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Characterization and enhancement of genetic resources for the improvement of nutritional and organoleptic quality in *Solanaceae* vegetable crops

Ph.D. dissertation by

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Instituto de Conservación y Mejora
de la Agrodiversidad Valenciana

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“Caminante, no hay camino,
se hace camino al andar.”

Antonio Machado

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ABSTRACT

Domestication and development of high-yielding, uniform modern varieties led to a loss of genetic variability for fruit composition and quality attributes in the cultivated background of fruits and vegetables. Consumers are now increasingly complaining about that loss, demanding products with better flavour and higher health-promoting potential, which has become a major goal for breeders. On that regard, the *Solanaceae* vegetable crops tomato, eggplant and pepper are highly valued as a source of fibre, vitamins, minerals and antioxidant compounds to the human diet. The fact that they are amongst the top ten vegetables most produced worldwide and are present in the cuisine of almost every culture, makes them an attractive target for the improvement of fruit quality. On the other hand, intensive production of these vegetables has caused a negative environmental footprint during the last decades. The need for reducing fertilization inputs, the advent of climate change effects and food security are major new challenges that agriculture will have to tackle in the next few generations. Within this scenario, new varieties henceforth will need to be more diverse, resilient to abiotic stresses and adapted to a more sustainable agriculture, with higher yields while providing higher nutritional content and health benefits.

Therefore, in the present doctoral thesis, we focused on studying the variability and providing new useful resources for the improvement of nutritional and organoleptic quality of fruits of tomato, eggplant and pepper, while adapting the crops to lower nitrogen (N) inputs. To achieve this purpose, we carried out a comprehensive characterization of fruit composition, along with important agronomic traits, of a set of genetic resources scarcely explored up to date.

In the first chapter of this thesis, we aimed at assessing the diversity within species and providing an accurate comparison of fruit quality determinants among tomato, eggplant and pepper. Thus, a comprehensive characterization of 36 fruit composition traits, encompassing sugars, organic acids, antioxidants and minerals, was carried out in a set of 10 accessions per crop for tomato, eggplant and pepper, grown under the same cultivation conditions and organic farming. The results showed a considerably distinct composition profile among tomato, eggplant and pepper. The main differences were observed for the antioxidant profile. In this way, pepper had an outstanding content in vitamin C, tomato accumulated high vitamin C, although at a lower level than pepper, and was also rich in the flavonoid rutin, and eggplant accumulated high concentrations of chlorogenic acid. Furthermore, pepper had the highest contents in sugars and organic acids and was the richest in Fe, although eggplant was the species with higher content in most minerals, particularly for K, Mg and Cu. Due to their complementary nutritional profiles, a combined regular consumption of the three vegetables would supply more than 20% of the Dietary Reference Intake of several of the analysed phytochemicals. In addition, a large variability was found within each species, which is of interest for

selecting varieties with better nutritional and organoleptic profiles, as well as for breeding new cultivars.

In the second chapter of this thesis, we addressed the characterization of eggplant introgression lines (ILs) as a potential genetic resource for introducing new genetic diversity from crop wild relatives into the cultivated genetic background. In this way, the wild species *Solanum incanum* L. has been used as donor parent for the development of a set of eggplant ILs, which have already showed their interest for breeding for stress tolerance and relevant morpho-agronomic traits. However, the use of this eggplant interspecific IL population requires ensuring that glycoalkaloids levels are below safety limits. In a two-environment trial, we evaluated 25 fruit composition traits, including proximate composition, sugars, acids, phenolics, glycoalkaloids and minerals in a set of 16 eggplant ILs with *S. incanum*, along with the recipient and donor parents and the hybrid. A strong influence of the environment accounted for the variation of most of the traits evaluated, which confirmed that the growing environment is a major factor affecting nutritional content. Overall, the results obtained showed that fruits produced by the ILs were safe for consumption, with similar composition to the recipient parent. These findings encourage the utilization of these ILs as pre-breeding materials.

Lowering N inputs is a major goal for sustainable agriculture, and *S. incanum* is a resilient species of interest for eggplant breeding. Therefore, once the safety of consumption of the fruits produced by the ILs with *S. incanum* was verified, we proposed to evaluate a set of 10 ILs, together with the recipient *S. melongena* parent, under two N fertilization doses supplied with the irrigation system, which corresponded to 8.25 mM NH_4NO_3 added to the intake water (high N treatment), and no external N supply to the intake water (low N treatment). A soil composition analysis before and after cultivation showed N contents slightly lower in the high N treatment, probably as a consequence of higher nutrient removal from soil by plants in the latter. Although a significant limitation in plant growth, N and C distribution, fruit load and dry matter occurred as a response of minimizing N inputs, the ILs reached, on average, similar final yields under both N treatments, as well as produced fruits with similar morphology, weight and phenolics content. These results enhance the ILs as potential materials for breeding for the eggplant adaptation to low N inputs.

Thanks to the availability of the genotyping of the eggplant ILs, the investigations carried out using this population enabled the identification of several putative QTLs associated to morpho-agronomic and fruit composition traits, which included stable QTLs across environments, as well as N treatment-specific QTLs. Potential candidate genes were also spotted for some of them. These provide new relevant information for broadening eggplant genetic knowledge and for the development of powerful tools for breeding.

In the final chapter of this thesis, we addressed the utilization of local varieties of ‘de penjar’ long shelf-life tomato for breeding for the crop adaptation to low N inputs. They are a group of resilient local varieties as they have been selected across generations under low inputs. Herein, we present the first evaluation on fruit quality and yield parameters of a collection of 44 varieties of ‘de penjar’ tomato under two N fertilization levels, provided by doses of manure equivalent to 162 kg N ha⁻¹ in the high N treatment and 49 kg N ha⁻¹ in the low N treatment. A large variation was observed, with lycopene content showing the highest relative range of variation (over 4-fold) under both N treatments. Reducing N inputs had a significant effect on hue, whose average slightly increased, and sweetness-related compounds and parameters, whose average decreased by 11-16%, while yield and most quality traits were not affected. These are promising results that encourage the conservation of the ‘de penjar’ tomato and their utilization in breeding programs. In addition, highest yielding varieties under both conditions displayed wide variation in the composition and quality profiles, which may allow the selection of specific ideotypes with high quality under low N conditions. Our study also reinforces the appreciation of local varieties for their organoleptic and nutritional quality as they had higher values than commercial varieties for sweetness to acidity ratio and for vitamin C.

With these works, we were able to characterize the diversity for fruit quality within and among tomato, eggplant and pepper, which is a fundamental task for the conservation and improvement of genetic resources. We enhanced the gene pool of crop wild relatives and local varieties to broadening and improving the cultivated genetic background of eggplant and tomato. In addition, we demonstrated that reducing the usual N inputs for both eggplant and tomato cultivation is possible, without jeopardising yields and most quality traits. We believe these works will provide new useful phenomic and genomic resources for future breeding programs of the *Solanaceae* vegetables.

RESUMEN

La domesticación y el desarrollo de variedades modernas, uniformes y de alto rendimiento, condujeron a una pérdida de variabilidad genética para caracteres de composición de fruto y atributos de calidad en el fondo genético cultivado de frutas y verduras. Actualmente, los consumidores se quejan cada vez más de esta pérdida y exigen productos con mejor sabor y que reporten un mayor beneficio a la salud, lo que se ha convertido en uno de los principales objetivos de los mejoradores. En este sentido, las hortalizas de la familia *Solanaceae*, tomate, berenjena y pimiento, son muy apreciadas como fuente de fibra, vitaminas, minerales y compuestos antioxidantes en la dieta. El hecho de que se encuentren entre las diez hortalizas más producidas a nivel mundial y estén presentes en la cocina de casi todas las culturas, las convierte en un objetivo atractivo para la mejora de la calidad de sus frutos. Por otro lado, la producción intensiva de estas hortalizas ha provocado una grave huella medioambiental durante las últimas décadas. La necesidad de reducir los insumos en la fertilización, la llegada de los efectos del cambio climático y la seguridad alimentaria son nuevos e importantes retos que la agricultura deberá afrontar en las próximas generaciones. Con este escenario, las nuevas variedades tendrán que ser a partir de ahora más diversas, resilientes a los estreses abióticos y adaptadas a una agricultura más sostenible, con mayores rendimientos a la vez que proporcionando un mayor contenido nutricional y beneficios para la salud.

Por ello, en la presente tesis doctoral nos hemos centrado en estudiar la variabilidad y aportar nuevos recursos útiles para la mejora de la calidad nutricional y organoléptica de los frutos de tomate, berenjena y pimiento, adaptando los cultivos a menores aportes de nitrógeno (N). Para lograr este propósito, llevamos a cabo una caracterización exhaustiva de la composición de los frutos, junto con importantes rasgos agronómicos, de un conjunto de recursos genéticos escasamente explorados hasta la fecha.

En el primer capítulo de esta tesis, nos propusimos evaluar la diversidad dentro de las tres especies y proporcionar una comparación precisa de los determinantes de la calidad del fruto entre el tomate, la berenjena y el pimiento. Así, se llevó a cabo una caracterización exhaustiva de 36 compuestos y parámetros de composición del fruto, que incluían azúcares, ácidos orgánicos, antioxidantes y minerales, en un conjunto de 10 accesiones por cultivo de tomate, pimiento y berenjena, cultivados en las mismas condiciones y prácticas ecológicas. Los resultados mostraron un perfil de composición considerablemente distinto entre el tomate, la berenjena y el pimiento. Las principales diferencias se observaron en el perfil antioxidante. Así, el pimiento destacó por su elevado contenido en vitamina C, el tomate acumuló también un alto contenido en vitamina C, aunque a un nivel inferior al del pimiento, y era también rico en el flavonoide rutina, mientras que la berenjena acumuló altas concentraciones de ácido clorogénico. Además, el pimiento tuvo los contenidos más altos en azúcares y ácidos orgánicos y fue el más rico en Fe, aunque la berenjena fue la especie con mayor contenido en la mayoría de los minerales, particularmente en K, Mg y Cu. Debido a sus perfiles nutricionales complementarios, un consumo regular combinado de las tres

verduras aportaría más del 20% de la Ingesta Dietética diaria de Referencia de varios de los fitoquímicos analizados. Además, se encontró una gran variabilidad dentro de cada especie, lo cual es de interés para la selección de variedades con mejores perfiles nutricionales y organolépticos, así como para la obtención de nuevos cultivares mejorados.

En el segundo capítulo de esta tesis, se abordó la caracterización de unas líneas de introgresión (ILs) de berenjena como potencial recurso genético para introducir nueva diversidad genética a partir de especies silvestres relacionadas en el fondo genético cultivado. De este modo, la especie silvestre *Solanum incanum* L. se ha utilizado como parental donante para el desarrollo de un conjunto de ILs de berenjena, que ya han mostrado su interés para la mejora de la tolerancia al estrés y de rasgos morfo-agronómicos relevantes. Sin embargo, el uso de esta población de ILs interespecíficas de berenjena requiere asegurar que los niveles de glicoalcaloides estén por debajo de los límites de seguridad. En un ensayo en dos ambientes, evaluamos 25 rasgos de composición del fruto, incluyendo composición proximal, azúcares, ácidos, compuestos fenólicos, glicoalcaloides y minerales en un conjunto de 16 ILs de berenjena con *S. incanum*, junto con los padres recurrente y donante, y el híbrido. La variación encontrada para la mayoría de los rasgos evaluados estuvo fuertemente influenciada por el ambiente, lo que confirmó que este es un factor importante que afecta al contenido nutricional. En general, los resultados obtenidos mostraron que los frutos producidos por las ILs eran seguros para el consumo, con una composición similar a la del parental recurrente. Estos resultados promueven la utilización de estas ILs como materiales de pre-breeding.

La reducción de los aportes de N es un objetivo esencial para una agricultura sostenible, y *S. incanum* es una especie resiliente de interés para la mejora de la berenjena. Por lo tanto, una vez verificada la seguridad para el consumo de los frutos producidos por las ILs con *S. incanum*, nos propusimos evaluar un conjunto de 10 ILs, junto con el parental recurrente *S. melongena*, bajo dos dosis de fertilización de N suministradas con el sistema de riego, que correspondían a 8.25 mM de NH_4NO_3 añadido al agua de riego (tratamiento de alto N), y ningún suministro de N externo al agua de riego (tratamiento de bajo N). Un análisis de la composición del suelo antes y después del cultivo mostró contenidos de N ligeramente inferiores en el tratamiento de alto N, probablemente como consecuencia de una mayor absorción de nutrientes del suelo por parte de las plantas en este último. Aunque se produjo una limitación significativa en el crecimiento de la planta, en la distribución de N y C, en la carga de frutos y en la materia seca como respuesta a la minimización de los aportes de N, las ILs alcanzaron, en promedio, rendimientos finales similares bajo ambos tratamientos de N, así como produjeron frutos con similar morfología, peso y contenido en compuestos fenólicos. Estos resultados potencian las IL como materiales de mejora útiles para la adaptación de la berenjena a bajos aportes de N.

Gracias a la disponibilidad del genotipado de las ILs de berenjena, las investigaciones realizadas con esta población permitieron identificar varios QTLs putativos asociados a

caracteres morfo-agronómicos y de composición del fruto, que incluían QTLs estables a través de los ambientes, así como QTLs específicos de tratamiento de N. También se detectaron posibles genes candidatos para algunos de ellos. Esto proporciona nueva información relevante para ampliar el conocimiento genético de la berenjena y para el desarrollo de potentes herramientas de mejora.

En el último capítulo de esta tesis, abordamos la utilización de variedades locales de tomate de larga vida ‘de penjar’ para la mejora para la adaptación del cultivo de tomate a bajos aportes de N. Se trata de un grupo de variedades locales resilientes, ya que han sido seleccionadas a lo largo de las generaciones en condiciones de bajos insumos. En este trabajo se presenta la primera evaluación de parámetros de calidad de fruto y rendimiento de una colección de 44 variedades de tomate ‘de penjar’ bajo dos niveles de fertilización nitrogenada, proporcionados por dosis de estiércol equivalentes a 162 kg N ha⁻¹ en el tratamiento de alto N y 49 kg N ha⁻¹ en el tratamiento de bajo N. Se observó una gran variación, siendo el contenido de licopeno el que mostró el mayor rango relativo de variación (más de 4x) bajo ambos tratamientos de N. La reducción de los aportes de N tuvo un efecto significativo en el hue, cuya media aumentó ligeramente, y en los compuestos y parámetros relacionados con el dulzor, cuya media disminuyó en un 11-16%, mientras que el rendimiento y la mayoría de los rasgos de calidad no se vieron afectados. Estos son resultados prometedores que promueven la conservación del tomate ‘de penjar’ y a su utilización en programas de mejora. Además, las variedades de mayor rendimiento en ambas condiciones mostraron una amplia variación en los perfiles de composición y calidad, lo que puede permitir la selección de ideotipos específicos de alta calidad en condiciones de bajo N. Nuestro estudio también refuerza la apreciación de las variedades locales por su calidad organoléptica y nutricional, ya que presentaron valores más altos que las variedades comerciales para la relación dulzor-acidez y para la vitamina C.

Con estos trabajos, hemos podido caracterizar la diversidad para la calidad de fruto dentro y entre las especies de tomate, berenjena y pimiento, lo cual es una tarea fundamental para la conservación y la mejora de los recursos genéticos. Hemos potenciado los recursos genéticos de las especies silvestres relacionadas y de las variedades locales para ampliar y mejorar el fondo genético cultivado de la berenjena y el tomate. Además, se ha demostrado que es posible reducir los aportes habituales de N para el cultivo de la berenjena y el tomate, sin poner en peligro el rendimiento y la mayoría de los caracteres de calidad. En definitiva, creemos que estos trabajos proporcionarán nuevos recursos fenómicos y genómicos útiles para futuros programas de mejora de las hortalizas de la familia *Solanaceae*.

