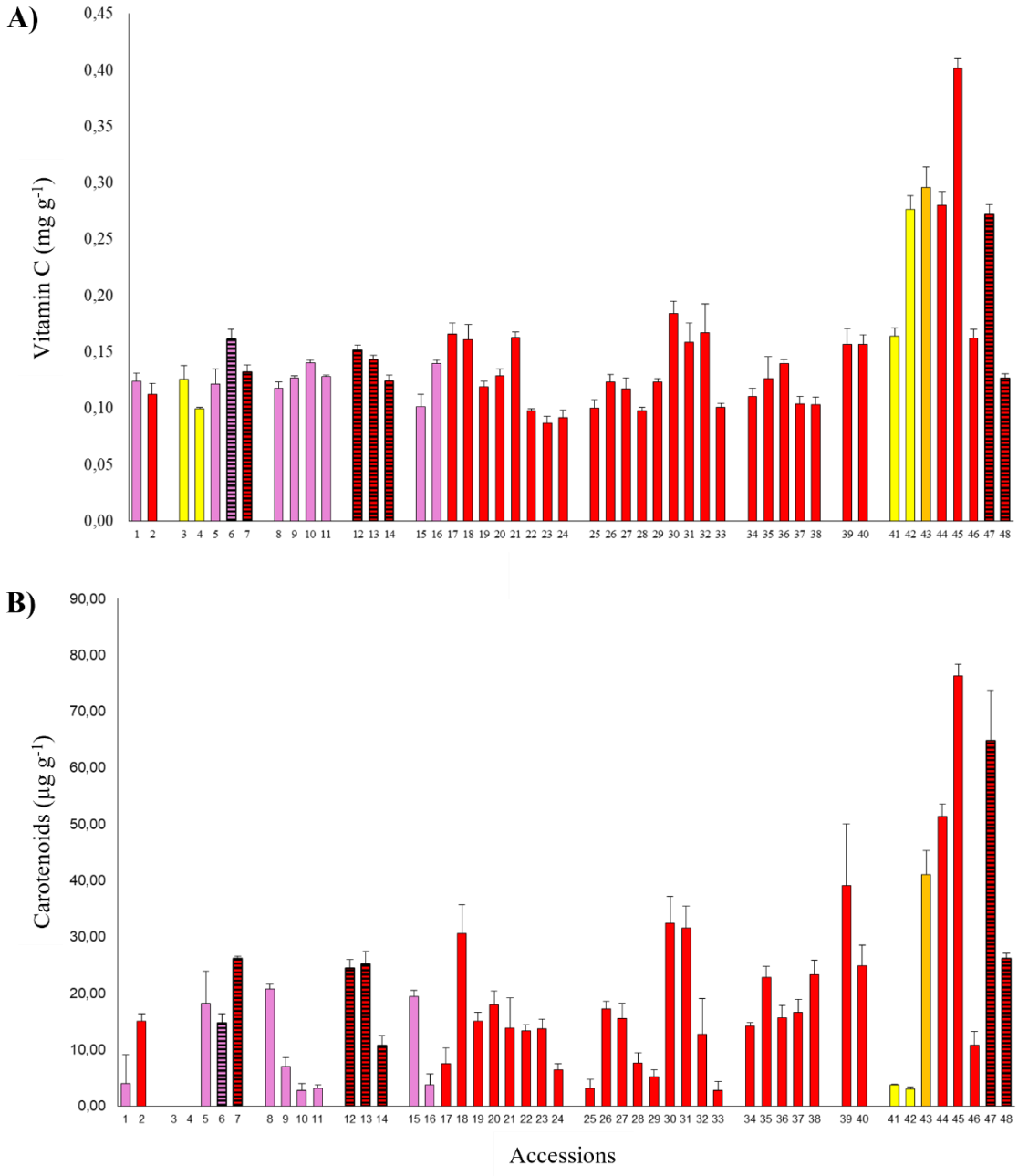

Figure 4. Concentration of vitamin C (A, mg g^{-1} FW) and carotenoids (B, µg g^{-1} FW), in tomato accessions classified from 1 to 48 (according to the key in Table 1) and coloured according to the external colour of the fruit. Bars represent mean ± SE (n=4).

3.4. Discussion

The development and valorisation of new varieties must take into account many factors, from yield to resistance to environmental changes, from taste to storage or transport characteristics, and of course consumer preferences and/or market requirements (Sinesio et al., 2021; Ribes-Moya et al., 2018). In this respect, landraces can represent a portfolio of desirable crop characteristics, as they are best adapted to local growing conditions, require less

agrochemical resources compared to modern varieties, and maintain a high diversity of yields, flavours, colours and shapes (Moreno-Peris et al., 2020; Ribes-Moya et al., 2018). The latter makes them very attractive to consumers and markets.

This study analysed the diversity present in a number of local tomato varieties, taking into account different physical (weight, colour, size, shape), organoleptic (total soluble solids, acidity, sugars and organic acids) and nutritional (vitamin C and carotenoids) characteristics. The analysis of fruit weight and colour gave an idea of the variability present both between and within varieties. This variability was also observed in the nutritional and organoleptic composition of the fruits. It is well known that consumers often select tomatoes based on their appearance, and colour and size are two of the most important quality factors that influence the appearance of tomatoes and therefore their acceptability (Yahia and Brecht, 2012).

In our study, tomato fruits have different external colours, such as pink, yellow, orange and red, as a result of the colour of the flesh and skin. Thus, a pink tomato is due to a colourless skin and red flesh, while an orange tomato is due to the presence of a yellow skin and red flesh (Figàs et al., 2015). Genotypes with reddish-black and pinkish-black colouring are also included in our study because they carry the STAY-GREEN (*SGR*) mutation. These genotypes are currently widely accepted by consumers and growers, partly due to their distinctive colour and characteristic flavour (Manoharan et al., 2017). In addition to their organoleptic quality, these varieties have a high nutraceutical value due to their proven properties against cancer and other diseases (Fahey et al., 2005). In this sense, and considering the role of nutrition in health, it is interesting to include in the diet products rich in antioxidants such as vitamins, carotenoids and chlorophylls (Cruz et al., 2013). For example, the vitamin C content of commercial tomatoes is around 0.13 mg g^{-1} (Bonachela et al., 2022; George et al., 2017) and the carotenoid content is 40-60 µg g^{-1} (Flores et al., 2017; Figàs et al., 2015; Gonzalez-Cebrino et al., 2011), while in our study 50% of the varieties studied reached or exceeded these concentrations, so they can be considered good sources of healthy compounds and can be defined as varieties with "high added value".

3.5. Conclusions

An integrated description of traditional tomato varieties from south-eastern Spain was carried out, including morphological, organoleptic and nutritional parameters. The aim of this study was to characterise and subsequently exploit local tomato varieties particularly adapted to low-input cultivation. The results showed a high variability and the presence of varieties with high nutritional quality. The red-black and pink-black varieties, regardless of their size, stood out for their organoleptic composition and flavour, mainly due to their fructose and citric acid content. Muchamiel tomatoes also stood out for their glutamic acid content, one of the components of the *umami* flavour. In terms of bioactive compounds, the cherry tomatoes were once again outstanding, but the 'Tomate cuarenteno' and 'Tomate pimiento' also showed very interesting values for vitamin C and carotenoids. After this study, we hope that these varieties can be used either for direct exploitation ("*per se*") or for the improvement and development of new hybrid varieties of high quality and taste, supporting the interest of farmers and consumers.

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4. Chapter II

Fruit agronomic and quality traits of tomato F_1 hybrids derived from traditional varieties

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Abstract

The high genetic diversity of the tomato and its high micronutrient content make this fruit very interesting from an economic and nutritional point of view. The genetic erosion suffered by this crop, due to breeding objectives based on yield and marketing, makes it necessary to return to the origins in search of the nutritional and organoleptic quality lost in traditional varieties. In this study, the agronomic, physical, organoleptic, and nutritional characteristics of eighteen F_1 hybrids, obtained by crossing fourteen traditional varieties, previously selected for their quality, were studied in order to select genotypes of superior quality that could be candidates for new varieties. All the parameters studied were strongly influenced by genotype, with a wide range between varieties. Most of the experimental hybrids showed higher quality scores than the commercial hybrids used as controls, due to the extensive selection process carried out on the parents in previous work. Principal component analysis revealed the characteristics of each hybrid that distinguished it from the others. Some hybrids (H1, H2, and H4) stood out for their high concentration of active compounds, others (H14, H13, H8, H15, H7, and H9) for their agronomic performance and high β-carotene content, and H3 was the only one to contain chlorophyll in its ripe fruits. Finally, the evaluation index allowed the selection of five hybrids with interesting characteristics, combining good yield performance and high quality. The results of this work have allowed for the selection of a group of hybrids with high organoleptic and nutritional quality which will be used as parents in a breeding programme, in which their characteristics will be fixed and their resilience will be increased through the introduction of virus resistance.

4.1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely consumed vegetables in the world all year round. The annual production of fresh tomatoes amounted to approximately 186 million tons in 2022 (Food and Agriculture Organization, 2023). In addition to its economic relevance, tomato is a nutritionally well-balanced food that presents a high content of bioactive compounds (Flores et al., 2016, 2017; Kavitha et al., 2014; Morales-Soto et al., 2014). Among the chemical compounds that can be found in ripe tomatoes, the most significant are carotenes (such as lycopene, *β*-carotene, and lutein), vitamin C, and phenolic compounds, which are considered health-promoting agents (Wang et al., 2023; Felföldi et al., 2022; Ali et al., 2021). Specifically, carotenoids (lycopene and *β*-carotene) found in tomatoes are precursors of vitamin A and provide ripe tomatoes with their red colour (Anđelini et al., 2023; Flores et al., 2017; Adalid et al., 2010). Additionally, it has been reported that these bioactive compounds can inhibit the proliferation of many types of cancer cells and potentially serve as antioxidants to prevent the development of cardiovascular diseases, age-related macular degeneration, and other eye conditions (Friedman, 2013). Moreover, phenolic compounds may have beneficial effects on inflammation-based illnesses and various forms of cancer (Tommonaro et al., 2012). Finally, vitamin C demonstrates significant antioxidant and electron donation capabilities, thus protecting DNA from damage caused by oxidation (Scarano et al., 2020). The quality of tomatoes is not only determined by their nutritional value, but also by their organoleptic and flavour characteristics. These properties of the fruit result from the combination of taste and aromas created by various volatile and non-volatile compounds (Figàs et al., 2015). The balance and interaction between organic acids (malic, citric, and glutamic) and soluble sugars (fructose and glucose) contribute to the distinct flavours of tomatoes (Anđelini et al., 2023; Natalini et al., 2021). However, different studies have demonstrated that the production and accumulation of these compounds are influenced by various factors, including agronomic and environmental conditions, as well as genetic variations among cultivars (Ali et al., 2021; Dumas et al., 2003).

Modern agriculture has prioritised the development of new tomato cultivars with high yield, uniform appearance, disease tolerance, and long shelf-life, aimed at enhancing crop management and market distribution. However, due to the limited number of commercial varieties used for tomato production across the globe, genetic erosion has become increasingly prevalent (Ficiciyan et al., 2018; Messmer et al., 2015). The decrease in biodiversity causes a decrease in genetic diversity, resulting in the loss of distinctive traits related to aroma, taste, and appearance that are characteristic of local varieties due to a lack of attention in recent times (Casals et al., 2021; Ribes-Moya et al., 2018; Figàs et al., 2015). Specifically, research shows that breeding efforts have altered the metabolome of tomato fruit, leading to the loss of numerous genes

associated with fruit quality in favour of higher yields (Zhu et al., 2018). Currently, the nutritional and organoleptic quality of fresh tomatoes is a vital parameter for growers and tomato breeders due to the rising consumer interest in healthy and flavourful food (Sinesio et al., 2021; Flores et al., 2017; Oltman et al., 2014). Landraces could serve as a workable solution, as they may possess beneficial allelic combinations that are absent in modern varieties (Arca et al., 2023; Caramante et al., 2023). Although these traditional types often possess exceptional organoleptic and nutritional characteristics, their agronomic performance in terms of yield or resistance to the prevailing biotic stresses is often unsatisfactory, rendering them inappropriate for widespread commercial cultivation under most circumstances (Baldina et al., 2016).

In Spain, certain traditional varieties are arriving on the market and becoming viable alternatives for farmers in terms of economic profitability. This is mainly due to the successful implementation of breeding and valorisation programmes. Notably, the "Valenciano d'El Perelló" and "de Penjar d'Alcalà de Xivert" tomatoes in the Valencian Community are interesting examples in this regard (Figàs et al., 2015). A valorisation programme is currently underway, focusing on improving resistance to viruses and fungi, which have a significant impact on these tomato varieties (Figàs et al., 2015; Soler et al., 2015). Similarly, significant progress has been made in the development of high-performance materials for the "De la Pera" and "Mutxamel" tomatoes grown in Alicante, where resistance to viruses has been incorporated (García-Martínez et al., 2015). In addition, breeding efforts with "Pera de Girona" and "Montserrat" tomatoes in Catalonia have yielded remarkable results (Casals, 2014; Casals et al., 2010). The successful implementation of these breeding programmes has the potential to offer farmers profitable alternatives in an increasingly competitive and complex agricultural landscape, thus ensuring the sustainability and development of the agricultural industry.

The aim of tomato breeding is to produce heterozygous F_1 hybrid cultivars with specific disease resistance, quality, and/or yield characteristics. This is achieved by crossing two carefully selected divergent parental lines, resulting in a combination of desirable traits from both parents and promoting heterosis (Liu et al., 2021; Carli et al., 2011), which improves fruit performance. Due to their valuable-quality gene pools, traditional tomato varieties can be used as good parents to develop high-quality F_1 hybrid cultivars that can improve the quality of commercial varieties and be economically competitive (Kumar and Yadav, 2023; Fortuny et al., 2021). This strategy could effectively revalue traditional varieties as sources of nutritious food while increasing tomato production. In our group and in recent years, extensive evaluation and characterisation work has been carried out on more than 60 traditional tomato varieties from the regional germplasm bank (Sánchez et al., 2023; Flores et al., 2018). As a result of this previous work, fourteen of these landraces were selected for their high nutritional quality and adequate agronomic response to act

as parents in the breeding of new hybrids. In view of the above, this work proposes the characterisation of new tomato hybrids in terms of agronomic (total yield, average fruit weight, and number of fruits), physical (polar diameter, equatorial diameter, and colour parameters), organoleptic (glucose, fructose, total soluble solids, and acidity), and nutritional (vitamin C, carotenoids, and phenolic compounds) traits, with the aim of selecting new genotypes of superior quality to add to the genetic diversity currently present in the agricultural sector.

4.2. Materials and methods

4.2.1. Cultivation conditions and plant material

Fourteen pure lines of traditional Murcian tomato from the germplasm bank (BAGERIM) of the Instituto Murciano de Investigación y Desarrollo Agrario y Medioambiental (IMIDA) were used as parentals to obtain high-quality hybrids (Figure S1). These parental lines were the result of a selection in which priority was given to their organoleptic and/or nutritional quality (Table S1; Figure S2) (Sánchez et al., 2023; Flores et al., 2018, 2017). Eighteen biparental crosses were made by combining these parental lines according to their specific characteristics (Figure S1; Table S2). Plants were grown in a greenhouse at an experimental farm ("Torreblanca") located in Torre Pacheco (Murcia, SE Spain, 37°46′33.564″ N, 0°53′47.225″ W), on soil classified as clay loam and irrigated with water from the Tajo-Segura Transfer System (0.8–1.3 dS m^{-1}). The area has a Mediterranean climate. Transplanting was conducted in December according to a randomised block design with three replicate plots and ten plants per replication. The distance between rows was 1.0 m, and the distance between adjacent plants within a row was 0.4 m. Plants were maintained until the end of the production phase (130–250 days after transplanting, depending on the genotype). Plots were managed according to a low-input tomato production management system, using integrated pest management with the support of flower margins to promote the presence of auxiliary fauna. In addition, two commercial F_1 hybrids (Mongo and Pasadena) were included as controls.

4.2.2. Agronomic and phenotypic evaluation

At the end of the growing season, three yield-related traits (total yield (TY), fruit number (FN), and mean fruit weight (NF)), and three morphological traits (equatorial (ED) and longitudinal diameters (LD), as well as external colour) were evaluated. For total yield, fruit number, and average fruit weight, all of the fruits from 3 plants per replicate were collected and individually weighed. Of these, 10 fruits from each block were randomly selected for morphological characterisation. Equatorial and longitudinal diameters and external colour were then measured using a Mitutoyo 500−196-30 Digimatic calliper (Kanagawa, Japan) and a Minolta CR-200 Chroma Meter (Ramsey, NJ, USA), respectively. The colour parameters L* (lightness),

a* (red-ness), and b* (yellowness) were measured in three different areas of the fruit surface. The hue angle (HUE, h° = $\tan^{-1}$ [b*/a*]) and the saturation or chroma (CH, C = [a*2 + b*2]/2) were calculated from the primary L*, a*, and b* measurements.

4.2.3. Analysis of fruit quality and nutritional value traits

For total soluble solids (TSS) and titratable acidity (TA), about 20 fruits from the same replicate were then cut into small pieces, mixed, and frozen at −80 °C, then homogenised using a Thermomix and frozen at −80 °C until further analysis. TSS was measured by refractive index (expressed as °Brix) using a digital hand-held "pocket" refractometer PAL−1 (Atago, USA), and TA was determined by automatic titration (Mettler-Toledo DL15 automatic titrator) with 0.1 N sodium hydroxide and expressed as g of citric acid per L of juice.

4.2.4. Determination of soluble sugars

Triplicate aliquots of frozen tomatoes were used for the determination of soluble sugars (glucose (GL) and fructose (FR)). Compounds were extracted with deionised water, purified with C18 Sep-Pak cartridges, and analysed by molecular exclusion chromatography using an Agilent 1100 liquid chromatograph (Waldbronn, Germany) equipped with a refractive index detector and a CARBOSep CHO-682 LEAD (Concise Separations, San Jose, CA, USA) 300 × 7.8 mm ID column using deionised water as a mobile phase at a flow rate of 0.4 mL min^{-1} (Flores et al., 2016). The results are expressed as mg g^{-1} FW.

4.2.5. Determination of carotenoids and chlorophylls

Carotenoids and chlorophylls were extracted according to (Böhm, 2001) using *β*-apo-8′-carotenal as an internal standard and determined on a Hewlett–Packard 1200 HPLC system (Santa Clara, CA, USA) equipped with an UV-visible photodiode array detector (spectral range from 250 to 800 nm), according to Hernández et al. (2015). Separation was carried out at a flow rate of 1.0 mL min^{-1} in a 250 mm × 4.6 mm, i.d., 3 µm Prontosil C30 column (Bischoff, Leonberg, Germany), using methanol and methyl tert-butyl ether as a mobile phase. The main compounds were quantified as µg g^{-1} using the external standards, lycopene (LY), and *β*-carotene (BC) (Sigma-Aldrich, Steinheim , Germany) and lutein (LU), violaxanthin (VIO), and chlorophyll (CHL) (DHI LAB, Hoersholm, Denmark). Phytoene (PHE) and phytofluene (PHF) are expressed as *β*-carotene equivalents.

4.2.6. Determination of vitamin C

For the extraction of vitamin C (ascorbic and dehydroascorbic acids), samples were homogenized with EDTA, 0.05% (*w/v*) and dithiothreitol (Sigma, Steinheim, Germany). Vitamin C was analysed using liquid chromatography equipped with a triple quadrupole mass spectrometer detector (Agilent Series 1200, Santa Clara, CA, USA) according to the methodology

developed by Fenoll et al. (2011). Vitamin C (VC) was quantified as mg g^{-1} using commercial ascorbic acid as a standard (Sigma-Aldrich, Steinheim, Germany).

4.2.7. Determination of phenolic compounds

Phenolic compounds were extracted with methanol:formic acid (97:3) and identified as described by Vallverdú-Queralt et al. (2011). Analysis was performed by HPLC-MS/MS on a Lichrosphere C18 analytical column (250 mm × 4 mm and 5 µm particle size) according to Flores et al. (2019). Phenolic compounds were quantified against their corresponding standards purchased from Sigma-Aldrich. If no standards were available, the corresponding isomer, hydroxycinnamic acid, or aglycone was used. The LC-QqQ analysis led to the identification of 18 compounds, which were grouped into different families: flavanones (FA), calculated as the sum of naringenin and naringenin-*O*-hexoside; flavonols (FO), calculated as the sum of rutin-O-hexoside and kaempferol-3-*O*-rutinoside; and hydroxycinnamic acids (HA), determined as the sum of chlorogenic acid, *p*-coumaric acid, ferulic acid, caffeic acid, homovanillic acid, and their respective isomers and derivatives. All of the compounds were quantified as µg g^{-1}.

4.2.8. Statistical analysis

Results were statistically analysed using IBM SPSS Statistic 25. Measures of dispersion were calculated (mean, standard deviation, coefficient of variation, range). Pearson's linear correlation coefficients were calculated between pairs of traits, and the significance ($p < 0.05$) of the correlations was assessed using the Bonferroni test. Principal component analysis (PCA) was performed on the normalised compositional data using pairwise Euclidean distances between accession means. Eigenvalues and percent variance of each principal component were calculated, as well as the correlation coefficients between compositional traits and principal components.

Finally, in order to identify hybrids with a high fruit quality, an evaluation index (EI) was estimated according to the previously published method (Scarano et al., 2020), assigning to each trait a score ranging from a maximum of 18 to a minimum of 1, descending from the highest to the lowest value for all of the traits related to organoleptic (total soluble solids, total acidity, soluble sugars) and bioactive (vitamin C, phenolic compounds, carotenoids, and chlorophylls) characteristics.

4.3. Results

Eighteen hybrid varieties were obtained by crossing fourteen traditional varieties belonging to eight tomato types (Corazón de Toro, de la Sierra, Flor de Baladre, Kumato, Mesa Murciano, Muchamiel, de la Pera, and Pimiento) from the IMIDA germplasm bank (Table 1). Progenitors included fruits of different sizes (medium, large, and extra-large), colours (pink, red,

and red-black) and shapes (heart-shaped, pyriform, oblong, and rounded). A wide variability in size, shape, and colour was also observed among the hybrid varieties.

Table 1. List tested of plant material, parent lines (P), F_1 hybrids (H), and commercial controls (C), type of traditional variety, colour, and size. In the case of developed hybrids, the crosses are indicated, and the code assigned to each hybrid is given in parentheses.

Genotypes	Type	Colour	Size *
Parents			
BGMU01010600 (P1)	Corazón de toro	Pink	L
BGMU01010922 (P2)	de la Sierra	Red-Black	XL
BGMU01010639 (P3)	Flor de Baladre	Pink	XL
BGMU01010640 (P4)	Flor de Baladre	Pink	XL
BGMU01010609 (P5)	Kumato	Red-Black	M
BGMU01010661 (P6)	Mesa Murciano	Pink	XL
BGMU01010643 (P7)	Mesa Murciano	Red	XL
BGMU01010675 (P8)	Mesa Murciano	Red	L
BGMU01010646 (P9)	Muchamiel	Red	L
BGMU01010665 (P10)	Muchamiel	Red	L
BGMU01010672 (P11)	Muchamiel	Red	XL
BGMU01010683 (P12)	de la Pera	Red	M
BGMU01010602 (P13)	Pimiento	Red	L
BGMU01010633 (P14)	Pimiento	Red	L
Hybrids			
Pasadena (C1)	Tomate gordo	Red	L
Mongo (C2)	Marmande	Red	L
P3 × P2 (H1)	Flor de baladre × de la Sierra	Red	XL
P3 × P4 (H2)	Flor de baladre × Flor de baladre	Pink	XL
P5 × P2 (H3)	Kumato × de la Sierra	Red-Black	M
P6 × P2 (H4)	Mesa murciano × de la Sierra	Red	XL
P7 × P6 (H5)	Mesa murciano × Mesa murciano	Red	XL
P7 × P11 (H6)	Mesa murciano × Muchamiel	Red	XL
P8 × P2 (H7)	Mesa murciano × de la Sierra	Red	L
P8 × P5 (H8)	Mesa murciano × Kumato	Red	M
P8 × P6 (H9)	Mesa murciano × Mesa murciano	Red	L
P8 × P7 (H10)	Mesa murciano × Mesa murciano	Red	L
P9 × P6 (H11)	Muchamiel × Mesa murciano	Red	L
P9 × P7 (H12)	Muchamiel × Mesa murciano	Red	L
P9 × P10 (H13)	Muchamiel × Muchamiel	Red	L
P9 × P11 (H14)	Muchamiel × Muchamiel	Red	L
P12 × P10 (H15)	de la Pera × Muchamiel	Red	M
P12 × P13 (H16)	de la Pera × Pimiento	Red	M
P13 × P1 (H17)	Pimiento × Corazón de toro	Red	L
P13 × P14 (H18)	Pimiento × Pimiento	Red	L

* Size according to: M (75–150 g), L (150–300 g) and XL (>300 g).

4.3.1. Description of agronomic, physical, organoleptic, and bioactive traits in parental lines and hybrids

Descriptive data confirmed the diversity of the accessions used as parental lines, which were subsequently used to obtain F_1 hybrids. The evaluated parameters showed coefficients of variation (CV) ranging from 9% for total soluble solids to 54% for fruit number, displaying a sufficient variation for the different breeding objectives (Table 2). In both the parental and hybrid lines, the highest CV values were observed in plant yield traits, such as mean fruit weight (MW), with 44 and 36%, respectively, and fruit number (FN), with 54 and 51%, respectively, whereas those related to the physical parameters of colour (Chroma (CH) and hue (HUE)) and total soluble solids (organoleptic trait) showed the lowest CV percentages. In hybrids, the coefficients of variation were lower than in the parental lines, except for the yield and the total soluble solids. In addition, as expected, control varieties showed very low CVs in the parameters related to agronomic yield and organoleptic characteristics of the fruit, with percentages ranging between 0.7 and 12.5, i.e., lower than those determined in the parental lines and hybrids. On the other hand, these varieties showed higher coefficients of variation than the traditional varieties for two parameters related to bioactive quality: vitamin C (VC) and total phenolic compounds (TPC).

Table 2. Descriptive statistics for agronomic (total yield (TY), mean fruit weight (MW), and fruit number (FN)), physical (equatorial diameter (ED), longitudinal diameter (LD), Chroma (CH), and hue (HUE)), organoleptic (total soluble solids (TSS), titratable acidity (TA), glucose (GL) and fructose (FR)) and bioactive (vitamin C (VC), total phenolic compounds (TPC), and total carotenoids (TC)) analysed traits of 18 hybrids, 14 parental lines, and 2 commercial controls. Data are expressed in mean ± SD (n = 3).

	Parental Lines			**Hybrids**			**Control**		
	Mean ± SD	**CV**	**Range**	**Mean ± SD**	**CV**	**Range**	**Mean ± SD**	**CV**	**Range**
TY (kg)	9.65 ± 1.33	25	5.97–15.07	12.35 ± 0.67	29	3.66–18.72	17.35 ± 1.35	3	16.99–17.70
MW (g)	243.47 ± 23.37	44	76.89–365.14	254.84 ± 11.32	36	140.47–436.69	232.68 ± 19.53	7	207.31–258.05
FN	48.60 ± 5.82	54	23.67–104.33	59.63 ± 1.85	51	19–137	87.00 ± 10.39	4	84.67–89.33
ED (mm)	80.33 ± 2.64	21	51.56–100.90	83.44 ± 1.47	17	61.22–108.31	81.17 ± 2.80	1	80.72–81.62
LD (mm)	68.51 ± 1.13	26	50.71–113.16	62.95 ± 0.86	17	50.34–90.66	57.00 ± 1.32	7	54.26–59.75
CH	34.49 ± 0.42	13	24.42–41.63	35.78 ± 0.24	10	26.25–40.53	37.13 ± 0.10	13	33.63–40.62
HUE	49.44 ± 0.25	16	37.28–64.09	49.38 ± 0.40	8	36.81–56.31	49.62 ± 2.07	0	49.47–49.77
TSS (°Brix)	6.34 ± 0.11	9	5.20–7.20	6.38 ± 0.08	12	5.3–8.1	5.84 ± 0.18	9	5.50–6.20
TA (g L^{-1})	4.02 ± 0.15	22	2.90–6.20	4.25 ± 0.02	16	2.7–5.2	4.22 ± 0.26	12	3.90–4.60
GL (mg g^{-1})	16.64 ± 0.40	16	13.60–24.60	15.24 ± 0.37	15	10.4–17.8	15.91 ± 0.23	5	15.40–16.40
FR (mg g^{-1})	17.16 ± 1.46	20	10.10–25.00	14.99 ± 0.81	24	9.1–20.4	17.82 ± 0.35	4	17.30–18.40
VC (mg g^{-1})	0.16 ± 0.00	15	0.10–0.20	0.16 ± 0.00	13	0.1–0.2	0.15 ± 0.00	21	0.10–0.20
TPC (µg g^{-1})	116.28 ± 5.56	33	8.10–130.40	102.79 ± 3.54	28	14.4–92.8	89.70 ± 5.81	38	36.50–57.90
TC (µg g^{-1})	34.92 ± 0.18	24	21.60–51.10	29.98 ± 0.60	14	24.00–40.20	46.94 ± 2.27	7	44.60–49.30

4.3.1.1. Agronomic and physical traits

A high phenotypic variability was observed among the hybrids of the traditional varieties for the studied parameters. In terms of agronomic traits, the total yield of the hybrids ranged from 3.7 kg (H16) to 18.7 kg (H7); that of the traditional parents, from 6.0 to 15.1 kg; and that of the commercial hybrids, from 17.0 to 17.7 kg (Table 3). Results showed that 85% of the hybrids equalled or exceeded the yield of their parents (Table S1), and that only two of the new hybrids (H7 and H14) equalled or exceeded the yield of the control varieties. In hybrids, the number of fruits ranged from 19 (H18) to 137 (H8), with no observed correlation with the mean fruit weight. For this parameter, the largest fruits were observed in H1 and H2 (349 and 395 g, respectively), whose common parent (P3) is a variety of the Flor de Baladre type (MW > 350 g), and the smallest fruits were observed in H8, H15, and H16 (around 120 g), derived from the crosses Mesa Murciano × Kumato, de la Pera × Muchamiel and de la Pera × Pimiento, respectively. On the other hand, the longitudinal and equatorial diameters of the fruits varied between 51 cm (H8) and 91 cm (H18) and between 61 cm (H17) and 108 cm (H2), respectively. In this sense, hybrids (H17 and H18) derived from crosses with pepper varieties (traditional variety, characterised by its elongated shape) had the highest longitudinal diameter. Finally, Chroma and HUE ranged from 41 (C2) to 26 (H3) and 56 (H3) to 37 (H2), respectively.

Table 3. Total yield (TY), mean fruit weight (MW), fruit number (FN), longitudinal diameter (LD), equatorial diameter (ED), Chroma (CH), hue (HUE), total soluble solids (TSS), and titratable acidity (TA) for the hybrids (H1 to H18) and commercial controls (C1 and C2). Data are expressed in mean ± SD (n=3).

Hybrids	TY (kg)	MW (g)	FN	LD (mm)	ED (mm)	CH	HUE	TSS (°Brix)	TA (g L^{-1})
C1	17.7 ± 0.6	211 ± 12	85 ± 6	60 ± 2	82 ± 4	34 ± 1	49 ± 3	6.2 ± 0.4	4.6 ± 0.3
C2	17.0 ± 3.1	192 ± 11	89 ± 17	54 ± 1	81 ± 4	41 ± 1	50 ± 1	5.5 ± 0.1	3.9 ± 0.3
H1	9.6 ± 1.6	349 ± 33	27 ± 2	60 ± 2	93 ± 2	39 ± 2	50 ± 2	5.5 ± 0.3	3.0 ± 0.3
H2	12.4 ± 2.2	395 ± 27	31 ± 4	63 ± 1	108 ± 4	29 ± 4	37 ± 1	6.6 ± 0.5	4.2 ± 0.2
H3	10.6 ± 0.6	123 ± 5	86 ± 3	50 ± 2	67 ± 2	26 ± 0	56 ± 2	6.8 ± 0.3	5.2 ± 0.2
H4	11.7 ± 1.7	301 ± 33	39 ± 5	58 ± 5	88 ± 9	35 ± 2	49 ± 1	5.5 ± 0.1	4.1 ± 0.1
H5	9.9 ± 0.4	307 ± 20	33 ± 3	57 ± 0	91 ± 1	36 ± 1	51 ± 2	6.9 ± 0.4	4.7 ± 0.3
H6	13.8 ± 1.1	319 ± 34	45 ± 7	59 ± 2	99 ± 5	34 ± 1	50 ± 2	6.0 ± 0.2	4.1 ± 0.1
H7	18.7 ± 1.7	238 ± 10	79 ± 10	58 ± 2	87 ± 6	33 ± 1	51 ± 1	5.9 ± 0.1	5.0 ± 0.3
H8	16.4 ± 3.1	118 ± 4	137 ± 22	51 ± 3	70 ± 3	32 ± 0	52 ± 1	6.2 ± 0.3	5.0 ± 0.8
H9	14.9 ± 1.2	247 ± 20	62 ± 9	62 ± 1	93 ± 3	31 ± 1	51 ± 2	5.3 ± 0.2	3.6 ± 0.5
H10	14.7 ± 1.9	256 ± 6	57 ± 6	60 ± 0	90 ± 2	34 ± 2	51 ± 1	6.6 ± 0.2	4.0 ± 0.4
H11	8.7 ± 2.3	275 ± 33	31 ± 7	58 ± 4	86 ± 8	35 ± 4	49 ± 1	7.1 ± 0.4	4.7 ± 0.7
H12	12.0 ± 2.2	274 ± 3	44 ± 9	56 ± 1	95 ± 2	38 ± 1	49 ± 1	6.2 ± 0.2	4.1 ± 0.0
H13	14.5 ± 0.6	230 ± 17	64 ± 6	61 ± 2	80 ± 2	39 ± 1	48 ± 1	5.3 ± 0.2	2.7 ± 0.3
H14	17.7 ± 2.0	278 ± 18	65 ± 12	62 ± 2	96 ± 2	39 ± 1	53 ± 3	7.3 ± 0.2	5.2 ± 0.8
H15	12.1 ± 2.8	115 ± 11	105 ± 21	68 ± 3	62 ± 4	37 ± 1	48 ± 1	6.8 ± 0.4	3.5 ± 0.2
H16	11.2 ± 4.7	119 ± 25	83 ± 30	76 ± 2	68 ± 3	38 ± 1	46 ± 1	8.1 ± 0.6	4.6 ± 0.3
H17	9.7 ± 0.6	154 ± 19	66 ± 11	83 ± 3	61 ± 3	37 ± 1	47 ± 1	5.9 ± 0.3	4.2 ± 0.6
H18	3.7 ± 0.2	195 ± 14	19 ± 2	91 ± 3	70 ± 2	31 ± 4	50 ± 1	6.4 ± 0.3	4.4 ± 0.2

4.3.1.2. Organoleptic and bioactive traits

Commercial controls and hybrids were characterised for organoleptic (TSS, TA, and soluble sugars) and bioactive (vitamin C, carotenoids, and phenolic compounds) traits. In hybrids, TSS ranged from 5.3 °Brix (H13) to 8.1 °Brix (H16) (Table 3), and four hybrids (H5, H11, H14, and H16) were characterised by higher TSS values than those of their progenitors (Table S1). In addition, 40% of the hybrids exceeded the TSS of the commercial varieties. Acidity ranged between 2.7 and 5.2 (H14 and H13). Among new hybrids, 30% exceeded the TA of commercial controls, and 35% had higher values than their progenitors.