RESUM

La domesticació i el desenvolupament de varietats modernes, uniformes i d'alt rendiment, van conduir a una pèrdua de variabilitat genètica per a caràcters de composició de fruit i atributs de qualitat en el pool genètic cultivat de fruites i verdures. Actualment, els consumidors es queixen cada vegada més d'aquesta pèrdua i exigeixen productes amb millor sabor i que reporten un major benefici a la salut, la qual cosa s'ha convertit en un dels principals objectius dels milloradors. En aquest sentit, les hortalisses de la família *Solanaceae*, tomaca, albergínia i pimentó, són molt apreciades com a font de fibra, vitamines, minerals i compostos antioxidants en la dieta. El fet que es troben entre les deu hortalisses més produïdes a nivell mundial i siguen presents en la cuina de quasi totes les cultures, les converteix en un objectiu atractiu per a la millora de la qualitat dels seus fruits. D'altra banda, la producció intensiva d'aquestes hortalisses ha provocat una greu petjada mediambiental durant les últimes dècades. La necessitat de reduir els inputs en la fertilització, l'arribada dels efectes del canvi climàtic i la seguretat alimentària són nous i importants reptes que l'agricultura haurà d'afrontar en les pròximes generacions. En aquest escenari, les noves varietats hauran de ser a partir d'ara més diverses, resilents als estressos abiòtics i adaptades a una agricultura més sostenible, amb majors rendiments alhora que proporcionant un major contingut nutricional i beneficis per a la salut.

Per això, en la present tesi doctoral ens hem centrat en estudiar la variabilitat i aportar nous recursos útils per a la millora de la qualitat nutricional i organolèptica dels fruits de tomaca, albergínia i pimentó, adaptant els cultius a menors aportacions de nitrogen (N). Per a aconseguir aquest propòsit, duem a terme una caracterització exhaustiva de la composició dels fruits, juntament amb importants trets agronòmics, d'un conjunt de recursos genètics escassament explorats fins hui.

En el primer capítol d'aquesta tesi, ens vam proposar avaluar la diversitat dins de les tres espècies i proporcionar una comparació precisa dels determinants de la qualitat del fruit entre la tomaca, l'albergínia i el pimentó. Així, es va dur a terme una caracterització exhaustiva de 36 compostos i paràmetres de composició del fruit, que incloïen sucres, àcids orgànics, antioxidants i minerals, en un conjunt de 10 accessions per cultiu de tomaca, pimentó i albergínia, cultivats en les mateixes condicions i pràctiques ecològiques. Els resultats van mostrar un perfil de composició considerablement diferent entre la tomaca, l'albergínia i el pimentó. Les principals diferències es van observar en el perfil antioxidant. Així, el pimentó va destacar pel seu elevat contingut en vitamina C, la tomaca va acumular també un alt contingut en vitamina C, encara que a un nivell inferior al del pimentó, i va anar també ric en el flavonoide rutina, mentre que l'albergínia va acumular altes concentracions d'àcid clorogenic. A més, el pimentó va tindre els continguts més alts en sucres i àcids orgànics i va ser el més ric en Fe, encara que l'albergínia va ser l'espècie amb major contingut en la majoria dels minerals, particularment en K, Mg i Cu. A causa dels seus perfils nutricionals complementaris, un consum regular combinat de les tres verdures aportaria més del 20% de la Ingesta Dietètica diària de Referència de diversos dels fito químics analitzats. A més, es va

trobar una gran variabilitat dins de cada espècie, la qual cosa és d'interès per a la selecció de varietats amb millors perfils nutricionals i organolèptics, així com per a l'obtenció de noves cultivars millorades.

En el segon capítol d'aquesta tesi, es va abordar la caracterització d'unes línies d'introgressió (ILs) d'albergínia com a potencial recurs genètic per a introduir nova diversitat genètica a partir d'espècies silvestres relacionades en el fons genètic cultivat. D'aquesta manera, l'espècie silvestre *Solanum incanum* L. s'ha utilitzat com a parental donant per al desenvolupament d'un conjunt de ILs d'albergínia, que ja han mostrat el seu interès per a la millora de la tolerància a l'estrès i de trets morf-agronòmics rellevants. No obstant això, l'ús d'aquesta població de ILs interespecífiques d'albergínia requereix assegurar que els nivells de glucoalcaloides estiguen per davall dels límits de seguretat. En un assaig en dos ambients, avaluem 25 trets de composició del fruit, incloent composició proximal, sucres, àcids, compostos fenòlics, glucoalcaloides i minerals en un conjunt de 16 ILs d'albergínia amb *S. incanum*, juntament amb els pares recurrent i donant, i l'híbrid. La variació trobada per a la majoria dels trets avaluats va estar fortament influenciada per l'ambient, la qual cosa va confirmar que aquest és un factor important que afecta el contingut nutricional. En general, els resultats obtinguts van mostrar que els fruits produïts per les ILs eren segurs per al consum, amb una composició similar a la del parental recurrent. Aquests resultats promouen la utilització d'aquestes ILs com a materials de pre-breeding.

La reducció de les aportacions de N és un objectiu essencial per a una agricultura sostenible, i *S. incanum* és una espècie resilient d'interès per a la millora de l'albergínia. Per tant, una vegada verificada la seguretat per al consum dels fruits produïts per les ILs amb *S. incanum*, ens vam proposar avaluar un conjunt de 10 ILs, juntament amb el parental recurrent *S. melongena*, sota dues dosis de fertilització de N subministrades amb el sistema de reg, que corresponien a 8.25 mm de NH_4NO_3 afegit a l'aigua de reg (tractament d'alt N), i cap subministrament de N extern a l'aigua de reg (tractament de baix N). Una anàlisi de la composició del sòl abans i després del cultiu va mostrar continguts de N lleugerament inferiors en el tractament d'alt N, probablement a conseqüència d'una major absorció de nutrients del sòl per part de les plantes en aquest últim. Encara que es va produir una limitació significativa en el creixement de la planta, en la distribució de N i C, en la càrrega de fruits i en la matèria seca com a resposta a la minimització de les aportacions de N, les ILs van aconseguir, en mitjana, rendiments finals similars sota tots dos tractaments de N, així com van produir fruits amb similar morfologia, pes i contingut en compostos fenòlics. Aquests resultats potencien les ILs com a materials de millora útils per a l'adaptació de l'albergínia a baixes aportacions de N.

Gràcies a la disponibilitat del genotipat de les ILs d'albergínia, les investigacions realitzades amb aquesta població van permetre identificar diversos QTLs putatius associats a caràcters morf-agronòmics i de composició del fruit, que inclouen QTLs estables a través dels ambients, així com QTLs específics de tractament de N. També es van detectar possibles gens candidats per a alguns d'ells. Això proporciona nova

informació rellevant per a ampliar el coneixement genètic de l'albergínia i per al desenvolupament de potents eines de millora.

En l'últim capítol d'aquesta tesi, abordem la utilització de varietats locals de tomaca de llarga vida 'de penjar' per a la millora per a l'adaptació del cultiu de tomaca a baixes aportacions de N. Es tracta d'un grup de varietats locals resilients, ja que han sigut seleccionades al llarg de les generacions en condicions de baixos inputs. En aquest treball es presenta la primera avaluació de paràmetres de qualitat de fruit i rendiment d'una col·lecció de 44 varietats de tomaca 'de penjar' sota dos nivells de fertilització nitrogenada, proporcionats per dosi de fem equivalents a 162 kg N ha^{-1} en el tractament d'alt N i 49 kg N ha^{-1} en el tractament de baix N. Es va observar una gran variació, sent el contingut de licopèl el que va mostrar el major rang relatiu de variació (més de 4x) sota tots dos tractaments de N. La reducció de les aportacions de N va tindre un efecte significatiu en el hue, la mitjana del qual va augmentar lleugerament, i en els compostos i paràmetres relacionats amb la dolçor, la mitjana de la qual va disminuir en un 11-16%, mentre que el rendiment i la majoria dels trets de qualitat no es van veure afectats. Aquests són resultats prometedors que promouen la conservació de la tomaca 'de penjar' i a la seua utilització en programes de millora. A més, les varietats de major rendiment en totes dues condicions van mostrar una àmplia variació en els perfils de composició i qualitat, la qual cosa pot permetre la selecció de idiotips específics d'alta qualitat en condicions de baix N. El nostre estudi també reforça l'apreciació de les varietats locals per la seua qualitat organolèptica i nutricional, ja que van presentar valors més alts que les varietats comercials per a la relació dolçor-acidesa i per a la vitamina C.

Amb aquests treballs, hem pogut caracteritzar la diversitat per a la qualitat de fruit dins i entre les espècies de tomaca, albergínia i pimentó, la qual cosa és una tasca fonamental per a la conservació i la millora dels recursos genètics. Hem potenciat els recursos genètics de les espècies silvestres relacionades i de les varietats locals per a ampliar i millorar el fons genètic cultivat de l'albergínia i la tomaca. A més, s'ha demostrats que és possible reduir les aportacions habituals de N per al cultiu de l'albergínia i la tomaca, sense posar en perill el rendiment i la majoria dels caràcters de qualitat. En definitiva, creiem que aquests treballs proporcionaran nous recursos fenòmics i genòmics útils per a futurs programes de millora de les hortalisses de la família *Solanaceae*.

GENERAL INTRODUCTION

1. Why breeding for quality in *Solanaceae*?

The *Solanaceae* family encompasses over 3000 species, almost half of which belong to the hyper-diverse *Solanum* genus (Knapp et al., 2004; Olmstead et al., 2008). *Solanaceae* species have been exploited by humans for an array of purposes, such as ornamental, for their medicinal and psychotropic properties and as model species for biological and genetic research, although their main usage has been as food (Afroz et al., 2020; Bombarely et al., 2016; Daunay et al., 2007; Gebhardt, 2016) (Figure 1). In this context, within the *Solanaceae* family we can find some of the most economically important vegetables worldwide, such as tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.) and pepper (*Capsicum annum* L.) (Figure 1).

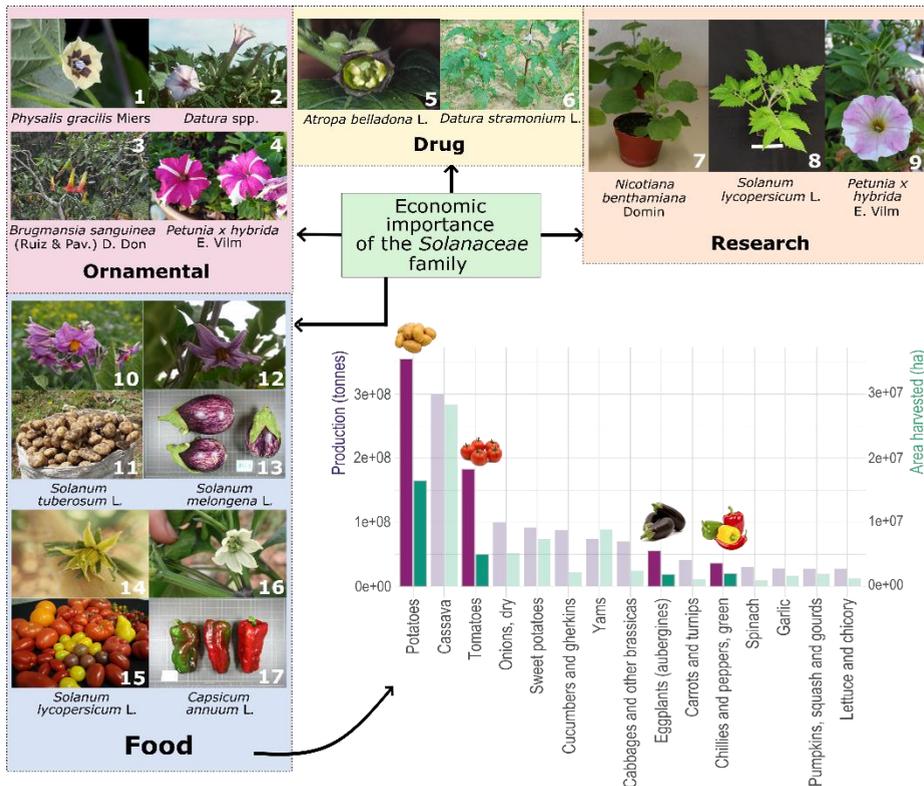


Figure 1. Overview of the economic importance of the *Solanaceae* family showing their main usages. The bar plot on the lower right represents the top 15 vegetable crops (including tubers) for world production, according to FAOSTAT (2019). The total production is represented by purple bars, and the total area harvested by crop, by green bars. The bars corresponding to *Solanaceae* crops are highlighted. Images 1-3, 5, 6 and 10 were downloaded from ‘The World Flora Online’ (WFO, 2021), licensed by [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/). Image 7 was downloaded from ‘Wikimedia commons’ (Wikimedia, 2021), licensed by [CC BY-SA 3.0](https://creativecommons.org/licenses/by-sa/3.0/). Authors: (1), O. M. Montiel; (2), E. Denison; (6) Steve R. Turner; (7), Chandres; (10) and (11), Sandra Knapp; (3) and (5), unknown; (4), (8), (9) and (12) – (17), own elaboration (Elena Rosa Martínez).

These species are widely produced for their edible berries and consumed in almost every continent in a variety of culinary formats. According to FAO, in 2019 they ranked first ($181 \cdot 10^6$ t), fifth ($55 \cdot 10^6$ t), and seventh ($38 \cdot 10^6$ t), respectively, among vegetables for global production. Also, the total area harvested of tomato, eggplant and pepper surpassed 5, 1.8 and 1.9 million ha in that same year, respectively (Figure 1). For all three vegetables, China stood as the world's largest producer, and Spain was found among the world's top 10 producers, ranking eighth, tenth and fifth for each vegetable, respectively (FAOSTAT, 2019).

In a very simplified vision, both tomato and pepper are native to South America, more precisely to Peru (Blanca et al., 2015) and Bolivia (Carrizo-García et al., 2016), respectively, and were domesticated in Mesoamerica before being introduced in Europe through Spain, which is considered a secondary centre of diversity (Arnoux et al., 2021). On the other hand, eggplant is an Old-World species, which originated in South-East Asia, although the exact centre of domestication has not yet been unravelled, and the possibility of at least three different domestication centres is being discussed (Arnoux et al., 2021; Meyer et al., 2012; Page et al., 2019b). Then, eggplant germplasm spread first eastward into Japan, and, during the fourteenth century, westward into Western Asia, Europe and, lastly, to the rest of the world (Taher et al., 2017).

Domestication events are associated to diversity bottlenecks due to several selection rounds of only a subset of genotypes, usually those producing more palatable non-toxic fruits (Page et al., 2019a; Van De Wouw et al., 2009), from which the new cultivated species are emerged (Figure 2). For instance, scientific evidence has proved that the early selection of five major loci during tomato domestication was responsible for the dramatic reduction of toxic glycoalkaloids accumulation in fruits (G. Zhu et al., 2018). Also, the indirect negative selection for bioactive compounds and stress tolerance during domestication likely resulted in the elimination of alleles that contribute to the high content in phenolics (Meyer et al., 2015), as well as genes associated to cell-wall thickening, which influences tolerance to biotic and abiotic stresses (Gao et al., 2019). Besides that, since the 1960's, agriculture has faced the challenge of feeding an exponentially growing population, which led to the development of modern, highly inbred varieties with higher yields and resistance or tolerance to major biotic stresses for each crop, producing bigger fruits with attractive and uniform external appearance and longer shelf-life. The gradual replacement of landraces, adapted to specific agroclimatic conditions, for a reduced number of modern varieties used under an intensive agriculture model has also resulted in loss of variation in crops, which has been defined as genetic erosion (Fu and Dong, 2015; Lin et al., 2014; Van De Wouw et al., 2009) (Figure 2). Some of the neglected traits in the development of modern varieties have been those related to fruit quality, and consumers have been complaining about that lack for the last few decades (Causse et al., 2010, 2003; Klee and Tieman, 2018; Tieman et al., 2017).

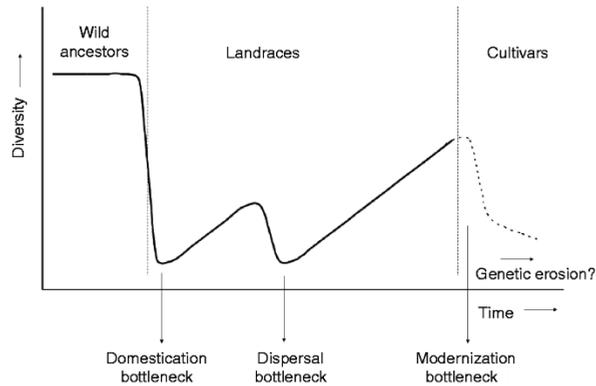


Figure 2. Model of diversity bottlenecks of crops from wild ancestors to modern cultivars. Adapted from Van De Wouw et al. (2009).

Quality of fruits and vegetables is a complex trait, or rather a set of traits, that encompasses several aspects, and which varies according to each specific crop, to its use and to the market preferences at any given moment (Asensio et al., 2019; Causse et al., 2010; Eggink et al., 2012; Jürkenbeck et al., 2019; Kyriacou and Rouphael, 2018; Roch et al., 2019). In this way, the most relevant aspects of fruit quality in current breeding strategies are organoleptic or sensory quality, which refers to traits that consumers perceive through their senses (size, shape, colour, flavour, texture) (Causse et al., 2010; Kader, 2008; Klee and Tieman, 2018), and nutritional quality, which refers to the ability of food to provide the basic nutrients for maintaining a good health. Moreover, the latter aspect has also embraced the currently highly attention-grabbing nutraceutical quality or functional potential, which refers to the role of some phytochemicals present in fruits and vegetables as bioactive molecules, producing a benefit on human health beyond their nutritional value (Table 1). In this context, a wide array of secondary metabolites produced by plants (Figure 3), such as phenolics, carotenoids, isoprenoids, nitrogenous and sulphur compounds, as well as micronutrients, such as vitamins and minerals, are currently in the spotlight as they have shown antioxidant, anti-diabetic, anti-carcinogenic, immunomodulatory, anti-atherosclerotic, or neuroprotective properties in several assays (D'Amelia et al., 2018; Hounsome et al., 2008; Kyriacou and Rouphael, 2018; Pott et al., 2019; Vincente et al., 2014; Wahyuni et al., 2013; Weng and Yen, 2012; Yahia et al., 2019).

Consumers in developed countries, where daily nutrient and energy needs are fully covered, have been complaining about the “missing” flavour of fruit and vegetables and longing for the so-called “taste of the past” (Casals et al., 2011; Causse et al., 2010). In addition, an increasing concern for maintaining a healthy lifestyle has led to growing interest for increased nutraceutical quality and functional foods (Hansen and Thomsen, 2018). Since the development of new varieties goes hand in hand with consumer preferences, the interest in quality improvement of fruits and vegetables has risen among

researchers and breeders over the last few years. Nevertheless, the importance of improving fruit quality not only lies in serving the preferences of a middle-to-upper class consumer, but also to address malnutrition in a large part of the world's population, mainly in underdeveloped countries, which cannot be solved by increasing crop production. In this way, the term 'biofortification' has been coined to refer to an increased nutrient density in crops by means of plant breeding strategies and agronomic practices without altering their inherent characteristics preferred by farmers and consumers. Biofortification is mainly directed towards the improvement of staple crops in impoverished areas for the mitigation of micronutrient deficiencies, such as vitamin A, iron or zinc (Bouis et al., 2011; Gaikwad et al., 2020).

Table 1. Major factors, components and metabolite determinants of the two main aspects of quality of fruits and vegetables.

	Factors	Components	Metabolite determinants
Organoleptic/ Sensory quality	Size	Weight, volume, dimension	
	Shape	Diameter/Depth ratio	
	Colour	Uniformity, intensity	Carotenoids, phenolic compounds
	Flavour	Sweetness, sourness, astringency, aroma	Sugars, organic acids, volatiles
	Texture	Firmness, crispness, juiciness	Fibre, water
Nutritional quality	Nutrition		Carbohydrates, proteins, vitamins, minerals
	Functional/ Nutraceutical properties		Phenolic compounds, terpenes, nitrogenous and sulphur compounds, vitamins, minerals

Increasing the accumulation of secondary metabolites in plants not only would benefit human health and consumer satisfaction, but would also improve plant tolerance to an array of biotic and abiotic stresses, since the synthesis of several of these compounds is induced under stress, and they are involved in the plant signalling response or have protective effects (Caretto et al., 2015; Yang et al., 2018). In this way, anthocyanins have been reported to play two roles against high light stress (Zheng et al., 2021). On one hand, they act as scavengers of radical oxygen species (ROS) accumulation induced by the stress (Xu et al., 2017). On the other hand, they act as a light barrier since they intercept yellow-green light, improving the quality and intensity of light reaching the plant, thus reducing chloroplasts overexcitation and damage (Zhang et al., 2010; Zheng et al., 2021). Phenolic compounds have been observed to reduce insect oviposition and larval growth on the host plant (Bentivenha et al., 2018; Puri et al., 2022). Tannins also prevent insect proliferation on plants since they cause an astringent taste and reduced digestibility and act as enzyme inactivators (Barbehenn et al., 2009). The well-known hydroxybenzoic acid salicylic acid has an essential role as signalling molecule in the

plant systemic acquired resistance, pathogen-associated molecular pattern-triggered immunity and effector-triggered immunity (Zhang and Li, 2019).

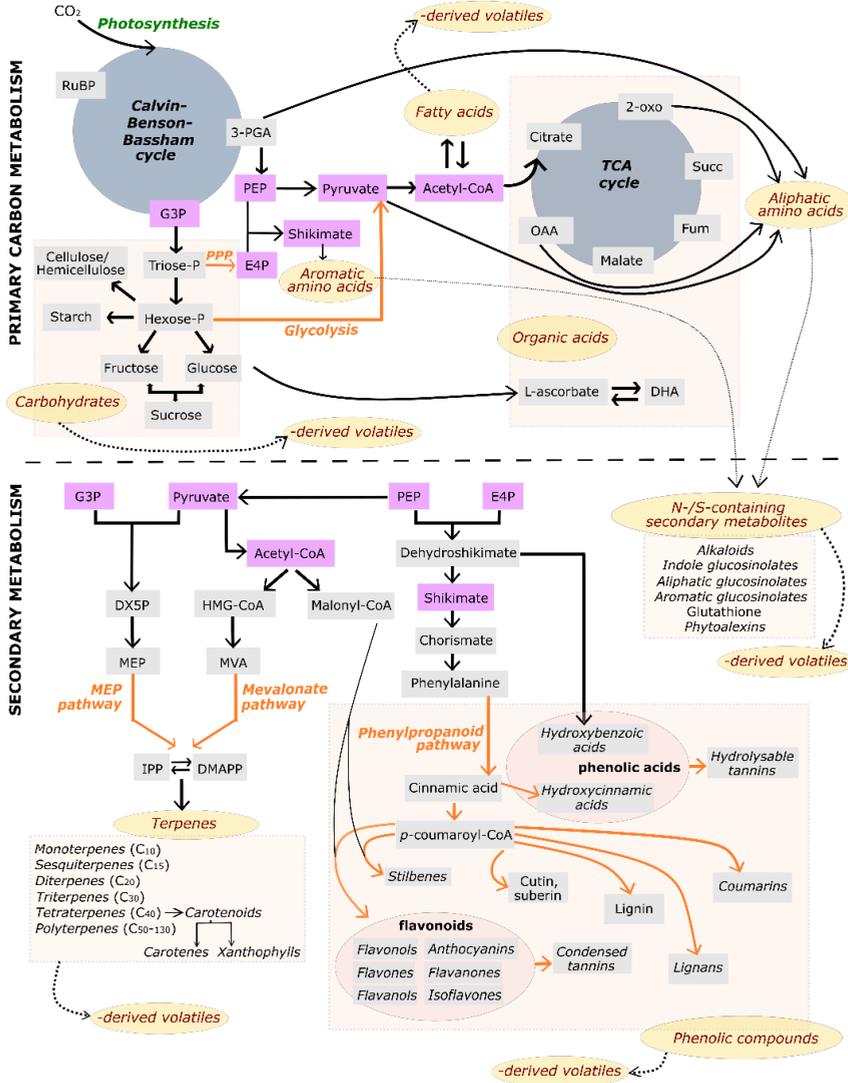


Figure 3. Simplified schematic overview of primary and secondary plant metabolism. The main classes of metabolites produced by plants are emphasized with red lettering and enclosed in a yellow circle. The main metabolites that link primary and secondary metabolism are highlighted in pink. 3-PGA, 3-phosphoglycerate; G3P, glyceraldehyde-3-phosphate; RuBP, ribulose-1,5-biphosphate; PEP, phosphoenolpyruvate; PPP, pentose phosphate pathway; E4P, erythrose-4-phosphate; 2-oxo, 2-oxoglutarate; Succ, succinate; Fum, fumarate; OAA, oxaloacetate; DHA, dehydroascorbate; DX5P, 1-deoxy-D-xylulose-5-phosphate; MEP, 2-C-methyl-D-erythritol-4-phosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MVA, mevalonate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate.

Notwithstanding, improving fruit quality is not a straightforward process. The great complexity of the term ‘quality’ (Kyriacou and Roupheal, 2018), the lack of adequate, objective, simple, rapid and sensitive methods of evaluation for highly subjective traits such as flavour (Chambers and Koppel, 2013), the polygenic control (Tieman et al., 2017), with strong environmental influence and low heritability (Panthee et al., 2012), and the negative correlation between fruit quality and important agronomic traits, are some of the factors that make improving fruit quality challenging. In the last years, a great effort has been made for increasing the knowledge on available germplasm resources and existing variability, mode of inheritance, genomic tools, and environmental influence on quality traits (e.g., Figàs et al., 2015; Fratianni et al., 2020; Gramazio et al., 2014; Ribes-Moya et al., 2020, 2018; San José et al., 2014, 2013; Stommel et al., 2015; Zhao et al., 2019) which is a necessity for developing efficient breeding strategies. Among the most economically important vegetables worldwide, tomato is undoubtedly the most studied, serving as a model species for research, particularly for other *Solanaceae* (de Vos et al., 2018). Despite this, much of the variability remains to be explored and a greater understanding of the genetics of quality traits is necessary, as well as reducing the large knowledge gap between other important *Solanaceae* vegetables, such as eggplant or pepper, and tomato. Moreover, as agriculture faces new challenges such as climate change and the need of reducing fertilizer and pesticide use (Raza et al., 2019; Stevens, 2019), there is still a need for broaden this knowledge. In this framework, in the present thesis we have focused on studying the variability and providing new useful resources for the improvement of nutritional, organoleptic quality and functional potential of fruits of tomato, eggplant and pepper, in the context of a more sustainable agriculture.

2. Breeding for fruit quality in tomato

Tomato is produced in all tropical, subtropical and temperate regions worldwide and presents many forms of consumption. Breeding for tomato fruit quality will primarily depend on its destination after harvest. In this way, we can clearly distinguish between two main uses of tomato: for fresh market and for industrial processing (dried, tomato paste, diced and whole canned tomatoes, tomato sauces, tomato juice, tomato soup and ketchup) (Rubatzky and Yamaguchi, 1997). In the present thesis we are focusing on quality of fresh market tomatoes.

Improving organoleptic quality of fresh market tomato will be based on consumer preferences. Although commercial tomatoes are mainly based on modern high-yielding F₁ hybrids, capable of producing fruits with uniform appearance and longer shelf-life, in the Mediterranean region, which is a secondary centre of diversity for tomato, a wide phenotypic variability can be found for fruit size, shape, colour and flavour (Figure 4), in the form of dozens of landraces or local varieties (Bauchet and Causse, 2012; Díez and Nuez, 2008; Rodríguez-Burruezo et al., 2015). This is the result of several generations of cultivation and conservation by farmers, of an array of varieties adapted

to very specific agroclimatic conditions throughout this region, which produce fruits with unique characteristics that are deeply rooted into the consumer preferences of each specific area. With the consumers' demand for diversification of tomato types and for fruits with better flavour, the appreciation for those varieties has significantly increased. In fact, researchers and breeders have recognized these local varieties as important genetic resources, as they are often associated to a better flavour (“taste of the past”) and to a higher content in bioactive compounds compared to commercial varieties (Brugarolas et al., 2009; D’Angelo et al., 2018; Figàs et al., 2015; Meza et al., 2020).



Figure 4. Display of the existing diversity of fruits of tomato.

Besides the external and internal appearance, organoleptic quality includes flavour, which basically consists in the combination of taste, aroma and texture. Taste is mainly given by the balance between sweetness and sourness or acidity, while a wide range of volatile compounds is responsible for the aroma (Carli et al., 2011; Kader, 2008; Martina et al., 2021; Tieman et al., 2017). Improving flavour is currently one of the main objectives in tomato breeding (Figure 5). The first studies on this topic in tomato focused on tomato taste, and found a highly positive correlation between overall satisfaction and balanced sugars to organic acids ratio (Baldwin et al., 1998; Malundo et al., 1995). Thus, increasing contents of both compounds would be preferable as breeding goals. In this context, the major carbohydrates in tomato are fructose and glucose, which are usually present at 1:1 ratio, while the major organic acid in tomato is citric acid, followed by malic acid (Beckles, 2012). However, other compounds involved in tomato flavour have

been gaining interest in recent years. On the one hand, free amino acids glutamic acid, and aspartic acid, the latter at a lesser extent, play a role in taste-enhancement, being responsible for the ‘umami’ taste (Casals et al., 2019b, 2015; Lioe et al., 2010). Therefore, there is interest in increasing their content, not only in terms of taste improvement but also because glutamic acid acts as a precursor of GABA (γ -aminobutyric acid), which has been associated with relaxation, stress reduction, pain relief and improved sleep in humans (Gramazio et al., 2020b) (Figure 5). Although tomato has been reported to accumulate several-fold higher content of glutamic acid than other vegetables such as onion, pepper, or carrot (Haytowitz et al., 2011), the existing variability in tomato collections has not been yet extensively evaluated for the accumulation of those compounds, which would enable to find new materials to improve both the organoleptic and nutritional quality. On the other hand, more than 400 volatile compounds (VOCs) have been reported in tomato (Baldwin et al., 2000; Martina et al., 2021; Tieman et al., 2017). VOCs, and their interaction with non-volatile taste-related chemicals, are essential to the perception of tomato aroma and flavour (Tieman et al., 2017) (Figure 5). The biggest challenge for breeders and researchers has lied in detecting which individual volatiles correlated to the like or dislike of consumers, in order to select specific key breeding targets. In this way, a recent 5-year study, in which 150 different tomato varieties were evaluated by a 100-people consumer panel, resulted in the identification of 29 major volatiles positively or negatively involved in general liking (Tieman et al., 2017). Furthermore, the current availability of several tomato genome sequences and other relevant genomic tools, together with the development of sensitive metabolomic methods of analysis, has led to a significant progress in understanding the genetic basis underlying the synthesis of VOCs, which is a very useful tool for efficient improvement of tomato flavour (Bauchet et al., 2017; Martina et al., 2021; Saliba-Colombani et al., 2001; Tieman et al., 2017, 2006).