The main sugars determined in ripe tomato fruits were glucose and fructose, with the latter being slightly more abundant in most of the hybrids, except for H1, H7, and H9 (Figure 1A). With regards to the values determined in the fruits of the hybrids, glucose concentration ranged from 10.4 mg g^{-1} (H15) to 17.7 mg g^{-1} (H5), and fructose concentration ranged from 9.1 mg g^{-1} (H9) to 20.4 mg g^{-1} (H5). Compared to their parents, H4 and H5 had higher glucose and fructose values than their best progenitor (P2 and P7, respectively), and H7, H13, and H16 had lower values than their worst progenitor (P8, P9, and P13, respectively) (Figure S2A). Compared to commercial hybrids, 15% and 35% of the hybrids exceeded their fructose and glucose content, respectively.

With regard to the bioactive compounds present in the fruit (vitamin C, carotenoids, and phenolic compounds), a large intervarietal variability was observed. In traditional hybrids, vitamin C content ranged from 0.12 mg g^{-1} (H7) to 0.20 mg g^{-1} (H12) (Figure 1B); total carotenoids (calculated as the sum of lycopene, β-carotene, violaxanthin, lutein, phytoene, and phytofluene) ranged from 26.0 µg g^{-1} (H7 and H8) to 40.2 µg g^{-1} (H3) (Figure 1C); and total phenolic content (calculated as the sum of hydroxycinnamic acids, flavonols, and flavanones) ranged from 52.4 µg g^{-1} (H9) to 174.6 µg g^{-1} (H4) (Figure 1D). In commercial hybrids, content of bioactive compounds ranged from 0.12 mg g^{-1} to 0.17 mg g^{-1}, 65.3 µg g^{-1} to 114.1 µg g^{-1}, and 44.6 µg g^{-1} to 49.3 µg g^{-1}, and in progenitors, from 0.12 mg g^{-1} (P11) to 0.21 mg g^{-1} (P5), 21.6 µg g^{-1} (P11) to 51.1 µg g^{-1} (P14), and 55.4 µg g^{-1} (P6) to 195.3 µg g^{-1} (P2) for vitamin C, total carotenoids, and total phenolic compounds, respectively (Figure S2B–D). Thus, hybrids exceeded or equalled commercial hybrids by 20% and 25%, and their parents by 5% and 35%, for phenolic compounds and vitamin C, respectively. On the other hand, none of the hybrids exceeded the total carotenoid content of the commercial hybrids and their parents.

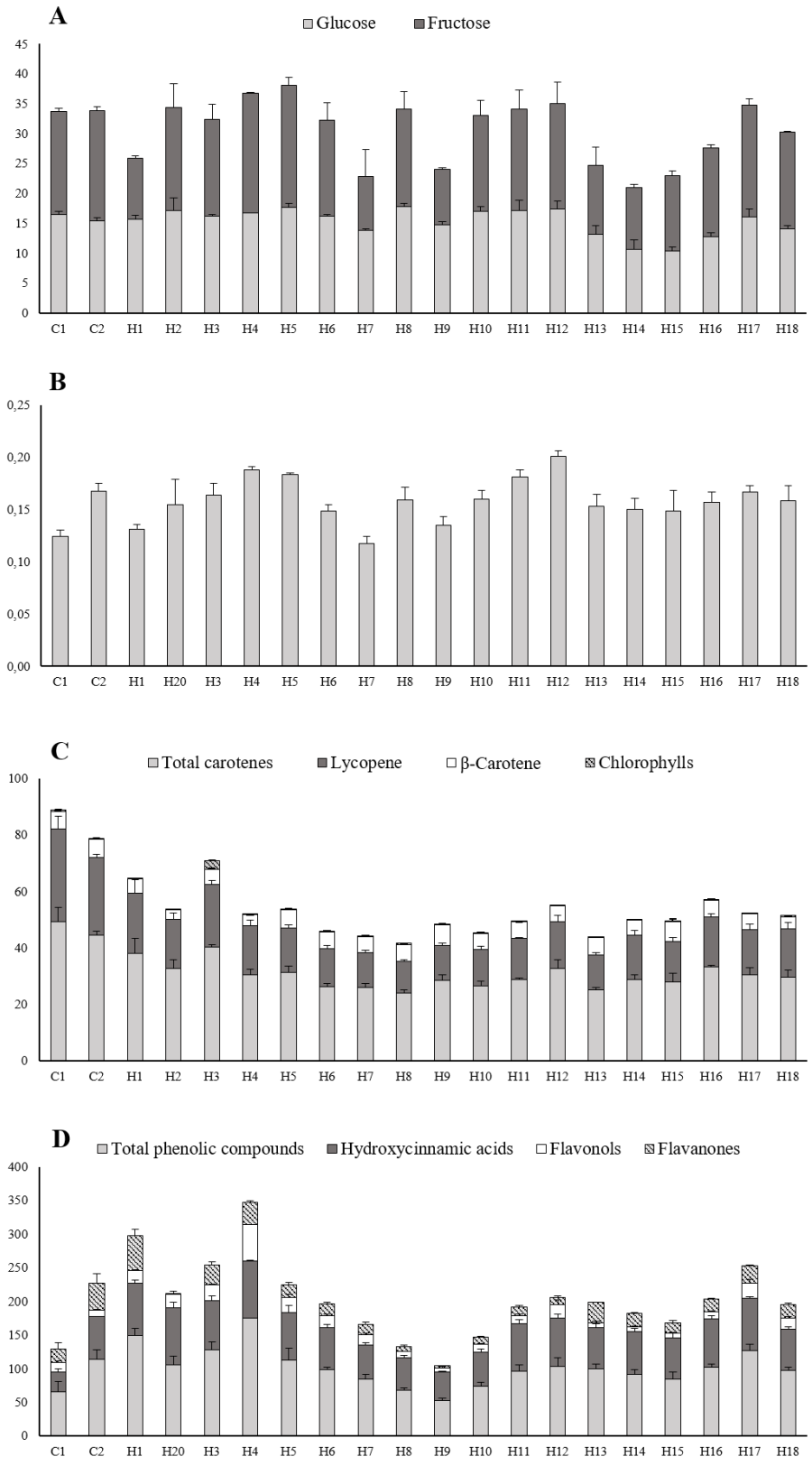

Figure 1. Nutritional and bioactive composition in tomato hybrids (H1 to H18) and commercial varieties C1 (Pasadena) and C2 (Mongo). (A) Soluble sugars (mg g^{-1}); (B) vitamin C (mg g^{-1}); (C) total carotenoids, lycopene, *β*-carotene and chlorophylls (µg g^{-1}); (D) total phenolic compounds, hydroxycinnamic acids, flavonols, and flavanones (µg g^{-1}). The bars represent the mean ± SE (n = 3).

Lycopene, followed by *β*-carotene, were the main carotenoids present in hybrids, parents, and commercial varieties (Figures 1C and S2C). Thus, lycopene ranged from 11.1 µg g^{-1} (H8) to 22.2 µg g^{-1} (H3), and *β*-carotene, from 3.5 µg g^{-1} to 7.2 µg g^{-1} for H2 and H9, respectively. Lycopene concentrations were found to be higher in the commercial varieties and parents than in the hybrids, whereas 19% and 38% of these hybrids exceeded the *β*-carotene concentrations found in the commercial varieties and parents. In addition, two compounds with carotenoid characteristics, violaxanthin and lutein, and the precursors of lycopene, phytoene, and phytofluene, were identified and quantified. Thus, the highest levels of violaxanthin (VIO) and lutein (LU) were found in H3 (Kumato × de la Sierra), whereas phytoene (PHE) and phytofluene (PHF) were higher in H1 and H2, whose common parent is of the Flor de Baladre type. On the other hand, and as expected due to the recessive nature of the chlorophyll retainer (*cl*) gene, of the five combinations on which at least one parent contained this gene, only H3, the result of the black Kumato (P3, *cl*+) × de la Sierra (A14, *cl*+) cross, showed a black colour and a significant chlorophyll concentration (2.9 µg g^{-1}).

Finally, twenty-three phenolic compounds were identified. They belonged to three groups: hydroxycinnamic acids (chlorogenic, *p*-coumaric, ferulic, and caffeic acids and their derivatives), flavonols (rutin, kaempferol, and quercetin and their derivatives) and flavanones (naringenin and its derivatives). Hydroxycinnamic acids were the major group (50–80%) determined in both hybrids and commercial and parental varieties, followed by flavonols (15–60%) and flavanones (0–45%) (Figures 1D and S2D). The content and proportion of each group of compounds depended on the genotype studied. Thus, H2 and H9 stood out for their high percentage of hydroxycinnamic acids (about 81% of the total phenolic compounds), whereas H4 and H13 stood out for their percentage of flavonols (31.4%) and flavanones (30.8%), respectively. In terms of content, H4 stood out for its high content in all three groups: 84.9 µg g^{-1}, 54.2 µg g^{-1}, and 33.8 µg g^{-1} for hydroxycinnamic acids, flavonols, and flavanones, respectively.

4.3.2. Correlation between agronomic, physical, organoleptic, and bioactive traits and similarities between hybrids

Among agronomic traits, the number of fruits correlated positively with total production (r = 0.6) and negatively with mean fruit weight (r = −0.4), so that hybrids with larger fruits had a lower number of fruits per plant (Figure 2). These two parameters, mean weight and number of fruits, correlated with equatorial diameter in such a way that, as the latter increased, the mean weight increased (r = 0.9) and the number of fruits decreased (r= −0.4). In general, agronomic parameters were negatively correlated with the organoleptic and bioactive characteristics of the fruit, except for *β*-carotene, although in this case, correlation was very low (r = 0.2). As expected, positive and highly significant correlations (r = 0.6 and r = 0.7, respectively) were observed for

the pairs TSS and TA and GL and FR, due to their relationship within fruit metabolism. The correlation between TC and LY was close to 1 (r = 0.97), as this compound is the main carotenoid that is present in ripe tomato fruit. Chlorophyll (CHL) was not included in this statistical analysis because only one of the studied hybrids contained chlorophylls.

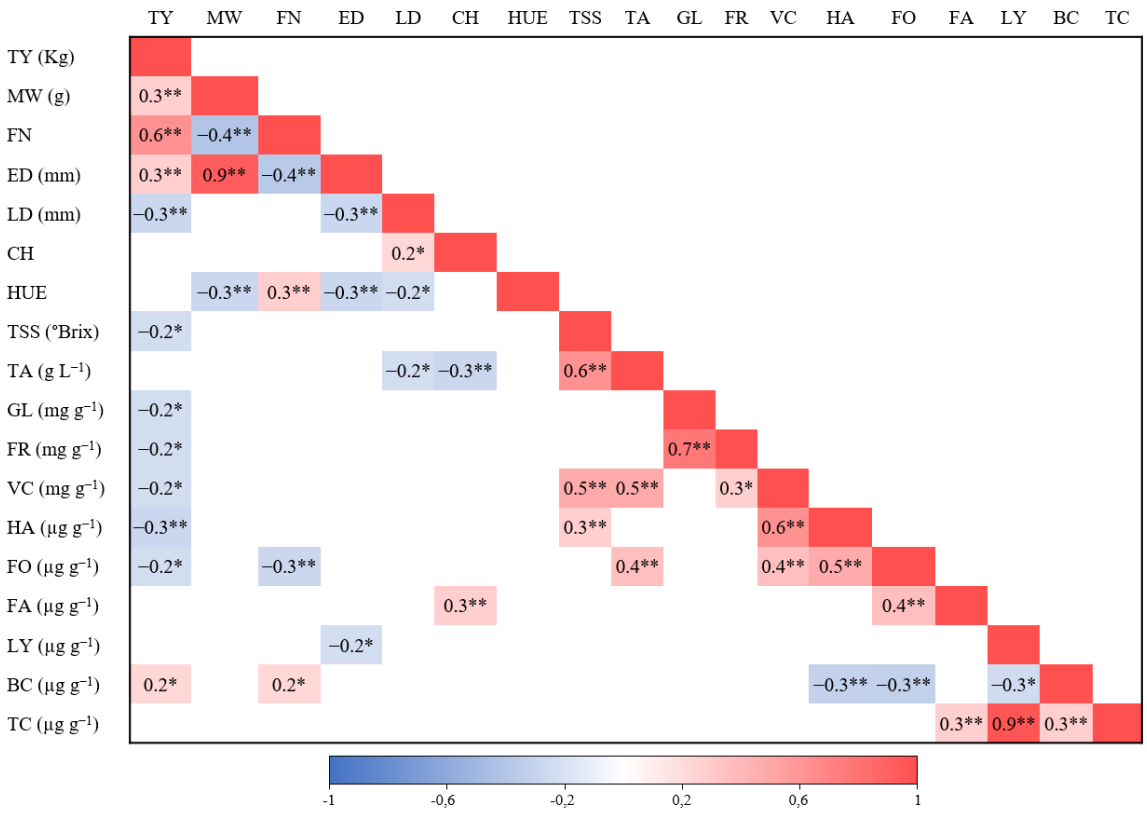

Figure 2. Pearson's correlation coefficient (r) of agronomic, physical, organoleptic, and bioactive traits analysed in tomato F_1 hybrids. Data are expressed as mean ± SD (n = 3). *$p < 0.05$, ** $p < 0.01$.

Data were analysed using principal component analysis (PCA) to select those hybrids that, in addition to showing a good agronomic performance, produced fruit with high levels of key flavour compounds and health-promoting phytochemicals. PCA was applied to agronomic (TY, MW, and FN), physical (ED, LD, CH, and HUE), organoleptic (TSS, TA; GL, and FR), and bioactive (VC, phenolic compounds (FA, FO, and HC), carotenoids (LU, VIO, LY, BC, PHF, and PHE) and chlorophyll (CHL)) traits of eighteen hybrids and two commercial controls. PCA details are shown in Table S2. According to the principle of an eigenvalue greater than 1, the first two components (PC1 and PC2) were used for further comprehensive evaluation. PC1 explained 24.8% of the total variation, and PC2 explained 19.5% of it, with a cumulative contribution rate of 44.3% (Figure 3). The first principal component (PC1) was positively correlated with several descriptors, such as MW, ED, CH, and carotenoid precursors (PHF and PHE), and negatively correlated with FN, HUE, TSS, TA, BC, and VIO. The second principal component (PC2) was positively correlated with VC, phenolic compounds (HA, FA, FO), GL, FR, LYC, LU, and CHL.

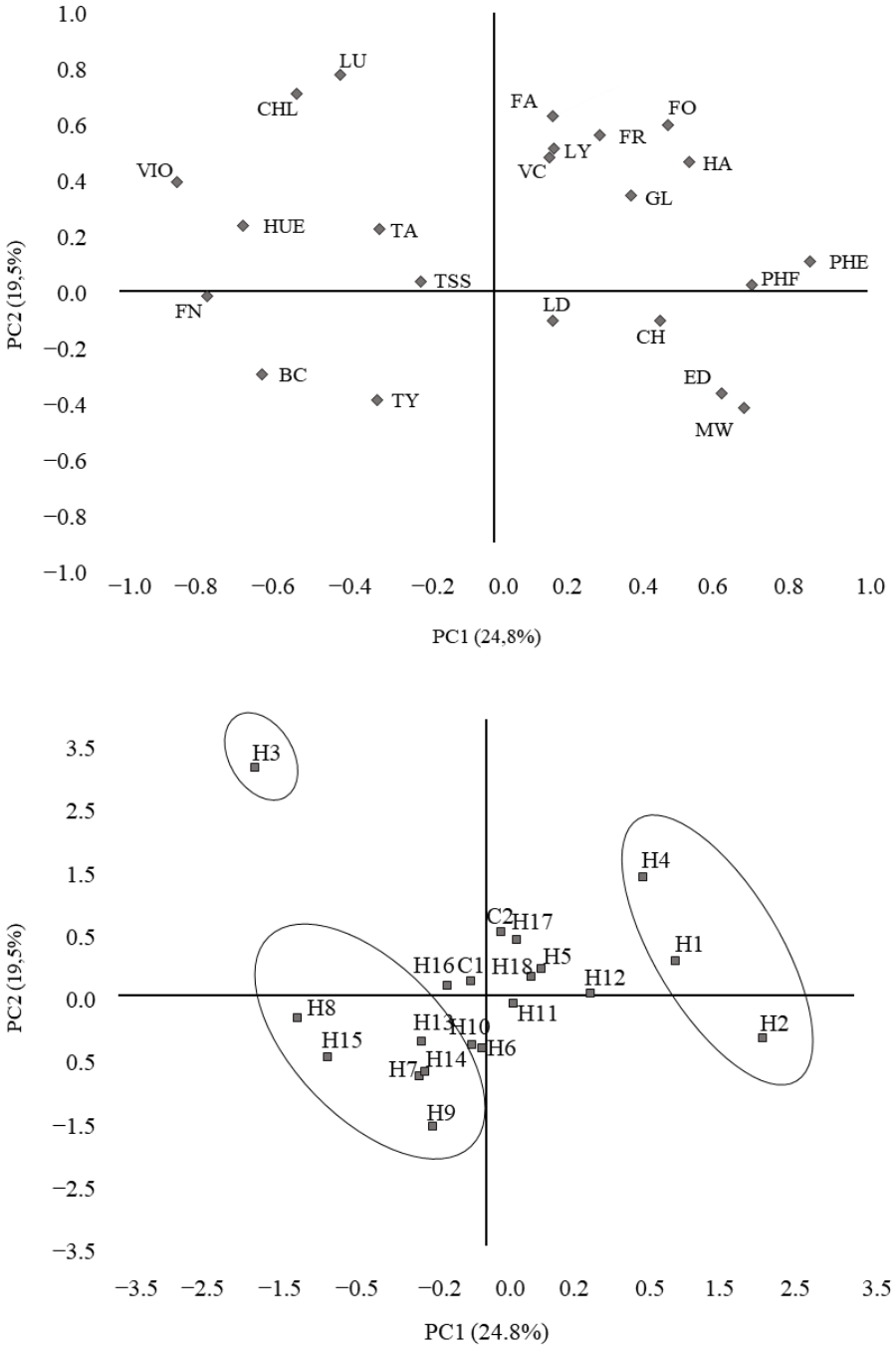

Figure 3. Principal component analysis (PCA) of agronomic, physical, organoleptic, and bioactive traits analysed in eighteen experimental F_1 hybrids (H1 to H18) and two commercial F_1 hybrids (C1 and C2).

Although the PCA dot plot showed a wide range of hybrids, some with outstanding characteristics could be identified. In the graphical representation of the PCA, three main clusters were spotted (Figure 3). The first one showed positive PC1 values and included hybrids H4 (P6 × P2), H1 (P3 × P2), and H2 (P3 × P4), which shared different parents from local varieties Flor de Baladre and de la Sierra type. Accessions included in this cluster were characterised by a higher content of bioactive compounds in their fruits, such as vitamin C, total phenolic compounds,

phytoene, phytofluene, flavonols, and hydroxycinnamic acids, as well as a high content of fructose. Along with having a better quality, the average fruit weight was higher, and the equatorial diameter was larger. The second cluster was identified by negative PC1 and PC2 values, and included genotypes H7, H8, H9, H13, H14, and H15. According to their position in the diagram, these accessions were characterised by a high number of fruits with a high β-carotene content compared to the other hybrids. The third cluster, with negative PC1 values and positive PC2 values, consisted of a single hybrid (H3), the only one with a red-black colour, due to the crossing between two traditional landraces with black fruits. This accession was therefore distinguished for its high chlorophyll and lutein content. All of the other genotypes had intermediate characteristics.

The evaluation index was calculated considering the organoleptic and bioactive traits and was estimated assigning increasing values to each of the parameters for each of the hybrids. In our hybrids, EI ranged from a minimum of 96 (H9) to a maximum of 219 (H3), with a mean value of 162. Figure 4 shows the distribution of the eighteen hybrids developed according to their EI value and total yield. This analysis clearly showed that five hybrids (H3, H16, H5, H4, and H17) were distinguished by good organoleptic and nutritional characteristics (EI > 200) and an acceptable yield (TY > 10 Kg). Moreover, the results also showed that the hybrids with the best agronomic performance (TY > 15 kg) had low evaluation index values (EI < 150). For example, H7 and H14 would be a good hybrid in terms of agronomic performance (TY = 19 kg), but their EI was 113 and 140, respectively. Unfortunately, no hybrids with high quality (EI > 200) and a high total yield (>15 kg) were obtained, but using this index, some hybrids with very low values of EI (H9) or yield (H18) could be discarded.

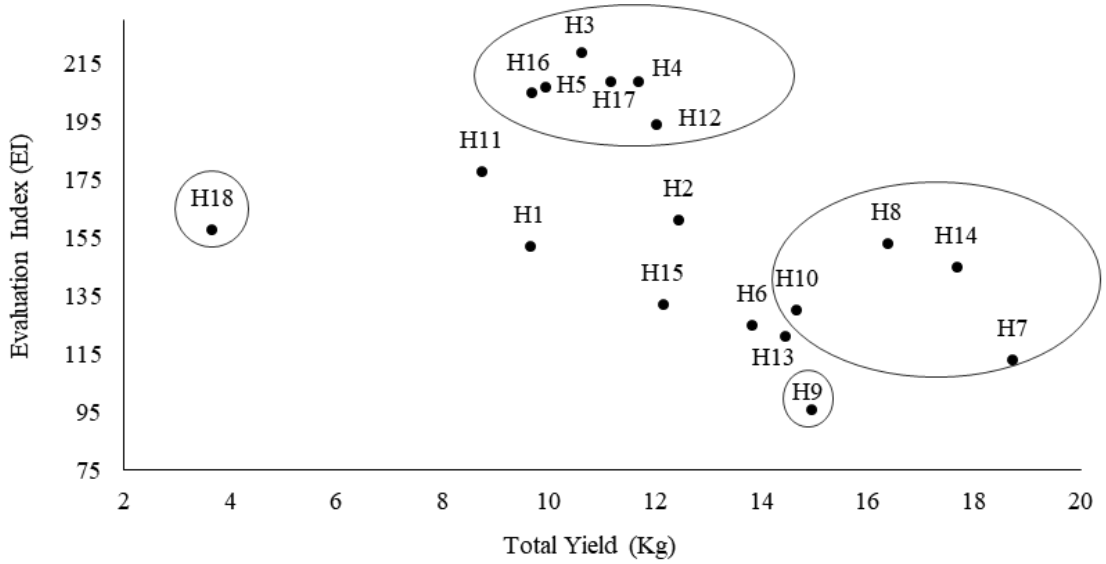

Figure 4. Scatter diagram of the eighteen F_1 hybrids (H1 to H18) according to their evaluation index and total yield production.

4.4. Discussion

Growing consumer interest in healthy and tasty food has led tomato breeding, traditionally based on yield and resistance improvement, to focus on organoleptic and nutritional traits (Sinesio et al., 2021). With this in mind, the outstanding quality traits of certain landraces can be a useful tool for the development of new commercial tomato varieties that incorporate these quality traits (Caramante et al., 2023; Casals et al., 2021; Zhu et al., 2018). Currently, F_1 hybrid tomato cultivars are the most widely grown due to advantages such as the heterosis factor, especially in the case of agronomic traits. Therefore, this study is based on the use of Spanish landraces, previously selected for their high quality (Sánchez et al., 2023; Flores et al., 2018, 2017), to develop F_1 hybrids by means of a conventional breeding technique (natural cross-pollination). We evaluated their agronomic, physical, organoleptic, and nutritional performance with regards to commercial hybrids. In addition, genotypes of superior quality were selected considering their yield.

During the development of the hybrids, a special emphasis was placed on agronomic performance, in addition to fruit quality. A high variability in yield, mean fruit weight, and number of fruits was observed among the eighteen hybrids (Table 2). An important consideration is that hybrids should be superior to their parents in terms of agronomic traits to be commercially advantageous (Liu et al., 2021). Generally speaking, the yield of the new hybrid varieties was higher than that of their parents, except for two hybrids. This could be attributed to a smaller genetic distance between these cultivars, as existing literature suggests that the greater the genetic distance between cultivars, the higher the hybrid vigour (Liu et al., 2021; Carli et al., 2011). In addition, a high variability was found between fruit diameters (equatorial and longitudinal) due to the high diversity of the parents used in this study (Table 2). The colour attribute was studied using parameters such as the hue angle, which refers to the HUE, and the Chroma (CR), which represents the quantitative attribute of colourfulness, so that as the CR increases, the colour becomes more intense (Nour et al., 2015). Most of the hybrids showed a red colour at the ripening stage, except for H2, a cross between two traditional pink varieties, which resulted in maintaining its pink fruit colour, and H3, a cross between two traditional red-black varieties. It has been described that carotenoid biosynthesis and chlorophyll degradation pathways are closely related to colour development in tomato fruit (Kang et al., 2017). The presence of pink colour in tomato fruit is controlled by a recessive (*y*) locus, which results in a colourless peel due to the absence of ripening-induced accumulation of yellow-coloured flavonoids (Ballester et al., 2010). In the case of red-black coloured fruits, this phenotype is observed due to the chlorophyll retainer (*cl)* gene (Hu et al., 2011), which prevents normal chlorophyll degradation, resulting in a darker colouration of the fruit when combined with carotenoids. These mutations in these genes are recessive; thus,

hybrids with only one red-black (H1, H4, H8) or pink (H1, H4, H5, H9, H11) parent resulted in fully red fruits. The analysed red tomato varieties developed a similar colour at the deep red stage, with average colour angles ranging between 46 and 53 degrees.

Consumer preferences have an influence over the commercial value of tomatoes, with consumers generally tending to prefer sweet and sour tomato fruit (Casals et al., 2019). In this sense, soluble solids and acidity are important determinants of tomato fruit flavour. In the developed hybrids and compared to their parents, a good increase in TSS and acidity was achieved in three hybrids (H11, H14, and H16), which would be associated with a high consumer acceptance (Casals et al., 2019). Similar results were reported for traditional landraces (Glogovac et al., 2022; Kavitha et al., 2014). Approximately half of the soluble solids content corresponds to the sugar fraction, which in tomato fruit consists of the monosaccharide-reducing sugars, glucose, and fructose, and their content can be influenced by many factors, including genotype, harvest period, and environmental conditions (Anđelini et al., 2023; Felföldi et al., 2022). In our study, H5 and H4 had the highest content of reducing sugars and, in addition, H4 had a higher fructose content, with a glucose–fructose ratio of 1:1.3. The accumulation of fructose in the tomato fruit is advantageous in terms of flavour, as this soluble sugar is approximately twice as sweet as glucose and affects the taste of the fruit in a significant way.

In addition to the above-mentioned factors, in recent years, the nutritional value of agri-food products has received increasing attention from consumers due to the health benefits derived from their consumption (Scarano et al., 2020; Flores et al., 2017; Frusciante et al., 2007). Traditionally, tomatoes have been known for their high and varied composition of bioactive compounds and antioxidants (Collins et al., 2022). In this sense, lycopene is the best-known antioxidant in tomatoes and the focus of many cancer studies, as its antioxidant properties protect against cancer processes by preventing DNA and protein damage and inducing apoptosis in cancer cells (Collins et al., 2022). However, tomatoes also contain other interesting carotenoids such as *β*-carotene, lutein, violaxanthin, phytoene, and phytofluene (Collins et al., 2022; Meléndez-Martínez et al., 2019; Jayappriyan et al., 2013); vitamins such as vitamin C (Felföldi et al., 2022; Kavitha et al., 2014); and phenolics such as flavanones, flavonols, and hydroxycinnamic acids (Sánchez et al., 2023; Flores et al., 2016; Hernández et al., 2015).

In the developed hybrids, a wide variability was observed with regards to their bioactive components (Figure 1) and, in general, the values determined for these bioactive compounds are within the ranges previously described for different tomato cultivars; for example, vitamin C levels ranged from 0.12 to 0.20 mg g^{-1} in our hybrids, and values between 0.06 and 0.37 mg g^{-1} are described in literature (Rosa-Martínez et al., 2021; Martí et al., 2018; Figàs et al., 2015), lycopene and *β*-carotene ranged from 11.1 to 22.2 µg g^{-1} and 3.5 to 7.3 µg g^{-1}, respectively, in

our hybrids, and from 11.6 to 65.0 µg g^{-1} and 0.5 to 15 µg g^{-1}, respectively, in different tomato varieties (Hellín et al., 2023; Hernández et al., 2019; Flores et al., 2016; Martí et al., 2016; Hallmann, 2012; Martínez-Valverde et al., 2002). In addition, and in relation to the phenolic composition of the hybrids, the major group was hydroxycinnamic acids, followed by flavanols and flavonols. The main phenolics found in our developed hybrids were chlorogenic acid, rutin, kaempferol, and naringenin. For these compounds, and for our crosses, the range of variation described in literature for the different compounds is very large because of the strong influence of factors like genotype, crop management, and environmental conditions (Hellín et al., 2023; Hernández et al., 2019; Hallmann, 2012).

The analysis of correlations was really useful to determine the relationship between the measured parameters. Highly significant positive correlations were observed between total yield and average fruit weight, number of fruits, and fruit equatorial diameter, whereas it was negative with longitudinal diameter (Figure 2). The "large fruit" or large average fruit weight trait is easily identifiable by the breeder, and the selection of hybrid plants based on it, selecting the elites with the largest fruit size, will simultaneously ensure an improvement in productivity (Liu et al., 2021). In addition, fruit size has been found to be positively correlated with the concentrations of phytofluene and phytoene, precursors of carotenoids, suggesting that fruit size should not be a limiting factor to increase nutritional compounds in breeding. Changes in tomato fruit colour during ripening from light green to bright red are consistent with the breakdown of chlorophyll and the synthesis of carotenoid pigments (Felföldi et al., 2022). In this study, HUE and Chroma are not correlated with the main carotenoids (lycopene and *β*-carotene). Contrary to our results, a positive correlation was observed between lycopene and Chroma, with it being negative between lycopene and HUE angle (Nour et al., 2015). These data suggest that simple colour measurements cannot be used to estimate the amount of carotenoids in these essays and that direct HPLC measurements of lycopene should be performed, as observed by Frusciante et al. (2007).

Principal component analysis (PCA) was applied to the combined agronomic, physical, organoleptic, and nutritional traits to identify the characteristics of each of the hybrids that made them stand out from the others (Figure 3). In particular, the PCA analysis identified some outstanding hybrids, grouped in three clusters according to the different traits that were studied. Cluster I (H4, H1, and H2), which includes varieties with a higher concentration of bioactive compounds in their fruits, such as vitamin C, phytoene, phytofluene, flavonols, and hydroxycinnamic acids, as well as a high fructose content; cluster II (H14, H13, H8, H15, H7, and H9), which includes genotypes with a high total yield related with a high number of fruits and a high content of *β*-carotene; and cluster III (H3), related to chlorophyll and lutein content. This latter hybrid was the only one to contain chlorophyll, being the result of a cross between two

parentals expressing the chlorophyll retainer (*cl*) gene (Hu et al., 2011). These results have facilitated the identification of promising hybrids within all of the combinations of traditional varieties tested and the selection of those that stand out due to specific characteristics, such as bioactive quality (cluster I), agronomic yield (cluster II), or high content of specific compounds that are beneficial to human health due to their ability to absorb UV radiation, prevent oxidative stress, induce cancer cell apoptosis, and reduce cancer cell proliferation (Collins et al., 2022), such as phenolic compounds (H1 and H4), phytoene and phytofluene (H2), *β*-carotene (H9), chlorophyll (H3), etc. In addition, the analysis makes it possible to determine which traits have an important genetic basis in this collection of tested traditional varieties, since in many cases hybrids have retained or improved the characteristics of their parents. This is supported by the literature indicating that the predominant gene action for traits such as lycopene content is additive (Carli et al., 2011).