Another major objective in tomato breeding is the improvement of nutritional quality. Tomatoes do not stand out among fruits and vegetables for having exceptional nutritional characteristics; however, since it is one of the most consumed vegetables daily by large part of the world population, this component acquires great importance in tomato breeding programs. In this context, tomatoes are the main dietary source of lycopene, which is a carotenoid pigment responsible for the tomato red colour of ripe fruits (Siddiqui et al., 2015), but which has also shown ROS scavenging capacity (Kelkel et al., 2011). Other relevant carotenoid, β -carotene, is also present in tomato fruits, although at much lower concentrations than lycopene (Cortés-Olmos et al., 2014) (Figure 5). Both carotenoids have demonstrated to play a role in prevention of several types of cancer, cardiovascular diseases and photosensitivity disorders (Das et al., 2005; Eggersdorfer and Wyss, 2018; Ford et al., 2011; Imran et al., 2020; Jeong et al., 2019; Stahl and Sies, 2012). In addition, β -carotene constitutes the precursor of vitamin A, which is an essential component for a healthy vision (Weber and Grune, 2012). Tomato is also considered a rich source of vitamin C (ascorbic and dehydroascorbic acid) (Figure

5), insofar as a serving size (200 g) of fresh tomato usually contributes to around 30% of the daily Dietary Reference Intake (DRI) value (García-Closas et al., 2004; Institute of Medicine, 2006). Humans lack the ability to synthesize vitamin C, thus we only get it through our diet. Serious deficiency of this acid could cause the fatal disease scurvy (Padayatty and Levine, 2016), which makes vitamin C an essential nutrient. In addition to its primary goal in the human body, vitamin C can also act as a potent antioxidant and its beneficial effects on human health have been broadly studied (Carr and Maggini, 2017; Granger and Eck, 2018; Lee et al., 2019; Pullar et al., 2017). In this way, suboptimal concentrations of vitamin C in plasma were associated to higher risk of suffering cardiovascular diseases (Langlois et al., 2001; Moser and Chun, 2016; Odermarsky et al., 2009). Lastly, phenolics are also gaining attention in tomato. The major phenolic compounds present in the tomato peel are the flavonoids naringenin chalcone and rutin (quercetin-3-*O*-rutinoside) (Slimestad et al., 2008; Slimestad and Verheul, 2009) (Figure 5). Although at a lower concentration, other phenolics commonly found in tomato are chlorogenic and caffeic acids and kaempferol (Chassy et al., 2006; García-Valverde et al., 2013; Hallmann, 2012; Martínez-Valverde et al., 2002). Numerous health benefits have been linked to those phenolics (Dabeek and Marra, 2019; Frutos et al., 2019; Sato et al., 2011; Zeng et al., 2018). For instance, naringenin chalcone showed *in vitro* anti-allergic activity in a histamine-release assay (Yamamoto et al., 2004), while rutin and the hydrolysed quercetin have demonstrated a potential neuroprotective role (Khan et al., 2019; Wang et al., 2012).

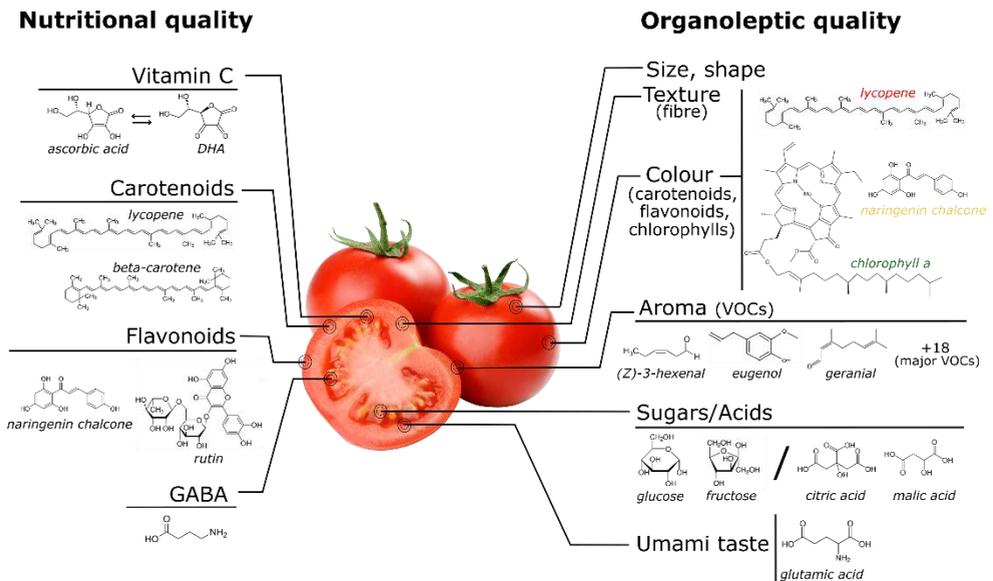


Figure 5. Main breeding targets and most relevant metabolites involved in the organoleptic and nutritional quality of tomato. DHA, dehydroascorbic acid; VOCs, volatile organic compounds; GABA, γ -aminobutyric acid.

In the last decades, many studies have found large variation for all mentioned nutritional and health-promoting compounds, among and within several cultivar groups of tomato. In this respect, in a recent study, Coyago-Cruz et al. (2019) found average contents in lycopene between 47 and 1171 mg kg⁻¹ on a dry weight basis among red tomatoes from different cultivars. Figàs et al. (2015) detected genotypic coefficients of variation of 25%, 18%, 11% and 15%, and phenotypic coefficients of variation of 64%, 44%, 19% and 24% for lycopene, β -carotene, ascorbic acid and total phenolics, respectively, in a collection of 69 accessions from eight different cultivar groups. The strong environmental influence on nutritional and organoleptic compounds has been extensively studied in tomato (e.g., Araya et al., 2021; Carli et al., 2011; Cebolla-Cornejo et al., 2011; Figàs et al., 2018; Iglesias et al., 2015; Martí et al., 2018; Wei et al., 2018). In this way, a significant interaction has been reported between 42 tomato genotypes and three different locations, with lycopene being the trait most influenced by environment, while titratable acidity was the least influenced (Panthee et al., 2012). Similarly, significant effects of location and production system, as well as their interaction with genotype were detected for the accumulation of β -carotene and some phenolic compounds in fruits of the local variety 'Rosa de Barbastro' (Asensio et al., 2019). More genotype \times environment interaction studies are necessary to evaluate the capacity of developing tomato varieties for multi-environment cultivation or for specific target environments (Roselló et al., 2011).

Since the end of the 20th century, the interest in the presence of health-promoting anthocyanins in fruits and vegetables has increased among consumers. Anthocyanins are pigments responsible for the purple colour, thus purple or black fruits are often associated to healthier properties by the consumer. There are nowadays purple fruit tomato varieties on the market, which are highly appreciated for having higher nutritional content (Blando et al., 2019). Due to the lack of the ability of synthesizing and accumulating anthocyanins of cultivated tomato, the variability for this trait was introgressed from crop wild relative species (CWRs) or using genetic engineering methods such as transgenesis. In this way, the light intensity-induced anthocyanin synthesis, due to the action of the dominant gene *Anthocyanin fruit* (*Aft*), was introgressed by an interspecific cross between cultivated tomato and *S. chilense* (Dunal) Reiche (Jones et al., 2003; Mes et al., 2008). Moreover, another recessive gene, *atrorosea* (*atr*), responsible of the strong stimulation of anthocyanin production in the entire plant, was introgressed from *S. cheesmaniae* (L. Riley) Fosberg (Maligeppagol et al., 2013; Mes et al., 2008; Povero et al., 2011). In this context, many authors have underlined the advantages of using CWRs, not only for the introgression of biotic and abiotic resistances or tolerances, but also of superior alleles of genes controlling the synthesis of health-promoting secondary metabolites, such as phenolics (Prohens et al., 2017). On the other hand, Butelli et al., (2008) reported the development of a transgenic purple tomato with enhanced levels of anthocyanins. The authors also showed an increased lifespan by 28% of cancer-susceptible mice fed with a diet

supplemented with those purple tomatoes. Despite being a more efficient breeding strategy, the use of genetic engineering for the development of new varieties for human consumption is currently banned in Europe and other regions of the world.

Finally, the early availability of numerous genomic tools in tomato, such as several genome sequences with annotated genes, a pan-genome, genetic maps, several intra- and interspecific mapping populations, a well-characterized monogenic mutant library, genome editing tools or gene expression databases (100 Tomato Genome Sequencing Consortium, 2014; Bostan and Chiusano, 2015; Eshed and Zamir, 1995; Fulton et al., 2000; Gao et al., 2019; Gramazio et al., 2020a; Menda et al., 2004; Monforte and Tanksley, 2000; Pascual et al., 2015; Suresh et al., 2014; The Tomato Genome Consortium, 2012; Tieman et al., 2017; Zhao et al., 2019), allowed a rapid advance in the knowledge of the genetic basis of the biosynthetic pathways of most of the mentioned metabolites, which is very useful for an efficient improvement of this widely important vegetable. Furthermore, the advanced knowledge of the tomato genetics has facilitated research studies in eggplant and pepper, as well as the comprehension of the genome evolution in the *Solanaceae* family, through synteny studies (Rinaldi et al., 2016; Wu et al., 2009).

3. Breeding for fruit quality in eggplant

Eggplants, also called aubergines or brinjals, produce white-fleshy fruits with a firm glossy edible peel. The external appearance of fruits is very variable (Figure 6). Depending on the variety they can show different sizes and shapes, from round to ovoid to elongated (Mangino et al., 2021; Rubatzky and Yamaguchi, 1997). The peel colour can be mainly green, purple, black or white at commercial ripeness, according to the presence of chlorophylls, anthocyanins, both or neither, respectively, and may also present a patterned mix of those colours (stripped, mottled) (Daunay et al., 2004) (Figure 7). Eggplants are consumed in many countries worldwide, but have been traditionally essential in the Mediterranean and Southeast Asian cuisine. Fruits are harvested and consumed at immature state, when seeds are not completely formed yet, and they are usually cooked, due to the bitter, astringent taste of raw eggplant (Chapman, 2019).

Breeding for eggplant appearance, as for tomato, would depend on the market preferences. In this way, many authors divided eggplant varieties into “Oriental” (or Asian) and “Occidental” (or Western) types, names that fit their main cultivation areas, showing particularly distinctive phenotype for morphological traits as well as different genetic background (Cericola et al., 2013; Hurtado et al., 2012; Vilanova et al., 2012). Whereas “Oriental” eggplants are usually slim, cylindrical, elongated fruits with thinner skin and lighter purple colour, most commercialised “Occidental” cultivars produce thicker fruits, especially at the bottom, adopting a teardrop or egg shape, with black or purple-white striped colours (Muñoz-Falcón et al., 2009, 2008) (Figure 6). Notwithstanding, a large diversity exists among eggplants for fruit appearance, which

can be found in some cultivars but mainly “hidden” in local varieties (Figure 6). With the increasing consumers’ demand for a diversification of eggplant types, some new cultivars are breaking through, like the white or the pickling eggplants (Prohens et al., 2003) (Figure 6).



Figure 6. Display of the existing diversity of fruits of eggplant.

As for eggplant internal appearance, a major breeding goal has been reducing flesh browning (Figure 7). This process is mainly the result of the action of polyphenol oxidase enzymes (PPOs), which are stored in the vacuoles and released when the eggplant is cut and cellular compartmentation destroyed, catalysing the oxidation of phenolic compounds accumulated in the cytosol, into quinones, which in turn in contact with oxygen form brown compounds that are visually unpleasant (Docimo et al., 2016; Mishra et al., 2013; Prohens et al., 2007). Since the increase of phenolics in eggplant is also an important target in breeding for nutritional quality due to their proven health-promoting capacity, it is therefore essential for breeders to find a good balance between phenolic content and low flesh browning when developing new varieties. Correlation studies between phenolic content or PPO activity and flesh browning have found a moderately positive link, suggesting the involvement of other physiological factors in this process (Docimo et al., 2016; Mishra et al., 2013; Plazas et al., 2013; Prohens et al., 2007). In that way, increasing the content in some organic acids has been reported to decrease browning (Zhou et al., 2020). Reduced-browning in apple has also been recently associated to the activity of antioxidant enzymatic complexes (T. Tang et al.,

2020), providing new insight into fruit browning and new interesting targets that could be further investigated for internal quality improvement in eggplant.

Most common commercialised eggplants usually have a mild or slightly bitter taste with soft, creamy texture once cooked. However, the existing variability for the texture, sugar, acid and especially the volatile profiles has been rarely explored in eggplant, because enhancing flavour is not a primary breeding objective for this vegetable. However, greater effort in the study of this diversity would be very interesting when developing and releasing new types of eggplant varieties or new eggplant-derived food products (Mieles-Gómez et al., 2021).

The presence of nitrogen-containing secondary metabolites, namely glycoalkaloids and saponins, are responsible for the bitter taste of the fruit flesh (Figure 7). Glycoalkaloids are compounds commonly found in the *Solanaceae* family. The solasodine derivatives α -solamargine and α -solasoinine are the major glycoalkaloids in eggplant fruits (Friedman, 2015). They are considered anti-nutritional compounds due to their toxic effect when ingested by humans above certain level, which has been internationally accepted at 200 mg kg⁻¹ fresh weight (fw) (OECD, 2020). In this regard, cultivated eggplant has been found to show much lower glycoalkaloid content than the global safety limit, with reported average values of 7.5-55.6 mg kg⁻¹ fw (Sánchez-Mata et al., 2010) However, this content needs to be monitored when releasing new varieties to the market, especially when wild species, with reported higher glycoalkaloid concentration, are incorporated into breeding pipelines (Mennella et al., 2012, 2010). On the other hand, glycoalkaloids are gaining interest for their use in pharmacology, especially as new anticancer drugs, since they have shown significant growth inhibition ability of several human cancer cell lines (Ding et al., 2012; Liang et al., 2004).

Regarding nutritional quality, eggplant is known to be a good source of fibre, minerals, mainly potassium, and antioxidants (Ayaz et al., 2015; Luthria et al., 2010; Morales-Soto et al., 2014; Plazas et al., 2013; Raigón et al., 2010, 2008; San José et al., 2014, 2013; Singh et al., 2017, 2009) (Figure 7). With the increasing consumers' demand for functional foods, the interest in health benefits of eggplant consumption has burgeoned among researchers and breeders because of its high content in phenolic compounds (Figure 7). In this way, chlorogenic acid in the fruit flesh is the major contributor to the antioxidant activity, and to total phenolics in eggplant by more than 70% (Prohens et al., 2013; Singh et al., 2009). The beneficial effects on human health of chlorogenic acid have been widely reported in the literature, as it showed *in vitro* and *in vivo* antioxidant, anti-inflammatory and anti-microbial activity (Chen et al., 2018; Liu et al., 2019; Sato et al., 2011). In addition, associated to these biological activities, chlorogenic acid has demonstrated potential anti-obesity, anti-diabetic, anti-carcinogenic, neuroprotective and cardioprotective properties (Heitman and Ingram, 2017; Huang et al., 2015; Ong et al., 2013; Tian et al., 2019; Zeng et al., 2021). Besides chlorogenic acid, anthocyanins in the fruit peel, mainly delphinidin-3-rutinoside, are the second most important

phenolic compound in eggplant (Singh et al., 2017; Toppino et al., 2016) (Figure 7). Anthocyanins have also shown strong antioxidant activity (Tena et al., 2020), and several studies underlined their potential health-promoting effect preventing or reducing chronic and degenerative diseases (Alvarez-Suarez et al., 2014; Jang et al., 2010; Li et al., 2015; Wu et al., 2013). The development of new varieties with enhanced antioxidant activity, linked to increased phenolic content has thus become an overriding goal in eggplant breeding. Several studies in the last decades have demonstrated that a wide intraspecific diversity exists for phenolic content, thus proving that there is still room for improvement. In this way, ranges of variation from 1.6-fold up to 6-fold on average were found for chlorogenic acid among different varieties of cultivated eggplant in various studies (Luthria, 2012; Luthria et al., 2010; Plazas et al., 2013; Singh et al., 2009; Stommel and Whitaker, 2003; Whitaker and Stommel, 2003). In addition, the use of crop wild relatives (CWR) has been reported as a potential strategy for increasing phenolic content in eggplant (Prohens et al., 2017, 2013). Efforts have been made towards the evaluation of chlorogenic acid and total phenolics in several eggplant wild relatives of the primary, secondary and tertiary genepool (Kaushik et al., 2017; Mennella et al., 2012, 2010; Plazas et al., 2016; Prohens et al., 2013; Villanueva et al., 2021), which confirmed the higher phenolic concentration in their fruits compared to cultivated eggplant.