Considering the above and the difficulty of selecting new "balanced" genotypes with an acceptable agronomic performance and excellent nutritional, organoleptic, and antioxidant properties to satisfy consumer/producer preferences (Sinesio et al., 2021), an evaluation index was estimated by assigning increasing values to each of the parameters for each of the hybrids and relating the obtained value to the total yield of each of the hybrids (Scarano et al., 2020). Thus, the combined use of the EI, which considers organoleptic and nutritional quality traits, with the total yield may facilitate the selection of hybrids with a good nutritional quality, since it has been shown that obtaining varieties with a high fruit quality has been hampered by the negative correlation between this trait and plant yield (Klee and Tieman, 2013; Chassy et al., 2006). In this sense, five hybrids (H3, H16, H5, H4, and H17) were characterised by very good organoleptic, nutritional, and bioactive performance (EI > 200) and also by an acceptable total yield (TY ≈ 10 kg), which makes them very interesting to start a line of improvement based on their quality. In particular, H3 is one of the most interesting hybrids developed in this work, because it conserves the chlorophyll retention gene inherited from its parents (de la Sierra × Kumato). Additionally, it has high levels of acidity, flavonols, lycopene, and lutein, which make it a good variety in terms of nutritional quality. Another very interesting hybrid was H4, which stands out for its content of vitamin C, a powerful antioxidant that is thought to reduce the risk of gastric carcinogenesis by controlling the levels of ROS that can lead to DNA damage or by stopping the development of carcinogenic nitrosamines introduced as part of the diet (Collins et al., 2022), and for its high content of phenolic compounds, some of which also have antioxidant functions in human health, and H17, which does not stand out in any particular trait, but presents high scores on most of the nutritional, phenolic, and carotenoid traits, which make it one of the highest-scoring varieties overall. Results also showed that the hybrids with the best agronomic performance (TY > 15 kg) had low evaluation index values (EI < 150). In this sense, the negative relationship between the

organoleptic and nutritional quality of tomato and its agronomic yield had already been observed by several authors (Klee and Tieman, 2013). In our case, this negative relationship was observed in six of the hybrids. Unfortunately, no hybrids with high quality (EI > 200) and high total yield (>15 kg) were obtained, but using this index, some hybrids with very low values of EI (H9) or yield (H18) could be discarded. Our study could have granted the identification and promotion of hybrid tomato cultivars with good yield, fruit quality, and nutritional value, as tomato breeding needs to meet the increasing consumer demand for high quality fruit and chemical value (Tommonaro et al., 2012).

4.5. Conclusions

The need to obtain new tomato varieties that meet market expectations is closely linked to crop sustainability and genetic variability. The aim of this study was to create new hybrids with high organoleptic and nutritional characteristics from traditional inbred lines, which have been selected for years on the basis of their genetic distances and their agronomic and quality characteristics. Genetic crosses of these varieties have resulted in 18 hybrid varieties, some of which improve the agronomic characteristics of their parents and the commercial hybrids used as controls, while maintaining the quality and flavour of the traditional varieties from which they derive. On the premise of good agronomic performance, and using a combined multivariate analysis based on these three quantitative yield components and thirteen qualitative physical, organoleptic, and nutritional parameters, six F_1 hybrids (H3, H16, H5, H4, H17, and H12) have been selected in this work, which combine good agronomic performance, due to hybrid vigour, with high fruit quality, due to the selection of phenotypic characteristics of their parents. These selected hybrids will be improved by the introduction of virus resistance genes and low-input cultivation.

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4.7. Supplementary material

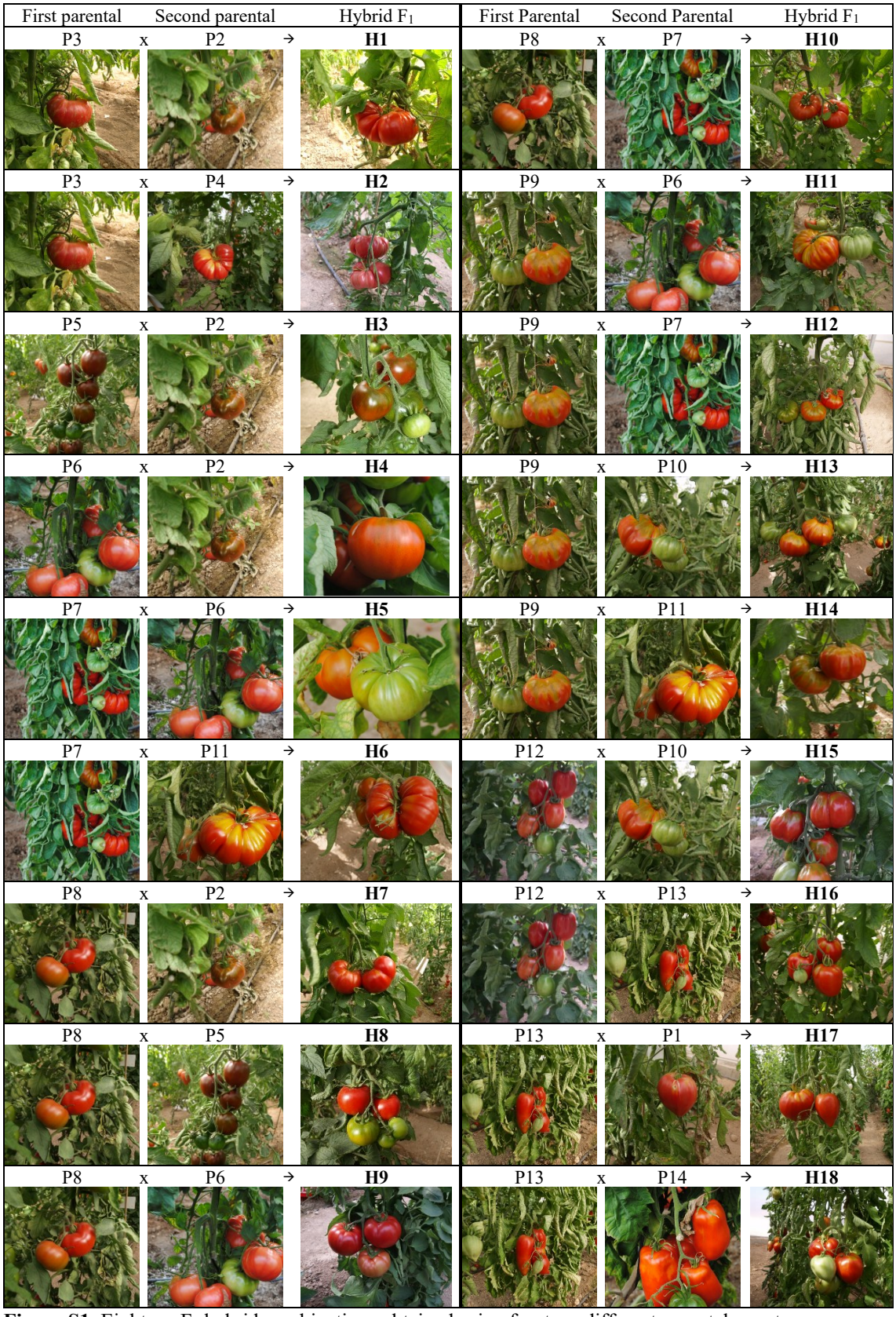

Figure S1. Eighteen F_1 hybrid combinations obtained using fourteen different parental genotypes.

Table S1. Total yield (TY; kg), mean fruit weight (MFW; g), fruit number (FN), longitudinal diameter (LD; mm), equatorial diameter (ED; mm), chroma (CH), hue (HUE), total soluble solids (TSS; °Brix), and titratable acidity (TA; g L^{-1}), for the parental lines.

Parental lines	Y	MFW	FN	LD	ED	CH	HUE	TSS	TA
P1	10,6 ± 1.8	295 ± 18	40 ± 6	87 ± 1	85 ± 2	26 ± 6	38 ± 2	6,1 ± 0.6	4,1 ± 0.7
P2	9,1 ± 1.7	291 ± 35	24 ± 3	56 ± 2	92 ± 4	23 ± 5	59 ± 1	6,2 ± 0.2	4,8 ± 0.1
P3	9,6 ± 1.3	365 ± 97	26 ± 5	60 ± 6	101 ± 11	27 ± 4	40 ± 2	7,0 ± 0.6	4,4 ± 0.5
P4	10,7 ± 2.5	321 ± 52	30 ± 4	60 ± 3	96 ± 6	33 ± 1	37 ± 1	6,9 ± 0.6	4,4 ± 0.7
P5	7,7 ± 1.2	77 ± 7	104 ± 11	51 ± 1	52 ± 2	24 ± 1	64 ± 2	7,2 ± 0.2	6,2 ± 0.7
P6	8,2 ± 2.0	285 ± 34	24 ± 4	68 ± 4	86 ± 7	30 ± 1	45 ± 3	5,8 ± 0.2	3,5 ± 0.1
P7	9,0 ± 1.0	291 ± 38	27 ± 2	58 ± 3	94 ± 4	36 ± 1	49 ± 1	6,5 ± 0.2	4,7 ± 0.4
P8	15,1 ± 1.9	288 ± 31	90 ± 26	64 ± 1	87 ± 4	34 ± 2	51 ± 1	5,2 ± 0.1	3,0 ± 0.1
P9	11,5 ± 2.9	189 ± 15	62 ± 11	58 ± 2	78 ± 3	38 ± 1	52 ± 1	6,0 ± 0.6	3,4 ± 0.4
P10	13,2 ± 1.5	178 ± 16	80 ± 9	55 ± 1	81 ± 3	42 ± 1	51 ± 1	6,3 ± 0	4,1 ± 0.2
P11	8,9 ± 1.1	341 ± 35	28 ± 2	66 ± 7	98 ± 3	38 ± 1	58 ± 2	5,7 ± 0.2	3,5 ± 0.3
P12	6,0 ± 1.3	113 ± 6	53 ± 10	68 ± 1	56 ± 3	30 ± 6	50 ± 2	7,0 ± 0.6	2,9 ± 0.3
P13	7,6 ± 1.5	194 ± 8	46 ± 8	113 ± 3	59 ± 2	39 ± 0	49 ± 1	6,7 ± 0.2	3,6 ± 0.3
P14	7,9 ± 0.8	179 ± 28	47 ± 6	96 ± 3	60 ± 6	38 ± 1	49 ± 1	6,2 ± 0.3	3,6 ± 0.3

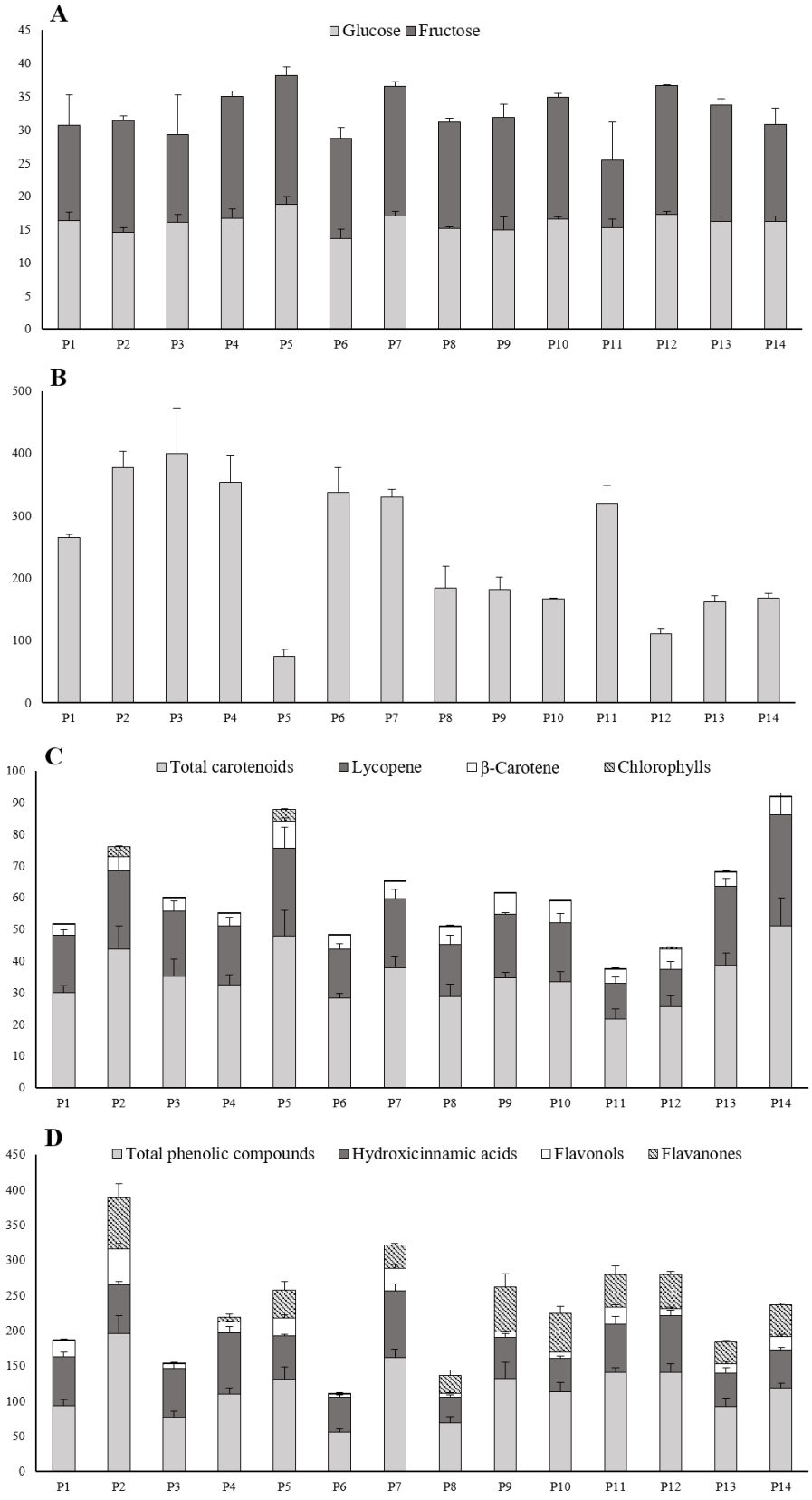

Figure S2. Concentration of soluble sugars (A; mg g^{-1}); vitamin C (B; mg g^{-1}); total carotenoids, lycopene, β-carotene, and chlorophylls (C; µg g^{-1}); and total phenolic compounds, hydroxycinnamic acids, flavonols, and flavanones (D; µg g^{-1}) in traditional parental lines (P1 to P14).

Table S2. Selection characteristics of tomato traditional parental lines used in the development of F_1 hybrids.

Genotypes	Type	Number of Crosses		Characteristics
		Male	Female	
BGMU01010600 (P1)	Corazón de toro	1	-	• Very good taste • Equilibrate flavour
BGMU01010922 (P2)	de la Sierra	4	-	• Good taste • Chlorophyll content • High phenolic compounds content • High lycopene content
BGMU01010639 (P3)	Flor de Baladre	0	2	• Agronomic performance • Good taste
BGMU01010640 (P4)	Flor de Baladre	1	-	• Agronomic performance • Very good taste
BGMU01010609 (P5)	Kumato	1	1	• Chlorophyll content • High vitamin C content • High carotenoids content
BGMU01010661 (P6)	Mesa Murciano	3	1	• Agronomic performance • Very good taste
BGMU01010643 (P7)	Mesa Murciano	2	2	• Very good taste • High lycopene content • High phenolic compounds content
BGMU01010675 (P8)	Mesa Murciano	-	4	• Agronomic performance • Very good taste
BGMU01010646 (P9)	Muchamiel	-	4	• Agronomic performance • Good taste • High vitamin C content • High lycopene content
BGMU01010665 (P10)	Muchamiel	2	-	• Agronomic performance • High lycopene content
BGMU01010672 (P11)	Muchamiel	2	-	• Very good taste • High phenolic compounds content
BGMU01010683 (P12)	de la Pera	-	2	• Agronomic performance
BGMU01010602 (P13)	Pimiento	1	2	• Agronomic performance • Very good taste
BGMU01010633 (P14)	Pimiento	1	-	• Very good taste • High lycopene content

Table S3. Principal Component Analysis in tomato fruit for different agronomic, physical, organoleptic, and bioactive traits

	PC1	**PC2**	PC3	PC4	PC5	PC6
Eigenvalue	**5,952**	**4,686**	3,157	2,572	2,044	1,47
Variability (%)	**24,802**	**19,524**	13,153	10,715	8,517	6,123
Cumulative (%)	**24,802**	**44,326**	57,479	68,194	76,711	82,835
Factor loading						
Y	**-0,311**	**-0,391**	0,638	0,160	-0,101	-0,105
MFW	**0,669**	**-0,420**	0,284	0,374	-0,243	0,249
FN	**-0,766**	**-0,019**	0,234	-0,025	0,206	-0,227
ED	**0,609**	**-0,367**	0,335	0,412	-0,315	0,171
LD	**0,158**	**-0,105**	-0,576	-0,446	0,406	0,172
CH	**0,446**	**-0,105**	-0,200	-0,608	0,103	-0,122
HUE	**-0,669**	**0,236**	0,172	-0,031	-0,341	0,040
TSS	**-0,194**	**0,034**	-0,363	0,277	0,581	0,438
TA	**-0,304**	**0,221**	-0,072	0,601	0,300	0,295
VC	**0,150**	**0,480**	-0,502	0,248	0,189	-0,438
HC	**0,523**	**0,465**	-0,436	-0,026	-0,144	0,203
FO	**0,467**	**0,595**	-0,163	0,140	-0,367	-0,017
FA	**0,159**	**0,630**	0,289	-0,599	-0,261	0,033
GLC	**0,366**	**0,344**	0,123	0,584	-0,081	-0,511
FRT	**0,285**	**0,560**	-0,201	0,327	0,284	-0,483
LY	**0,161**	**0,514**	0,635	-0,196	0,439	0,057
PHF	**0,689**	**0,021**	0,516	-0,010	0,401	-0,005
CHL	**-0,525**	**0,709**	0,139	0,229	-0,080	0,283
PHE	**0,844**	**0,108**	0,350	0,010	0,226	0,096
BC	**-0,619**	**-0,299**	0,278	-0,191	0,111	-0,336
VIO	**-0,845**	**0,392**	0,102	-0,073	-0,086	0,047
LU	**-0,409**	**0,779**	0,009	0,235	-0,087	0,220

5. Chapter III

Effect of *Tm-2a*, *Sw-5* and *Ty-1* gene introduction on the agronomic performance and metabolic profile of traditional Muchamiel-type tomato varieties

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Abstract

The introduction of virus resistance genes into traditional tomato varieties offers a strategy to preserve genetic diversity and enhance commercial viability. However, the homozygous presence of these genes has been associated with negative effects on yield and fruit quality. This two-year study evaluated the impact of introducing the *Tm-2a*, *Sw-5* and *Ty-1* genes, which are associated with resistance to ToMV, TSWV and TYLCV, respectively, on the agronomic yield, fruit characteristics and metabolic profile of Muchamiel-type cultivars. Four hybrids were obtained by crossing two breeding lines carrying the resistance genes in homozygosis (UMH1139 and UMH1200) with two traditional susceptible varieties (MC1 and MC2). Hybrids matched or exceeded the agronomic performance of their parents. Fruit morphology of the hybrids was similar to traditional parents. The presence of *Ty-1* correlated with reduced organic acid concentration, though hybrids exhibited higher levels than the homozygous line, UMH1200. No negative effects on soluble sugars or secondary metabolites were observed. Genotypes carrying resistance genes, breeding lines and hybrids exhibited higher flavonoid contents, suggesting a potential role in virus response. Hybrids maintained or improved the bioactive profile of traditional varieties. These findings support the development of Muchamiel-type hybrids that combine the presence of virus resistance genes in heterozygosity with the desirable traits of traditional tomatoes.

5.1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically valuable horticultural crops in the world. According to the Food and Agriculture Organization, it is the second biggest crop in terms of cultivated area, accounting for 14% of total vegetable production (excluding potatoes) in 2023 (Food and Agriculture Organization, 2023). Since the advent of modern plant breeding, programs have prioritized the development of varieties with high yields and disease resistance, as well as a longer postharvest life. This has resulted in a genetic bottleneck and a significant loss of crop and variety diversity (Ficiciyan et al., 2018). The recovery of traditional varieties is a promising strategy for increasing agricultural biodiversity, particularly in the Mediterranean basin, a secondary centre of tomato diversification (Pons et al., 2022). In addition to reducing genetic erosion, many of these varieties are notable for their flavour and fruit quality, attributes that are gaining increasing importance among consumers (Casals et al., 2021; Sinesio et al., 2021). The flavour-related quality is determined by primary metabolites, such as sugars and organic acids, while nutritional quality is associated with secondary metabolites, such as carotenoids, phenolic compounds, and vitamins (Quinet et al., 2019; Tamasi et al., 2019). The flavour profile is influenced by the interaction and balance between organic acids, such as malic, citric, and glutamic acids, and soluble sugars, including fructose and glucose (Villena et al., 2023). Secondary metabolites are bioactive compounds that have been shown to promote health. Lycopene, for instance, has been associated with a reduced risk of cardiovascular disease, macular degeneration, and cancer (Friedman, 2013).

Despite their outstanding quality, traditional varieties are limited by their susceptibility to viral diseases, which are among the leading causes of economic losses in tomato cultivation (Picó et al., 2002). The tomato mosaic virus (ToMV), the tomato spotted wilt virus (TSWV), and the tomato yellow curl virus (TYLCV) are three viral agents that cause significant economic losses in crops. Integrated pest management can reduce populations of thrips and whiteflies, the main vectors of TSWV and TYLCV, to levels that limit direct damage, but it is often insufficient to prevent virus transmission. In the case of ToMV, mechanical spread further complicates disease control in greenhouse systems (Picó et al., 2002). The development of breeding programs to introduce resistance genes is considered to be an effective and sustainable strategy, particularly in the Mediterranean basin, where intensive agriculture contributes to high virosis pressure that compromises tomato production (Carbonell et al., 2018). The dominant genes that confer resistance to ToMV and TSWV, as well as tolerance to TYLCV, are *Tm-2a, Sw-5*, and *Ty-1*, respectively. These genes were isolated and characterized in previous studies. These genes have been identified in wild tomatoes, specifically *S. peruvianum* (*Tm-2a* and *Sw-5*) and *S. chilense* (Dunal) accession LA1969 (*Ty-1*) (Verlaan et al., 2013; Lanfermeijer et al., 2003;

Brommonschenkel et al., 2000). Due to the high incidence of mixed infections, breeding programs have focused on introducing multiple genes using technologies such as marker-assisted selection (MAS), which accelerates the selection process (Carbonell et al., 2018).

The Muchamiel tomato variety, a traditional cultivar with significant value in local markets in southeastern Spain, is limited by its susceptibility to virosis (García-Martínez et al., 2011). Like other traditional varieties, its cultivation has declined despite being highly appreciated for its flavour, due to its replacement by commercially available varieties that are resistant to viruses, particularly virosis (Picó et al., 2002). In order to address this issue, in 1998, the CIAGRO-UMH plant breeding group initiated a program intending to enhance traditional varieties by introducing virus-resistance genes into local varieties from southeastern Spain. These varieties include the Muchamiel variety, as well as others such as the De la Pera and Moruno varieties. Breeding lines incorporating the *Tm-2a*, *Sw-5,* and *Ty-1* resistance genes in homozygosis have been developed and officially registered within the Spanish Plant Variety Office since 2011 (Carbonell et al., 2018). However, the introgression of resistance genes from wild species has been shown to have a negative impact on fruit quality and yield. This can be attributed to either the genes themselves or to linkage drag. A decline in yield and fruit quality has been observed in the processing of tomatoes resistant to ToMV (Tanksley et al., 1998), as well as in tobacco plants that possess the N gene from the *Nicotiana glutinosa* L. species, which confers resistance to TMV (Lewis et al., 2007). As described by Verlaan et al. (2011), the recombination of the *Ty-1* gene was restricted in the *S. chilense* region. This was attributed to the presence of two chromosomal inversions in S. chilense LA1969 and *S. lycopersicum*. According to Alonso et al. (2008), the introgression of *Ty-1* had a negative effect on yield and quality traits in De la Pera breeding lines. In the Muchamiel and De la Pera breeding lines, yield was reduced by up to 50% due to the presence of *Ty-1* and in the absence of TYLCV (García-Martínez et al., 2011, 2012). Rubio et al. (2016), also reported that *Ty-1* homozygosity significantly compromised most of the measured parameters in near-isogenic lines (NILs), with homozygosity for one, two or all of the three introgressed genes (*Tm-2a*, *Sw-5*, and *Ty-1*), resulting in a 40–50% yield decrease.

Although the Muchamiel-type breeding lines developed by the CIAGRO-UMH carry resistance genes targeting the major viruses affecting tomatoes, improving quality and productivity remain a challenge. Therefore, alternative strategies must be explored to preserve their exceptional fruit quality. One promising approach is to use resistance genes in the heterozygous state, as this maintains resistance through dominant inheritance while reducing yield and quality losses (Tanksley et al., 1998). A hybrid resulting from a cross between a Muchamiel breeding line and a US heirloom variety was evaluated in a previous study (Carbonell et al., 2020).

While a reduction in the negative impact of gene introgression on yield and quality was observed, this hybrid could not be categorized as Muchamiel-type, since one of its parents belonged to a different cultivar. In this context, the CIAGRO-UMH and the IMIDA have developed new Muchamiel tomato hybrids using varietal-type Muchamiel parents carrying resistance genes in the homozygous state for ToMV, TSWV, and TYLCV (García-Martínez et al., 2011, 2015), as well as traditional varieties that were previously selected for their high quality yet remain sensitive to these viruses (Sánchez et al., 2023, 2024; Flores et al., 2017). The present study aims to evaluate the impact of introducing the *Tm-2a*, *Sw-5*, and *Ty-1* genes into Muchamiel-type hybrids on their agronomic performance, fruit morphology, and metabolic composition. This approach seeks to increase the available biodiversity for tomato cultivars and to provide viable alternatives without compromising the distinctive traits that are particularly valued by consumers.

5.2. Materials and methods

5.2.1. Plant materials

A total of eight Muchamiel-type tomato cultivars were evaluated, including two traditional varieties, two breeding lines, and four hybrids developed by the CIAGRO-UMH Plant Breeding Group and the IMIDA (Figure 1 and Table 1). The MC1 (BGMU0753) and MC2 (BGMU0672) traditional varieties were selected from the IMIDA germplasm bank (BAGERIM) for their outstanding quality traits and agronomic performance (Sánchez et al., 2023; Flores et al., 2017). The UMH1200 and UMH1139 breeding lines were developed through a backcrossing program involving a Muchamiel variety and six backcrosses, as described in (García-Martínez et al., 2011, 2015). Each breeding line carries a distinct combination of virus-resistance genes that were introgressed during the breeding program. UMH1200 and UMH1139 carry the *Tm-2a* and *Sw-5* genes, respectively associated with resistance to ToMV and TSWV. Additionally, UMH1200 carries the *Ty-1* gene, linked to tolerance of TYLCV. The four hybrids were obtained through simple crosses between the two breeding lines and two traditional varieties, each of which was heterozygous for the corresponding resistance genes. The Anairis commercial variety (Seminis, Bayer S.L., Barcelona, Spain), whose size is comparable to that of Muchamiel, was included as a yield reference, but was excluded from the statistical analysis due to its different varietal group. The virus resistance performance of hybrid varieties was evaluated in laboratory, using agroinoculation for TYLCV and mechanical inoculation for ToMV and TSWV.

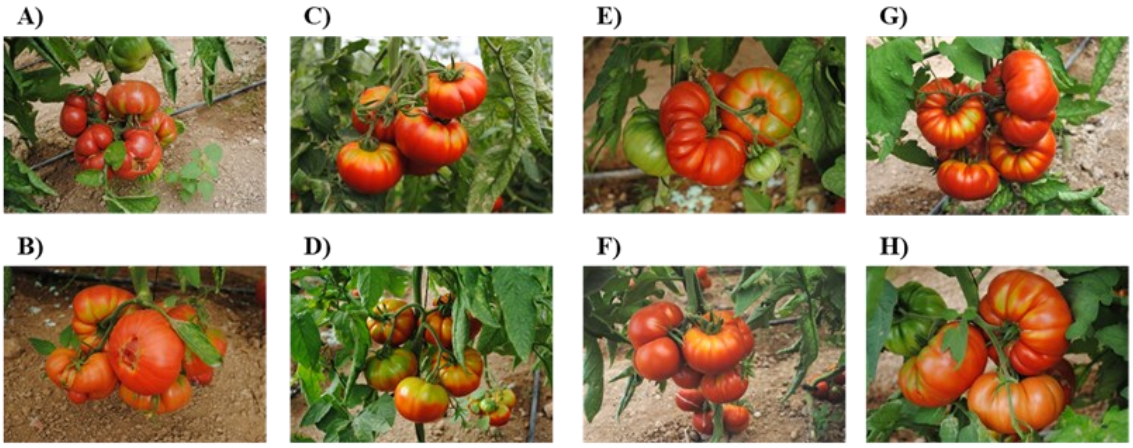

Figure 1. Images of eight cultivars: traditional varieties (A) MC1, (B) MC2; breeding lines (C) UMH1139, (D) UMH1200; developed hybrids (E) UMH1139×MC1, (F) UMH1200×MC1, (G) UMH1139×MC2, and (H) UMH1200×MC2.

Table 1. Genotypes of the resistance genes *Tm-2a* (tomato mosaic virus), *Sw-5* (tomato spotted wilt virus) and *Ty-1* (tomato yellow leaf curl virus) in traditional varieties, breeding lines and developed hybrids (RR: resistant homozygous; rr: susceptible homozygous; Rr: heterozygous).

Type	Cultivar	Genotype		
		Tm-2a	***Sw-5***	***Ty-1***
Traditional variety	MC1 (BGMU0753)	rr	rr	rr
	MC2 (BGMU0672)	rr	rr	rr
Breeding line	UMH1200	RR	RR	RR
	UMH1139	RR	RR	rr
Developed hybrid	UMH1200×MC1	Rr	Rr	Rr
	UMH1200×MC2	Rr	Rr	Rr
	UMH1139×MC1	Rr	Rr	rr
	UMH1139×MC2	Rr	Rr	rr

5.2.2. Experimental design and crop conditions

The above-described cultivars were grown in a polyethylene multi-tunnel greenhouse at the "Torreblanca" experimental farm in Torre Pacheco, Murcia, southeastern Spain (37°46′33.564″ N, 0°53′47.225″ W), which is characterized by an arid to semi-arid Mediterranean-type climate. Water for irrigation was supplied from the Tajo-Segura transfer system (electrical conductivity: 0.8–1.3 dS m^{-1}). Mineral fertilization was carried out following standard agronomic practices adapted to the soil and climatic conditions of the region, in compliance with the technical guidelines for integrated tomato production set out in the Order of 10 May 2012 (BORM, 2012).

Cultivars were grown during the spring season (March–July) of two consecutive years (2022 and 2023). Transplanting was performed using 40-day-old seedlings on 22 March 2022 and 14 March 2023. The planting frame was 0.4 m between plants and 1 m between rows, resulting in a transplant density of 2.5 plants per square meter. The experimental design included three

randomized blocks, each comprising three rows containing eight cultivars, with ten plants per cultivar (a total of eighty plants per block). The same experimental design was used in both years. Plants were grown vertically with one stem after axillary bud removal and maintained until the fourth truss.

During the two-year study, average daily temperature and accumulated radiation were recorded by the TP42 weather station in the experimental farm of the SIAM (Murcia Agricultural Information System). Temperature was measured using a 50Y thermo-hygrometer (serial number W0940091; Vaisala, Vantaa, Finland), while solar radiation was monitored with a CMP7 pyranometer (serial number 980198; Kipp & Zonen, Delft, The Netherlands), both integrated into the station. Whereas the cycles had a similar length in both years (120 days in the first year and 112 days in the second year), environmental conditions varied between years (Supplementary Figure S1). The vegetative development phase occurred within the first 60 days after transplanting. In the first year, the maximum temperatures during this period were slightly higher than in the second year. After the onset of fruit set (60 days after transplanting), minimum temperatures remained similar in both years, whereas higher maximum temperature and cumulative radiation values were recorded during the second year. Four harvests were made: 98, 105, 113, and 120 DAT, and 91, 98, 105, and 112 DAT in the first and second years, respectively. Climatic conditions were similar in both years during this period of fruit production.

5.2.3. Agronomic and fruit morphology evaluation

To evaluate the agronomic performance of the studied cultivars, all the fruit from ten plants per replicate (thirty plants per cultivar) was harvested and weighed. Yield was determined as the total weight of the fruit harvested per plant (kg/plant). To characterize the fruit and analyse the metabolites, three replicates were established for each cultivar, with each replicate consisting of ten fully ripe, homogeneously coloured and defect-free fruits selected from the ten plants corresponding to each experimental block. The equatorial and longitudinal diameters, as well as the external colour, were measured using a Mitutoyo 500–196–30 Digimatic caliper (Kawasaki, Japan) and a Minolta CR-200 Chroma Meter (Ramsey, NJ, USA), respectively. The L* (lightness), a* (redness), and b* (yellowness) colour parameters were measured in three areas on the surface of each fruit. Subsequently, the hue angle ($h° = \tan^{-1}$ [b*/a*]) and saturation or chroma (C* = [a*2 + b*2]/2) were calculated from these primary measurements.

5.2.4. Metabolite analysis

To analyse the metabolites, ten mature, uniform fruits from the same replicate were cut into small pieces, mixed together, and frozen at −80 °C. This mixture was then homogenized using a Thermomix and frozen at −80 °C until further analysis.

Individual soluble sugars, glucose (GLU) and fructose (FRU), were extracted using deionized water and purified with C18 Sep-Pak cartridges. These were subsequently analysed using molecular exclusion chromatography on an Agilent 1100 liquid chromatograph (Waldbronn, Germany). The device was equipped with a refractive index (RI) detector and a CHO-682 LEAD 300 × 7.8 mm ID CARBOSep column (Concise Separations, San Jose, CA, USA). Deionized water was used as the mobile phase at a flow rate of 0.4 mL/min (Flores et al., 2016). Results are expressed as milligrams per gram of fresh weight (FW).

Organic acids were analysed as described in Flores et al. (2016), using liquid chromatography (Agilent 1200; Agilent Technologies, Santa Clara, CA, USA) with a G6410A triple quadrupole mass spectrometer detector (Supplementary Table S1, HPLC-MS-MS). Organic acids were quantified relative to their corresponding standards (Sigma-Aldrich, Steinheim, Germany). Results are expressed as mg g^{-1} FW.

Vitamin C (ascorbic acid and its derivative, dehydroascorbic acid) was extracted using a solution of 0.05% (w/v) ethylenediaminetetraacetic acid (EDTA) and dithiothreitol (Sigma-Aldrich, Steinheim, Germany). The extract was analysed using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS-MS), according to the methodology developed in Fenoll et al. (2011). Vitamin C (VC) is expressed as µg g^{-1} FW, using commercial ascorbic acid (Sigma-Aldrich, Steinheim, Germany) as a standard.

Two separate extractions were performed to analyse the carotenoid profile, as detailed in Supplementary Table S2. Polar carotenoids were extracted using the method described in Flores et al. (2016), entailing the utilization of a methanol–tetrahydrofuran (1:1, v/v) solution containing 200 mg of MgO and 0.1% BHT. This was followed by evaporation and dissolution in a methanol–tetrahydrofuran (1:1, v/v) solution containing 2% BHT. Non-polar carotenoids were extracted using the method of Olives-Barba et al. (2006), involving the use of a mixture of hexane, acetone, and ethanol (2:1:1) containing 0.1% BHT. The hexane layer was then evaporated, and the residue was dissolved in a mixture of methanol and tetrahydrofuran (1:1, v/v) containing 2% BHT. The polar and non-polar carotenoids were analysed using the methodology validated in Motilva et al. (2014), with a Hewlett-Packard 1100 HPLC system (Waldbronn, Germany) equipped with a photodiode array UV/Vis detector operating in the 250–800 nm spectral range. Separation was achieved using a 250 mm × 4.6 mm, 3 µm Prontosil C30 column (Bischoff, Leonberg, Germany), with a mobile phase consisting of methanol (solvent A) and methyl tertbutyl ether (solvent B). Elution was performed at a flow rate of 1.3 mL min^{-1} with an injection volume of 20 µL, with detection performed at 287 nm for phytoene, 347 nm for phytofluene, and 444 nm for lutein, neoxanthin, and violaxanthin. The carotenoids in the samples were identified by comparing their

retention times (min) and UV/Vis absorptions with the corresponding standards reported in the literature (Flores et al., 2016). Compounds were quantified in μg g^{-1} FW.

Phenolic compounds were extracted using a methanol–formic acid solution (97:3, v/v) and identified as described in Vallverdú-Queralt et al. (2011). Analysis was performed using HPLC-MS/MS (Agilent Series 1100; Agilent Technologies) with an ESI interface operating in negative ion mode, and the following operating parameters: capillary voltage of 2000 V, nebulizer pressure of 60 psi, drying gas flow of 13 L min^{-1}, and drying gas temperature of 350 °C. Separation was achieved using an analytical column (250 mm × 4 mm, 5 μm particle size) of Lichrosphere C18 (Agilent Technologies, Waldbronn, Germany), with a mobile phase consisting of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) at a flow rate of 1 mL min^{-1}. Full-scan, neutral loss scan, and precursor ion scan experiments were performed to confirm the identity of some compounds for which standards were unavailable (Supplementary Table S3). Polyphenols were quantified relative to their corresponding standards, which were purchased from Sigma-Aldrich. When standards were unavailable, quantification was performed relative to the corresponding isomers, hydroxycinnamic acids or aglycones. Compounds were quantified in μg g^{-1} FW.

5.2.5. Statistical analysis

All of the statistical analyses were conducted using IBM SPSS Statistics 21. First, normality of the data distribution was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated through the Levene's test based on the median. A two-way analysis of variance (ANOVA) was performed separately for each F_1 hybrid and its two parental lines to evaluate the effect of variety (V), harvesting year (Y), and their interaction (V × Y). Duncan's multiple range test was applied to each group (hybrid and parents) when significant differences were found.

5.3. Results

5.3.1. Yield and fruit characterization

Table 2 shows the yield per plant and fruit characteristics for the Muchamiel-type hybrids (UMH1139×MC1 and UMH1200×MC1) and their respective parents. The two-way ANOVA data included the effects of year and variety, as well as the interaction between these two factors. The yield per plant of the hybrids ranged from 3.2 to 4.4 kg per plant in 2022 and from 3.0 to 3.5 kg per plant in 2023. UMH1200×MC1 exhibited the lowest yield in both years, whereas UMH1200×MC2 attained the highest yield. No variety × year interaction was found for this parameter, and no significant differences were observed between years either, except for UMH1200×MC2, whose yield in 2023 was 12% lower than in 2022. However, significant

differences were found for the variety factor in hybrids with UMH1200 as the parent; these were more productive than their parents.

Regarding fruit morphology, the year factor did not affect the longitudinal or equatorial diameters of the studied hybrids, except for UMH1200×MC2 and UMH1139×MC1, where the fruit was slightly smaller in 2023 than in 2022 (−5% in both cases). The variety effect was significant in all of the hybrids compared to their parents, with the hybrids retaining the morphology of the traditional varieties. The UMH1139×MC1 hybrid exhibited a comparable longitudinal diameter and a larger equatorial diameter than its parents. In contrast, the other three hybrids exhibited diameters comparable to the traditional parent and exceeding those of the breeding line, which generally produced smaller fruit. No variety × year interaction was observed for these parameters. When colour was evaluated using the chroma (C*; saturation) and hue angle (h°; hue) parameters, variability was observed between cultivars and years. However, the variety × year interaction was not significant. In general terms, hybrids exhibited more intense, reddish colours (higher C* and h°) than their traditional parents, with a decrease in C* and an increase in h° observed in 2023 compared to 2022.

Table 2. Yield (kg plant^{-1}), longitudinal diameter (LD; mm), equatorial diameter (ED; mm), chroma (C*) and hue angle (h°) and the *p*-values from ANOVA for the effects of variety, year and variety × year interaction in the developed hybrids and their respective parents during the years 2022 and 2023.

	Yield		LD		ED		C*		h°	
	2022	**2023**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
UMH1139	2.8 ± 0.2	2.5 ± 0.2	58 ± 1	56 ± 1	93 ± 4 ^{a}	85 ± 1 ^{a}	42 ± 1 ^{c}	39 ± 1 ^{c}	48 ± 1 ^{c}	54 ± 1 ^{c}
MC1	2.6 ± 0.3	2.5 ± 0.1	57 ± 1	59 ± 1	92 ± 3 ^{a}	92 ± 2 ^{a}	29 ± 1 ^{a}	24 ± 1 ^{a}	36 ± 1 ^{a}	37 ± 1 ^{a}
UMH1139×MC1	3.2 ± 0.4	3.1 ± 0.4	59 ± 2	57 ± 1	100 ± 2 ^{b}	95 ± 3 ^{b}	34 ± 1 ^{b}	32 ± 1 ^{b}	46 ± 1 ^{b}	45 ± 1 ^{b}
Variety	0.105		0.654		0.003 **		0 ***		0 ***	
Year	0.465		0.568		0.024 *		0 ***		0.001 **	
Variety × Year	0.934		0.307		0.542		0.074		0 ***	
UMH1200	2.5 ± 0.1 ^{a}	2.5 ± 0.3 ^{a}	56 ± 1 ^{a}	54 ± 1 ^{a}	87 ± 3 ^{a}	81 ± 2 ^{a}	44 ± 1 ^{c}	37 ± 1 ^{c}	50 ± 1 ^{c}	50 ± 1 ^{c}
MC1	2.6 ± 0.3 ^{a}	2.5 ± 0.1 ^{a}	57 ± 1 ^{b}	59 ± 1 ^{b}	92 ± 3 ^{b}	92 ± 2 ^{b}	29 ± 1 ^{a}	24 ± 1 ^{a}	36 ± 1 ^{a}	37 ± 1 ^{a}
UMH1200×MC1	3.2 ± 0.3 ^{b}	3.0 ± 0.1 ^{b}	58 ± 1 ^{b}	57 ± 1 ^{b}	95 ± 3 ^{b}	93 ± 2 ^{b}	35 ± 1 ^{b}	28 ± 1 ^{b}	46 ± 1 ^{b}	47 ± 1 ^{b}
Variety	0.015 *		0.012 *		0.001 **		0 ***		0 ***	
Year	0.547		0.691		0.153		0 ***		0.133	
Variety × Year	0.945		0.228		0.828		0.353		0.138	
UMH1139	2.8 ± 0.2	2.5 ± 0.2	58 ± 1 ^{a}	56 ± 1 ^{a}	93 ± 4 ^{a}	85 ± 1 ^{a}	42 ± 1 ^{c}	39 ± 1 ^{c}	48 ± 1 ^{c}	54 ± 1 ^{c}
MC2	3.4 ± 0.4	2.5 ± 0.2	62 ± 2 ^{b}	63 ± 1 ^{b}	104 ± 3 ^{b}	106 ± 3 ^{b}	29 ± 1 ^{a}	27 ± 1 ^{a}	38 ± 1 ^{a}	43 ± 1 ^{a}
UMH1139×MC2	3.5 ± 0.4	3.2 ± 0.2	62 ± 2 ^{b}	60 ± 1 ^{b}	107 ± 2 ^{b}	101 ± 2 ^{b}	36 ± 1 ^{b}	32 ± 1 ^{b}	46 ± 1 ^{b}	50 ± 1 ^{b}
Variety	0.129		0 ***		0 ***		0 ***		0 ***	
Year	0.055		0.207		0.030 *		0 ***		0 ***	
Variety × Year	0.505		0.356		0.144		0.732		0.723	
UMH1200	2.5 ± 0.1 ^{a}	2.5 ± 0.3 ^{a}	56 ± 1 ^{a}	54 ± 1 ^{a}	87 ± 3 ^{a}	81 ± 2 ^{a}	44 ± 1 ^{c}	37 ± 1 ^{c}	50 ± 1 ^{c}	50 ± 1 ^{b}
MC2	3.4 ± 0.4 ^{a}	2.5 ± 0.2 ^{a}	62 ± 2 ^{b}	63 ± 1 ^{b}	104 ± 3 ^{b}	106 ± 3 ^{b}	29 ± 1 ^{a}	27 ± 1 ^{a}	38 ± 1 ^{a}	43 ± 1 ^{a}
UMH1200×MC2	4.0 ± 0.2 ^{b}	3.5 ± 0.1 ^{b}	63 ± 1 ^{b}	60 ± 1 ^{b}	109 ± 3 ^{b}	101 ± 2 ^{b}	37 ± 1 ^{b}	34 ± 1 ^{b}	47 ± 1 ^{b}	49 ± 1 ^{b}
Variety	0.001 *		0 ***		0 ***		0 ***		0 ***	
Year	0.024 **		0.145		0.092		0 ***		0.009 **	
Variety × Year	0.213		0.257		0.249		0.005 **		0.019 *	

*, **, *** Significant differences between means at a 5, 1 or 0.1% probability levels, respectively. Different letters in the same column indicate significant differences between genotypes within each year according to Duncan's test at $p < 0.05$. Values are means ± SE (n = 6).

5.3.2. Primary metabolites

The characterization of flavour-related traits in ripe fruit was performed based on the presence of primary metabolites, thus determining the concentrations of soluble sugars (mainly fructose and glucose; Figure 2) and organic acids (mainly citric, glutamic, and malic acids; Figure 3), as well as isocitric and quinic acids at lower concentrations (Supplementary Table S4). Two-way ANOVA was used to analyse the effects of year and variety, as well as the interaction between the two factors (Table 3).

Table 3. *p*-values from a two-way ANOVA assessing the effects of variety, year and their interaction on concentrations of soluble sugars and organic acids. Each analysis was performed separately for each hybrid and its two corresponding parental lines, which are grouped under the hybrid name in the table.

	Effect	Glucose	Fructose	Citric Acid	Glutamic Acid	Malic Acid
UMH1139×MC1	*Variety*	0 ***	0 ***	0 ***	0 ***	0.001 **
	Year	0.05 **	0 ***	0.012 *	0 ***	0.609
	Variety × Year	0.785	0.517	0.033 *	0.085	0.001 **
UMH1200×MC1	*Variety*	0 ***	0 ***	0 ***	0 ***	0 ***
	Year	0.025 *	0 ***	0.022 *	0 ***	0.933
	Variety × Year	0.539	0.770	0 ***	0.190	0.026 *
UMH1139×MC2	*Variety*	0 ***	0 ***	0 ***	0.001 **	0.024 *
	Year	0.024 *	0 ***	0 ***	0 ***	0 ***
	Variety × Year	0.819	0.249	0 ***	0.315	0.035 *
UMH1200×MC2	*Variety*	0 ***	0 ***	0 ***	0 ***	0 ***
	Year	0 ***	0 ***	0 ***	0 ***	0 ***
	Variety × Year	0.256	0.099	0.114	0.359	0.042 *

*, **, *** Significant differences between means at a 5, 1 or 0.1% level of probability, respectively.

5.3.2.1. Soluble sugars

Significant differences were found in the variety and year factors of the four developed hybrids concerning this parameter, with no variety × year interactions (Table 3). Among the parents, MC1 exhibited higher fructose and glucose concentrations than MC2. Among the breeding lines, UMH1200 exhibited lower values than UMH1139 (Figure 2). The developed hybrids outperformed their traditional parents in terms of concentration of these sugars, with glucose and fructose increases ranging from 13% to 34%, depending on the hybrid and year. The glucose concentration in the hybrids ranged from 9.7 to 9.9 mg g^{-1} in 2022 and from 10.2 to 11.9 mg g^{-1} in 2023. Meanwhile, the fructose concentration ranged from 10.1 to 10.9 mg g^{-1} in 2022 and from 12.7 to 14.9 mg g^{-1} in 2023. A significant overall increase was observed in the second year.

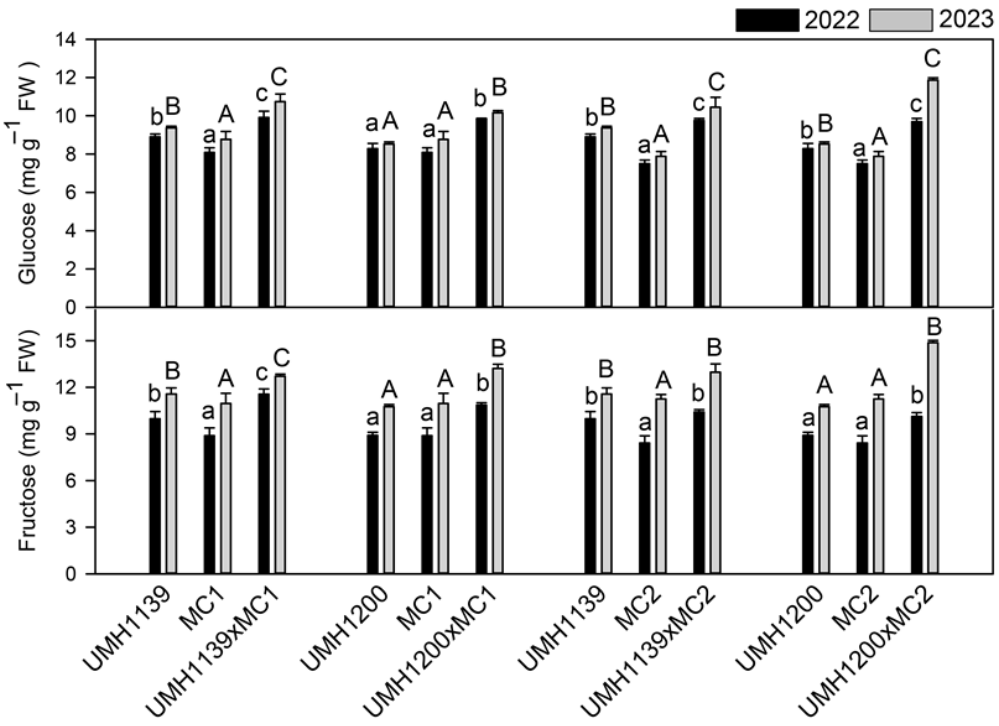

Figure 2. Concentrations of glucose and fructose (mg g^{-1} FW) in the developed hybrids and their respective parents in 2022 and 2023. Different letters indicate significant differences between genotypes within each year according to Duncan's test at $p < 0.05$: lowercase letters for 2022 and uppercase letters for 2023. Statistical significance of the main effects (variety, year, and interaction) is provided in Table 3. Values are means ± SE (n = 6).

5.3.2.2. Organic acids

Eleven organic acids were identified and quantified in the analysed cultivars (Supplementary Table S1). This study assessed the three major acids: citric acid, representing 60% of the total acid concentration on average; glutamic acid, constituting 25% on average; and malic acid, accounting for 13% on average. These percentages varied according to variety and year (Figure 3). Significant differences in the concentrations of these metabolites were observed between hybrids and their parents, and between years of cultivation, as well as between these two factors (Table 3).

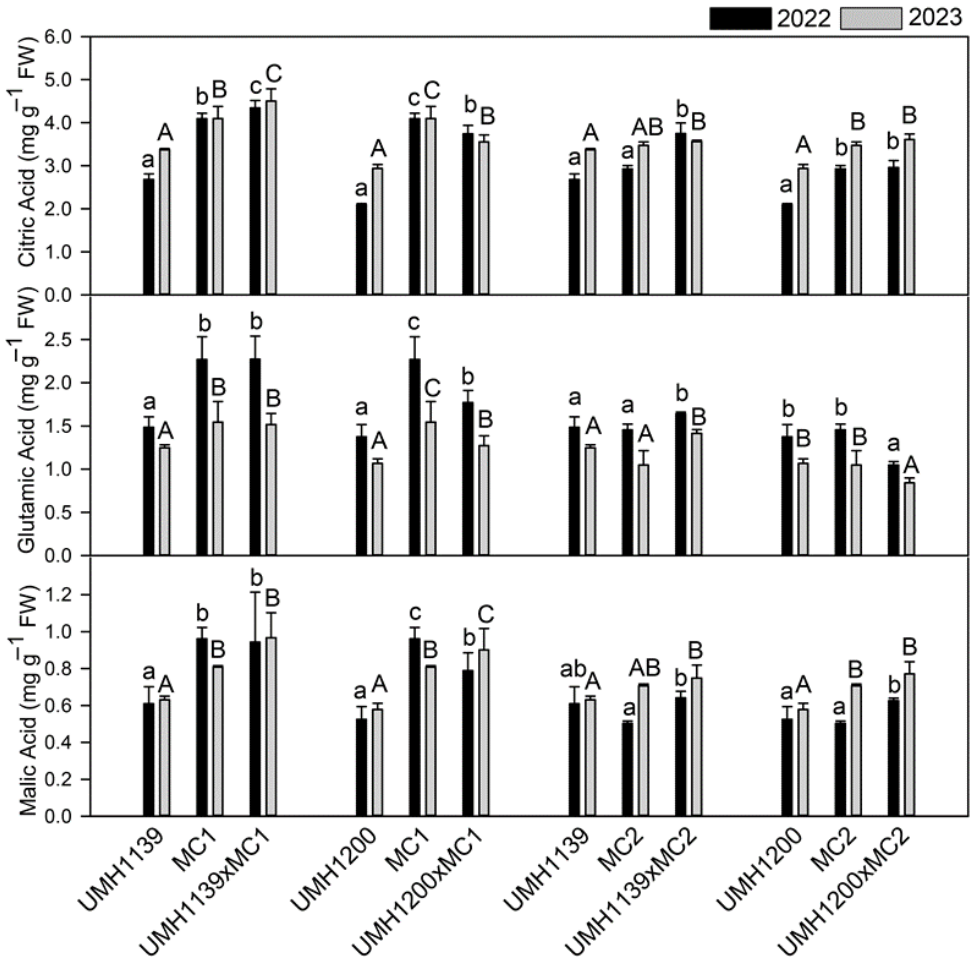

Figure 3. Concentration of the main organic acids (citric, glutamic, and malic acids) (mg g^{-1} FW) in the developed hybrids and their respective parents for 2022 and 2023. Different letters indicate significant differences between genotypes within each year according to Duncan's test at $p < 0.05$: lowercase letters for 2022 and uppercase letters for 2023. Statistical significance of the main effects (variety, year, and interaction) is provided in Table 3. Values are means ± SE (n = 6).

Of the four hybrids evaluated in terms of citric acid concentration, the UMH1139×MC1 hybrid performed exceptionally well, exceeding its traditional parent (MC1) by 6% and 9% in 2022 and 2023, respectively. By contrast, the values determined for UMH1200×MC1 were lower than those obtained for MC1. Regarding glutamic acid, crosses with the UMH1200 resistance line exhibited lower values than those obtained with MC1 and MC2. However, hybrids with the UMH1139 resistance line outperformed or equalled their traditional parent (MC2 or MC1). Regarding malic acid, all of the hybrids, except UMH1139×MC2 in 2022, exhibited a superior performance than the respective breeding lines used as their parents. The effect of the year varied according to the compound and hybrid versus parental combination. Whereas the concentration of glutamic acid decreased in all of the cultivars in 2023, the concentrations of citric and malic acids were influenced by the interaction between variety and year. An increase in citric acid concentration was recorded in the breeding lines and MC2 in 2023, whereas MC1 maintained a

constant concentration of citric acid in its fruits. Regarding malic acid, the breeding lines exhibited stable concentrations in both years, whereas MC1 decreased and MC2 increased in 2023 compared to 2022. A heterogeneous response was observed among the hybrids concerning citric acid: an increase was observed in UMH1200×MC2, a decrease in UMH1139×MC2 and UMH1200×MC1, and stability in UMH1139×MC1. All of the hybrids exhibited an increase in malic acid concentration by 2023, except for UMH1139×MC1, which remained stable over both years.

5.3.3. Secondary metabolites

5.3.3.1. Vitamin C

The concentration of vitamin C in the studied cultivars was significantly affected by variety and year, with no meaningful interactions between the two factors (Table 4). The developed hybrids had higher vitamin concentrations than the breeding lines, which were similar to those observed in the traditional parents in both years of the study (Figure 4). In 2022, the values in the developed hybrids ranged from 120 µg g^{-1} (UMH1139×MC1) to 130 µg g^{-1} (UMH1139×MC2), and in 2023, they ranged from 94 µg g^{-1} (UMH1139×MC1) to 112 µg g^{-1} (UMH1200×MC2). There was an overall decrease in vitamin C concentration in 2023 compared to 2022.

Table 4. *p*-values from a two-way ANOVA assessing the effects of variety, year and their interaction on vitamin C and carotenoid concentrations. Each analysis was performed separately for each hybrid and its two corresponding parental lines, which are grouped under the hybrid name in the table.

	Effect	**Vitamin C**	**Lycopene**	***β*-Carotene**	**Phytoene**	**Phytofluene**
	Variety	0 ***	0 ***	0 ***	0 ***	0 ***
UMH1139×MC1	*Year*	0 ***	0 ***	0 ***	0 ***	0 ***
	Variety × Year	0.439	0.002 **	0.552	0.112	0.002 **
	Variety	0 ***	0 ***	0 ***	0 ***	0 ***
UMH1200×MC1	*Year*	0 ***	0 ***	0 ***	0 ***	0 ***
	Variety × Year	0.440	0.225	0.454	0.469	0.039 *
	Variety	0 ***	0 ***	0 ***	0 ***	0 ***
UMH1139×MC2	*Year*	0 ***	0 ***	0 ***	0 ***	0 ***
	Variety × Year	0.116	0.159	0.459	0.034 *	0.008 **
	Variety	0 ***	0 ***	0 ***	0 ***	0 ***
UMH1200×MC2	*Year*	0.002 **	0 ***	0 ***	0 ***	0 ***
	Variety × Year	0.137	0.169	0.460	0.048 *	0.020 *

*, **, *** Significant differences between means at a 5, 1 or 0.1% level of probability, respectively.

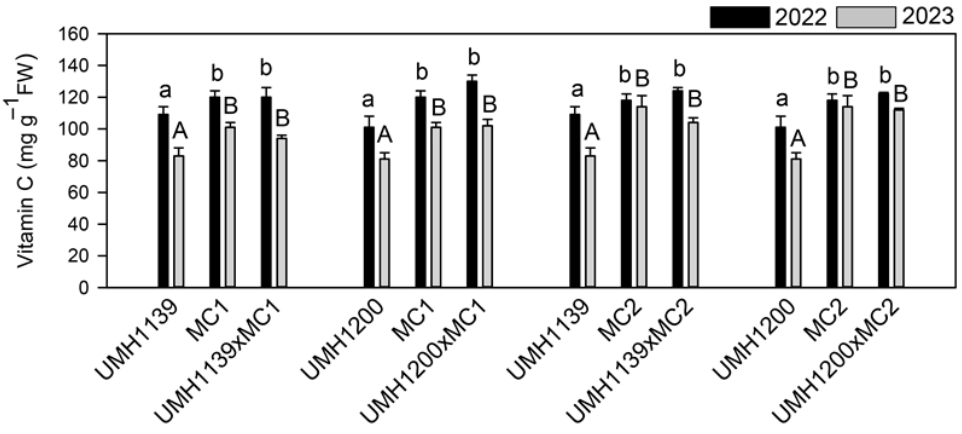

Figure 4. Concentration of vitamin C (µg g^{-1} FW) in the developed hybrids and their respective parents in 2022 and 2023. Different letters indicate significant differences between genotypes within each year according to Duncan's test at $p < 0.05$: lowercase letters for 2022 and uppercase letters for 2023. Statistical significance of the main effects (variety, year, and interaction) is provided in Table 4. Values are means ± SE (n = 6).

5.3.3.2. Carotenoids

A total of nineteen compounds were identified, which together constituted the carotenoid profiles of the studied cultivars (Supplementary Table S2). Eight compounds of interest were considered in this study: phytofluene (calculated as the sum of phytofluene I and II), phytoene, lycopene (the sum of the *all-trans*, 13-cis, 13′-cis, 9-cis, 9′-cis, 5-cis, and 5′-cis isomers), *β*-carotene, *δ*-carotene, and three xanthophylls (lutein, neoxanthin, and violaxanthin). As expected, lycopene was identified as the dominant component, followed by the carotenoid precursors, phytoene and phytofluene.

The concentrations of the major carotenoids lycopene, *β*-carotene, phytoene, and phytofluene were found to be significantly affected by the variety and year (Table 4). The interaction between variety and year was found to be significantly associated with phytofluene concentration in cultivars. For phytoene, however, this interaction was only observed in hybrids whose traditional parent was MC2. A significant variety × year interaction was also detected for lycopene concentration in the UMH1139×MC1 hybrid compared to its parents. In both years, hybrids exhibited concentrations equal to or greater than those of their traditional parents, except for UMH1139×MC1, which had a lower phytoene concentration. Compared to breeding lines, hybrids exhibited similar or lower lycopene and *β*-carotene concentrations. However, phytoene and phytofluene values were higher than those observed in the breeding lines. Regarding the effect of the year, the concentration of these carotenoids decreased in 2023 compared to 2022, except for *β*-carotene, which increased in all of the studied cultivars (Figure 5). The concentrations of *δ*-carotene (a lutein precursor), lutein, and xanthophylls (neoxanthin and violaxanthin) were significantly affected by variety (except for neoxanthin in the UMH1139×MC1 hybrid and its

parents) and crop year (except for lutein concentration in all of the cultivars, and for violaxanthin concentration in the UMH1139×MC1 hybrid and its parents) (Table 5.) The interaction between variety and year was only significant for violaxanthin in the UMH1200×MC1 hybrid compared to its parents. In terms of variety, hybrids exhibited equal or higher concentrations of *δ*-carotene, violaxanthin, and neoxanthin than their traditional parents. Lutein concentration was lower than that found in the traditional parent in hybrids derived from MC1, and the same as in hybrids derived from MC2, in both years. The year had a significant effect on the concentrations of *δ*-carotene and neoxanthin, with a decrease in *δ*-carotene and an increase in neoxanthin observed in all of the cultivars in 2023 compared to 2022. A significant year effect was observed for violaxanthin, with a generalized decrease in 2023, except for the UMH1200×MC1 hybrid, in which case an increase was recorded.

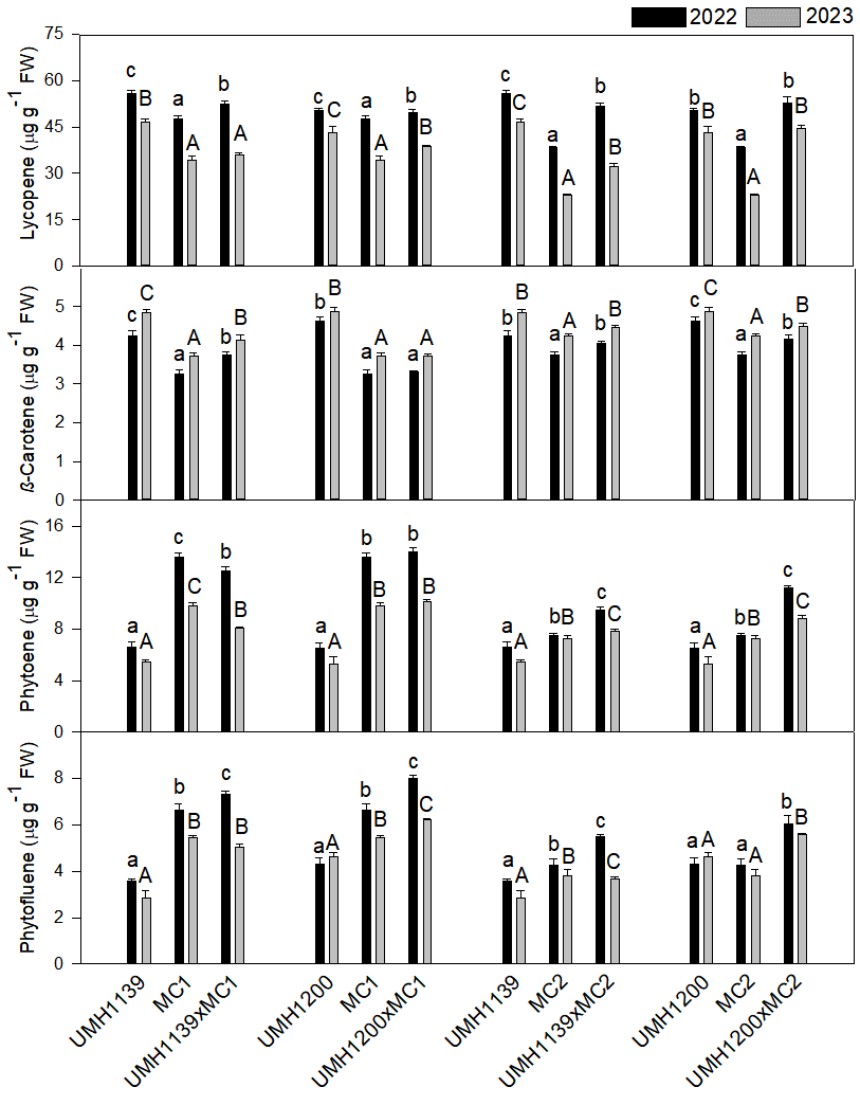

Figure 5. Concentrations of lycopene, *β*-carotene, phytoene and phytofluene (µg g^{-1} FW) in the developed hybrids and their respective parents in 2022 and 2023. Different letters indicate significant differences between genotypes within each year according to Duncan's test at $p < 0.05$: lowercase letters for 2022 and uppercase letters for 2023. Statistical significance of the main effects (variety, year, and interaction) is provided in Table 4. Values are means ± SE (n = 6).

Table 5. Concentrations of *δ*-carotene, lutein, neoxanthin, and violaxanthin (μg g^{-1} FW) and the *p*-values from a two-way ANOVA assessing the effects of variety, year and their interaction in the developed hybrids and their respective parents in 2022 and 2023 years.