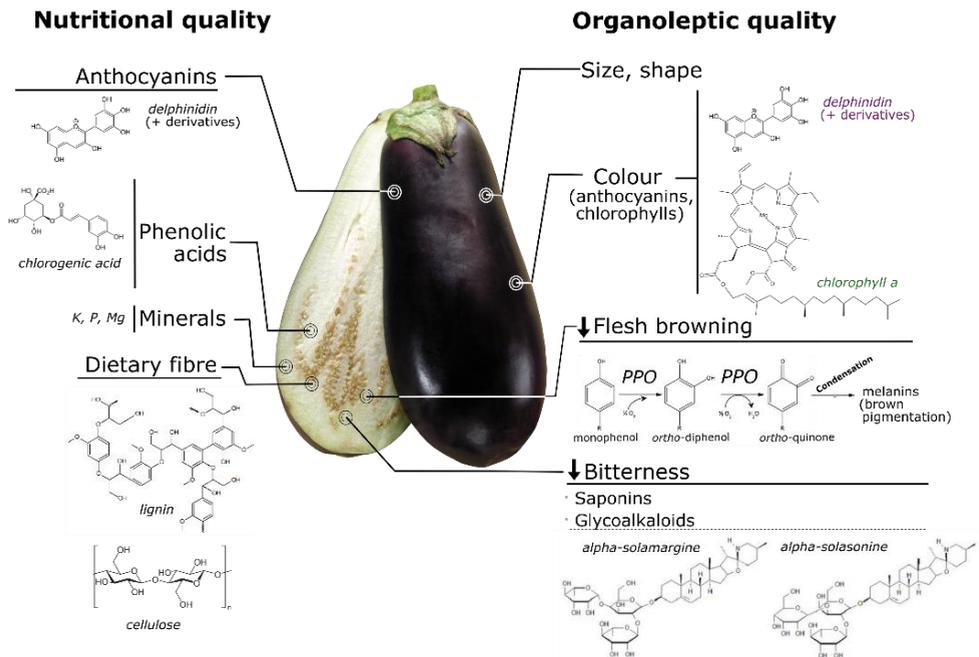


Figure 7. Main breeding targets and most relevant metabolites involved in the organoleptic and nutritional quality of eggplant. PPO, polyphenol oxidase.

Breeding for quantitative traits, such as phenolic content, is a difficult task, due to their polygenic control and the strong environmental influence (Gramazio et al., 2014; Stommel et al., 2015). In this context, some studies have reported important variation in phenolics content along with the eggplant maturity stage (Mennella et al., 2012), year (Raigón et al., 2010), location (Stommel et al., 2015; Toppino et al., 2016) and agricultural practices (Raigón et al., 2010). This variation could be very interesting for the development of new varieties for their cultivation in places with specific agroclimatic conditions, and for their adaptation to a future more sustainable agriculture in a context of climate change. Therefore, more studies evaluating genotype × environment interaction are essential to select the best individuals under certain conditions, as well as to estimate the response to selection if these conditions were to vary.

The availability of diversity studies coupled with the development of genomic tools in the last decades, such as mapping populations, elite materials, molecular markers associated to quantitative trait loci (QTLs) and candidate genes of traits of interest (Barchi et al., 2019; Docimo et al., 2016; Doganlar et al., 2002; Gramazio et al., 2017, 2014; Li et al., 2019; Sulli et al., 2021; Toppino et al., 2016; Wei et al., 2020), constitute a set of useful resources for rapid and efficient breeding of eggplant quality. In this sense, the genes encoding key enzymes of both chlorogenic acid and anthocyanin synthesis pathway, as well as genes encoding different polyphenol oxidases, have already been described and mapped in the eggplant genome (Barchi et al., 2019; Gramazio et al., 2014). However, further studies are necessary for the development of molecular markers linked to genetic elements controlling the traits of interest.

4. Breeding for fruit quality in pepper

Pepper, ‘chile’ or ‘aji’ are terms that refer to distinct forms within the *Capsicum* genus. Although there are five cultivated species in the world, the most popular among occidental consumers and the most economically important is *Capsicum annuum* L. Consistently, this species has largely monopolised the efforts of researchers and breeders. The fruits of pepper are ubiquitous to many cultures and used in countless dishes worldwide under two main culinary formats: as fresh vegetable or as a spice. As in the case of tomatoes, this differentiation will lead to distinct objectives in pepper breeding in terms of fruit quality. In the present thesis we are focusing on fresh market peppers.

Nowadays, uniform, highly-productive, and resistance-carrying F₁ hybrids of the ‘California’, ‘Lamuyo’ and ‘Italian Sweet’ types comprise most of occidental pepper production, including Spain (Figure 8). In that regard, Spain markets generally prefer large ‘Lamuyo’ type fruits and not necessarily uniform. Strikingly, this drastically contrasts with the fact that Spain is a secondary centre of diversity for pepper, as a result of its introduction during the Discovery Era that led to the creation of a bunch of

landraces with different shapes, sizes, colours, flavours and forms of consumption that can still be found today in different regions of the country (Figure 8), including numerous EU Protected Designations of Origin (PDOs) and Protected Geographical Indications (PGIs) such as ‘Pimiento del Piquillo de Lodosa’, ‘Pimiento asado del Bierzo’, ‘Pemento de Herbón’ or ‘Pimiento Riojano’ (MAPA, 2020; Rodríguez-Burruezo et al., 2016). Thus, there is a strong preference among Spanish consumers for these local or traditional varieties of pepper. Pepper fruits can be consumed mature or immature. In this way, some varieties have been selected for only one of these forms of consumption. This is, for instance, the case of the variety ‘Pimiento de Padrón’, whose fruits are only consumed at immature green state (López-Hernández et al., 1996). In terms of flavour, mature red peppers are known for their sweet taste, while green peppers have a grassier taste.



Figure 8. Display of the existing diversity of fruits of pepper.

Internal quality, including sugars and organic acid contents as taste-related factors, have not been a primary goal in programs for improving pepper quality. Instead, pungency and, more recently, the profile of volatile compounds (VOCs) have been the dominant flavour-related factors in research and breeding (Lopez-Ortiz et al., 2020; Moreno-Peris et al., 2020; B. Sun et al., 2020; Taiti et al., 2019; Yoo et al., 2019) (Figure 9). Pungency results from the capsaicinoids accumulation in the fruit placenta, which are a group of more than 20 secondary metabolites unique to the genus *Capsicum* (de Sá Mendes and Branco de Andrade Gonçalves, 2020; Kollmannsberger et al., 2011; Naves et al., 2019). For this specific trait, consumer preferences are divided. In this way, in Spain and in most occidental cultures, the hot flavour deters most consumers from eating peppers,

thus sweet varieties have become the most popular. Even so, some breeding programs were aimed at developing sweet forms out of traditionally pungent types, such as ‘NuMex Sweet’ and ‘NuMex Garnet’ cultivated in South USA (Srivastava and Mangal, 2019). On the other hand, in Southeast Asia and in the American continent, pungent varieties are extremely appreciated and high-capsaicin varieties are a constant pursue in breeding. The ‘Hot Wax’, ‘Chile poblano’ and ‘Chile jalapeño’ are some of those. Furthermore, capsaicinoids are currently gaining relevance in the nutraceutical quality of pepper as they have showed anti-inflammatory (Ghiasi et al., 2019), pain-relief (Derry et al., 2017), anticarcinogenic (Clark and Lee, 2016), and weight-loss properties (Tremblay et al., 2016; Whiting et al., 2014). Despite their important role in pepper aroma and flavour intensity, the volatile fraction of fruits has only been intensively explored during the last decade. In this period, more than 300 VOCs have been reported, although only for a small set of varieties within the cultivated genepool (Bianchi and Scalzo, 2018; Kollmannsberger et al., 2011; Moreno et al., 2012; Rodríguez-Burruezo et al., 2010). The pepper volatile fraction has been found to be highly dependent on the species, genotype, environmental conditions and ripening stage (Bianchi and Scalzo, 2018; Eggink et al., 2012; Kim et al., 2020; Lo Scalzo et al., 2020; Moreno et al., 2012; Parisi et al., 2017; Pino et al., 2006; Yoo et al., 2019). This great variation observed among the studied materials along with new insights into the volatiles mode of inheritance are paving the way for developing new pepper varieties with both new aromas combinations and intensities (Moreno-Peris et al., 2020).

Peppers have a high nutritional value and their consumption has been associated to a multitude of health benefits (Bonaccio et al., 2019; de Sá Mendes and Branco de Andrade Gonçalves, 2020; Hassan et al., 2019; Hernández-Pérez et al., 2020; Hervert-Hernández et al., 2011; Mennella et al., 2018; Wahyuni et al., 2013; Yahia et al., 2019). The most relevant, researched and targeted traits in pepper breeding regarding nutritional content are vitamin C, carotenoids and phenolics (Figure 9). The high antioxidant capacity of pepper fruits (Morales-Soto et al., 2014) is mainly due to their outstanding content in vitamin C (Fратиanni et al., 2020; García-Closas et al., 2004; Howard et al., 2000; Sánchez et al., 2018; Wahyuni et al., 2013). In fact, 100 g fruit portion of most of the varieties studied have been reported to exceed the DRI value of this nutrient, ranking well above other fruits and vegetables commonly known for their vitamin C content, such as orange or kiwi (Howard et al., 2000; Wahyuni et al., 2013). A greater branching of the carotenoid synthesis pathway compared to other vegetables and different key structural genes expressed result in the accumulation of a wide range of these compounds in pepper. More than 20 carotenoids have been identified in peppers, among which β -carotene, capsanthin, capsoroubin, lutein and zeaxanthin are the predominant in ripe fruits and collectively contribute to their final colour as well as to their antioxidant capacity (Giuffrida et al., 2013; Hassan et al., 2019; Wahyuni et al., 2013). Finally, fruits of pepper also constitute a good source of phenolic compounds. According to several studies (Chassy et al., 2006; Frатиanni et al., 2020; Morales-Soto

2004; Martínez-Ispizua et al., 2021b; Mennella et al., 2018; Morales-Soto et al., 2013; Ribes-Moya et al., 2020, 2018), providing useful resources for breeding for increased contents. In this context, Denev et al. (2019) found significant differences of up to 60-fold in the flavonoid content among 63 pepper accessions from five cultivar types. Although research has been performed in order to determine the range of variation of nutritional and organoleptic compounds with the ripeness stage (Howard et al., 2000; Kim et al., 2020; Martínez et al., 2005; Navarro et al., 2006; Pino et al., 2006), the environment, and different production systems (Guilherme et al., 2020; Lo Scalzo et al., 2020; Ribes-Moya et al., 2018; Tripodi et al., 2018; Yoo et al., 2019), more efforts would be required in order to better understand the interaction of those factors with the genotype.

Despite its great economic relevance, genetic research in pepper has lagged behind other vegetables such as tomato and eggplant. Nevertheless, the availability of a high-quality reference genome for *C. annuum* (Acquadro et al., 2020; Hulse-Kemp et al., 2018; Kim et al., 2017, 2014) and two other cultivated species of pepper (Qin et al., 2014), genetic maps (Lee, 2019; Tanksley et al., 1988), gene expression analyses (B. Tang et al., 2020; Wahyuni et al., 2014), and highly informative genome-wide molecular markers (Colonna et al., 2019; Lee et al., 2020; Mohan and Paran, 2019; Park et al., 2020; Wahyuni et al., 2014), together with metabolomic analyses and an extensive understanding of the biosynthetic pathways and key enzymes associated to nutritional and bioactive compounds, will boost pepper breeding towards the improvement of fruit quality parameters.

5. Breeding for quality for future agricultural requirements. Towards reducing the use of N-enriched fertilizers

Since the Green Revolution we have seen dramatic advances in agriculture that have enabled sufficient and varied food for a large part of the population, decreasing hunger and malnutrition (Godfray et al., 2010). The exponential increase in crop yields that we have witnessed has been mainly due to the advent and quick widespread of inorganic fertilizers and pesticides and their excessive utilization, coupled with the development of modern cultivars highly responsive to the application of fertilizers (Prohens, 2011). In this way, the agricultural use of the three major nutrients of enriched inorganic fertilizers, nitrogen (N), phosphorus (P) and potassium (K), has experienced a 7.1-fold, 3.0-fold and 2.5-fold increase, respectively, between 1961 and 2000. The trend in the last 20 years has also been on the rise, albeit at a slightly lower level (FAOSTAT, 2019), since governments are increasingly adopting sustainable fertilizer management policies (Vero et al., 2018).

N is the major essential nutrient for the growth and development of plants, as it is part of proteins and nucleic acids (Hawkesford et al., 2012). N-enriched fertilizers are, therefore, the most consumed by growers worldwide. The environmental damage caused

by the overuse of this chemical on lands has been observed for several decades now (Figure 10, 11). Firstly, producing inorganic N-enriched fertilizers consumes about 1.2% of the global primary energy demand through combustion of fossil fuels causing, not only an increase in fertilizer prices, but also a release of greenhouse gases to the atmosphere, contributing to climate change (Figure 10) (Tallaksen et al., 2015). Secondly, about 50-80% of the N supplied to crops is lost in the form of pollutant gases or leached out rapidly to aquifers and lakes where it causes eutrophication and hypoxia (Figure 10) (Martínez-Dalmau et al., 2021; Stevens, 2019; Sylvestre et al., 2019; Thompson et al., 2007).

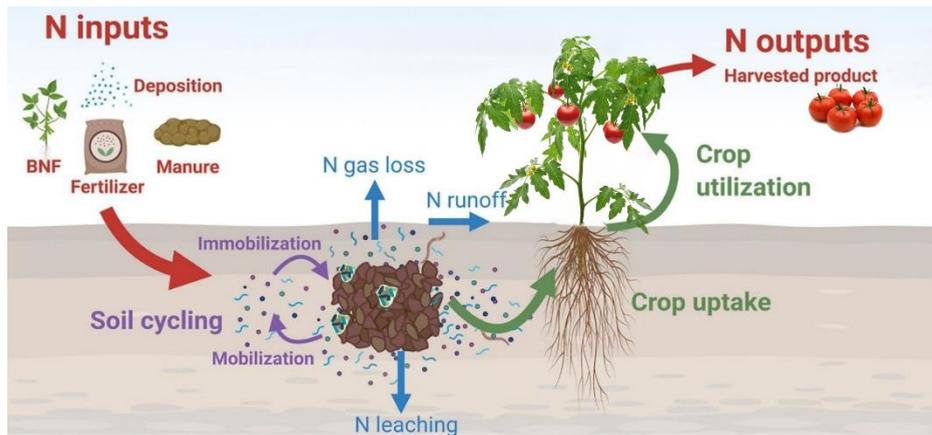


Figure 10. Schematic representation of N cycle as a result of agriculture practices. Blue arrows represent environmental N losses. Adapted from Udvardi et al. (2021).

During the 2012-2015 period, 13.2% of European groundwater stations exceeded the 50 mg nitrates per litre, which was the limit established by the EU in the Council Directive 91/676/EEC (European Commission, 1991) to consider nitrates polluted waters and to be designated as Nitrate Vulnerable Zones (NVZ) (Figure 11). Other associated problems that over-fertilization is causing include greenhouse gas emissions of P-mining, necessary to P-enriched fertilizer production (Cordell et al., 2009; Tilman et al., 2002), loss of biodiversity in flora and fauna of water bodies (Martínez-Dalmau et al., 2021; Stevens, 2019; Sylvestre et al., 2019), soil acidification, mineral depletion (Lv et al., 2020; Song et al., 2016) and even reduced productivity and fruit or crop quality (Albornoz, 2016) (Figure 11). The current environmental situation has prompted research studies to understand the effects of decreasing N inputs on different crops and developing new varieties with improved N use efficiency (Gojon, 2017). Yield, photosynthetic parameters and some quality compounds have been analysed in several crops under different rates of N fertilization (Duran et al., 2016; Fernandez et al., 2020; Fontes et al., 2010; Pérez-Jiménez et al., 2019; Sylvestre et al., 2019; Tosti et al., 2016; Wang and Li, 2004; Zhu et al., 2016). In general terms, N shortage is associated with a limitation of plant growth, photosynthesis rate, and synthesis and accumulation of

bioactive compounds in fruits. Studies agreed that the response was N level and species/cultivar dependent. Among the major *Solanaceae* vegetables, almost all existing studies exploring the variability for N use efficiency (NUE) and the effects in fruit quality of reducing N fertilization have been carried out in tomato (Bénard et al., 2009; De Pascale et al., 2016, 2006; Djidonou et al., 2013; Du et al., 2017; Elia and Conversa, 2012; Hernández et al., 2020; Montemurro et al., 2007; Sainju et al., 2001; Truffault et al., 2019; Wang and Xing, 2017; Wang et al., 2007; Warner et al., 2004), while this knowledge is much scarce in eggplant (Mauceri et al., 2021, 2019; Pal et al., 2002; Radicetti et al., 2016; Villanueva et al., 2021) and pepper (Benincasa et al., 2011; Candido et al., 2009; da Silva et al., 2020; Flores et al., 2007; Van, 2007; Yasuor et al., 2013). Therefore, more information is needed in order to exploit variability for N use efficiency without deteriorating the nutritional and organoleptic quality of fruits of eggplant, tomato and pepper. Within this context, European public funding in the framework of the H2020 call SusCrop-ERA-Net (ID#47) has been assigned to the SOLNUE project, entitled “Tomato and eggplant nitrogen utilization efficiency in Mediterranean environments”, currently underway, in which four research centres from three European countries (Italy, Spain and France) are participating (<https://www.suscrop.eu/projects-first-call/solnue>). The aims of this project are “the identification of high NUE tomato and eggplant genotypes, and the identification of their contrasting physiological and molecular traits, that could be used to provide tools for developing genomic-based breeding strategies”.



Figure 11. Pictures of eutrophication and fish dead from hypoxia as a consequence of excess N fertilization in the ‘Mar Menor’, Murcia, Spain. Pictures downloaded from El País Semanal digital (2021) (left), and La Verdad digital (2021) (right). Authors: Raúl Belinchón (left), © Ediciones EL PAÍS, and Vicente Vicéns (right), © LA VERDAD MULTIMEDIA, S.A.

Within this environmental background, agriculture production systems are also changing towards a more sustainable crop management. The organic farming model is growing in developed countries (Figure 12). In fact, in Spain a new state government department, ‘Ministerio para la Transición Ecológica y el Reto Demográfico’ has been recently created with the aim of mitigating climate change and supporting a more environmentally conscious agricultural production and social model, among other goals. Furthermore, European public funding has been assigned to the BRESOV international

initiative, entitled “Breeding for resilient, efficient and sustainable organic vegetable production”, in which 22 partners from 13 countries are participating (<https://www.bresov.eu>). The aim of this project was “to explore the genetic diversity of three of the economically most significant vegetable crops (broccoli, snap bean and tomato) and to improve the competitiveness of these three crops in an organic and sustainable environment, in order to increase the plants’ tolerance to biotic and abiotic stresses and adapt the varieties to the specific requirements of organic and low-input production processes”.

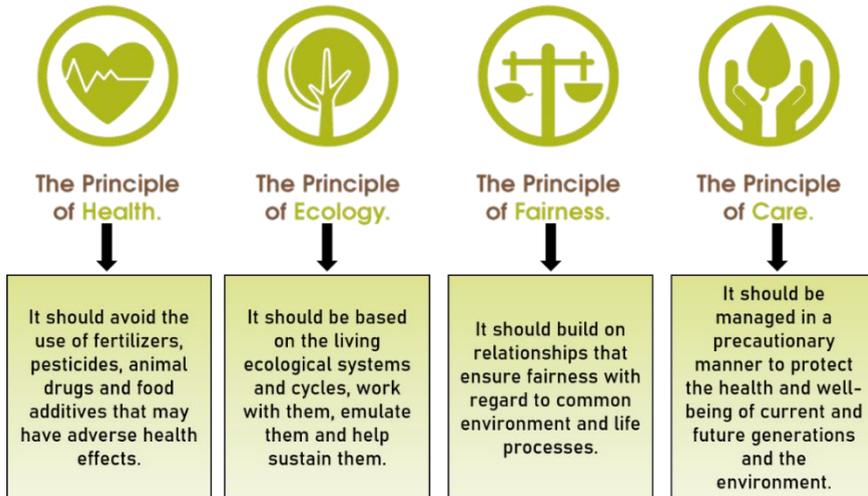


Figure 12. The four principles of organic agriculture, according to IFOAM-Organics International (IFOAM, 2021).

Several studies have arisen in order to compare the effects of organic and conventional farming on important agronomic traits and the organoleptic and nutritional quality of eggplant, tomato and pepper (Chassy et al., 2006; Guilherme et al., 2020; Hallmann, 2012; Hallmann and Rembalkowska, 2012; Lo Scalzo et al., 2020; Luthria et al., 2010; Raigón et al., 2010; Ribes-Moya et al., 2020, 2018; Singh et al., 2017). Although more research is necessary, data analyses seem to indicate that the organic farming system favours a higher accumulation of nutritional and bioactive compounds in the fruits of tomato, eggplant and pepper, albeit sometimes at the cost of lower yields. In addition, some authors suggested that organic cultivation practices might be a better approach than conventional methods for the identification of the best genotypes for yield and quality breeding under limiting N and water conditions (De Pascale et al., 2016).

With such a forward-looking perspective, an exhaustive germplasm screening among cultivated eggplant, tomato and pepper varieties, as well as wild relatives, would be of great importance to identify promising materials for the development of new improved climate-smart varieties adapted to the new agricultural requirements. Linking phenotype

to genotype is also of paramount importance for better understanding the genetic basis of NUE as well as for developing useful molecular tools for an efficient selection and breeding process (Mauceri et al., 2021; Villanueva et al., 2021).

6. Genetic resources for breeding

The existence of variability and their exploitation is the main principle on which plant breeding is based. This is even more relevant in areas where genetic engineering techniques are not legally permitted for the development and marketing of new varieties for human consumption. In this sense, the interest in crop wild relatives (CWRs) and local varieties as a source of variation has greatly increased in recent years for the improvement of nutritional and organoleptic quality in horticultural species, as well as of other important agronomic traits such as the adaptation to climate change and to new production systems for a more sustainable agriculture. The conservation and public access to these resources, which, except in the case of tomato has been limited, is becoming imperative (Barchenger et al., 2019; Syfert et al., 2016).

6.1. Introgression lines as elite materials for breeding

Introgression lines (ILs) consist of bi-parental populations obtained by means of several repeated backcrosses of the hybrid with the recurrent parent, and final steps of selfing in order to bring the introgressed fragment into homozygosity (Figure 13) (Prohens et al., 2017; Zamir, 2001). The immense potential of ILs has been reported in numerous works (Ballester et al., 2016; Mangino et al., 2021, 2020; Naz et al., 2014; Perpiñá et al., 2016; Schauer et al., 2006; Szymański et al., 2020; Tripodi et al., 2020; Zahn et al., 2020). In this way, ILs are a useful and powerful resource for the elucidation of the genetic control of traits of interest, with the advantage over other mapping populations, such as F₂, double haploids or RILs, of minimizing the linkage drag, since each line carries only a small percentage of the introgressed donor genome (Lippman et al., 2007; Pratap et al., 2021). This makes it easier to narrow down the chromosomal region associated to a trait of interest and even find potential candidate genes within this region. Furthermore, ILs can be directly introduced in breeding pipelines as they are elite materials with a cultivated genetic background (Prohens et al., 2017) (Figure 13). Regarding that, the construction of ILs and their use in breeding is of particular interest for the introduction of attractive traits from wild species. CWRs are reservoirs of genetic variation, although their direct use in breeding programs is difficult or simply not possible due to the presence of undesirable traits, such as prickles, anti-nutritional compounds, lower yields, etc. (Bauchet and Causse, 2012; Mennella et al., 2010; Prohens et al., 2017; Zamir, 2001; Zhang et al., 2017). Using ILs with a CWR as a donor parent instead of directly CWRs in breeding programs usually ensure that undesirable traits are not dragged along (Prohens et al., 2017; Zamir, 2001). The new breeding approach consisting of “mass scale development of plant materials and populations with

introgressions from CWRs into the genetic background of crops” has been called ‘introgressomics’ (Prohens et al., 2017).

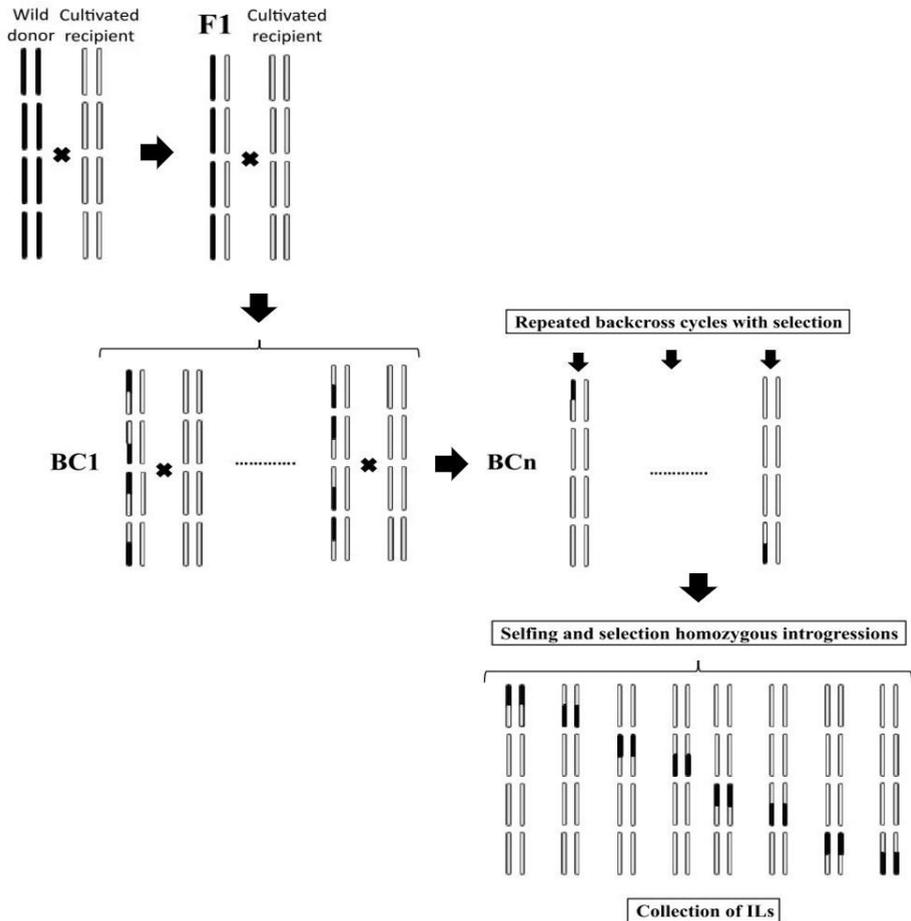


Figure 13. Schematic representation of the development of an introgression line (IL) population. Adapted from Prohens et al. (2017).

Several traits have been introgressed in tomato from their wild relatives, such as different resistance genes from *Solanum peruvianum* L., *Solanum cheesmanii* (L. Riley) Fosberg, *Solanum pennellii* Correll or *Solanum chilense* (Dunal) Reiche, (Lin et al., 2014). In the work of Lin et al. (2014) was also found that, in terms of fruit quality, an introgression from *Solanum habrochaites* S. Knapp & D.M. Spooner on chromosome 1 enhanced soluble solids content in mature fruits of some modern varieties. In addition, several interspecific IL populations have been developed in tomato since mid-90’s (Ballester et al., 2016; Barrantes et al., 2014; Brog et al., 2019; Chetelat et al., 2019; Eshed and Zamir, 1995; Finkers et al., 2007) and used for the identification and mapping of more than 1000 QTLs associated to traits related to organoleptic and nutritional

quality (Alseekh et al., 2015; Ballester et al., 2016; Gürbüz Çolak et al., 2020; Hanson et al., 2014; Ökmen et al., 2011; Tripodi et al., 2020). Among them, probably the most widely used for this purpose has been the IL population from *S. pennelli* (LA716) into the cultivated tomato M82 background (Almeida et al., 2011; Alseekh et al., 2015; Di Matteo et al., 2013; Eshed and Zamir, 1995; Quadrana et al., 2014; Rousseaux et al., 2005; Schauer et al., 2006; Szymański et al., 2020). Some of these ILs have been recognised as potential resources for fruit quality improvement, and even utilised as donors in breeding programs (Dariva et al., 2021; Rigano et al., 2014; Sacco et al., 2013).

Although more recently than tomato, the first eggplant IL population covering the whole genome of a wild relative (*Solanum incanum* L.) was developed by Gramazio et al. (2017) and has already been reported to be useful for improving fruit morphological and important agronomic traits in eggplant (Mangino et al., 2021, 2020). The in-depth study of these ILs for fruit quality traits might lead to the identification of useful materials for breeding, since several eggplant CWRs, including *S. incanum*, have already been reported to present higher levels of phenolics than cultivated eggplant (Kaur et al., 2014; Mennella et al., 2012, 2010; Prohens et al., 2013). Furthermore, the *S. melongena* research and breeding group at the COMAV (Universitat Politècnica de València, Spain) is currently working in the development of three new sets of eggplant ILs using the CWRs *Solanum insanum* L. (primary genepool and wild ancestor of eggplant), *Solanum dasyphyllum* Schumach. & Thonn. (secondary genepool) and *Solanum elaeagnifolium* Cav. (tertiary genepool) as donor parents, and using high-throughput genotyping for marker-assisted selection (García-Forteza et al., 2019; Kouassi et al., 2016; Plazas et al., 2016; Villanueva et al., 2021). These ILs will contribute to broadening the genetic base of eggplant and may be useful materials for the development of improved eggplant cultivars for traits of interest.