	***δ*-Carotene**		**Lutein**		**Neoxanthin**		**Violaxanthin**	
	2022	**2023**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
UMH1139	4.42 ± 0.08 ^{ab}	3.22 ± 0.05 ^{ab}	1.80 ± 0.03 ^{a}	1.57 ± 0.03 ^{a}	0.15 ± 0.01	0.22 ± 0.01	0.71 ± 0.01 ^{c}	0.67 ± 0.02 ^{c}
MC1	4.17 ± 0.08 ^{a}	3.04 ± 0.15 ^{a}	2.21 ± 0.05 ^{c}	2.33 ± 0.11 ^{c}	0.16 ± 0.01	0.19 ± 0.02	0.55 ± 0.01 ^{a}	0.52 ± 0.02 ^{a}
UMH1139×MC1	4.74 ± 0.05 ^{b}	3.22 ± 0.03 ^{b}	2.05 ± 0.04 ^{b}	2.04 ± 0.05 ^{b}	0.18 ± 0.01	0.21 ± 0.01	0.61 ± 0.02 ^{b}	0.61 ± 0.01 ^{b}
Variety	0.007 **		0 ***		0.089		0 ***	
Year	0 ***		0.370		0 ***		0.098	
Variety × Year	0.198		0.221		0.222		0.500	
UMH1200	4.45 ± 0.13 ^{b}	4.11 ± 0.17 ^{b}	1.78 ± 0.05 ^{a}	1.78 ± 0.02 ^{a}	0.19 ± 0.01 ^{b}	0.25 ± 0.02 ^{b}	0.84 ± 0.01 ^{c}	0.69 ± 0.03 ^{b}
MC1	4.17 ± 0.08 ^{a}	3.04 ± 0.15 ^{a}	2.21 ± 0.05 ^{c}	2.33 ± 0.11 ^{c}	0.16 ± 0.01 ^{a}	0.19 ± 0.02 ^{a}	0.55 ± 0.01 ^{a}	0.52 ± 0.02 ^{a}
UMH1200×MC1	4.54 ± 0.12 ^{b}	3.88 ± 0.07 ^{b}	2.10 ± 0.04 ^{b}	2.06 ± 0.05 ^{b}	0.19 ± 0.01 ^{b}	0.24 ± 0.01 ^{b}	0.59 ± 0.01 ^{b}	0.63 ± 0.03 ^{b}
Variety	0 ***		0 ***		0 ***		0 ***	
Year	0 ***		0.606		0 ***		0.009 **	
Variety × Year	0.112		0.441		0.430		0 ***	
UMH1139	4.42 ± 0.08 ^{b}	3.22 ± 0.05 ^{b}	1.80 ± 0.03 ^{a}	1.57 ± 0.03 ^{a}	0.15 ± 0.01 ^{c}	0.22 ± 0.01 ^{c}	0.71 ± 0.01 ^{b}	0.67 ± 0.02 ^{b}
MC2	3.95 ± 0.09 ^{a}	2.63 ± 0.15^{a}	2.01 ± 0.05 ^{b}	2.04 ± 0.06 ^{b}	0.10 ± 0.01 ^{a}	0.13 ± 0.01 ^{a}	0.63 ± 0.01 ^{a}	0.55 ± 0.01 ^{a}
UMH1139×MC2	4.61 ± 0.05 ^{c}	3.44 ± 0.02 ^{c}	1.94 ± 0.03 ^{b}	2.00 ± 0.07 ^{b}	0.13 ± 0.01 ^{b}	0.18 ± 0.01 ^{b}	0.63 ± 0.03 ^{a}	0.53 ± 0.01 ^{a}
Variety	0 ***		0 ***		0 ***		0 ***	
Year	0 ***		0.214		0 ***		0 ***	
Variety × Year	0.629		0.069		0.559		0.304	
UMH1200	4.45 ± 0.13 ^{b}	4.11 ± 0.17 ^{b}	1.78 ± 0.05 ^{a}	1.78 ± 0.02 ^{a}	0.19 ± 0.01 ^{c}	0.25 ± 0.02 ^{c}	0.84 ± 0.01 ^{b}	0.69 ± 0.03 ^{b}
MC2	3.95 ± 0.09 ^{a}	2.63 ± 0.15 ^{a}	2.01 ± 0.05 ^{b}	2.04 ± 0.06 ^{b}	0.10 ± 0.01 ^{a}	0.13 ± 0.01 ^{a}	0.63 ± 0.01 ^{a}	0.55 ± 0.01 ^{a}
UMH1200×MC2	4.90 ± 0.11 ^{c}	4.72 ± 0.06 ^{c}	1.99 ± 0.03 ^{b}	1.95 ± 0.03 ^{b}	0.13 ± 0.01 ^{b}	0.17 ± 0.01 ^{b}	0.62 ± 0.03 ^{a}	0.57 ± 0.01 ^{a}
Variety	0 ***		0 ***		0 ***		0 ***	
Year	0 ***		0.937		0 ***		0 ***	
Variety × Year	0.071		0.708		0.072		0.062	

*, **, *** Significant differences between means at a 5, 1 or 0.1% probability levels, respectively. Different letters in the same column indicate significant differences between genotypes within each year according to Duncan's test at $p < 0.05$. Values are means ± SE (n = 6).

5.3.3.3. Phenolic Compounds

In this study, 29 phenolic compounds were identified (Supplementary Table S3), the most important of which were phenolic acids (Table 6), followed by flavonols and flavanones (Table 7). Among the former were chlorogenic acid and its derivatives (chlorogenic acid-like 1 and 2 and chlorogenic acid), followed by homovallinic acid-*O*-hexoside (1 and 2), caffeic acid and its derivatives (caffeic acid, caffeic-acid-*O*-hexoside 1, 2, and 3, and dicaffeoylquinic acid 1, 2, and 3), ferulic acid and its derivatives (ferulic acid and ferulic-acid-*O*-hexoside 1 and 2), and cryptochlorogenic acid. Among the flavonols, rutin and its derivatives (rutin and rutin-*O*-pentoside), together with kaempferol-3-*O*-ruthinoside, were predominant, whereas flavanones were represented only by naringenin and its derivatives (naringenin and naringenin-*O*-hexoside 1, 2, 3, 4, and 5). The presented results correspond to the total concentration of the compounds and their derivatives (Tables 6 and 7).

All of the tested phenolic compounds were significantly affected by variety, whereas the effect of the year depended on the tested compound and cultivar. Homovanillic acid-O-hexoside and ferulic acid concentrations were significantly affected by the year in all of the hybrid–parent comparisons. However, chlorogenic acid showed significant differences only in UMH1139×MC1 and its respective parents. In those cases where the effect of the year was significant, an overall decrease in these compounds was observed in 2023 compared to 2022. A significant variety × year interaction was observed for rutin, kaempferol-3-*O*-rutinoside (K-3-*O*-R), naringenin, and phloretin. These compounds exhibited a decline in the hybrids in 2023, except for K-3-*O*-R, whose concentrations remained stable. Additionally, phloretin concentrations in UMH1139×MC1 and naringenin concentrations in UMH1200×MC1 and UMH1200×MC2 showed no variation between years. The concentrations of chlorogenic acid, the most abundant phenolic acid, in the hybrids were similar to those of the traditional parent and higher than the ones found in the breeding line in both years, except for UMH1139×MC1 in 2023, which showed significantly higher values than both parents (67% higher than UMH1139 and 38% higher than MC1). The concentrations of homovanillic acid-*O*-hexoside and caffeic acid in all of the hybrids were comparable to or greater than those found in their respective traditional parents. The hybrids showed higher concentrations of cryptochlorogenic and ferulic acids than the breeding lines. However, contrasting responses were observed when compared with their traditional counterparts. All of the hybrids exhibited higher levels of cryptochlorogenic acid. In the case of ferulic acid, however, only UMH1139×MC1 and UMH1200×MC2 attained concentrations that were comparable to those of MC1 and MC2, respectively; lower concentrations were observed in the rest of the hybrids.

Table 6. Concentration of the main phenolic acids (μg g^{-1} FW) and the *p*-values from a two-way ANOVA assessing the effects of variety, year and their interaction in the developed hybrids and their respective parents in 2022 and 2023 years.

	Chlorogenic Acid		H-*O*-H		Caffeic and Der		Ferulic and Der		Cryptochlorogenic Acid	
	2022	**2023**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
UMH1139	5.3 ± 0.4 ^{a}	5.6 ± 0.1 ^{a}	4.9 ± 0.1 ^{a}	2.3 ± 0.1 ^{a}	1.8 ± 0.1 ^{a}	2.1 ± 0.1 ^{a}	1.3 ± 0.2 ^{a}	1.1 ± 0.1 ^{a}	1.2 ± 0.2 ^{ab}	1.0 ± 0.1 ^{a}
MC1	8.6 ± 1.3 ^{b}	6.8 ± 0.2 ^{b}	5.5 ± 0.6 ^{b}	3.5 ± 0.4 ^{b}	2.0 ± 0.1 ^{a}	1.9 ± 0.2 ^{a}	2.2 ± 0.4 ^{b}	1.8 ± 0.1 ^{b}	1.0 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}
UMH1139×MC1	8.6 ± 0.9 ^{b}	9.4 ± 0.6 ^{c}	7.4 ± 1.4 ^{c}	5.4 ± 0.6 ^{c}	2.7 ± 0.1 ^{b}	3.0 ± 0.3 ^{b}	2.1 ± 0.1 ^{b}	1.8 ± 0.1 ^{b}	1.4 ± 0.1 ^{b}	1.7 ± 0.3 ^{b}
Variety	0 ***		0 ***		0 ***		0 ***		0 ***	
Year	0.572		0 ***		0.033 *		0.005 **		0.390	
Variety × Year	0.021 *		0.860		0.069		0.618		0.013 *	
UMH1200	5.9 ± 0.9 ^{a}	4.6 ± 0.4 ^{a}	6.3 ± 0.6 ^{b}	2.6 ± 0.2 ^{a}	1.2 ± 0.1 ^{a}	1.5 ± 0.1 ^{a}	1.0 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}	1.3 ± 0.1 ^{b}	0.9 ± 0.1 ^{b}
MC1	8.6 ± 1.3 ^{b}	6.9 ± 0.2 ^{b}	5.5 ± 0.6 ^{a}	3.5 ± 0.4 ^{b}	2.0 ± 0.1 ^{b}	1.9 ± 0.2 ^{b}	2.2 ± 0.4 ^{c}	1.8 ± 0.1 ^{c}	1.0 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}
UMH1200×MC1	8.7 ± 0.4 ^{b}	7.2 ± 0.1 ^{b}	7.1 ± 0.7 ^{b}	3.3 ± 0.3 ^{b}	2.1 ± 0.3 ^{b}	2.3 ± 0.1 ^{b}	1.7 ± 0.1 ^{b}	1.5 ± 0.1 ^{b}	1.3 ± 0.2 ^{b}	1.1 ± 0.1 ^{b}
Variety	0 ***		0.050 *		0 ***		0 ***		0.004 **	
Year	0 ***		0 ***		0.181		0.010 *		0 ***	
Variety × Year	0.880		0.013 *		0.149		0.329		0.224	
UMH1139	5.3 ± 0.4 ^{a}	5.6 ± 0.1 ^{a}	4.9 ± 0.1 ^{a}	2.3 ± 0.1 ^{a}	1.8 ± 0.1 ^{b}	2.1 ± 0.1 ^{b}	1.3 ± 0.2 ^{a}	1.1 ± 0.1 ^{a}	1.2 ± 0.2 ^{a}	1.0 ± 0.1 ^{a}
MC2	8.0 ± 1.9 ^{b}	7.2 ± 0.9 ^{b}	7.0 ± 0.5 ^{b}	2.6 ± 0.2 ^{a}	1.6 ± 0.1 ^{a}	1.7 ± 0.2 ^{a}	3.1 ± 0.2 ^{c}	1.8 ± 0.1 ^{c}	1.3 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}
UMH1139×MC2	8.0 ± 0.8 ^{b}	7.9 ± 0.8 ^{b}	6.9 ± 0.3 ^{b}	3.5 ± 0.2 ^{b}	2.2 ± 0.1 ^{c}	2.2 ± 0.1 ^{b}	1.9 ± 0.1 ^{b}	1.5 ± 0.1 ^{b}	1.5 ± 0.1 ^{b}	1.2 ± 0.1 ^{b}
Variety	0.001 **		0 ***		0 ***		0 ***		0 ***	
Year	0.707		0 ***		0.038 *		0 ***		0 ***	
Variety × Year	0.713		0 ***		0.017 *		0 ***		0.199	
UMH1200	5.9 ± 0.9 ^{a}	4.6 ± 0.4 ^{a}	6.3 ± 0.6 ^{a}	2.6 ± 0.2 ^{a}	1.2 ± 0.1 ^{a}	1.5 ± 0.1 ^{a}	1.0 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}	1.3 ± 0.1 ^{a}	0.9 ± 0.1 ^{a}
MC2	8.0 ± 1.9 ^{b}	7.2 ± 0.9 ^{b}	7.0 ± 0.5 ^{a}	2.6 ± 0.2 ^{a}	1.6 ± 0.1 ^{b}	1.7 ± 0.2 ^{b}	3.1 ± 0.2 ^{c}	1.8 ± 0.1 ^{b}	1.3 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}
UMH1200×MC2	6.8 ± 1.9 ^{b}	8.9 ± 0.8 ^{b}	8.2 ± 0.4 ^{b}	4.2 ± 0.4 ^{b}	1.8 ± 0.1 ^{c}	2.2 ± 0.1 ^{c}	2.1 ± 0.1 ^{b}	1.5 ± 0.1 ^{b}	1.5 ± 0.1 ^{b}	1.0 ± 0.1 ^{b}
Variety	0.005 **		0 ***		0 ***		0 ***		0 ***	
Year	0.980		0 ***		0.002 **		0 ***		0 ***	
Variety × Year	0.100		0.401		0.204		0 ***		0.895	

*, **, *** Significant differences between means at a 5, 1 or 0.1% probability levels, respectively. Different letters in the same column indicate significant differences between genotypes within each year according to Duncan's test at $p < 0.05$. Values are means ± SE ($n = 6$)

Table 7. Concentration of the main flavonols and flavanones (μg g^{-1} FW) and the *p*-values from a two-way ANOVA assessing the effects of variety, year and their interaction in the developed hybrids and their respective parents in 2022 and 2023 years.

	Rutina and Der		**K-3-*O*-R**		**Naringenin and Der**		**Phloretin**	
	2022	**2023**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
UMH1139	1.37 ± 0.02 [c]	0.73 ± 0.11 [c]	1.25 ± 0.08 [c]	0.88 ± 0.12 [c]	0.21 ± 0.02 [b]	0.21 ± 0.02 [b]	0.37 ± 0.01 [c]	0.38 ± 0.03 [c]
MC1	0.09 ± 0.02 [a]	0.08 ± 0.01 [a]	0.09 ± 0.01 [a]	0.09 ± 0.01 [a]	0.06 ± 0.01 [a]	0.04 ± 0.01 [a]	0.02 ± 0.01 [a]	0.00 ± 0.01 [a]
UMH1139×MC1	0.35 ± 0.09 [b]	0.26 ± 0.01 [b]	0.38 ± 0.03 [b]	0.37 ± 0.01 [b]	0.20 ± 0.03 [b]	0.18 ± 0.03 [b]	0.11 ± 0.02 [b]	0.09 ± 0.01 [b]
Variety	0 ***		0 ***		0 ***		0 ***	
Year	0 ***		0.001 **		0.205		0.483	
Variety × Year	0 ***		0 ***		0.726		0.425	
UMH1200	1.91 ± 0.12 [c]	0.61 ± 0.02 [c]	1.71 ± 0.13 [c]	0.69 ± 0.02 [c]	0.26 ± 0.06 [c]	0.20 ± 0.03 [c]	0.50 ± 0.09 [b]	0.38 ± 0.07 [b]
MC1	0.09 ± 0.02 [a]	0.08 ± 0.01 [a]	0.09 ± 0.01 [a]	0.09 ± 0.01 [a]	0.06 ± 0.01 [a]	0.04 ± 0.01 [a]	0.02 ± 0.01 [a]	0.00 ± 0.01 [a]
UMH1200×MC1	0.24 ± 0.02 [b]	0.28 ± 0.01 [b]	0.27 ± 0.03 [b]	0.26 ± 0.01 [b]	0.15 ± 0.02 [b]	0.13 ± 0.02 [b]	0.09 ± 0.01 [a]	0.05 ± 0.01 [a]
Variety	0 ***		0 ***		0 ***		0 ***	
Year	0 ***		0 ***		0.048 *		0.001 **	
Variety × Year	0 ***		0 ***		0.471		0.019 *	
UMH1139	1.37 ± 0.02 [c]	0.73 ± 0.11 [c]	1.25 ± 0.08 [c]	0.88 ± 0.12 [c]	0.21 ± 0.02 [c]	0.21 ± 0.02 [c]	0.37 ± 0.01 [c]	0.38 ± 0.03 [c]
MC2	0.12 ± 0.03 [a]	0.09 ± 0.01 [a]	0.05 ± 0.02 [a]	0.07 ± 0.01 [a]	0.06 ± 0.01 [a]	0.03 ± 0.01 [a]	0.01 ± 0.01 [a]	0.00 ± 0.01 [a]
UMH1139×MC2	0.43 ± 0.04 [b]	0.30 ±0.04 [b]	0.34 ± 0.01 [b]	0.33 ± 0.02 [b]	0.20 ± 0.01 [b]	0.15 ± 0.03 [b]	0.11 ± 0.01 [b]	0.09 ± 0.02 [b]
Variety	0 ***		0 ***		0 ***		0 ***	
Year	0 ***		0.001 **		0.011 *		0.238	
Variety × Year	0 ***		0 ***		0.146		0.192	
UMH1200	1.91 ± 0.12 [c]	0.61 ± 0.02 [c]	1.71 ± 0.13 [c]	0.69 ± 0.02 [c]	0.26 ± 0.06 [c]	0.20 ± 0.03 [c]	0.50 ± 0.09 [c]	0.38 ± 0.07 [c]
MC2	0.12 ± 0.03 [a]	0.09 ± 0.01 [a]	0.05 ± 0.02 [a]	0.07 ± 0.01 [a]	0.06 ± 0.01 [a]	0.03 ± 0.01 [a]	0.01 ± 0.01 [a]	0.00 ± 0.01 [a]
UMH1200×MC2	0.40 ± 0.07 [b]	0.27 ± 0.06 [b]	0.29 ± 0.04 [b]	0.34 ± 0.03 [b]	0.18 ± 0.04 [b]	0.17 ± 0.01 [b]	0.12 ± 0.01 [b]	0.07 ± 0.01 [b]
Variety	0 ***		0 ***		0 ***		0 ***	
Year	0 ***		0 ***		0.075		0.001 **	
Variety × Year	0 ***		0 ***		0.471		0.018 *	

*, **, *** Significant differences between means at a 5, 1 or 0.1% probability levels, respectively. Different letters in the same column indicate significant differences between genotypes within each year according to Duncan's test at $p < 0.05$. Values are means ± SE (n = 6).

Regarding the main flavonols (rutin and kaempferol-3-*O*-rutinoside), flavanones (naringin), and phloretin, the hybrids exhibited intermediate concentrations that were higher than those of the traditional parent, but lower than those of the breeding line. The exception was UMH1139×MC1, which exhibited values comparable to those of the UMH1139 breeding line in naringenin, and UMH1200×MC1, which reached similar results to the ones found in the traditional parent in phloretin.

5.4. Discussion

The recovery of traditional varieties is limited by their susceptibility to viruses, such as ToMV, TYLCV, and TSWV, which leads to a substantial reduction in profitability for farmers (Picó et al., 2002). To address this issue and maintain genetic diversity, in 1998, the CIAGRO-UMH plant breeding group initiated a program to introduce three dominant genes (*Tm-2a*, *Sw-5*, and *Ty-1*), previously described as conferring resistance to these viruses into traditional varieties from southeastern Spain, including the Muchamiel type (Carbonell et al., 2018). However, the breeding lines with the *Ty-1* gene in a homozygous state obtained through this program exhibited a reduced yield and, in certain instances, an inferior fruit quality compared to the original variety. These losses are attributed to the introgression of linked genes that cannot be eliminated during the backcrossing process (Verlaan et al., 2011). One strategy to minimize these adverse effects is to use resistance genes in a heterozygous state (Tanksley et al., 1998). This study sought to evaluate the impact of introducing the *Tm-2a*, *Sw-5*, and *Ty-1* genes on the agronomic performance, fruit morphology, and metabolic composition of Muchamiel-type hybrids. This approach aimed at enhancing the biodiversity available for tomato cultivars and providing viable alternatives without compromising the distinctive traits that are particularly valued by consumers.

Although the introduction of the *Ty-1* gene has been reported to negatively affect yield (Verlaan et al., 2011; Alonso et al., 2008), no comparable effects were detected in the present study, either in the breeding lines or the hybrids. These results are consistent with those obtained by Carbonell et al. (2020), who also found no yield differences between the UMH1200 and UMH1139 breeding lines used in the present study. However, it is essential to note that these breeding lines do not originate from identical Muchamiel cultivars, which could result in minor genetic variations masking the negative impact of *Ty-1* on yield. Indeed, a study previously carried out by the same research group observed a 48% reduction in yield in Muchamiel NILs that were homozygous for *Ty-1* compared to susceptible lines (Rubio et al., 2016). In contrast, the hybrids that were developed in this study had a higher yield than their parents, even though this was only significant for UMH1200×MC1 and UMH1200×MC2. This increase could be attributed

to the vigour of the hybrid, albeit moderated by the reduced genetic distance between the parental lines, which are both of the Muchamiel type (Fortuny et al., 2021). The yield of the evaluated hybrids (3 to 4.4 kg per plant) is within the range reported for traditional Muchamiel varieties, with values ranging from 2 to 2.8 kg (Rubio et al., 2016) and up to 4.5 kg per plant (García-Martínez et al., 2015). Resistance genes were found to influence fruit morphology, as determined by longitudinal and equatorial diameters and parameters directly correlated with fruit weight. The breeding lines exhibited the smallest diameters, consistent with prior research indicating a potential adverse effect of these genes on fruit size (Carbonell et al., 2020; Rubio et al., 2016; Alonso et al., 2008). However, the hybrids that were heterozygous for the resistance genes recovered the longitudinal and equatorial diameter values of their traditional parents. This confirms that the introduction of virus-resistance genes in a heterozygous state can help to avoid the negative effects observed in homozygous lines (Carbonell et al., 2020).

Consumer preferences shape the commercial value of tomatoes, as fruits with a balance between acidity and sweetness are often preferred (Casals et al., 2019). Primary metabolites play a key role in determining the flavour. The Muchamiel variety is characterized by intermediate concentrations of glucose, fructose, and citric acid, as well as high concentrations of malic acid and glutamic acid. The latter is closely linked to the *umami* flavour, which is important for the sensory quality of the fruit (Sánchez et al., 2023, 2024). Whereas the impact of resistance genes' introductions on the primary metabolite profile of tomatoes remains largely unexplored, the literature does offer insights into their influence on parameters such as titratable acidity and total soluble solids, which are primarily determined by organic acids and soluble sugars, respectively. Rubio et al. (2016) observed that the presence of the *Ty-1* gene in homozygosis in NILs of the traditional Muchamiel and De la Pera varieties reduced titratable acidity without affecting the total soluble solids. This effect was subsequently confirmed in (Cabrera et al., 2024; Carbonell et al., 2020). The *Ty-1* locus from S. chilense is located within a non-recombinant genomic region of approximately 35.5 Mb on chromosome 6, where two chromosomal inversions suppress recombination. As a result, this large introgressed segment may retain wild tomato genes that negatively affect acidity (Verlaan et al., 2011). The results of this study are consistent with these findings. Hybrids derived from the UMH1139 line, in which the *Ty-1* gene is not present, were found to maintain or increase their organic acid concentration compared to the traditional parent line. In contrast, hybrids derived from the UMH1200 line, which carries the *Ty-1* gene, exhibited reductions in all of the three major organic acids (UMH1200×MC1) or the glutamic acid only (UMH1200×MC2). However, in the UMH1200×MC1 hybrid, this decrease did not reach the levels observed in the breeding line, instead presenting an intermediate concentration of the three acids, possibly due to the presence of a single copy of the *Ty-1* gene (Carbonell et al., 2020; Tanksley et al., 1998). No effect that could be attributed to the introduction of resistance genes

was detected with regard to soluble sugars, but it was noted that hybrids consistently outperformed their traditional parents (MC2 and MC1), with an average increase in glucose and fructose of 18% and 22%, respectively, over MC2 and MC1, and variations depending on the hybrid and year.

In recent years, consumer interest in the functional value of agrifood products, primarily driven by the secondary metabolites found in tomatoes, has increased due to the health benefits offered by these metabolites (Ali et al., 2021). The concentrations of different secondary metabolites in the cultivars examined in this study were consistent with those reported in previous studies on Muchamiel-type cultivars (Sánchez et al., 2023, 2024; Meza et al., 2020). However, the metabolic profiles of these breeding lines and their hybrids remain to be characterized. The adverse effect of the introduction of resistance genes, particularly *Ty-1*, observed on organic acid concentrations, was not detected in the profile of secondary metabolites. It was reported that the *Ty-1* introgression in De la Pera breeding lines did not affect the antioxidant activity of the fruit (Cabrera et al., 2024). In this study, the cultivars carrying resistance genes (both the homozygous breeding lines and the heterozygous hybrids) showed higher concentrations of rutin, kaempherol-3-O-rutinoside, naringenin, and phloretin than the susceptible cultivars (the traditional parents). The breeding lines showed the highest concentrations, whereas the hybrids exhibited intermediate values. This suggests a direct relationship between the number of copies of the resistance genes and the accumulation of these antioxidant compounds. A higher basal antioxidant level, induced by increased flavonoid synthesis, can enable a quick response to the virus without causing significant oxidative stress. Modulating ROS accumulation during viral infection enables a rapid defence response, such as a hypersensitive reaction, while preventing excessive oxidative damage to surrounding tissues (Hernández et al., 2016). Differences in the metabolome of virus-susceptible and virus-resistant tomato cultivars have been observed. For example, a 14.17-fold increase in naringenin in resistant cultivars following TSWV infection has been observed, compared to 2.81- and 1.57-fold increases in susceptible lines (Lv et al., 2023). Similarly, higher concentrations of secondary metabolites were reported in TYLCV-resistant cultivars prior to infection, which suggests that these metabolites play a role in conferring virus tolerance (Sade et al., 2015). Further studies with near-isogenic lines are recommended to confirm the effect of resistance genes on flavonoid accumulation, as these lines are genetically identical, except for the single introgressed region. This enables a more accurate evaluation of the impact of these introgressed regions, particularly the resistance genes, in this context (Rubio et al., 2016).

Maintaining an outstanding functional quality is crucial for Muchamiel cultivars. Therefore, it is important to note that hybrids have conserved or improved the functional characteristics of their parents. Depending on the compound, they present a higher concentration of each secondary metabolite in either the breeding line or the traditional parent. Previous studies

have suggested that the predominant gene action for traits such as secondary metabolite content is additive (Sánchez et al., 2024; Fortuny et al., 2021), which is consistent with these results. From a nutritional perspective, vitamin C is recognized for its potent antioxidant properties and its ability to donate electrons, thereby protecting DNA from oxidative damage (Scarano et al., 2020). The traditional parents were notable for their high vitamin C concentration, a trait that the hybrids have retained. Due to their antioxidant properties, carotenoids such as lycopene, *β*-carotene, and lutein play a crucial role in preventing chronic diseases, including cancer, cardiovascular disease, and macular degeneration (Friedman, 2013). Despite their limited bioavailability, xanthophylls are believed to bolster cellular defence and potentially reduce the risk of certain cancers and skin damage (Abdel-Aal et al., 2013). Breeding lines (UMH1139 and UMH1200) exhibited the highest concentrations of lycopene and *β*-carotene, consistent with their intense colour. They also showed elevated levels of neoxanthin and violaxanthin, whereas traditional parents had higher amounts of lutein, phytoene, and phytofluene. Hybrids showed intermediate values, even though some equalled their parents in terms of lycopene, *β*-carotene, and neoxanthin content. Additionally, they outperformed traditional parents in phytofluene and, in the case of those with MC2 as a parent, in phytoene. Phenolic acids have been associated with anticancer effects and protection against inflammation-induced diabetic nephropathy (Tommonaro et al., 2012). Naringenin, the primary flavanone in tomatoes, has been shown to play a role in preventing cancer and other diseases (Quinet et al., 2019). Flavonols have been extensively studied for their pharmacological properties, including anticancer, analgesic, and anti-arthritic effects, as well as their benefits to various bodily systems (Tieman et al., 2017). All of the hybrids maintained the high concentration of chlorogenic acid (the main phenolic acid found in tomatoes) characteristic of their traditional parents. Most of the hybrids outperformed their parents in terms of concentrations of caffeic and cryptochlorogenic compounds, except for UMH1200×MC1, which matched them. The ferulic acid concentration of UMH1139×MC1 is similar to that of MC1. Among the flavonols and flavanones, the breeding lines exhibited the highest concentrations, the traditional parents, the lowest, and the hybrids, intermediate values.

Finally, as most traits are quantitative and sensitive to environmental factors, analysing the effect of the year of culture on the development of new varieties is essential. Semi-arid zones are characterized by elevated levels of solar radiation and high summer temperatures, which can impact crop productivity and quality (Rosales et al., 2011). The yield of hybrids remained stable in both years, indicating a high productive stability. The only significant yield reduction observed in UMH1200×MC2 in 2023 is within the range of interannual variation previously reported (García-Martínez et al., 2015). Given the added value of this hybrid, such a reduction can be considered acceptable from the perspective of the farmer. However, visual (morphology and colour), flavour-related (primary metabolites), and nutritional (secondary metabolites) quality

parameters exhibited interannual variability, attributed to environmental differences between 2022 and 2023. Increased temperatures and cumulative irradiance during the fruiting period in 2023 compared to 2022 could have triggered variations in the composition of the studied fruit varieties (Supplementary Figure S1). Such conditions have been reported to favour an increase in sugars, as well as variations in organic acids (Hernández et al., 2015; Rosales et al., 2011; Gautier et al., 2008). Regarding the secondary metabolites, most compounds showed a reduction in 2023, which was consistent with previous studies (Hernández et al., 2015), although *β*-carotene increased in all of the cultivars (Delgado-Vargas et al., 2023). Other metabolites, such as lutein and violaxanthin in certain cases, chlorogenic acid in some cultivars, and caffeic acid, naringenin, and its derivatives under certain conditions, remained stable between years. The interannual response of these compounds varied according to the analysed cultivar and metabolite, explaining the diversity of patterns that were observed (Fibiani et al., 2022). Despite these variations, differences between parents and hybrids were maintained, indicating that the observed variability depended mainly on the genetic component, with annual environmental conditions having a minor effect.

5.5. Conclusions

This study demonstrates that the introduction of virus-resistance genes (*Tm-2a*, *Sw-5* and *Ty-1*) into traditional Muchamiel varieties in a heterozygous state did not compromise yield or fruit quality. In contrast to previous reports on homozygous breeding lines, the evaluated hybrids did not exhibit a reduced yield; in some instances, they even outperformed their traditional parents and breeding lines, likely due to hybrid vigour, highlighting their commercial potential. Fruit morphology and visual quality were preserved, with hybrids maintaining the characteristic diameters of the traditional variety. Flavour-related quality was also retained, as there were no adverse effects on soluble sugars; in fact, glucose and fructose levels increased. Although *Ty-1* negatively affected organic acid content, this effect was reduced in the heterozygous state. Furthermore, the introgression of resistance genes did not reduce the levels of secondary metabolites; instead, an increase in flavonoid concentration was observed, which could enhance the functional quality of the hybrids. Further studies using NILs are needed to clarify the specific metabolic effects of these resistance genes. Overall, the introduction of heterozygosity is a promising strategy for preserving the identity and quality of Muchamiel varieties while enhancing their resistance and market potential.

5.6. References

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5.7. Supplementary material

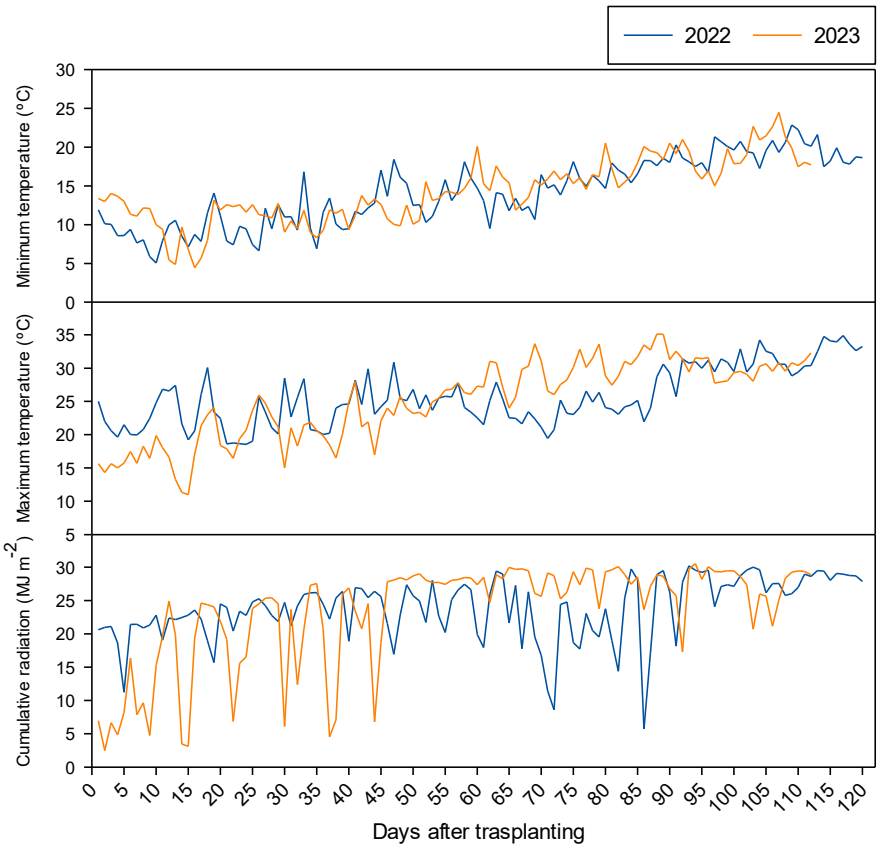

Figure S1. Evolution of minimum, maximum and cumulative radiation during the two cycles.

Table S1. Organic acids identified in the studied varieties by MS/MS approaches.

Peak no.	Compound	Rt (min)	$[M-H]^-$	ProdI*
1	Glutamic Acid	4,05	146	128, **102**
2	Quinic Acid	4,38	191	93, 87, **85**
3	Malic Acid	4,69	133	**115,** 71
4	Isocitric Acid	4,77	191	155, **111**, 73
5	Malonic Acid	4,87	103	**59**, 41
6	Shikimic Acid	5,02	173	**93**, 73
7	Ketoglutaric Acid	5,21	145	**101**, 57
8	Citric Acid	6,09	191	**111**, 87
9	Succinic Acid	6,66	117	99, **73**
10	Fumaric Acid	6,96	115	**71**
11	Glutaric Acid	10,97	131	113, **87**, 69

*Values in bold type are the most abundant ions.