The use of CWRs, interspecific hybrids and ILs populations in pepper has been extremely limited, as reviewed by Mongkolporn and Taylor (2011). Besides the need for more characterization studies of the existing variability beyond *C. annuum*, and the overall lack of access to wild *Capsicum* germplasm collections, the main limitation has been the existence of genetic reproductive barriers among different species, only overcome with difficulty through genetic bridge or *in vitro* culture techniques (Manzur et al., 2015; García-Forteza et al., 2020). Nevertheless, progress is being made. Thus, in terms of fruit quality, *Capsicum baccatum* L. has been reported to be a potential source for flavour and aroma improvement (Rodríguez-Burruezo et al., 2009; van Zonneveld et al., 2015). The development, through interspecific crossings combined with embryo rescue, and characterization of a set of BC2S1 plants and near-isogenic lines of *C. baccatum* into *C. annuum* background led to the detection and validation of loci affecting flavour, terpenoids and soluble solids content in the introgressions of *C. baccatum* (Eggink et al., 2014).

6.2. Local varieties as source of variation

Local varieties, landraces, heirlooms or traditional varieties, are gaining relevance as a potential genetic resource for breeding programs aimed to improving adaptation to climate change and sustainable agricultural practices, as well as improving nutritional and organoleptic fruit quality (Dwivedi et al., 2019). On the one hand, local varieties have been traditionally cultivated, selected and maintained by local small-scale farmers under a specific agroclimatic area and usually only using natural inputs, such as rain-fed and manure. Therefore, these materials are more resilient under low-inputs and abiotic stresses such as drought, salinity or flooding (Casañas et al., 2017; Conesa et al., 2020; Suarez et al., 2021). In this way, the evaluation of 165 tomato genotypes under water deficit showed promising results for landraces that may have different mechanisms allowing adaptation to water shortage through increased water use efficiency (Fullana-Pericàs et al., 2019). In addition, two local varieties cultivated in the Mediterranean basin, ‘Negro Yeste’ and ‘Verdal’, showed higher resilience against salt stress compared to the commercial cultivar ‘Moneymaker’, observed through a lower reduction in fruit load. These landraces also showed increased soluble solids content induced by salinity compared to ‘Moneymaker’ (Massaretto et al., 2018).

On the other hand, local varieties of vegetables have been associated to the “taste of the past” (Casals et al., 2011; Causse et al., 2010). In this way, they have been reported to produce more flavourful, nutritious fruits than modern varieties in several studies (Casals et al., 2019a; Celmeli et al., 2018; Cortés-Olmos et al., 2014; Medina-Lozano and Díaz, 2021; Mennella et al., 2018; Meza et al., 2020; Parisi et al., 2017; Raigón et al., 2008; San José et al., 2014, 2013; Sánchez et al., 2018). For instance, the *Almagro* eggplant landrace showed higher values for protein, vitamin C, glucose and total phenolics compared to commercial cultivars in San José et al. (2013). The horn-shaped sweet pepper ‘Friarello’ landrace from Italy has a strong characteristic flavour, thus it has been reported to be a good resource for improving aroma (Parisi et al., 2017).

In Spain, a vast heterogeneity of climatic conditions, soils and horticultural practices coexist through the landscape, which has led to the development of several hundred locally adapted varieties with distinctive characteristics (Rodríguez-Burruezo et al., 2016) (Figure 14). A large part of this wide variability has yet to be explored. In recent years, several research groups have been engaged in a comprehensive characterisation of phenotypic and genetic variability within local varieties of tomato (e.g., Carillo et al., 2019; Casals et al., 2015, 2012; Cortés-Olmos et al., 2014; Csambalik et al., 2017; Figàs et al., 2015; Sumalan et al., 2020), eggplant (e.g., Boyaci et al., 2015; Cericola et al., 2013; Martínez-Ispizua et al., 2021a; Muñoz-Falcón et al., 2009; Prohens et al., 2003; Raigón et al., 2008; Vilanova et al., 2014), and pepper (e.g., Martínez-Ispizua et al., 2021b; Parisi et al., 2017; Pereira-Dias et al., 2020, 2019; Ribes-Moya et al., 2018; Sánchez et al., 2018).

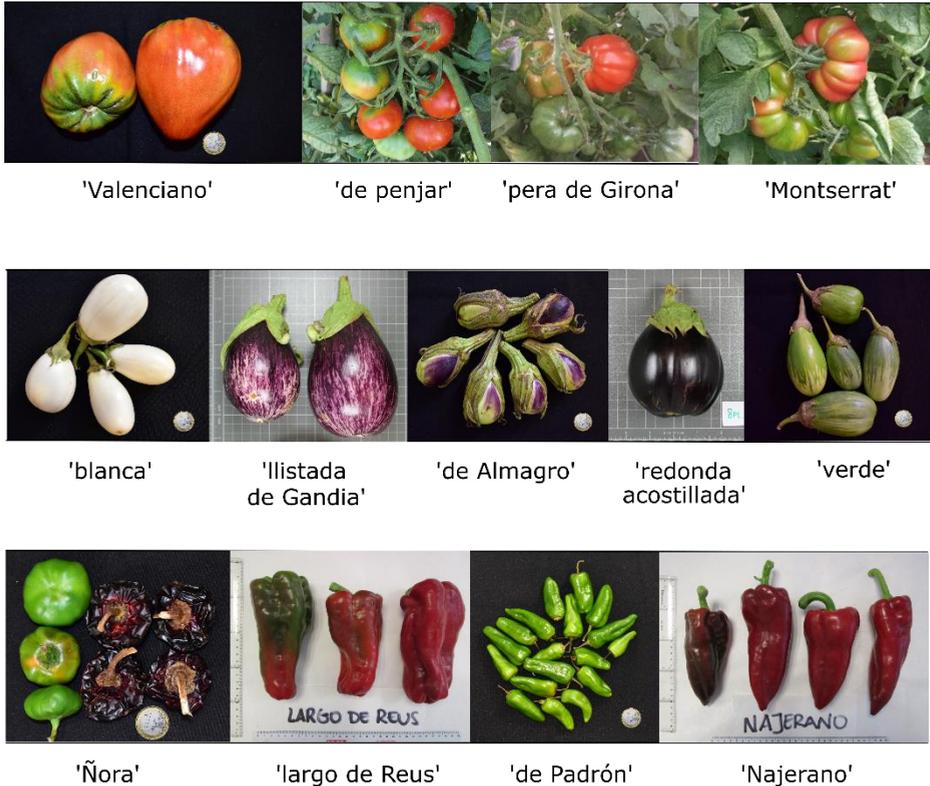


Figure 14. Illustration of traditional Spanish varieties of tomato (first row), eggplant (second row) and pepper (third row). Pictures of ‘pera de Girona’ and ‘Montserrat’ tomatoes were extracted from Soler et al. (2016).

This labour has led to a typification of several landraces, which is a first step for their conservation. Furthermore, several genotypes have been identified as promising materials for the improvement of one or several traits related to fruit quality and harsh environmental conditions. The widely recognised potential of local varieties as a source of variation makes their conservation, maintenance and improvement through participatory approaches essential (Hurtado et al., 2014). Further characterization studies under diverse conditions will be necessary for exploiting their potential.

7. Genetic and genomic tools for breeding: the case of phenolics

Review article

Genetics and breeding of phenolics content in tomato, eggplant and pepper fruits

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Ph.D. candidate contribution

E. R.-M. had a main role in the following activities: literature revision, data compilation, drafting manuscript, manuscript review and editing.

Abstract

Phenolic compounds are currently in the spotlight due to the numerous health benefits associated to a phenolic-rich diet, as well as for having a protective role against plant stresses. Tomato, eggplant and pepper are on the top 10 of most consumed vegetables in the world, and enhancing their phenolic content is of great interest for future breeding approaches. Phenolic acids and flavonoids are the main classes of phenolic compounds. They have been extensively characterised in tomato, eggplant and pepper fruits, which show a substantially different phenolic profile. In the last decades, a broad array of genetic and genomic tools developed for these three species have helped to identify several QTLs and candidate genes associated to the biosynthesis of phenolic acids and flavonoids in tomato, eggplant and pepper. The aim of this review is to synthesize the information in this area of knowledge making it easily available for researchers and breeders. The genetic basis of the relevant group of flavonoids anthocyanins is not discussed herein since it has been thoroughly analysed in recent reviews. We compiled the 191 QTLs found in the literature associated to phenolic acids, flavonoids and total phenolics content, and conducted a search throughout the most recent genome versions of tomato, eggplant and pepper at Sol Genomics Network database (<http://www.solgenomics.net>) in order to identify and locate the annotated putative orthologues encoding for the key enzymes involved in the phenylpropanoid pathway. Furthermore, genes and QTLs associated to the regulatory complexes that tightly control this pathway were discussed herein. Finally, we thoroughly and critically revised the existing literature proving the effectiveness of combining molecular tools and genetic variability through both conventional and genetic engineering strategies. We believe that this review provides vital information to research aiming at improving the content and profile of phenolic acids and flavonoids in tomato, eggplant and pepper.

Keywords: phenylpropanoid pathway, QTLs, structural and regulatory genes, flavonoids, phenolic acids, breeding strategies.

1. Introduction

The *Solanaceae* family includes some of the world most important crops (Olmstead et al., 2008). Tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.) and pepper (*Capsicum annuum* L.) are largely cultivated worldwide for their edible berries, providing a wealth of nutritional and health-promoting compounds to the human diet.

Over the last few decades, we have witnessed remarkable developments in plant breeding, particularly regarding yield, pest and disease resistance, and fruit apparent quality. However, traits like flavour and nutritional quality have been neglected for the sake of higher yields and longer shelf-life (Tieman et al., 2017). With the increasing amount of data linking a lower risk of disease incidence to a regular consumption of vegetables, consumer preferences are shifting towards healthier and tastier foods.

In this context, plant secondary metabolites have gained attention due to their bioactive role in the human body (Kaushik et al., 2016; Pott et al., 2019). Phenolic compounds are a large and diverse group of secondary metabolites ubiquitous to the plant kingdom. Characteristically, they share a phenol (aromatic) ring backbone with one or several hydroxyl groups or other substitutes such as sugar molecules or organic acids (Vogt, 2010). Depending on the number of aromatic rings and on the structural elements that bind these rings together, phenolic compounds may fall into one of several classes: phenolic acids, flavonoids, tannins, stilbenes, lignans, coumarins, chromones and xanthenes (Pott et al., 2019; Vogt, 2010). This myriad of compounds is synthesised through the shikimate and phenylpropanoid pathways and through the action of a complex network of oxygenases, ligases, oxidoreductases, and transferases (Vogt, 2010).

Herein we focused our efforts on two major groups, phenolic acids and flavonoids, as they are the most relevant and the most studied in fruits of tomato, eggplant and pepper.

Why breeding for higher levels of phenolic acids and flavonoids?

Phenolic compounds are of paramount importance to an array of plant biological processes and to the plant-environment interaction, such as protection against solar radiation, pathogens, herbivores, mechanical damage and wounding, attracting pollinators and seed dispersers, and abiotic stress signalling (Dong and Lin, 2021; Rutley et al., 2021; Szymański et al., 2020). In this way, the increased accumulation of these compounds in plants can have a positive effect on both its resilience and its development (Schijlen et al., 2007; Silva-Navas et al., 2016; Tohge and Fernie, 2016).

In addition, numerous health benefits have been linked to a phenolic-rich diet. These benefits are related to the antioxidant properties of phenolic compounds, which translate into their direct ability of reducing oxidant species, scavenging free radicals, and chelating metal ions (Cosme et al., 2020; Galano et al., 2016). In relation to their

antioxidant properties, phenolics have been reported to show anti-inflammatory, anti-microbial, anti-diabetic, anti-tumoral and cardioprotective activities. Several assays have been conducted in order to evaluate their properties *in vitro*, on cellular models, and on humans. For instance, a major circulating human metabolite of quercetin has been proved to decrease the transcription of genes encoding pro-inflammatory interleukins and enzymes involved in oxidative stress responses, thus reducing the effects of atherosclerosis (Derlindati et al., 2012); gallic acid presented a potent effect on Herpes simplex virus type 1 and parainfluenza type 3 at the therapeutic range of 0.8-0.05 $\mu\text{g ml}^{-1}$ using different cellular models (Özçelik et al., 2011); the flavanones naringin and naringenin have been reported to enhance the expression of the insulin receptor, glucose transporter GLUT4 and adiponectin in type II diabetic rats (Ahmed et al., 2017); caffeic acid has demonstrated anti-proliferative and apoptotic effect on human melanoma, colon, breast, liver, and other types of cancer cells (Pelinson et al., 2019; Santana-Gálvez et al., 2020; Weng and Yen, 2012); luteolin has been proven to prevent ischemia-reperfusion injury by reducing necrosis and apoptosis in rat cardiomyocytes and to induce arterial relaxation (Luo et al., 2017); and, human cohorts have shown that chlorogenic acid (25-400 mg d^{-1}) may significantly reduce blood pressure in mild hypertensive adults (Kajikawa et al., 2019; Mubarak et al., 2012).

Given that tomato, eggplant and pepper are among the 10 most consumed vegetables in the world, enhancing their phenolic content would lead to an indirect promotion of the consumers' health, mainly due to the preventive effect on different chronic, cardiovascular and metabolic diseases while generating more resilient crops.

Which phenolic acids and flavonoids are found in these crops?

Phenolic acids and flavonoids have been extensively characterised in tomato, eggplant and pepper fruits of cultivated varieties and wild relatives. Although these vegetables belong to the same family, their accumulation profile is substantially different (Rosa-Martínez et al., 2021).

In general, tomato accumulates more flavonoids than phenolic acids. The latter are mainly represented by caffeic acid and chlorogenic acid (Hallmann and Rembalkowska, 2012; Luthria et al., 2006; Martí et al., 2018), although gallic, ferulic, *p*-coumaric acids, and other derivatives, have been also identified in variable amounts in both tomato peel and pericarp (Alarcón-Flores et al., 2016; Martí et al., 2018; Rigano et al., 2014). Regarding flavonoids, tomato is known to be a good dietary source of naringenin chalcone and the flavonols rutin (quercetin-rutinoside), kaempferol-rutinoside and quercetin-trisaccharide (Muir et al., 2001; Slimestad et al., 2008), along with many other conjugated forms (Alarcón-Flores et al., 2016; Iijima et al., 2008; Moco et al., 2006). Within the tomato cultivated germplasm, flavonoids are accumulated almost exclusively in the fruit peel, while only trace amounts can be found in the fruit pericarp (Muir et al., 2001; Willits et al., 2005). Naringenin chalcone is the most

abundant flavonoid in tomato, reaching its peak at around 1% of the peel dry weight in ripening tomatoes, while rutin and kaempferol are accumulated at much lower amounts (Muir et al., 2001; Slimestad et al., 2008). Naringenin chalcone is also the pigment responsible for the transient yellow coloration of tomato fruit peel at breaker stage, during the transition from mature green to red ripe fruits (Adato et al., 2009; Ballester et al., 2010).

Eggplant is known to be one of the vegetables with the highest antioxidant capacity. Morales-Soto et al., 2014 ranked it among the top 5 fruits and vegetables out of 44 evaluated, in terms of total antioxidant capacity. Eggplant antioxidant capacity is empirically attributed to the phenolic acid profile in the fruit flesh, and especially to its high chlorogenic acid content, but also to the presence of anthocyanins in the purple fruit peel (Docimo et al., 2016; Rosa-Martínez et al., 2021). Some have also reported the presence of flavonol derivatives in the eggplant fruit peel. In this way, Singh et al. (2017) identified 11 flavonols, including different glycosides of quercetin, kaempferol and myricetin. Docimo et al. (2016) and Sulli et al. (2021) also quantified rutin in the fruit peel of a RIL population. However, usually present in trace amounts. The first comprehensive characterization of the eggplant phenolic acid profile was reported by Whitaker and Stommel (2003). The authors identified and quantified fourteen major hydroxycinnamic acid conjugates in the fruits of seven commercial cultivars. All the compounds identified were esters of caffeic acid with quinic acid and derivatives. Since then, others have evaluated the phenolic acids profile in fruits of cultivated and wild eggplant (Mennella et al., 2012, 2010; Mori et al., 2013; Plazas et al., 2014; Prohens et al., 2012; Singh et al., 2009; Stommel et al., 2015; Sunseri et al., 2010). All demonstrated that chlorogenic acid is the most abundant phenolic in eggplant fruits. Indeed, within the cultivated pool, it typically represents 80-95% of the total phenolic acids present in the fruit flesh.