Table S2. Carotenoids identified in the studied varieties by UV-vis HPLC.

Peak no.	Compound	Rt (min)	Wavelength (nm)	Type
1	*All-T*-Neoxanthin	7,05	444	Polar[1]
2	*All-T*-Violaxanthin	8,03	444	Polar[1]
3	Lutein	15,11	444	Polar[1]
4	Phytoene	18,02	287	Polar[1]
5	Phytofluene I	19,69	347	Polar[1]
6	Phytofluene II	21,15	347	Polar[1]
7	*All-trans*-Beta-carotene	24,63	444	Non-Polar[2]
8	Deltacarotene1	25,88	444	Non-Polar[2]
9	Deltacarotene2	26,25	444	Non-Polar[2]
10	Deltacarotene3	28,20	444	Non-Polar[2]
11	15 or 5' cis-Lycopene	29,50	444	Non-Polar[2]
12	*All-trans*-Gammacarotene	29,68	444	Non-Polar[2]
13	13 or 13' cis-Lycopene	30,17	444	Non-Polar[2]
14	13 or 13' cis-Lycopene	30,82	444	Non-Polar[2]
15	9 or 9' cis-Lycopene	32,20	444	Non-Polar[2]
16	9 or 9' cis-Lycopene	32,47	444	Non-Polar[2]
17	5 or 5' cis-Lycopene	33,01	444	Non-Polar[2]
18	*All-trans*-Lycopene	34,63	444	Non-Polar[2]
19	5 or 5' cis-Lycopene	35,02	444	Non-Polar[2]

[1]Carotenoids classified as polar were extracted using methanol:tetrahydrofuran (1:1, v/v) with 200mg MgO and 0.1% BHT (Flores et al., 2016). [2]Carotenoides classified as non-polar were extracted using hexane:acetone:ethanol (2:1:1) with 0.1% BHT (Olives-Barba et al., 2006).

Table S3. Phenolic compounds identified in the studied varieties by MS/MS approaches.

Peak no.	Compound	Rt (min)	$[M-H]^-$	ProdI*
1	Chlorogenic Acid-Like1	11.14	353	**191**, 179
2	Homovallinic Acid-*O*-Hexoside 1	13.32	343	**181**,121
3	Caffeic-Acid-*O*-Hexoside 1	13.38	341	**179**,135
4	Caffeic-Acid-*O*-Hexoside 2	14.31	341	**179**,135
5	Homovallinic Acid-*O*-Hexoside 2	14.39	343	**181**,121
6	Ferulic-Acid-*O*-Hexoside 1	16.04	355	**193**,178
7	Caffeic-Acid-*O*-Hexoside 3	16.17	341	**179**,135
8	Cryptochlorogenic Acid	17.02	353	179, **173,** 135
9	Chlorogenic Acid	17.06	353	**191**, 179
10	Ferulic-Acid-*O*-Hexoside 2	18.56	355	**193**,178
11	Caffeic Acid	19.06	179	**135**
12	Chlorogenic Acid-Like2	19.73	353	**191**,179
13	Coumaroylquinic Acid	22.28	337	**191**, 163
14	*p*-Coumaric Acid	25.89	163	**119**
15	Ferulic Acid	28.99	193	149, **134**
16	Rutin-*O*-Pentoside	30.00	741	**609,** 300
17	Phloretin-*C*-Diglycoside	32.70	597	477, 417, 387, **357**
18	Rutin	32.87	609	**300**
19	Dicaffeoylquinic 1	34.42	515	353, **191**
20	Naringenin-*O*-Hexoside 1	35.88	433	**271**
21	Dicaffeoylquinic 2	35.98	515	353, **191**
22	Kaempferol-3-*O*-Rutinoside	36.89	593	285
23	Naringenin-*O*-Hexoside 2	37.26	433	**271**
24	Dicaffeoylquinic 3	39.15	515	353, **191**
25	Naringenin-*O*-Hexoside 3	40.00	433	**271**
26	Naringenin-*O*-Hexoside 4	42.09	433	**271**
27	Naringenin-*O*-Hexoside 5	44.00	433	**271**
28	Quercetin	49.21	301	179, **151**
29	Naringenin	53.06	271	**151**, 119

*Values in bold type are the most abundant ions.

Table S4. Concentration of the isocitric and quinic acid (µg g^{-1} FW) and the *p*-values from a two-way ANOVA assessing the effects of variety, year and their interaction in the developed hybrids and their respective parents in 2022 and 2023 years.

	Isocitric Acid		**Quinic Acid**	
	2022	2023	2022	2023
UMH1139	79 ± 5^{a}	69 ± 2^{a}	60 ± 8	40 ± 3
MC1	90 ± 6^{b}	88 ± 6^{b}	66 ± 3	49 ± 5
UMH1139×MC1	84 ± 1^{b}	90 ± 9^{b}	62 ± 1	50 ± 5
Variety	0***		0.067	
Year	0.482		0***	
Variety x Year	0.099		0.498	
UMH1200	71 ± 4^{a}	59 ± 3^{a}	49 ± 1^{a}	29 ± 3^{a}
MC1	90 ± 6^{b}	88 ± 6^{b}	66 ± 3^{c}	49 ± 5^{c}
UMH1200×MC1	71 ± 4^{a}	65 ± 2^{a}	58 ± 3^{b}	40 ± 2^{b}
Variety	0***		0***	
Year	0.047*		0***	
Variety x Year	0.313		0.363	
UMH1139	79 ± 5^{b}	69 ± 2^{a}	60 ± 8	40 ± 3
MC2	70 ± 2^{a}	69 ± 3^{a}	56 ± 5	35 ± 1
UMH1139×MC2	78 ± 1^{b}	80 ± 3^{b}	57 ± 1	38 ± 2
Variety	0***		0.190	
Year	0.241		0***	
Variety x Year	0.004*		0.956	
UMH1200	71 ± 4^{a}	59 ± 3^{a}	49 ± 1	29 ± 3
MC2	70 ± 2^{a}	69 ± 3^{a}	56 ± 5	35 ± 1
UMH1200×MC2	74 ± 4^{b}	72 ± 2^{b}	54 ± 6	36 ± 2
Variety	0.001**		0.108	
Year	0.047*		0***	
Variety x Year	0.053*		0.768	

*, **, *** Significant differences between means at a 5, 1 or 0.1% probability levels, respectively. Different letters in the same column indicate significant differences according to Duncan's test at $p<0.05$. Values are means ± SE (n=6).

6. Chapter IV

Agronomic performance and characteristics of traditional tomato varieties grown with low input conditions

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Abstract

The adaptation to local agro-climatic conditions of traditional varieties renders them a valuable resource for low-input agriculture. This study evaluates the effects of fertilisation (100% and 50%) and irrigation (100% and 75%) doses on the agronomic performance and fruit quality of traditional tomato varieties. Flor de Baladre (FB), Muchamiel (MC), Pera Pinatar (PP), and Negro de Nerpio (NN) varieties were studied, along with two F_1 hybrids (H1 and H2) obtained by crossing NN with other traditional varieties. Mongo (MO) was used as a commercial reference. All traditional cultivars exhibited superior sensory quality and achieved yields similar to MO in the control treatment (7.4 kg plant^{-1}), except for NN (lower number of fruits) and PP (smaller fruit size). FB and MC showed reduced agronomic performance in the treatment combining high fertiliser dose with low irrigation rate (100F+75I), while fruit quality improved due to concentration effects. Despite this, both varieties maintained their yield under reduced fertilisation, with FB further enhancing phenolic compound concentration (17%). NN and H1 and H2 hybrids showed higher mineral requirements, as their yield was negatively affected by fertiliser reduction. H1 excelled under reduced irrigation treatments, maintaining its yield and improving fructose and phenolic concentration (10 and 11%, respectively). PP showed tolerance to low in-puts, retaining its yield in all of the treatments and increasing glucose, fructose and vitamin C concentration under reduced fertilisation (12, 8 and 9%, respectively). The variability of the response of this collection of traditional Spanish varieties represents a valuable resource for sustainable production, being each of the varieties adapted to specific low-input conditions.

6.1. Introduction

One of the key challenges that modern agriculture has to face is the environmentally sustainable production of healthy and high-quality food. In particular, the Mediterranean basin, one of the most densely populated and agriculturally important areas in Europe, confronts a high resource consumption due to intensive agriculture, especially in terms of water, energy, and fertilisers (Gao and Giorgi, 2008). This significant demand exerts a considerable pressure on local ecosystems, as the excessive use of water and macronutrients (such as nitrogen, phosphorus, and potassium) can precipitate serious ecological problems, including water scarcity, soil degradation, and contamination of surrounding ecosystems by runoff and leaching, as well as a reduction in agricultural profitability and competitiveness (Kim et al., 2020). The sharp increase in world population, coupled with frequent extreme weather events and environmental pollution, exacerbates the issue of water scarcity (Hein et al., 2021). In this context, attaining a balance between agricultural productivity and environmental protection is vital if the long-term viability of agroecosystems is to be maintained (Tian et al., 2021).

Nitrogen, potassium, and phosphorus are essential macronutrients for crop development. In conventional agriculture, nitrogen is often over-applied to increase yields, though excessive use can negatively affect yield and fruit quality (Cheng et al., 2021; Albornoz, 2016; Elia and Conversa, 2012). The effects of low nitrogen levels on fruit quality are still debated (Hernández et al., 2020; Bénard et al., 2009). Potassium plays a significant role in vegetative growth, yield, fruit quality, and stress resistance (Li et al., 2021; Liu et al., 2021; Wang et al., 2013). Phosphorus is critical for key biological functions such as photosynthesis, respiration, energy transfer, and nucleic acid synthesis (Sung et al., 2015). Along with mineral nutrition, irrigation is a key aspect in agricultural systems. The implementation of effective irrigation management strategies is essential to optimize crop yield and promote efficient use of water, a scarce and vital resource (Lu et al., 2021; Wang and Xing, 2017). Some studies have demonstrated that reduced irrigation results in a decline in tomato yield, although it does enhance fruit quality by increasing sugar content, titratable acidity, and levels of bioactive compounds such as vitamin C and carotenoids (Conti et al., 2023). Nevertheless, the influence of water deficit on yield and fruit quality characteristics is contingent on the specific tomato genotype.

Following the introduction of tomatoes in Europe in the early 16th century, these fruits spread primarily throughout the Mediterranean countries, leading to the development of a wide diversity of varieties. These were selected by local farmers over generations and are nowadays known as traditional varieties (García-Martínez et al., 2013). In contrast to commercial varieties developed for yield and uniformity, traditional varieties maintain a high genetic diversity and offer exceptional organoleptic qualities, which include a superior taste and aroma. These

attributes are becoming increasingly important to consumers and the industry (Sinesio et al., 2021; Figàs et al., 2015; Casals et al., 2011). Zhu et al. (2018) demonstrated how breeding resulted in alterations to the metabolome of tomato fruits, accompanied by a reduction in the number of genetic alleles associated with flavour and nutritional compounds in commercial cultivars. Previous studies have demonstrated the superior organoleptic and nutritional quality of certain traditional varieties compared to commercial ones, particularly in terms of enhanced taste, higher phenolic compound concentrations, and elevated carotenoid levels (Casals et al., 2021; Figàs et al., 2015). Moreover, traditional varieties have attracted attention for their potential contribution to sustainable agriculture, owing to the hypothesis that their evolution within traditional farming systems has conferred adaptations enabling them to perform effectively under resource-limited conditions (Casals et al., 2021). In the context of resource scarcity and increasing demand for sustainable food production, these varieties could offer the potential to reduce inputs such as fertilisers and water without compromising yield or fruit quality. While the performance of commercial tomato varieties under reduced-input conditions has been widely studied (Wang et al., 2019; Wang and Xing, 2017), research on traditional varieties has predominantly focused on their potential in abiotic stresses, such as under salt stress (Meza et al., 2020; Massaretto et al., 2018; Moles et al., 2016), or their suitability for organic management systems (Chea et al., 2021; Suárez et al., 2008). However, only a small subset of the available traditional material has been evaluated, and studies specifically addressing their responses to reduced irrigation and fertilisation remain notably limited.

Previous evaluations of traditional tomato varieties, focusing on both agronomic performance and fruit quality, enabled the identification of cultivars that combine exceptional fruit quality with robust agronomic traits, highlighting their potential for commercial cultivation (Hellín et al., 2023; Sánchez et al., 2023; Flores et al., 2017, 2020). Using these superior cultivars, hybrids were developed to introduce novel genetic combinations (Sánchez et al., 2024). Among the resulting hybrids, those exhibiting yield levels comparable to commercial varieties while retaining or enhancing the fruit quality characteristics of their traditional parental lines were selected for further evaluation.

The objective of this study, corresponding to the second phase of this research, is to evaluate the performance of the selected collection of traditional varieties, characterised by genetic diversity and valuable traits, under low-input conditions, a key factor in the advancement of sustainable agriculture. For that, a 50% reduction in fertiliser was applied to simulate moderate nutrient stress, sufficient to differentiate tomato variety tolerance without causing crop failure (Abdelhady et al., 2017; Wang and Xing, 2017). Additionally, to assess tolerance to water deficit, irrigation was reduced by 75% of crop evapotranspiration (ETc), based on studies showing this

level induces significant water deficit without severely impacting yield or quality in tolerant genotypes (Al-Selwey et al., 2021; Wu et al., 2021), thereby enabling the identification of varieties with improved adaptation to deficit irrigation. The traits of interest for this evaluation include agronomic performance, organoleptic quality parameters, and the concentration of key bioactive compounds in tomato, such as carotenoids, phenolic compounds, and vitamin C.

6.2. Materials and methods

6.2.1. Plant material

Four traditional tomato varieties from the IMIDA germplasm bank (BAGERIM), previously selected for their agronomic performance and/or high quality (Sánchez et al., 2023), were grown: Flor de Baladre de Espinardo (FB; noted for its strong agronomic performance and exceptional flavour), Pera Pinatar (PP; recognized for its good agronomic performance), Muchamiel (MC; characterized by its agronomic performance and accession variability), and Negro de Nerpio (NN; distinguished by its high lycopene content and remarkable flavour) (Table 1; Figure 1). Additionally, two experimental F_1 hybrids were included: "Flor de Baladre de Espinardo x Negro de Nerpio" (H1) and "Rosa de la Arboleja x Negro de Nerpio" (H2), both previously characterized by their good organoleptic and nutritional characteristics and an acceptable yield (Sánchez et al., 2024). A commercial control variety, Mongo (MO) (Ramiro Arnedo, S.A.), was also included in the study.

Table 1. Main characteristics of the studied cultivars.

Cultivar	Bank code	Type	Colour	Size[x]
MO		Commercial hybrid (Marmande)	Red	L
FB	BGMU0639	Traditional (Flor de Baladre)	Pink	XL
PP	BGMU0683	Traditional (Pera)	Red	M
MC	BGMU0753	Traditional (Muchamiel)	Pink	L
NN	BGMU0922	Traditional (De la Sierra)	Red-Black	L
H1	BGMU0639 x BGMU0922	Experimental hybrid	Red	XL
H2	BGMU0661 x BGMU0922	Experimental hybrid	Red	L

[x] Size corresponds to the following: M (75-150 g), L (150-300 g), and XL (>300 g).

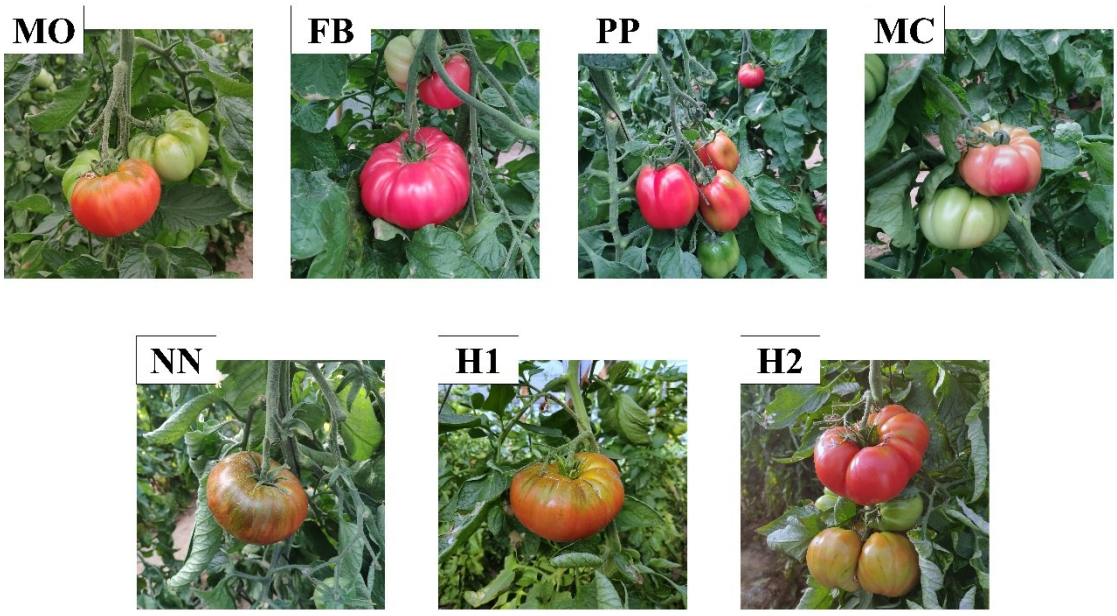

Figure 1. Images of the studied varieties (Mongo [MO], Flor de Baladre de Espinardo [FB], Pera Pinatar [PP], Muchamiel [MC], and Negro de Nerpio [NN]), and the experimental hybrids H1 and H2.

6.2.2. Experimental design and growth conditions

The cultivars described above were grown in a polyethylene multitunnel greenhouse at an experimental farm ("Torreblanca") located in Torre Pacheco (Murcia, SE Spain; 37° 46' 33.564" N, 0° 53' 47.225" W). The area has a Mediterranean climate, and its soil is classified as clay loam. The plants were irrigated with water from the Tajo-Segura transfer system (0.8-1.3 dS m^{-1}). The total amounts of fertilisers (F) applied during the entire growing season at the 100% fertilisation level (100F) consisted of 175 N, 180 P2O5, 276 K2O, 125 Ca, and 30 Mg (kg ha^{-1}). At the 100% irrigation level (100I), the total water volume applied was 0.24 m^3 m^{-2}. Table 2 provides detailed information regarding the distribution of the total dose of fertiliser and the total irrigation rate across the primary growth phases of the crop cycle. Both conditions were established on the basis of typical cultural practices and soil and climatic conditions of the growing area, as well as following the technical specifications for integrated tomato production, in accordance with the Order of 10 May 2012 (BORM, 2012). Both inputs were supplied through separate irrigation tanks for each treatment, using a drip irrigation system equipped with individual 2 L h^{-1} drippers for each plant. Fertiliser solutions were prepared and applied weekly, ensuring homogeneous distribution. Treatments consisted of a combination of two doses of fertilisation (F), 100% and 50%, with two rates of irrigation (I), 100% and 75%, resulting in a total of four treatments (100F+100I, 100F+75I, 50F+100I, 50F+75I) (Table 2). Based on the total amounts applied at the control treatment (Table 2), the total 50% fertilisation dose (50F) corresponded to 87.5 N, 90 P_2O_5, 138 K_2O, 62.5 Ca, and 15 Mg (kg ha^{-1}). Similarly, the total 75% irrigation rate (75I) corresponded to a total water volume of 0.18 m^3 m^{-2}. As a result of the decrease in water volume without reducing the fertiliser dose, the irrigation solutions of the

100F+75I and 50F+75I treatments were 33% more concentrated than those of 100F+100I and 50F+100I, respectively.

Table 2. Distribution of nutrients and irrigation throughout the crop growth cycle under the control treatment (100F and 100I). Nutrient values (N, P_2O_5, K_2O, Ca and Mg) are expressed as (kg ha^{-1}), and water is expressed as (m^3 m^{-2}).

Growth Phase	N	P_2O_5	K_2O	Ca	Mg	Water
Transplant - Appearance of third truss	15	30	25	5	0	0.02
Ending phase 1 - Colour turn of first fruits	137	120	211	90	25	0.19
Ending phase 2 - End of culture	23	30	40	30	5	0.03
Total crop cycle	**175**	**180**	**276**	**125**	**30**	**0.24**

Transplant was manually established in December using 40-day-old seedlings sourced from a commercial nursery. The planting frame was 0.44 m between plants and 1 m between rows, resulting in a transplanting density of approximately 2.27 plants per square meter. The experimental design included two randomized blocks per cultivar and treatment, resulting in a total of eight blocks. Within each block, the position of the seven cultivars was also randomized. Each block comprised a single row containing the seven cultivars described above, with five plants per cultivar (a total of 35 plants per block). The total area per block was 15.4 m^2. Boundary rows were placed along the edges of the test area and between the treated rows to avoid a "boundary effect". The plants grew vertically with one stem after axillary bud removal and were maintained until the end of the production phase (200–250 days after transplanting, depending on the genotype). A total of 5-6 trusses were harvested, depending on the variety, and fruits from the 2nd to the 4th truss used for sensory and fruit quality analyses. Integrated pest and disease management was carried out following the technical specifications for integrated tomato production, in accordance with the Order of 10 May 2012 (BORM, 2012). Bumblebee hives were employed to enhance pollination. Temperature evolution during this period is illustrated in Supplementary Figure 1, which shows the hourly average temperature (°C) recorded by the TP42 weather station in the experimental farm of the SIAM (Murcia Agricultural Information System). During the harvest period (from April to July), mean day and night temperatures ranged between 12–37 °C and 6–20 °C, respectively.

6.2.3. Sensory analysis

Sensory analysis was conducted following the methodology described by Hongsoongnern and Chambers IV (2008). A trained panel of 13 participants (9 women and 4 men) aged 24–43 years, carried out the methodology. Prior to the sensory session, references for extreme and intermediate values for each trait were established by consensus using a diverse set of commercial tomato samples. This preliminary step allowed the panel to align their criteria and scoring thresholds. The sensory evaluation was conducted in a single 1.5-hour session with all 13 panellists. For each of the analysis, a set of 10–15 commercially-ripe tomatoes was harvested

between the second and fourth truss of each variety. Selected fruits were washed with fresh tap water and dried with absorbent paper before analysis. In order to mitigate the impact of bias, the samples were anonymised using a coding system. These samples were then presented to the panellists in a randomised order. Panellists evaluated a set of six visual, five taste, and ten textural traits closely related to tomato consumer preferences, as detailed in Table 3, based on Hongsoongnern and Chambers IV (2008). Visual traits were assessed on whole tomatoes, whereas taste and texture traits were assessed on slices of the same tomatoes. Most of the traits were scored on a numerical scale ranging from 0 to 10 in 0.5-unit increments, with 0 representing no intensity and 10 representing an extremely strong intensity. As per the rest of the traits, they were scored on specific smaller scales agreed by the panellists prior to the analysis.

Table 3. Description and score scale for all of the evaluated traits.

Trait	Description	Scale
Visual		
Colour	Visual evaluation of the optimal skin colour of the tomatoes	0–10
Bicolour	Visual evaluation of two different colours	1–4
Homogeneity	General homogeneity of the sample	0–10
Brightness	Sensory attribute due to the natural waxes in skin of the tomato	1–4
Ribbing	Intensity of the ribs at calyx end	1–4
Green shoulder	Intensity of the green trips at calyx end	1–4
Flavour		
Sweet	A flavour stimulated by sugars	0–10
Acid	A flavour stimulated by acids	0–10
Tomato ID	Aromatics reminiscent tomato characteristic flavour	0–10
Fruity	Aromatics reminiscent fruity flavour	0–10
Aftertaste	Time that the tomato flavour remains in mouth after swallowing	0–10
Texture		
Hardness	Force required to bite completely through the sample with molar teeth	0–10
Crunchiness	Intensity of audible noise at first bite with molars	0–10
Juiciness	Sensation of moisture released by the tomatoes during the first bites	0–10
Juice density	Thickness of the tomato juice during the first bites	1-3
Flesh	Amount of pulp detected in mouth after the first bites	0–10
Peel	Thickness of the pericarp evaluated during the first bite	0–10
Seeds	Quantity of seeds of the sample	0–10
Solubility	Amount of tomato that remains after chewing 5 times	0–10
Residual peel	Amount of skin that remains on the teeth after swallowing the sample	0–10
Residual nerves	Degree of perception of the nerves of the tomato before the product is swallowed	0–10

6.2.4. Agronomic and fruit morphology evaluation

In order to determine the agronomic performance of the genotypes that were studied, all of the fruits from five plants per replicate (ten plants per treatment) were collected and individually weighed. Yield (Y), number of fruits (FN), and mean fruit weight (MFW) were

evaluated, and fruit uniformity (assessed by the coefficient of variation of individual fruit weight), precocity (weeks elapsed between transplanting and harvesting of 20% of the fruit), and temporality (T_{85}; weeks needed to reach an 85% yield) were calculated.

For fruit characterisation and metabolite analysis, three replicates per row (six per treatment) were established, each replicate consisting of ten fruits from two plants. Equatorial and longitudinal diameters were measured using a Mitutoyo 500-196-30 Digimatic calliper (Kanagawa, Japan), and fruit shape index was calculated as the ratio between both diameters. Fruit firmness was measured using a Bertuzzi FT011 hand penetrometer (T.R. Turoni Srl, Forlì, Italy) with an 8 mm-diameter plunger, being this expressed as kg mm^{-1}.

6.2.5. Metabolite analysis

For metabolite analysis, approximately 15 mature and uniform fruits from the same replicate were cut into small pieces and frozen at −80 °C. Subsequently, they were finely crushed using a Thermomix TM 60 and stored at −80 °C until further analysis. Each analysis was conducted in duplicate to ensure analytical reliability and reproducibility. Total soluble solids (TSS) and acidity were determined by refractive index (expressed as °Brix) using a PAL-1 digital hand-held "pocket" refractometer (Atago, USA) and by automatic titration using a Mettler-Toledo DL15 automatic titrator (Barcelona, Spain), expressed as g of citric acid per L of juice, respectively.

Individual soluble sugars, glucose (GLU), and fructose (FRU), were extracted with deionised water, purified with C18 Sep-Pak cartridges, and subsequently analysed by molecular-exclusion chromatography on an Agilent 1100 liquid chromatograph (Waldbronn, Germany). The device was equipped with an RI detector and a CHO-682 LEAD 300 × 7.8 mm ID CARBOSep column (Concise separations, San Jose, CA, USA), utilising deionised water as the mobile phase at a flow rate of 0.4 mL min^{-1} (Flores et al., 2016). Calibration curves were constructed using standard solutions of glucose and fructose (Sigma-Aldrich, Steinheim, Germany) within the range of 0 to 750 mg L^{-1}. Results are expressed as mg g^{-1} of fresh weight (FW).

Vitamin C (ascorbic and dehydroascorbic acids) was extracted with ethylenediaminetetraacetic acid (EDTA), 0.05% (w/v), and dithiothreitol (Sigma, Steinheim, Germany), and was analysed by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS-MS) according to the methodology developed by Fenoll et al. (2011). Calibration curves were constructed using standard solutions of ascorbic acid (Sigma-Aldrich, Steinheim, Germany) within the range of 0 to 100 mg L^{-1}. Vitamin C (VC) was quantified as µg g^{-1} FW.

Carotenoids and phenolic compounds were extracted using an acetone-hexane solvent (4:6, v/v). Briefly, following a five-minute shaking period, phase separation was achieved through the application of a 10 min, 12,000 rpm centrifugation process. The analysis of carotenoids was conducted in the solvent phase, following the methodology proposed by Nagata and Yamashita (1992). The absorbance of the acetone-hexane layer was measured at 450 nm using a quartz cuvette and a UV-Vis spectrophotometer. A *β*-carotene calibration curve, constructed using standard solutions in the range of 0 to 10 mg L^{-1}, was employed to express the total carotenoid content in µg g^{-1} FW. The analysis of phenolic compounds was conducted in the aqueous phase using the Folin and Ciocalteau (1927) method, with gallic acid for calibration (from 0 to 300 mg L^{-1}). Absorbance at 765 nm was measured, and results were expressed as µg gallic acid equivalents (GAE) g^{-1} FW.

6.2.6 Statistical analysis

The results of all the analysis were expressed as mean value ± standard error. Data were statistically analysed using IBM SPSS Statistics 25. Data were first checked for normality using the Shapiro-Wilk test and for homogeneity of variances using the Levene median test. A two-way analysis of variance (ANOVA) was then conducted, followed by Duncan's range test for multiple comparisons to establish significant differences between means with a confidence level of 95%. Principal component analysis (PCA) was performed on the normalised compositional data using pairwise Euclidean distances between accession means. Eigenvalues and percent variance of each principal component were calculated, as well as the correlation coefficients between compositional traits and principal components.

6.3. Results and discussion

6.3.1. General description of the cultivars

In the control treatment (100F+100I), the traditional varieties Muchamiel (MC) and Flor de Baladre (FB) exhibited yield values that were comparable to those found in Mongo (MO) (Table 4). These findings are consistent with those reported by Meza et al. (2020), which demonstrated that, under optimal conditions, certain traditional varieties can match or even surpass commercial varieties in terms of yield. In contrast, despite producing a greater number of fruits per plant, the Pera Pinatar (PP) cultivar exhibited lower yields compared to those found in the commercial variety. This lower yield can be attributed to the smaller fruit size that is characteristic of the PP cultivar. Notwithstanding the high fruit weight of the Negro de Nerpio (NN) variety, its overall yield was low due to the reduced number of fruits per plant. Nevertheless, this variety is highly valued for its fruit quality (Sánchez et al., 2023), which led to its selection as a parent for the development of the H1 and H2 hybrids in the study that was performed by

Sánchez et al. (2024). The yield of both hybrids was significantly higher than that of their common parent, NN, and comparable to that of the commercial variety. These results are consistent with the vigour of the hybrids that had been observed in the previous study, which supported their selection as promising varieties.

The parameters of precocity and temporality (T_{85}) provided valuable insights into key factors affecting the profitability of the crop. In order to accurately assess these temporal developmental traits across varieties with differing growth rates, all of the varieties were maintained until the conclusion of the trial, including those that reached maturity earlier. NN was the earliest maturing cultivar, reaching a 20% yield (precocity) in 4.1 weeks, and showed the lowest T_{85} value (10.8 weeks), which pinpoints a short growing cycle (Table 4). FB, MC, H1, and H2 exhibited a precocity that was similar to that found in the commercial variety MO, with T_{85} values that were comparable, except for FB, which had the highest T_{85} among the studied varieties. The prolonged development cycle of FB presents several disadvantages, including increased resource requirements (fertiliser, water, and labour), a heightened risk of diseases and pests, and a greater exposure to environmental stressors, all of which can negatively impact crop profitability. PP took 6.3 weeks to reach a 20% production, which made it the last cultivar to do so, whereas its T_{85} was similar to that found in the commercial variety. Regarding fruit uniformity, all of the varieties showed similar CV values, except for PP, which had the lowest one, making it the most homogeneous variety. The uniformity of this fruit, which is often variable in traditional varieties, makes PP particularly noteworthy, since it improves marketability and simplifies harvesting, packaging, and transport.

Table 4. Yield (kg plant^{-1}), mean fruit weight (MFW; g), number of fruits per plant (FN), precocity, temporality (T_{85}), fruit uniformity (CV), total soluble solids (TSS, °Brix), and acidity (g L^{-1}) of the selected varieties grown under control treatment (100F+100I).

Cultivar	Yield	MFW	FN	Precocity	T_{85}	CV	TSS	Acidity
MO	7.4^{bc}	193^{b}	40^{d}	5.1^{bc}	11.6^{b}	0.52^{b}	5.1^{a}	3.6^{a}
FB	7.2^{b}	400^{d}	19^{a}	5.1^{bc}	12.8^{c}	0.52^{b}	5.9^{bc}	4.3^{b}
PP	4.9^{a}	105^{a}	48^{e}	6.3^{d}	12.4^{bc}	0.35^{a}	6.6^{d}	3.7^{a}
MC	7.5^{bc}	234^{b}	37^{cd}	5.6^{c}	12.1^{bc}	0.52^{b}	6.2^{cd}	5.2^{c}
NN	3.9^{a}	227^{b}	18^{a}	4.1^{a}	10.8^{a}	0.61^{b}	5.8^{bc}	4.6^{b}
H1	8.1^{bc}	370^{d}	22^{ab}	5.1^{bc}	11.6^{b}	0.58^{b}	4.6^{a}	3.6^{a}
H2	9.0^{c}	312^{c}	29^{bc}	4.9^{b}	11.9^{b}	0.55^{b}	5.6^{b}	4.6^{b}
	***	***	***	***	***	***	***	***

*** Significant differences between means at a 0.1% level of probability. Different letters in the same column indicate the presence of significant differences between means according to Duncan's test at the 5% level. Data are means ± SE (n=10).

Regarding fruit composition, all of the varieties showed higher total soluble solids (TSS) and lower acidity compared to the commercial variety, except for the H1 hybrid, which exhibited values that were similar to those observed in Mongo for both parameters (Table 4). The evaluation

of commercial tomatoes is largely based on these parameters, and consumers tend to prefer a balance between sweet and sour flavour profiles (Casals et al., 2011). However, overall flavour is influenced by a combination of factors, which does not only include sugars and acids, but also volatile compounds and fruit texture (Felföldi et al., 2021). To evaluate sensory differences, a principal component analysis (PCA) was conducted on the quantitative sensory data. Detailed results are provided in Supplementary Table 1. According to the principle of an eigenvalue greater than 1, the first two components (PC1 and PC2) were used for further comprehensive evaluation. PC1 explained 41.3% of the total variation, and PC2 explained 25.6% of it, with a cumulative contribution rate of 66.9% (Figure 2). While the H2 hybrid showed intermediate characteristics, the other varieties clustered separately. MO and PP showed negative correlations with PC1 and PC2, indicating a high fruit homogeneity. FB and MC were positively correlated with PC2, signalling an acidic, fruity flavour and ribbed appearance. NN showed a strong positive correlation with PC1, indicating a sweet taste, good aftertaste, juicy texture, green-shouldered appearance, and high skin presence. H1 showed a positive correlation with both PC1 and PC2, and was characterized by acidity, sweetness, aftertaste, fruitiness, and a strong tomato ID.

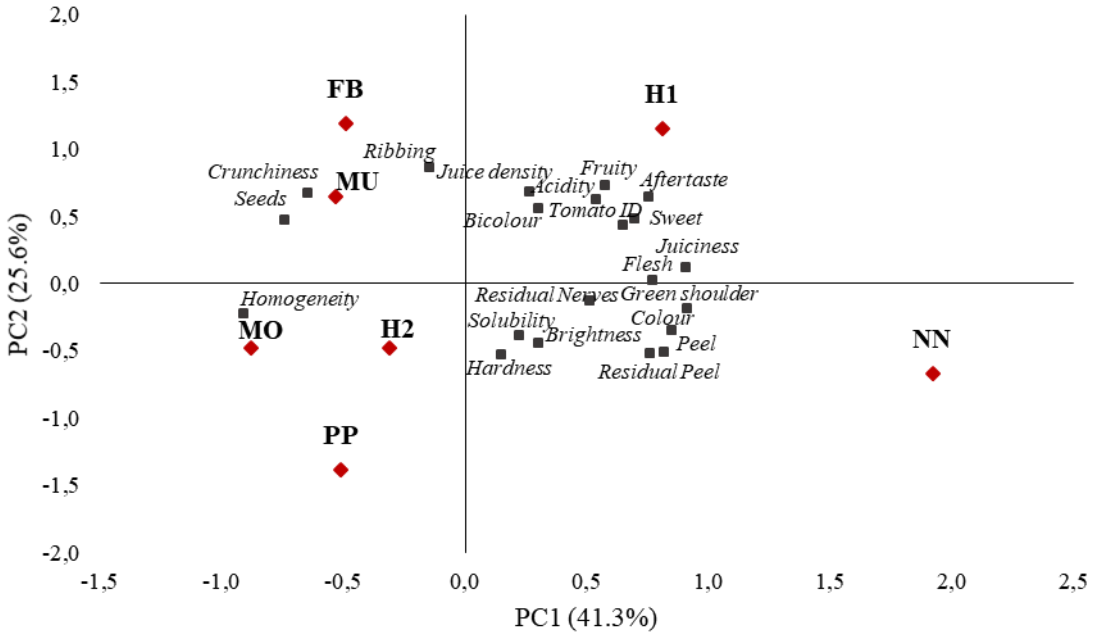

Figure 2. Bi-plot of the principal component analysis (PCA) of the sensory traits evaluated in the studied cultivars (Mongo [MO], Flor de Baladre [FB], Pera Pinatar [PP], Muchamiel [MC], and Negro de Nerpio [NN]) and the experimental hybrids (H1 and H2).

Several studies comparing the sensory properties of traditional and modern tomato varieties have shown that these varieties, especially the local ones corresponding to southern Italy and eastern Spain, tend to have a higher sensory quality and a higher concentration of aromatic volatiles (Casals et al., 2021; Alonso et al., 2009). However, traditional varieties do not consistently exhibit significantly superior sensory characteristics (Sinesio et al., 2021). To ensure

an accurate comparison, it is essential to select an appropriate commercial variety that can be used as a reference. MO was developed through modern breeding to improve consumer-driven quality traits; thus, it serves as an effective reference for comparison with traditional varieties that have previously exhibited a high quality (Sánchez et al., 2023, 2024; Flores et al., 2016, 2017). With regards to this reference, all of the traditional varieties (FB, MC, NN, and PP) that were selected for their outstanding characteristics in a previous study demonstrated superior organoleptic properties in both composition and sensory analyses. In addition, PP stood out for its fruit homogeneity, particularly appreciated in a commercial context (Casals et al., 2011). In spite of having lower TSS and acidity values than the other traditional varieties that were studied, H1 achieved exceptional results in the sensory analysis, likely due to other key factors influencing its sensory profile (Tieman et al., 2017).

6.3.2. Effect of the treatments

6.3.2.1. Agronomic response

After the evaluation of the impact of reduced fertilisation and irrigation on agronomic performance (measured as yield, number of fruits per plant, and mean fruit weight), PP was the only variety that did not show a significant change in response to these reductions (Table 5; Figures 3, 4, and 5). Additionally, the total yields of MO and MC were unaffected by a reduced fertilisation or irrigation. However, MO exhibited an increase in the number of fruits and a decrease in the mean fruit weight under reduced irrigation. MC showed a similar response to that found in MO, but only when fertilisation was maintained at 100% (100F+75I). In FB, a 37% yield decrease occurred with reduced irrigation despite maintaining fertilisation at 100% (100F+75I), primarily due to a smaller fruit size. Conversely, NN experienced a 35% yield reduction with decreased fertilisation and maintained irrigation (50F+100I), resulting from a lower number of fruits compared to the control. For the developed hybrids, H1 and H2, a reduced fertilisation resulted in a yield decrease, with a significant reduction in the number of fruits being observed in H2. Additionally, H2 experienced yield losses with decreased irrigation. H1 exhibited a yield reduction of approximately 20% under both fertilisation reduction treatments (50F+100I and 50F+75I), whereas H2 showed a more pronounced 40% decrease when both inputs were reduced (50F+75I), compared to a 20% reduction when only fertilisation was reduced (50F+100I) and a 15% reduction if only irrigation was reduced (100F+75I).

Table 5. Effect of fertilisation (100% vs 50%) and irrigation (100% vs 75%) on yield (Y; kg plant^{-1}), mean fruit weight (MFW; g), and number of fruits (FN) per plant in the studied varieties (Mongo [MO], Flor de Baladre [FB], Pera Pinatar [PP], Muchamiel [MC], and Negro de Nerpio [NN]) and the experimental hybrids (H1 and H2).

	MO			**FB**			**PP**			**MC**			**NN**			**H1**			**H2**		
	F	I	F×I	F	I	F×I	F	I	F×I	F	I	F×I	F	I	F×I	F	I	F×I	F	I	F×I
Y	ns	ns	ns	ns	*	**	ns	ns	ns	ns	ns	ns	*	ns	*	**	ns	ns	**	*	ns
MFW	ns	*	ns	*	*	**	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
FN	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns	*	ns	ns

*, ** Significant differences between means at a 5 or 1% level of probability, respectively; ns, non-significant at p=5%.

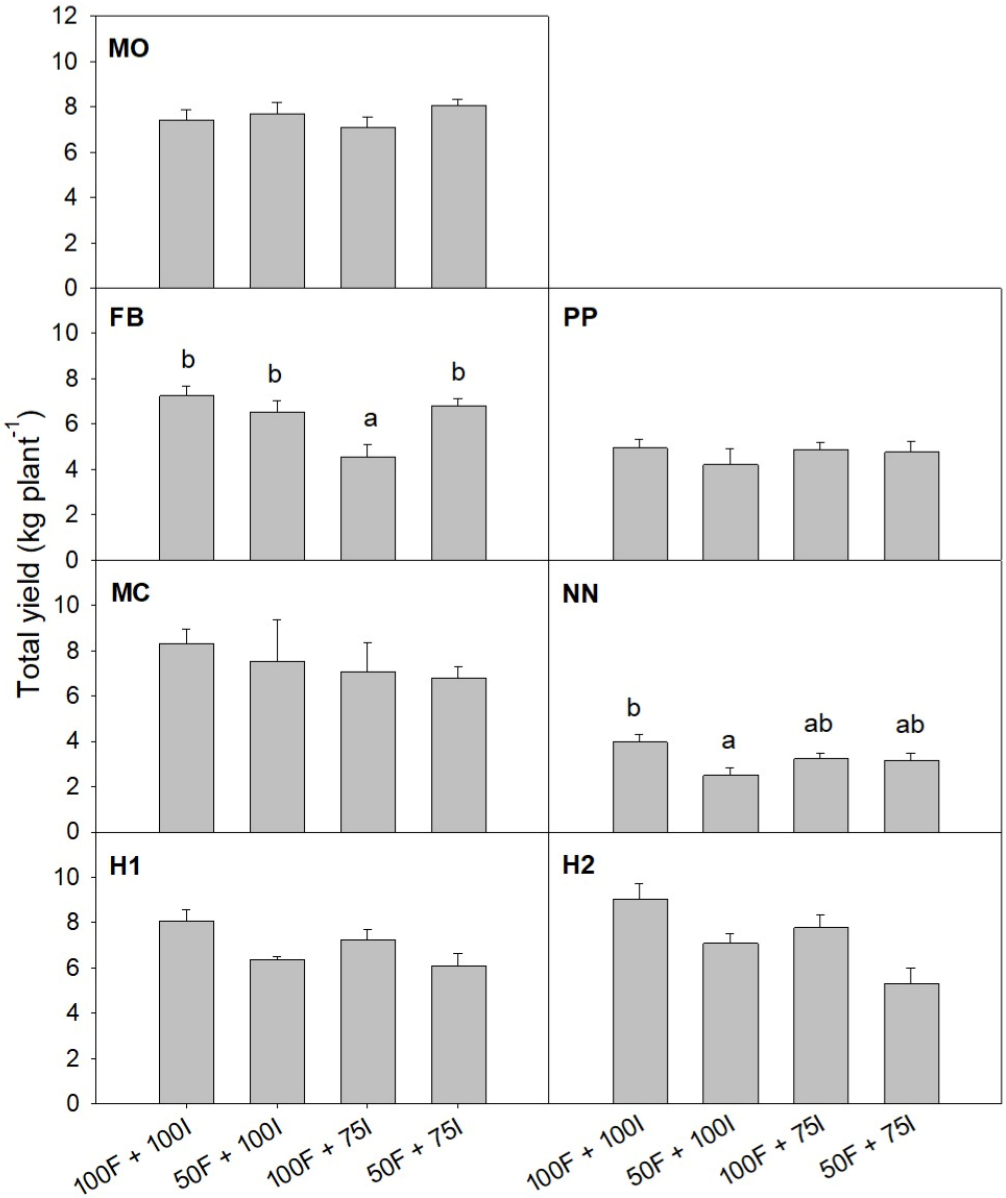

Figure 3. Effect of the fertilisation (100% vs 50%) and irrigation (100% vs 75%) doses on yield (kg plant^{-1}) of the studied varieties (Mongo [MO], Flor de Baladre [FB], Pera Pinatar [PP], Muchamiel [MC], and Negro de Nerpio [NN]) and the experimental hybrids (H1 and H2). Different letters within each variety indicate significant differences between treatments based on the Fertilisation × Irrigation interaction, according to Duncan's test at the 5% level. Data are means ± SE (n=10).

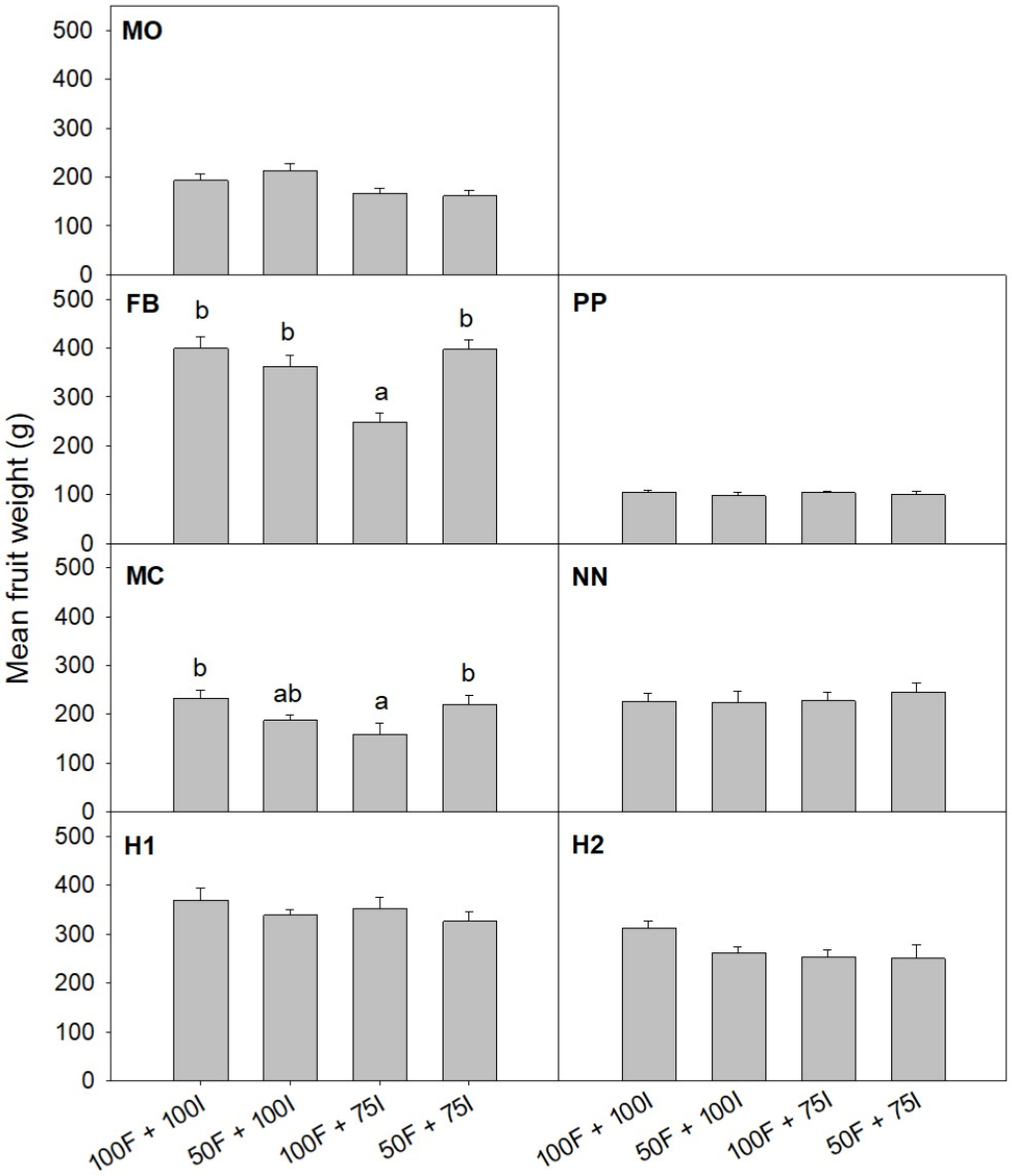

Figure 4. Effect of the fertilisation (100% vs 50%) and irrigation (100% vs 75%) doses on mean fruit weight (g) of the studied varieties (Mongo [MO], Flor de Baladre [FB], Pera Pinatar [PP], Muchamiel [MC], and Negro de Nerpio [NN]) and the experimental hybrids (H1 and H2). Different letters within each variety indicate significant differences between treatments based on the Fertilisation × Irrigation interaction, according to Duncan's test at the 5% level. Data are means ± SE (n=10).

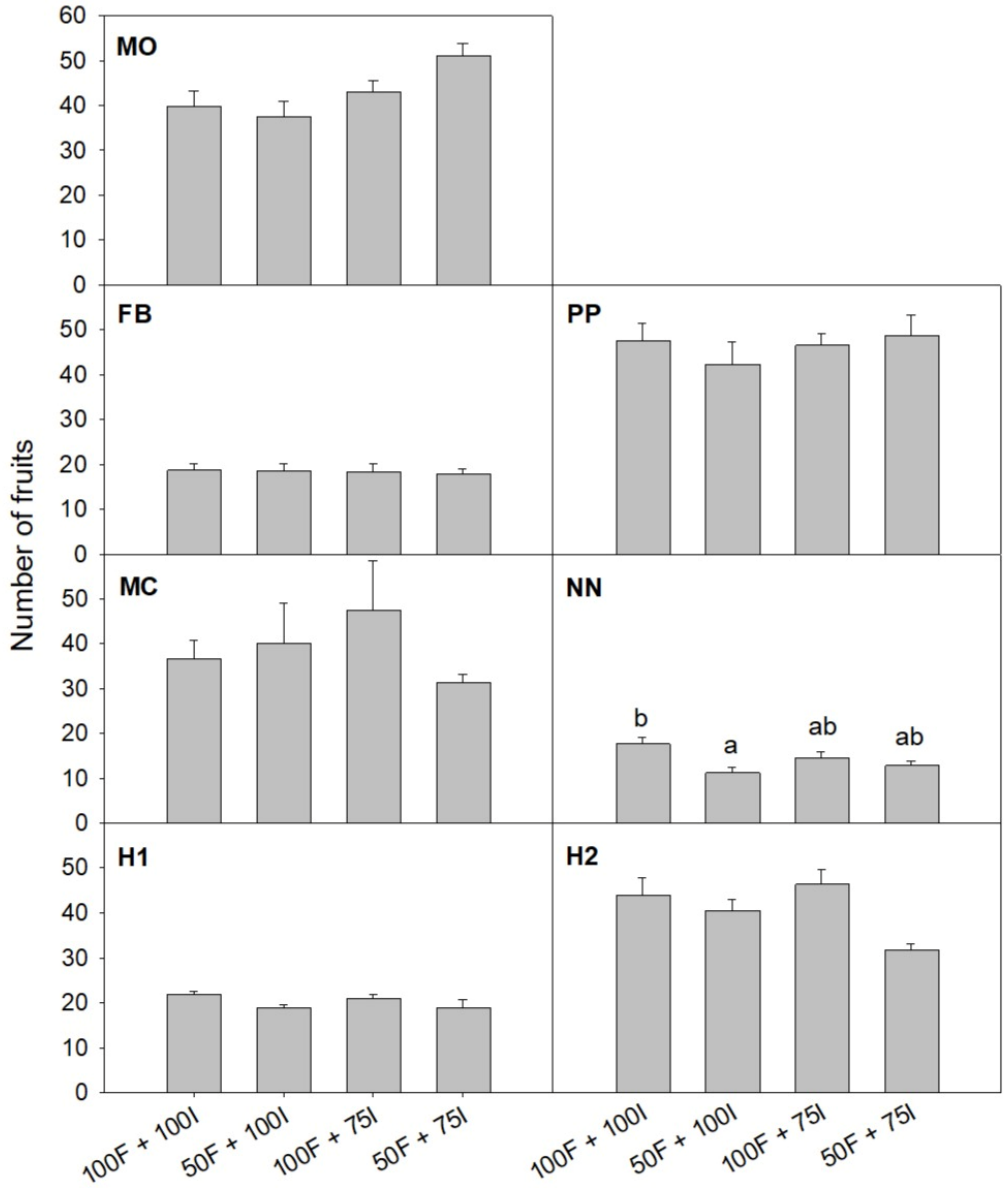

Figure 5. Effect of the fertilisation (100% vs 50%) and irrigation (100% vs 75%) doses on the number of fruits per plant in the studied varieties (Mongo [MO], Flor de Baladre [FB], Pera Pinatar [PP], Muchamiel [MC], and Negro de Nerpio [NN]) and the experimental hybrids (H1 and H2). Different letters within each variety indicate significant differences between treatments based on the Fertilisation × Irrigation interaction, according to Duncan's test at the 5% level. Data are means ± SE (n=10).

Previous studies suggest that low-input management has a variable effect on tomato agronomic performance that depends on the cultivar, with some cultivars showing better yield and quality under low-input conditions, and others performing better under conventional or high input systems (Chea et al., 2021; Meza et al., 2020). Among traditional varieties, PP performed consistently across all of the treatments, demonstrating adaptability to low-input conditions. This is substantiated by Carbonell et al. (2020), who observed no significant yield differences in Pera cultivars between low-input and conventional systems, suggesting the presence of efficient nutrient and water assimilation mechanisms, along with a greater stability compared to other

traditional varieties, such as Muchamiel. Historically, Pera cultivars have been cultivated in marginal and subsistence farming, having been selected and developed within low-input agricultural systems over time. In contrast, varieties such as Flor de Baladre, Negro de Nerpio, and Muchamiel, widely grown in south-eastern Spain, are likely more aligned with high-input systems and modern agricultural practices (Carbonell et al., 2020). The observed impact of a reduced nutrient and irrigation supply on yield for other traditional varieties could be explained by two key factors: the sensitivity of each variety to an elevated salinity in the irrigation solution and the specific nutritional requirements of each of the cultivars. In treatment 100F+75I, reducing the irrigation water volume while maintaining the fertiliser dose led to a more saline irrigation solution, with a 25% increase in concentration with regard to the control. Under these conditions, FB displayed yield losses, primarily due to a decrease in fruit size, probably indicating a lower tolerance to salinity despite full fertilisation. Literature suggests that saline irrigation generally reduces tomato fruit weight rather than the number of fruits, likely due to its impact on photosynthesis and the allocation of photoassimilates to the fruits (Meza et al., 2020; Flores et al., 2016). Under this treatment, MC showed an increase in the number of fruits along with a reduction in the mean fruit weight, while maintaining stable levels in overall yields. This ability to sustain yields suggests the presence of a compensatory mechanism that mitigates abiotic stress through the redistribution of photoassimilates (Nogueira et al., 2022; Osorio et al., 2014). On the other hand, a reduced fertilisation can decrease tomato yield by lowering the number of fruits rather than the fruit size, due to changes in the fruit set, but the extent of this effect depends on the requirements of the variety (Rosa-Martínez et al., 2021; Hernández et al., 2020; Elia and Conversa, 2012). The findings of the study on the effects of reduced fertilisation on the yield of NN and its derived hybrids, H1 and H2, indicated that these varieties have greater nutrient requirements than the reference and other traditional varieties. For the NN parent, yield loss due to a lower fertiliser dose was only observed in the most diluted nutrient solution treatment (50F+100I). In contrast, the yield of the hybrids decreased with any reduction in fertiliser, regardless of the irrigation rate, with H2 hybrid showing a particular sensitivity to both input reductions. This indicates that hybrid vigour is linked to a moderate to high demand for fertiliser (Carbonell et al., 2020).

6.3.2.2. Fruit morphology and composition

Concerning the morphological parameters, the shape index (SI), calculated as the ratio of longitudinal to equatorial diameters, was not influenced by the fertilisation dose in any of the varieties (Tables 6 and 7). However, a reduction in the irrigation rate decreased SI in the PP and H2 varieties. Although this decrease in SI was significant, it merely represented a reduction of approximately 6% and did not impact the visual appearance of the PP fruits, thus likely not affecting the perception of the consumer. Firmness was unaltered by lower fertilisation rates;

however, disparities were observed across certain varieties in response to a reduced irrigation. Specifically, a reduction in irrigation resulted in an increased firmness in MO, MC, and H2 varieties, as well as in the FB variety, but only under the 100F+75I treatment. The increased firmness could enhance long-distance storage and transportation, making it a valuable trait for the introduction of traditional varieties in the market (Lázaro and Ruiz-Aceituno, 2021). However, more studies are needed to rule out that this can affect the organoleptic quality and, therefore, consumer acceptance.

Regarding fruit composition, PP was the only variety in which the treatments did not affect the concentration of total soluble solids (TSS) and acidity (Tables 6 and 7). For the remaining varieties, a reduction in irrigation levels generally resulted in increased TSS and acidity, though several fertilisation × irrigation interactions were observed depending on the variety. Reducing the irrigation rate in the MO variety increased TSS regardless of the fertilisation treatment; however, acidity only increased with reduced irrigation when full fertilisation was applied (100F+75I). Similarly, in FB and MC varieties, by reducing irrigation at full fertilisation, only an increase in TSS and acidity was observed. In the experimental hybrid H2, a significant rise in TSS and acidity was observed with a reduced irrigation, regardless of the fertilisation treatment. In H1, reducing either the fertilisers (50F+100I), the water (100F+75I), or both inputs (50F+75I) resulted in an increase in TSS and acidity compared to the control (100F+100I). Finally, in the NN variety, the treatment with the most diluted nutrient solution (50F+100I) exhibited an increase in total soluble solids (TSS) and acidity compared to the other treatments. The results of agronomic performance and fruit composition for the PP variety indicate that this variety demonstrated a greater tolerance to low-input conditions compared to the other varieties. These findings align with the observations of Carbonell et al. (2020), where a reduction in NPK did not compromise the quality of PP-type cultivars, further supporting the perception that PP is better adapted to reduced fertiliser and water inputs. The increased TSS and acidity values observed in the other varieties (MO, FB, MC, NN, H1, and H2), resulting from different fertilisation × irrigation interactions, were in most cases accompanied by yield reductions (total yield, fruit weight, and/or number of fruits), suggesting a concentration effect likely driven by an increased source/sink ratio (Hernández et al., 2020, 2022). A reduction in fruit number or size decreases overall sink demand, resulting in the allocation of assimilates from source tissues to a smaller number of sinks. This redistribution increases the assimilate load per fruit, thereby elevating metabolite concentrations on a per-fruit basis (Osorio et al., 2014).

Table 6. Effect of the fertilisation (100% *vs* 50%) and irrigation (100% *vs* 75%) doses on the shape index (SI), firmness (F), total soluble solids (TSS; °Brix), and acidity (g L^{-1} of citric acid) in the studied varieties (Mongo [MO], Flor de Baladre [FB], Pera Pinatar [PP], and Muchamiel [MC]).

			SI	F	TSS	Acidity
MO	*F*	*I*				
	100	*100*	0.67	1.74	5.1	3.7[a]
	50		0.67	1.77	5.4	4.3[ab]
	100	*75*	0.64	1.87	5.9	4.8[b]
	50		0.66	2.17	6.0	4.4[b]
		F	ns	ns	ns	ns
		I	ns	**	**	**
		FxI	ns	ns	ns	*
FB	*F*	*I*				
	100	*100*	0.58	1.62[a]	5.9[a]	4.3[a]
	50		0.58	1.69[a]	5.7[a]	4.3[a]
	100	*75*	0.57	2.52[b]	7.1[b]	5.0[b]
	50		0.56	1.73[a]	6.0[a]	4.1[a]
		F	ns	**	**	*
		I	ns	***	**	ns
		FxI	ns	**	*	*
PP	*F*	*I*				
	100	*100*	1.24	2.77	6.6	3.7
	50		1.25	2.98	6.1	3.6
	100	*75*	1.16	2.82	6.3	3.6
	50		1.15	2.92	6.6	3.9
		F	ns	ns	ns	ns
		I	***	ns	ns	ns
		FxI	ns	ns	ns	ns
MC	*F*	*I*				
	100	*100*	0.61	1.84	6.2[a]	5.2[a]
	50		0.65	1.69	5.7[a]	4.5[a]
	100	*75*	0.63	2.10	7.8[b]	6.0[b]
	50		0.63	1.89	6.6[a]	4.8[a]
		F	ns	ns	ns	ns
		I	ns	*	***	*
		FxI	ns	ns	*	**

*, **, *** Significant differences between means at a 5, 1 or 0.1% level of probability, respectively; ns, non-significant at p=5%. Different letters in the same column indicate the presence of significant differences between means according to Duncan's test at the 5% level. Data are means (n=6).

Table 7. Effect of the fertilisation (100% vs 50%) and irrigation (100% vs 75%) doses on the shape index (SI), firmness (F), total soluble solids (TSS; °Brix), and acidity (g L^{-1} of citric acid) in the studied varieties (Negro de Nerpio [NN]) and the experimental hybrids (H1 and H2).

			SI	F	TSS	Acidity
NN	*F*	*I*				
	100	*100*	0.67	1.50	5.8[a]	4.7[b]
	50		0.65	1.51	6.5[b]	5.2[c]
	100	*75*	0.61	1.55	5.8[a]	4.4[b]
	50		0.65	1.69	5.5[a]	4.0[a]
		F	ns	ns	ns	ns
		I	ns	ns	**	***
		FxI	ns	ns	**	**
H1	*F*	*I*				
	100	*100*	0.59	1.67	4.6[a]	3.7[a]
	50		0.59	1.77	5.7[c]	4.9[c]
	100	*75*	0.60	1.67	5.9[c]	5.0[c]
	50		0.61	1.69	5.0[b]	4.4[b]
		F	ns	ns	ns	*
		I	ns	ns	*	*
		FxI	ns	ns	***	***
H2	*F*	*I*				
	100	*100*	0.66	1.58	5.6	4.6
	50		0.64	1.74	5.8	4.8
	100	*75*	0.61	1.79	6.2	5.1
	50		0.61	1.93	6.3	5.7
		F	ns	ns	ns	ns
		I	*	*	*	*
		FxI	ns	ns	ns	ns

*, **, *** Significant differences between means at a 5, 1 or 0.1% level of probability, respectively; ns, non-significant at p=5%. Different letters in the same column indicate the presence of significant differences between means according to Duncan's test at the 5% level. Data are means (n=6).

6.3.2.3. Fruit metabolites

Understanding the impact of fertiliser and water limitations on plant yield is critical for evaluating the feasibility of low-input cultivation of traditional varieties of interest. Additionally, assessing the effects of low-input cultivation on fruit composition is necessary to determine whether such restrictions influence organoleptic and functional quality. In order to determine the viability of these varieties in low-input systems, a comprehensive analysis was performed, evaluating three low-input conditions against the control treatment: reduced fertilisation with full irrigation (50F+100I), reduced irrigation with full fertilisation (100F+75I), and simultaneous reduction of both inputs (50F+75I). The analysis focused on agronomic traits and bioactive compounds and mean values for each treatment were categorized into three response categories

(neutral, positive, or negative) relative to the control, based on statistical significance determined by ANOVA tests (Table 10).

Table 8. Effect of the fertilisation (100% vs 50%) and irrigation (100% vs 75%) doses on the concentration of glucose (GLU; mg g^{-1}), fructose (FRU; mg g^{-1}), vitamin C (VC; µg g^{-1}), total phenolic compounds (TPC; µg g^{-1}), and total carotenoids (TC; µg g^{-1}) in the studied varieties (Mongo [MO], Flor de Baladre [FB], Pera Pinatar [PP], and Muchamiel [MC]).

			GLU	FRU	VC	TPC	TC
MO	*F*	*I*					
	100	*100*	13.8^{a}	17.0^{a}	138	104^{a}	62
	50		16.7^{b}	19.8^{b}	141	115^{b}	60
	100	*75*	18.1^{b}	20.2^{b}	132	115^{b}	59
	50		16.8^{b}	19.4^{b}	131	99^{a}	55
		F	ns	ns	ns	ns	ns
		I	**	*	*	ns	ns
		FxI	**	**	ns	***	ns
FB	*F*	*I*					
	100	*100*	16.6^{a}	19.8^{a}	135^{a}	104^{a}	45
	50		17.2^{a}	20.3^{a}	132^{a}	108^{a}	43
	100	*75*	20.6^{b}	23.4^{b}	156^{b}	122^{b}	53
	50		16.8^{a}	20.5^{a}	134^{a}	100^{a}	50
		F	ns	ns	*	ns	ns
		I	*	*	*	ns	**
		FxI	*	*	*	**	ns
PP	*F*	*I*					
	100	*100*	17.2	19.5	136	126	74
	50		18.1	20.2	144	130	72
	100	*75*	17.2	18.3	126	121	63
	50		20.0	21.2	145	118	72
		F	*	*	*	ns	ns
		I	ns	ns	ns	ns	ns
		FxI	ns	ns	ns	ns	ns
MC	*F*	*I*					
	100	*100*	16.2^{a}	19.2^{a}	133	117	56^{a}
	50		16.1^{a}	19.0^{a}	126	128	55^{a}
	100	*75*	18.7^{b}	21.2^{b}	145	127	63^{b}
	50		15.7^{a}	18.5^{a}	130	118	51^{a}
		F	*	*	ns	ns	ns
		I	ns	ns	ns	ns	ns
		FxI	*	*	ns	ns	**

*, **, *** Significant differences between means at a 5, 1 or 0.1% level of probability, respectively; ns, non-significant at p=5%. Different letters in the same column indicate the presence of significant differences between means according to Duncan's test at the 5% level. Data are means (n=6).

Table 9. Effect of the fertilisation (100% *vs* 50%) and irrigation (100% *vs* 75%) doses on the concentration of glucose (GLU; mg g^{-1}), fructose (FRU; mg g^{-1}), vitamin C (VC; µg g^{-1}), total phenolic compounds (TPC; µg g^{-1}), and total carotenoids (TC; µg g^{-1}) in the studied varieties (Negro de Nerpio [NN]) and the experimental hybrids (H1 and H2).

			GLU	FRU	VC	TPC	TC
NN	*F*	*I*					
	100	*100*	14.2	17.2	109	118	93^{ab}
	50		14.6	17.3	102	107	98^{b}
	100	*75*	14.1	17.1	119	110	103^{b}
	50		15.4	17.9	124	118	83^{a}
		F	ns	ns	ns	ns	ns
		I	ns	ns	***	ns	ns
		FxI	ns	ns	ns	ns	*
H1	*F*	*I*					
	100	*100*	14.5^{a}	17.7^{a}	122^{a}	107^{a}	64
	50		17.4^{b}	19.7^{b}	137^{b}	126^{b}	61
	100	*75*	15.6^{a}	18.4^{ab}	125^{ab}	119^{b}	63
	50		15.3^{a}	18.0^{a}	118^{a}	99^{a}	59
		F	*	ns	ns	ns	ns
		I	ns	ns	*	**	ns
		FxI	*	*	*	*	ns
H2	*F*	*I*					
	100	*100*	14.8^{a}	17.2^{a}	119^{a}	105	54
	50		17.0^{b}	19.3^{b}	133^{b}	114	59
	100	*75*	18.3^{b}	19.7^{b}	132^{b}	116	52
	50		15.7^{a}	16.8^{a}	116^{a}	109	58
		F	ns	ns	ns	ns	ns
		I	ns	ns	ns	ns	ns
		FxI	**	**	**	ns	ns

*, **, *** Significant differences between means at a 5, 1 or 0.1% level of probability, respectively; ns, non-significant at p=5. Different letters in the same column indicate the presence of significant differences between means according to Duncan's test at the 5% level. Data are means (n=6).

In the MO, PP, FB, and MC varieties, fertilisation without reducing the irrigation rate (50F+100I) did not affect the agronomic performance parameters, nor did it lessen the fruit concentration of the analysed metabolites related to flavour (glucose and fructose) and functional quality (vitamin C, phenolic compounds, and carotenoids) (Tables 8, 9, and 10). Moreover, under these limited fertilisation conditions, MO and PP fruits exhibited higher glucose and fructose levels compared to the control. Additionally, MO showed an increase in phenolic compound content, whereas PP displayed elevated vitamin C concentrations. Conversely, despite the observed increase in certain metabolites of interest under this fertilisation restriction, the NN variety and its hybrid progeny (H1 and H2) were not suitable candidates for these low-fertilisation

conditions, as it had a detrimental effect on their agronomic performance. The varieties that maintained a level in all of the productivity parameters that was comparable to that found in the control under reduced irrigation, without reducing fertilisation (100F+75I), were PP, NN, and H1. Additionally, this irrigation limitation led to an increased concentration of vitamin C in NN and higher levels of fructose and phenolic compounds in H1 compared to the control. MO, FB, MC, and H2 varieties exhibited an increase in the concentration of one or more metabolites of interest under this treatment; however, this increase was accompanied by a reduction in one of the yield parameters, either total yield and/or mean fruit weight. For PP, the promising results (which demonstrate its ability to maintain or even increase certain quality-related metabolites under reduced fertilisation or irrigation without compromising yield) were also made evident when both fertilisation and irrigation were reduced (50F+75I), resulting in an increase in glucose, fructose, and vitamin C concentration under this combined treatment (Table 8). Certain varieties that responded well to the reduction in the fertiliser dose, specifically FB and MC, as well as to a reduced irrigation, such as NN, also demonstrated a resilient response to the combined reduction of both inputs in terms of agronomic and quality considerations. Furthermore, under this combined low-input treatment, an increase in the phenolic compound concentration was observed for FB, whereas NN showed an increase in vitamin C concentration.

Irrigation and fertilisation are essential agronomic practices for optimizing tomato yield and fruit quality. In general, yield and fruit quality are more sensitive to changes in irrigation management than to modifications in fertilisation practices (Wang and Xing, 2017). Although many studies have shown that limited irrigation can be an effective strategy to improve tomato fruit composition while reducing water consumption, its impact on yield and quality is complex and varies significantly depending on the tomato variety (Lu et al., 2021). Numerous studies have also explored the effects of mineral nutrient supply on tomato yield and fruit quality, but the results have been highly variable, largely owing to differences in the experimental factors, cultivars, and environmental conditions (Cheng et al., 2021; Hernández et al., 2020).

Table 10. Comprehensive analysis of the response of the studied varieties (Mongo [MO], Flor de Baladre [FB], Pera Pinatar [PP], Muchamiel [MC], and Negro de Nerpio [NN]) and the experimental hybrids (H1 and H2) under 50F+100I, 100F+75I, and 50F+75I against the control treatment 100F+100I.

		Agronomic			Metabolites				
		Y	MFW	FN	FRU	GLU	VC	TPC	TC
50F+100I	MO				+	+		+	
	FB								
	PP				+	+	+		
	MC								
	NN	−		−					
	H1	−			+	+	+	+	
	H2	−		−	+	+	+		
100F+75I	MO		−	+	+	+	−	+	
	FB	−	−		+	+	+	+	+
	PP								
	MC		−		+	+			+
	NN						+		
	H1				+			+	
	H2	−			+	+	+		
50F+75I	MO		−	+	+	+	−		
	FB								+
	PP				+	+	+		
	MC								
	NN						+		
	H1	−							
	H2	−		−					

Mean values for each treatment and trait were classified into three response categories: neutral (yellow), positive (+ and green), and negative (− and red). Responses were determined according to ANOVA analyses and statistical significance of Tables 5, 8, and 9.

The mechanisms underlying the response to reduced irrigation and fertilisation of primary and secondary metabolites in fruits remain poorly understood. Some of the key responses that were observed in this study, consistent with previous research, include the following: (i) the ability to attract photoassimilates to the fruit is defined as sink strength, which influences metabolite distribution to fruit throughout the plant. A source-sink imbalance, often resulting from yield loss due to reduced fertilisation and irrigation, can lead to higher metabolite concentrations in the fruit (Flores et al., 2016; Osorio et al., 2014; Bénard et al., 2009); (ii) osmotic adjustment is an adaptive mechanism to maintain cell turgor under water stress. To enhance osmotic tolerance to water loss, plants increase the concentration of different metabolites in the cytoplasm, especially soluble sugars, such as fructose and glucose (Hou et al., 2020; Ripoll et al., 2014); (iii) reduced fertilisation and/or irrigation may decrease vegetative growth, resulting in an increased light reception on fruit surface. Enhanced light exposure facilitates the accumulation of primary and secondary metabolites by increasing fruit photosynthesis and fruit sugar content, while also upregulating enzymes associated with secondary metabolism that are activated by light (Hernández et al., 2020; Hou et al., 2020; Gautier et al., 2009); (iv) oxidative stress occurs as a secondary response to primary stress factors, such as low fertilisation or irrigation. Reduced

photosynthetic activity in leaves leads to an excessive production of reactive oxygen species (ROS), which damage cellular components and induce oxidative stress. To counteract this, plants activate biochemical strategies, including the enhanced synthesis of antioxidants such as carotenoids, vitamin C, and phenolic compounds (Egea et al., 2022; Ripoll et al., 2014). Vitamin C plays a central role in ROS detoxification, acting as an electron donor for ascorbate peroxidase (APX) in the reduction of H_2O_2 to H_2O, and also by directly regenerating tocopherol from its radical form (Mellidou et al., 2021). Additionally, the biosynthetic pathway of carotenoids and phenolic compounds appears to be under ROS/redox control, and their accumulation is frequently induced under abiotic stress conditions as part of the plant's adaptive response (Ripoll et al., 2014). Thus, the observed response of traditional germplasm to limited irrigation and fertilisation could be explained by a combination of various physiological and biochemical mechanisms. These mechanisms can differ significantly between varieties, influencing their growth and yield, as well as the synthesis and accumulation of metabolites that are linked to fruit quality, to different extents. The variable response makes these traditional varieties a valuable resource in the search for a germplasm that is suitable for low-irrigation and/or low-fertilisation conditions, or for enhancing organoleptic and nutritional quality without compromising the agronomic performance. The findings provide a framework for future research, with a particular focus on enhancing stress resilience through approaches such as grafting and plant-microbial interactions, which have been shown to improve plant responses to abiotic stress (Yuan et al., 2024; Gisbert-Mullor et al., 2020).

In summary, from the evaluation of the response to low-input cultivation of the studied varieties, which were previously selected for having a productivity that is comparable to that found in the commercial varieties and their outstanding quality, several conclusions can be inferred. PP variety exhibited the most favourable cost–benefit balance under reduced input conditions, as maintained yield while significantly increasing the concentration of primary (glucose and fructose) and secondary (vitamin C) metabolites. In contrast, hybrid H2 showed the least favourable response, as limitations in fertilisation, irrigation, or both led to yield reductions without consistently increasing the concentration of metabolites in the fruit. The yield and quality of FB and MC fruits were also maintained under low fertilisation, either alone or in combination with low irrigation. Notably, FB showed an increase in the phenolic compound concentration under the latter treatment, highlighting its potential for enhanced nutritional value in response to low-input cultivation. Finally, the H1 hybrid demonstrated an improved productivity compared to its parental variety NN and exhibited a strong tolerance to low irrigation conditions. It maintained a yield that was similar to that observed under control conditions, and showed an enhanced quality, particularly in terms of increased fructose concentration and phenolic compound content. These findings have significant implications for their integration into current

agricultural systems: farmers could adopt these varieties to reduce input dependency, lower production costs, and increase crop value because of their outstanding quality. Alternatively, future breeding programmes can build on these varieties to address new objectives, such as enhancing tolerance to abiotic and biotic stresses.

6.4. References

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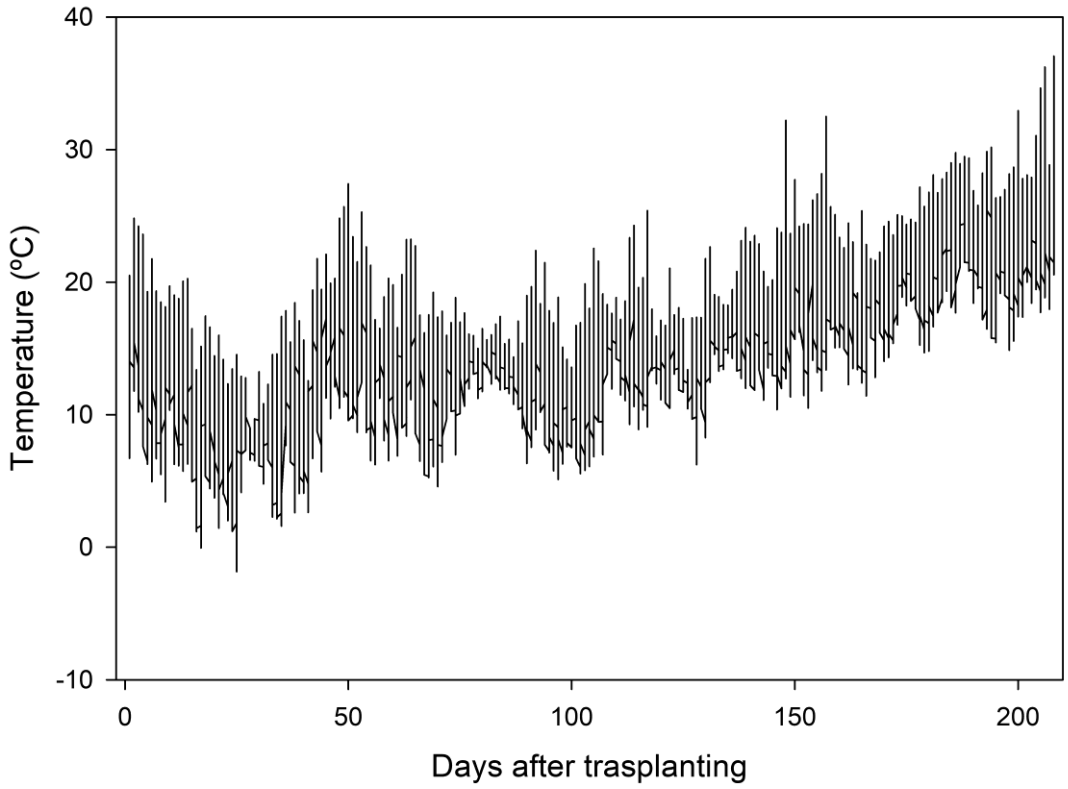
6.5. Supplementary material



Supplementary Figure 1. Hourly average temperature (°C) recorded by the TP42 weather station of SIAM (Murcia Agricultural Information System) throughout the growing season (December to July; 0 to 209 days after transplanting). Each point represents the average temperature at each hour of the day throughout the growing season. Data were collected using a VAISALA 50Y thermohygrometer (serial number W0940091) integrated in the station.

Supplementary Table 1. Principal component analysis in tomato fruit for different sensory traits.

	PC1	**PC2**	PC3	PC4	PC5
Eigenvalue	**8.683**	**5.380**	2.928	1.757	1.381
Variability (%)	**41.346**	**25.621**	13.945	8.368	6.574
Cumulative (%)	**41.346**	**66.967**	80.912	89.280	95.854
Factor loading					
Colour	**0.846**	**-0.342**	0.041	-0.180	0.314
Bicolour	**0.298**	**0.562**	0.671	-0.293	0.241
Homogeneity	**-0.909**	**-0.223**	0.257	-0.191	0.041
Brightness	**0.300**	**-0.441**	-0.048	0.395	-0.659
Ribbing	**-0.148**	**0.871**	0.169	0.057	-0.357
Green shoulder	**0.912**	**-0.182**	0.011	-0.196	0.172
Sweet	**0.695**	**0.481**	-0.428	0.044	0.224
Acidity	**0.536**	**0.632**	0.472	0.192	-0.232
Tomato ID	**0.649**	**0.439**	-0.416	0.072	-0.159
Fruity	**0.571**	**0.730**	-0.236	-0.271	-0.088
Aftertaste	**0.755**	**0.651**	-0.048	0.045	-0.042
Hardness	**0.149**	**-0.529**	-0.791	0.111	0.221
Crunchiness	**-0.647**	**0.674**	0.018	0.016	0.203
Juiciness	**0.904**	**0.121**	-0.050	0.390	-0.110
Juice density	**0.261**	**0.684**	-0.419	0.097	0.346
Flesh	**0.768**	**0.025**	0.516	0.333	0.174
Peel	**0.817**	**-0.509**	0.134	-0.099	-0.214
Seeds	**-0.745**	**0.477**	0.356	0.272	0.076
Solubility	**0.223**	**-0.378**	0.542	0.584	0.398
Residual Peel	**0.757**	**-0.520**	0.352	-0.032	0.055
Residual Nerves	**0.510**	**-0.123**	0.332	-0.758	-0.165

7. General discussion

The Mediterranean basin, considered a secondary centre of diversity for tomato, harbours a broad variability of traditional varieties of this species (Pons et al., 2022). In recent years, efforts to recover these varieties have been driven by the need to counteract the genetic erosion affecting most crops, as well as by the growing demand for differentiated products linked to traditional flavours and the cultural heritage of each region (Lázaro, 2018; Casañas et al., 2017). These varieties constitute a valuable genetic reservoir due to their remarkable diversity in fruit shape, size, and colour, as well as in their chemical composition (Tieman et al., 2017; Figàs et al., 2015b; Cortés-Olmos et al., 2014; Carli et al., 2011). Although several studies have demonstrated that specific traditional varieties display superior organoleptic and functional attributes compared with commercial cultivars (Casals et al., 2019; D'Angelo et al., 2018; Alonso et al., 2009), others have found no significant differences (Sinesio et al., 2007, 2021).

The main objective of this doctoral thesis was to explore and enhance the value of traditional tomato varieties by identifying those with outstanding quality traits, addressing their main productive limitations, and assessing their suitability for sustainable agricultural systems. The specific objectives were to identify traditional tomato varieties with superior fruit quality (Chapter I); evaluate the potential of developing F_1 hybrids by crossing these varieties to improve agronomic performance while maintaining quality (Chapter II); assess the effect of introducing resistance genes into traditional varieties on yield, morphology, and metabolic traits (Chapter III) and evaluate the performance of selected accessions under low-input conditions to identify genotypes adapted to sustainable cultivation (Chapter IV).

Extensive tomato germplasm collections are maintained worldwide, and several of the most relevant are located in Spain. Notable examples include the COMAV-UPV Germplasm Bank in Valencia, the CITA-Aragón Horticultural Germplasm Bank in Zaragoza, and the IMIDA Germplasm Bank in Murcia. The initial collection used in this thesis belongs to the latter and comprises forty-eight accessions selected for their contrasting phenotypic traits, including fruit size, colour, and shape. Chapter I describes the morphological, organoleptic, and functional characterisation of the fruits from these forty-eight accessions, revealing a substantial diversity in size, shape, colour, and composition. This variability was evident both among varietal groups and within varieties of the same group, consistent with findings reported in other Spanish (Villena et al., 2023; Figàs et al., 2015a, 2015b) and international germplasm collections (Tripodi et al., 2023; Nankar et al., 2020; Baldina et al., 2016). Such studies have enabled the identification of varieties with commercial potential. A clear example is that of red-black tomatoes, which have gained remarkable consumer interest in recent years due to their distinctive pigmentation and, in some cases, their characteristic flavour (Villena et al., 2023; Manoharan et al., 2017). In our study, the red-black coloration was not restricted to a single varietal type but appeared in landraces

belonging to the Sierra, Kumato, and Cherry groups, which, in general, excelled for their high concentration of sugars and/or organic acids, compounds closely linked to flavour perception. In addition, some accessions exhibited high levels of bioactive and flavour-related metabolites. In particular, Cherry-type tomatoes showed higher contents of sugars, organic acids, vitamin C, and carotenoids, in line with the observations of Casals et al. (2019), who highlighted the superior quality of this varietal type compared with common fresh-market cultivars. Among the medium- to large-fruited varieties evaluated in Chapter I, Muchamiel-type accessions were distinguished by their high glutamic acid content, while Pimiento-type tomatoes showed remarkable vitamin C concentrations. The results of this first study, together with information obtained from a participatory selection process in which farmers contributed their knowledge on adaptation and market preferences, enabled the identification of fourteen accessions combining acceptable agronomic performance with outstanding fruit quality. These selected varieties represented eight of the nine varietal groups initially considered, excluding the Cherry type, as the selection focused on medium- to large-fruited materials intended for fresh consumption. These varieties constitute the experimental foundation for Chapters II, III, and IV of this doctoral dissertation.

Traditional tomato varieties may still present challenges such as lower yield and shorter shelf life compared with commercial cultivars, which are key factors in their integration into modern production systems (Pérez-Caselles et al., 2020; Asensio et al., 2019). Current commercial varieties are mainly based on F_1 hybrids, which benefit from hybrid vigour or heterosis, particularly relevant to agronomic performance. Heterosis, defined as the superiority of hybrids over their parental lines, remains a subject of genetic debate, although it has been attributed to epistatic interactions, dominance complementation, and overdominance effects (Liu et al., 2020). While heterosis has been extensively documented in terms of yield improvement across numerous crops, its impact on fruit metabolites has been less investigated, and in the case of tomato, available studies remain limited (Fortuny et al., 2021). Chapter II of this thesis describes the development and characterization of F_1 hybrids obtained through direct crosses between the fourteen traditional varieties previously selected (Figure 1). This approach enabled the potential of hybrid vigour as a breeding tool to be evaluated while simultaneously combining favourable fruit-quality alleles from different parental lines (Kumar and Yadav, 2023; Fortuny et al., 2021; Liu et al., 2021; Olivieri et al., 2021). The strategy proved particularly effective in enhancing yield, as most hybrids outperformed their parental lines and some reached production levels comparable to the commercial hybrid controls, in agreement with Olivieri et al. (2021). Regarding fruit quality, a high degree of variability was observed in chemical composition, reflecting the diversity of the parental materials used. Among the obtained hybrids, several genotypes showed high concentrations of bioactive compounds such as vitamin C, carotenoids, and phenolic compounds. In all cases, this coincided with the presence of at least one parental

line with high levels of the same metabolite. This trend is consistent with findings on primary metabolites in tomato, where hybrids involving the wild parent *S. pimpinellifolium*, known for its high sugar content, also showed increased levels of these compounds (Fortuny et al., 2021). Although the results were promising, this strategy presented certain limitations. One major constraint was the partial loss of varietal identity resulting from crosses between distinct traditional cultivars, which may hinder their direct valorisation as landraces (Sinesio et al., 2021; Lázaro, 2018). Additionally, since F_1 hybrids require an external supply of seeds, this approach diverges from traditional farming practices involving on-farm seed saving. Despite these drawbacks, the directed crosses between traditional varieties successfully exploited their genetic value, generating new allelic combinations absent in modern cultivars. Consequently, the obtained hybrids represent not only promising materials for potential commercial exploitation but also a valuable genetic base for breeding programmes aimed at improving fruit quality (Arca et al., 2023). As a result of this work, six hybrids were selected due to their balanced profiles, combining high yield with outstanding organoleptic and functional quality. This achievement is particularly noteworthy, considering the negative correlation often reported in tomato between yield and quality traits (Chassy et al., 2006).

Another major limitation of traditional tomato varieties is their susceptibility to phytopathological problems, particularly viral diseases, which reduce productivity and compromise their commercial viability (Picó et al., 2002). The introgression of virus resistance genes into traditional varieties represents a promising strategy to control these diseases in a sustainable manner. In Spain, several breeding programmes have pursued this goal, including the incorporation of the *Tm-2a* gene into traditional varieties such as Tomaca Valenciana d'El Perelló (Figàs et al., 2017) and De Penjar (Figàs et al., 2018), as well as the introduction of resistance genes targeting ToMV (*Tm-2a*), TSWV (*Sw-5*), and TYLCV (*Ty-1*) into Muchamiel and De la Pera varieties (Carbonell et al., 2018). The main challenge of these breeding efforts lies in the fact that homozygous introgression of genes such as *Tm-2a*, *Sw-5*, and especially *Ty-1*, may negatively affect yield and fruit quality due to linkage drag associated with the introgressed region (Verlaan et al., 2011; Lewis et al., 2007; Tanksley et al., 1998). In this context, two Muchamiel-type accessions (Muchamiel aplastado and Muchamiel rizado) were selected to initiate a breeding programme aimed at introducing resistance genes against ToMV, TSWV and TYLCV (Figure 1). For this purpose, four hybrids were developed by crossing the two selected accessions with two Muchamiel-type breeding lines registered by CIAGRO-UMH, both homozygous for resistance genes: UMH1139, carrying *Tm-2a* and *Sw-5*, and UMH1200, carrying *Tm-2a*, *Sw-5* and *Ty-1* (García-Martínez et al., 2011, 2015). The development of hybrids has previously been proposed as a promising strategy to minimise the effects of linkage drag (Tanksley et al., 1998). Previous studies have obtained hybrids between Muchamiel breeding lines and a high-quality traditional

American variety, successfully reducing the negative impact on yield and quality. However, the varietal identity of Muchamiel could not be preserved because one of the parents belonged to a different cultivar (Carbonell et al., 2020, 2022).

Chapter III of this thesis analyses the effect of introducing these resistance genes on the agronomic performance and metabolic profile of the Muchamiel-type hybrids developed. The results obtained over two years of evaluation confirmed that hybrids carrying the resistance genes in heterozygosis retained the characteristic Muchamiel fruit morphology and metabolic profile. The concentration of secondary metabolites was not reduced by the introduction of these genes, in line with other studies reporting that phenolic compound concentration is not affected by the presence of *Ty-1* (Cabrera et al., 2024). Regarding primary metabolites, the introduction of *Ty-1* into the hybrids studied showed a limited negative effect restricted to organic acids, while no significant differences were observed in soluble sugar content, results consistent with those reported by Carbonell et al. (2020) and Rubio et al. (2016). In hybrid derived from Muchamiel aplastado that did not carry *Ty-1*, organic acid concentrations remained equal to or even higher than those of the traditional parent. However, in the hybrid carrying *Ty-1*, these concentrations were reduced to intermediate levels between the breeding line (lower values) and the traditional variety (higher values). Beyond the advantage of retaining the Muchamiel fruit morphology and an outstanding metabolic profile, the hybrids also exhibited yield increases attributable to hybrid vigour, particularly in those carrying *Ty-1* in heterozygosis. These results reinforce the potential of this strategy for the valorisation and improvement of traditional varieties, a conclusion also supported by the findings presented in Chapter II. One of the limitations that remain is that farmers cannot produce their own seed, since these are F_1 hybrids. However, considering the high market value of these varieties (Ruiz and García-Martínez, 2009), together with reduced virus-related losses and yield improvements, this approach can be regarded as economically viable for growers, thereby contributing to the revalorisation of traditional tomato varieties.

Beyond the challenges associated with integrating landraces into modern intensive production systems, it is equally important to consider the opportunities they offer under agronomic and environmental constraints. Modern agriculture faces significant challenges stemming from climate change and the intensive production model (Ali et al., 2022). In this context, selecting varieties with low input requirements emerges as a promising strategy. Traditional varieties, which have undergone generations of natural and farmer-led selection across diverse local environments, represent an ideal source for identifying genotypes capable of maintaining yield and fruit quality under low-input conditions (Fita et al., 2015).

Chapter IV of this thesis describes the study conducted to evaluate the effect of a 50% reduction in fertiliser application and a 75% reduction in irrigation, compared with conventional regional practices, on yield and fruit quality. The experiment included four traditional varieties, Negro de Nerpio, Flor de Baladre, Pera Pinatar and Muchamiel rizado, previously characterised and assessed in earlier chapters of this thesis (Figure 1). Two hybrids (H1 and H4) were also included, selected from among the eighteen developed in Chapter II due to their high bioactive compound content and marked hybrid vigour, which make them potentially advantageous from a commercial perspective. Consistent with previous reports indicating that the response of tomato to reduced inputs depends on the cultivar (Fullana-Pericàs et al., 2019, 2022; Chea et al., 2021; Carbonell et al., 2020), our results revealed distinct responses among both traditional varieties and hybrids, likely linked to their different genetic backgrounds. Pera Pinatar excelled due to its ability to maintain fruit yield across all low-input treatments compared with the control, a resilience that aligns with its origin in marginal production areas and with previous findings (Carbonell et al., 2020). By contrast, the agronomic performance of Negro de Nerpio, Flor de Baladre, and Muchamiel rizado, was negatively affected by the reduction in either fertilisation or irrigation, suggesting that these varieties may be better adapted to high-input systems and modern agronomic practices (Carbonell et al., 2020). Fruit quality remained stable, although lower yields in some genotypes led to higher concentrations of primary and secondary metabolites, likely due to a concentration effect (Hernández et al., 2020, 2022). Similar results have been reported with other landrace collections, with the identification of accessions tolerant to water deficit as well as to reduced irrigation and fertilisation (Grozeva et al., 2024; Rosa-Martínez et al., 2021; Carbonell et al., 2020; Fullana-Pericàs et al., 2019; Galmés et al., 2011). The origin of these varieties appears to be a key factor, as those from semi-arid Mediterranean regions characterised by limited water availability or from marginal farmlands with scarce access to mineral fertilisers tend to perform better under low-input conditions. Regarding the two hybrids evaluated, reduced fertilisation negatively affected yield in both cases, suggesting that hybrid vigour entails a greater demand for external resources (Carbonell et al., 2020). However, unlike the other hybrid, H1 maintained its yield under reduced irrigation and also exhibited increased concentrations of fruit metabolites.

The findings of this thesis suggest future research pathways aimed at strengthening ecological resilience and preserving the fruit quality of tomato landraces. Having confirmed the outstanding fruit metabolic profiles of some of these varieties and newly developed hybrids, it would be of interest to undertake further multi-year and multi-location evaluations of the selected genotypes, considering both agronomic performance and fruit quality, to examine potential genotype-environment interactions, and to evaluate their stability, adaptability, and consistency across different cultivation systems. In addition, the variability observed in the response to reduced inputs reveals a wide range of genotype-specific behaviours, highlighting the need to

continue characterising germplasm under low-input conditions to identify resilient genotypes. These findings also underline the importance of optimising irrigation and fertilisation regimes for each variety and evaluating other abiotic stresses typical of semi-arid regions, such as heat and salinity. Furthermore, gaining insight into the physiological, genetic, and biochemical mechanisms underlying tolerance in resilient varieties will provide key knowledge for their effective incorporation into future breeding programs aimed at enhancing agricultural sustainability. Finally, in order to enhance the competitiveness of landraces within intensive production systems, it is essential to continue the incorporation of virus resistance genes into a broader range of traditional varieties and strengthen tolerance to other diseases and pathogens affecting tomato.

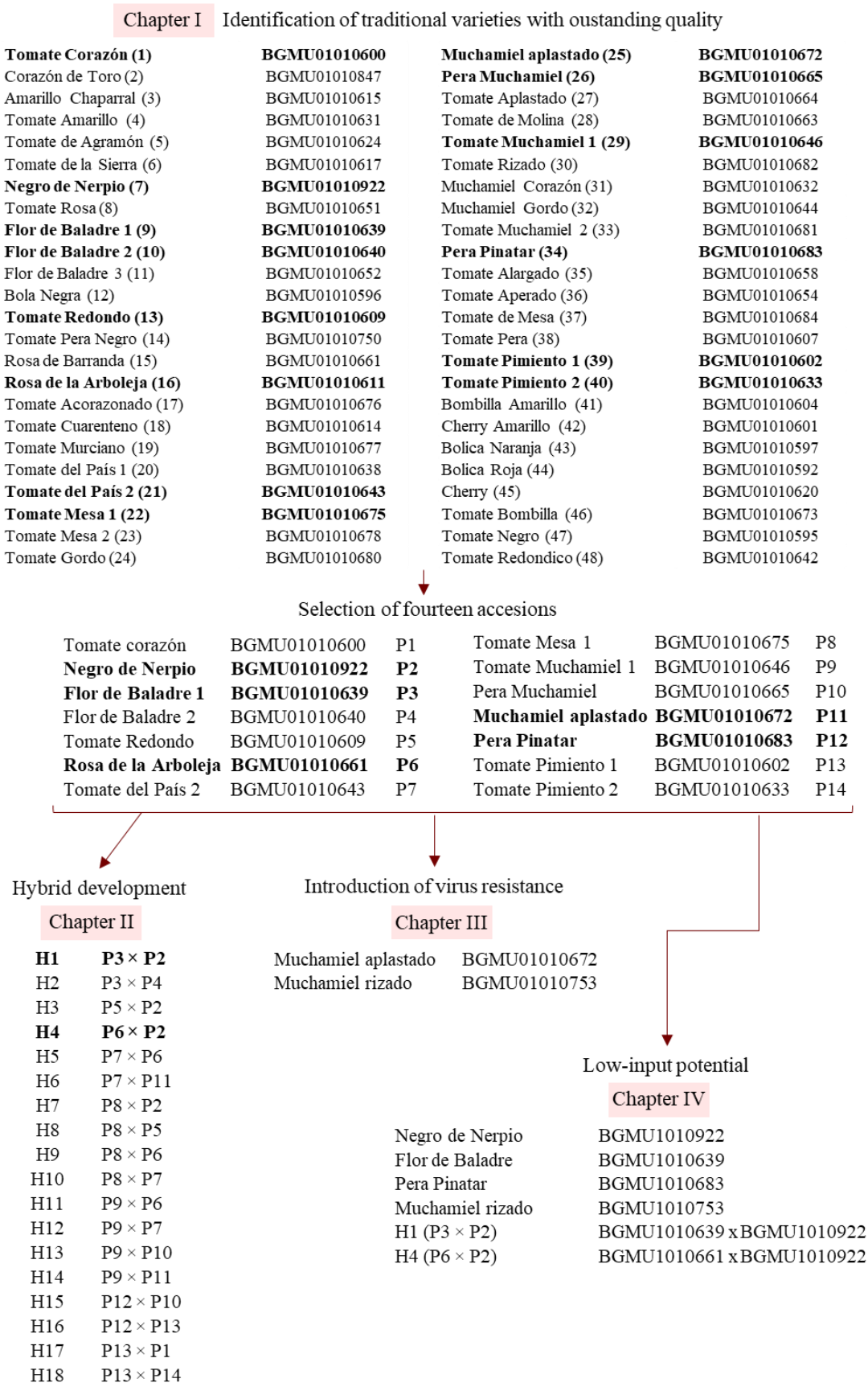


Figure 1. Selection flow of traditional tomato accessions throughout the thesis. Accessions selected for subsequent stages are highlighted in bold.

8. Thesis conclusions

1. The study of the organoleptic characteristics and fruit composition of forty-eight traditional tomato varieties from south-eastern Spain revealed a wide variability among genotypes in compounds related to flavour and functional value, including sugars, organic acids, vitamin C, carotenoids, and phenolic compounds. The selection of varieties with outstanding quality and good agronomic performance provided a valuable source of variability for subsequent studies.

2. The development of hybrids from selected traditional varieties produced new genetic combinations integrating the quality traits of landraces with the agronomic advantages of hybrid vigour, with two hybrids (H7 and H14) achieving performance levels comparable to those of commercial control varieties.

3. Six hybrids exhibited a balanced profile, combining high yield with outstanding fruit quality, both in terms of flavour-related and bioactive compounds. These characteristics make them competitive in the current market, and their cultivation may contribute to increasing the cultivated biodiversity of tomato.

4. The introduction of a single copy of the resistance genes *Tm-2a*, *Sw-5*, and *Ty-1* into traditional Muchamiel-type varieties preserved their varietal morphological identity, maintained or even enhanced fruit quality, and increased yield through hybrid vigour, confirming the effectiveness of this breeding strategy.

5. Although the *Ty-1* gene in homozygous breeding lines reduced fruit diameter and organic acid content, these effects were largely mitigated in heterozygous hybrids, which exhibited diameters similar to those of traditional varieties and intermediate organic acid levels between the traditional varieties and the breeding line.

6. Evaluation under low-input conditions revealed differential responses among traditional varieties. Pera Pinatar exhibited the most favourable cost–benefit balance, maintaining yield while sustaining or enhancing some metabolites. Flor de Baladre and Muchamiel rizado maintained yield under low-input treatments, except under the highest irrigation concentration (100F+75I), while sustaining or improving fruit quality. Negro de Nerpio showed a low yield compared to the other varieties and was particularly sensitive to the most diluted irrigation solution (50F + 100I). H1 and H2 hybrids exhibited hybrid vigour, achieving higher productivity than their common traditional parental variety, Negro de Nerpio. In addition, H1 maintained yield under low irrigation while improving the quality through increased concentrations of certain metabolites. In contrast, H2 was the least responsive, with reduced yield and no consistent metabolite improvements under low-input conditions.

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