Pepper has also been reported to have high antioxidant capacity, mainly due to its outstanding content in vitamin C (Morales-Soto et al., 2014). Nevertheless, phenolics of all classes have been identified and quantified in different varieties of pepper. Recently, Lemos et al. (2019) reviewed the data regarding pepper phenolic content and compiled it in a publication, making it readily accessible. Among hydroxybenzoic acids, the major compounds reported in pepper were gallic and vanillic acids (Mokhtar et al., 2015; Moreno-Ramírez et al., 2018; Mudrić et al., 2017) and the most abundant hydroxycinnamic acid was generally chlorogenic acid (Fратиanni et al., 2020; Hallmann and Rembialkowska, 2012), although *p*-coumaric, caffeic, ferulic, sinapic acids and several glycosides were also quantifiable in some works (Fратиanni et al., 2020; Marín et al., 2004; Mudrić et al., 2017). Regarding flavonoids, the most represented subfamilies in pepper fruits are flavonols and flavones. Within the first group, quercetin and its glycosides are the most common, followed by myricetin and kaempferol, while within flavones, luteolin and its derivatives are the most representative, followed by

apigenin and its derivatives (Chen and Kang, 2013; Fratianni et al., 2020; Hallmann and Rembialkowska, 2012; Jeong et al., 2011; Mokhtar et al., 2015; Morales-Soto et al., 2013; Ribes-Moya et al., 2020; Wahyuni et al., 2011). Another worth-mentioning group of health-promoting flavonoids usually found in pepper fruit is the flavan-3-ols, represented by catechin and epicatechi (Chen and Kang, 2013).

Genetic and genomic resources impact in phenolic acids and flavonoids studies

Recent advances in omics approaches and technologies have enhanced the knowledge regarding the genetics of complex traits like fruit quality. Notwithstanding, not all crops have the same resources available. Due to its diploid genome, short generation time, autogamy, routine transformation technology, fleshy fruits, compound leaves and sympodial shoot branching, tomato has been a model organism for basic and applied plant research for many years, especially a model for other *Solanaceae*. Consequently, numerous genetic and genomic tools have been developed for tomato since the late 80's, such as isogenic mutant libraries (Menda et al., 2004) and several intra and interspecific mapping populations (Eshed and Zamir, 1995; Fulton et al., 2000; Monforte and Tanksley, 2000; Pascual et al., 2015). The tomato reference genome sequence was first published in 2012 for the *S. lycopersicum* 'Heinz 1706' cultivar (The Tomato Genome Consortium, 2012) and has since then been re-sequenced, corrected and re-annotated to the current SL4.0 version with the ITAG4.0 annotation, which is available in the SolGenomics Network database (<https://solgenomics.net/>). Furthermore, more than 900 high-quality genome sequences of cultivated tomato and its wild relatives (Aflitos et al., 2014; Gramazio et al., 2020; Tieman et al., 2017), including a recently published pan-genome using 725 representative tomato accessions (Gao et al., 2019), have since been released, paving the way for numerous genome-wide association studies (Rothan et al., 2019).

In eggplant, studies devoted to the development of genomic tools started considerably later than in other major *Solanaceae*. However, in the last years, the available genomic information of eggplant has increased dramatically. Several factors have made this possible: (1) the development of the next-generation sequencing technologies in the first decade of the twenty-first century (Gebhardt, 2016); (2) the well-established synteny between the eggplant and tomato genomes (Barchi et al., 2019; Doganlar et al., 2002; Gramazio et al., 2014; Rinaldi et al., 2016; Wu et al., 2009); and, (3) the availability of recently developed intra and interspecific mapping populations with associated genetic linkage maps (Doganlar et al., 2002; Gramazio et al., 2014). The first point led to increasingly affordable whole-genome sequencing and in those terms, Hirakawa et al., 2014 published the first draft genome sequence using a common Asian eggplant cultivar. Moreover, in the last three years several high-quality eggplant genome assemblies with substantial increases in annotated genes have been released (Barchi et al., 2021; Li et al., 2021; Wei et al., 2020).

Despite its economic relevance, pepper remains a surprisingly understudied crop. For many years, pepper research relied almost entirely on F₂ mapping populations resulting from crosses between contrasting germplasm (Lee, 2019). The first interspecific genetic linkage map was published in 1988 using restriction fragment length polymorphisms (Tanksley et al., 1988). Since then, several other intra and interspecific maps, using different species and segregating populations, have been constructed to dissect different traits of interest (Lee, 2019). Thus, these first maps were of paramount importance to shed light into the synteny between the pepper and tomato genomes and enable marker-assisted selection and QTL dissection (Jaiswal et al., 2019; Mohan and Paran, 2019; Wahyuni et al., 2014). Recent technological advances have made it possible for researchers to have publicly-available high-quality genome sequences. The first pepper genomes were published in 2014 with the complete sequences of *C. annuum* cv. Serrano Criollo de Morelos 334 (CM334), *C. chinense* PI159236, *C. annuum* cv. Zunla-1, and *C. annuum* var. *glabriusculum* (Kim et al., 2014; Qin et al., 2014). Since then, new genomes and improved versions of the CM334 accession have been released (Acquadro et al., 2020; Hulse-Kemp et al., 2018; Kim et al., 2017), including the first pepper pangenome (Ou et al., 2018). The availability of such powerful genomic resources is of paramount importance to the precise dissection of gene function and genomic elements structure and has been successfully applied in many genome-wide association studies (Colonna et al., 2019; Lee et al., 2020; Siddique et al., 2019). However, pepper's unusually large genome size (~3.5Gb) and degree of repetitiveness (~80%), compared to other *Solanaceae*, a result of several duplication phenomena before speciation, have hampered the study of complex traits such as fruit nutritional content.

These genetic and genomic tools have helped to identify several QTLs and candidate genes associated to the synthesis of phenolic acids and flavonoids in tomato, eggplant and pepper. The aim of the following sections is to integrate and summarise the available literature on the genetics of phenolic acids and flavonoids in tomato, pepper and eggplant, making it easily available for researchers and breeders.

2. Genetic basis of phenolic compounds synthesis and accumulation

In addition to compiling the relevant QTLs found in the literature associated to phenolic acids and flavonoids, we have conducted a search throughout the most recent genome versions of tomato, eggplant and pepper at Sol Genomics Network database (<http://www.solgenomics.net>) for annotated putative orthologue genes encoding for the key enzymes involved in the phenylpropanoid pathway. For this purpose, we have performed a blastp using the sequence of each verified protein from *S. lycopersicum*, or *A. thaliana* when the former was not available, which were retrieved from the NCBI protein database (<https://www.ncbi.nlm.nih.gov/protein/>), and selected the best hits with an e-value threshold of 1e-10, higher score than 200 and identity higher than 70%.

2.1. Phenolic acids

Phenolic acids are a diverse group of molecules that share a benzene ring and a carboxyl group as a backbone (Figure 1). This group of secondary metabolites can be classified into derivatives of benzoic acid or cinnamic acid, depending on whether the aromatic ring has a carboxylic group (C6-C1 structure) or a propenoic acid (C6-C3 structure) attached to it, respectively (Figure 1).

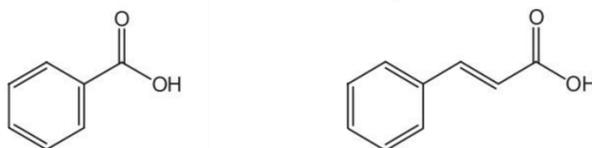


Figure 1. Basic structures of benzoic (left) and cinnamic (right) acids.

Different levels of hydroxylation and methoxylation of each basic structure will result in different compounds with different antioxidant capacity (Scarano et al., 2020). Hence, within the hydroxybenzoic derivatives, the most representative acids are gallic, ellagic, protocatechuic, *p*-hydroxybenzoic, vanillic and syringic acids. On the other hand, the major hydroxycinnamic acids are caffeic, ferulic, sinapic and *p*-coumaric acids, and their derivatives (Valanciene et al., 2020). Rather than in their free form, these compounds are usually found esterified with other organic acids (quinic, tartaric) or carbohydrates. The most relevant group of these conjugates are the esters of hydroxycinnamic acids with quinic acid, commonly called chlorogenic acids (Clifford et al., 2017). The also called chlorogenic acid (5-*O*-caffeoylquinic acid; CGA), along with other caffeic acid derivatives, constitute the most abundant type of phenolic acids in tomato, pepper and eggplant (García-Valverde et al., 2013; Luthria et al., 2010; Marín et al., 2004; Slimestad and Verheul, 2009; Whitaker and Stommel, 2003). In general, hydroxycinnamic acids are more abundant than hydroxybenzoic derivatives in plants, including the *Solanaceae* family, contributing at a larger extent to the human dietary phenolic acid intake (Valanciene et al., 2020). Consequently, more studies have been carried out in tomato, eggplant and pepper regarding the synthesis of hydroxycinnamic and chlorogenic acids than hydroxybenzoic acids. Accordingly, this review focuses on that group of phenolic acids.

2.1.1. Biosynthesis and genes encoding structural enzymes

All phenolic compounds ultimately stem from the shikimic acid pathway which starts with phosphoenolpyruvate and D-erythrose-4-phosphate forming the C6 core aromatic ring with one carboxyl and three hydroxyl substituents (Pott et al., 2019). Regarding the synthesis of hydroxybenzoic acids, two pathways have been described in plants. On one hand, the shikimate/chorismate pathway starts with two products of shikimic acid transformation: 3-dihydroshikimic acid (3-DHS) and chorismic acid. In this way, 3-

DHS serves as a precursor of protocatechuic acid and gallic acid, which in turn is transformed into ellagic acid and other derivatives; while from chorismic acid via the intermediate isochorismic acid, salicylic acid and a wide range of dihydroxybenzoic acids (DHBA) are synthesized (Wildermuth, 2006). Alternatively, hydroxybenzoic acids can also be synthesized from phenylalanine metabolism, in this case by means of the C3 side chain shortening of the hydroxycinnamic C6-C3 structure. In this case, the benzoic acid would be synthesized from cinnamic acid, salicylic acid from *o*-coumaric acid, 4-hydroxybenzoic acid from *p*-coumaric acid, protocatechuic acid from caffeic acid, vanillic acid from ferulic acid and syringic acid from sinapic acid. A network of multiple paths has been proposed within that second pathway, although the contribution of each one is still unclear. For further detailed information about the synthesis pathways of benzoic derived acids we refer to Widhalm and Dudareva (2015). Not all the enzymes involved in the synthesis of hydroxybenzoic acids have been identified. Nevertheless, there are several key enzymes, extensively studied in tomato, pepper and eggplant, which could be targeted to overexpress the phenylpropanoid pathway and thus increase the production of phenolic acids. These are described in the following paragraphs.

The synthesis of phenylalanine from chorismic acid is considered the branching point that links the shikimate pathway to the phenylpropanoid pathway. The synthesis of *p*-coumaroyl-CoA from phenylalanine in a three-step reaction is considered the starting point of the synthesis of both hydroxycinnamic acids and flavonoids (Vogt, 2010). These initial steps involve three key enzymes: phenylalanine ammonia-lyase (PAL), which catalyses the non-oxidative deamination of phenylalanine to cinnamic acid, cinnamate 4-hydroxylase (C4H), which catalyses the subsequent formation of *p*-coumaric acid, and 4-coumaroyl-CoA ligase (4CL), which is involved in the synthesis of the next branching element along the pathway, *p*-coumaroyl-CoA (Marchiosi et al., 2020). Several clusters of homologous genes encoding these key enzymes have been identified in tomato, pepper and eggplant genomes. Their location and distribution throughout the genomes are displayed in Figure 2.

From *p*-coumaric acid the other major hydroxycinnamic acids (caffeic, ferulic and sinapic acids) are synthesized through a multi-step chain reaction, either by direct transformation or by their CoA-conjugated intermediates. The pool of enzymes catalysing this series of reactions is also well characterized (Marchiosi et al., 2020). Again, three key enzymes are responsible for enhancing the content of the following hydroxycinnamic acids: 4-coumarate 3-hydroxylase (C3H), which catalyses the hydroxylation of *p*-coumaric acid to caffeic acid; caffeate O-methyltransferase (COMT), which is involved in both the subsequent formation of ferulic acid and, together with ferulate 5-hydroxylase (F5H), in the synthesis of sinapic acid. Besides, 4CL also catalyses the synthesis of each hydroxycinnamic acid-CoA conjugate (Figure 2).

Starting from *p*-coumaroyl-CoA, two synthesis pathways have been proposed for chlorogenic acid (CGA). Again, three key enzymes catalyse these steps (Gramazio et al., 2014). In one branch, *p*-coumaroyl-CoA is converted to *p*-coumaroyl quinic acid by the enzyme hydroxycinnamoyl-CoA:shikimate/quinic acid hydroxycinnamoyl transferase (HCT); then, the latter compound is hydroxylated to form chlorogenic acid with the action of *p*-coumaroyl ester 3'-hydroxylase (C3'H). In a second branch, *p*-coumaroyl shikimic acid is synthesized from *p*-coumaroyl-CoA via HCT, followed by two-step transformation to caffeoyl-CoA via the intermediate caffeoyl shikimic acid and catalysed by C3'H and HCT. Finally, caffeoyl-CoA is trans-esterified with quinic acid to form CGA via the enzyme hydroxycinnamoyl-CoA:quinic acid hydroxycinnamoyl transferase (HQT) (Figure 2).

Efforts have been made in tomato towards the characterisation of the chlorogenic acid pathway genes through transgenesis, gene expression analysis and enzymatic assays. In this way, gene silencing of a *HQT*-encoding gene resulted in a 98% reduction of CGA in tomato transgenic lines (Niggeweg et al., 2004), while no further soluble phenolics were affected by the gene silencing, demonstrating that the encoded enzyme constitutes the primary route for the synthesis of CGA and significantly controls the flux of the pathway (Niggeweg et al., 2004) (Figure 2).

Once the sequences of key genes in the chlorogenic acid synthesis pathway were known, gene expression analyses were performed in order to characterise them and to understand the tissue-specific accumulation of these health-promoting metabolites in eggplant. Thus, Docimo et al. (2016) found the higher contents of CGA in fruits, compared to other tissues, to be correlated to elevated transcript abundance of the structural genes *PAL*, *C4H*, *4CL* and *HQT*. They also isolated putative orthologs of the two CGA biosynthetic genes, *PAL* and *HQT*, from an Occidental *S. melongena* variety and demonstrated that both differed from homologs of Asiatic varieties. In addition, using a gene expression panel composed of 15 diverse *S. melongena* landraces and eight accessions of five related species of *Solanum* subgenus *Leptostemonum*, Meyer et al. (2019) characterized the genes encoding *HCT* and *HQT*. Their results suggested that in eggplant *SmHCT* was implicated in the synthesis of mono-caffeoylquinic acid (CQA) (CGA and its isomers 3-CQA, 4-CQA, 5-*cis*-CQA), while *SmHQT* was only catalysing di-CQA formation.

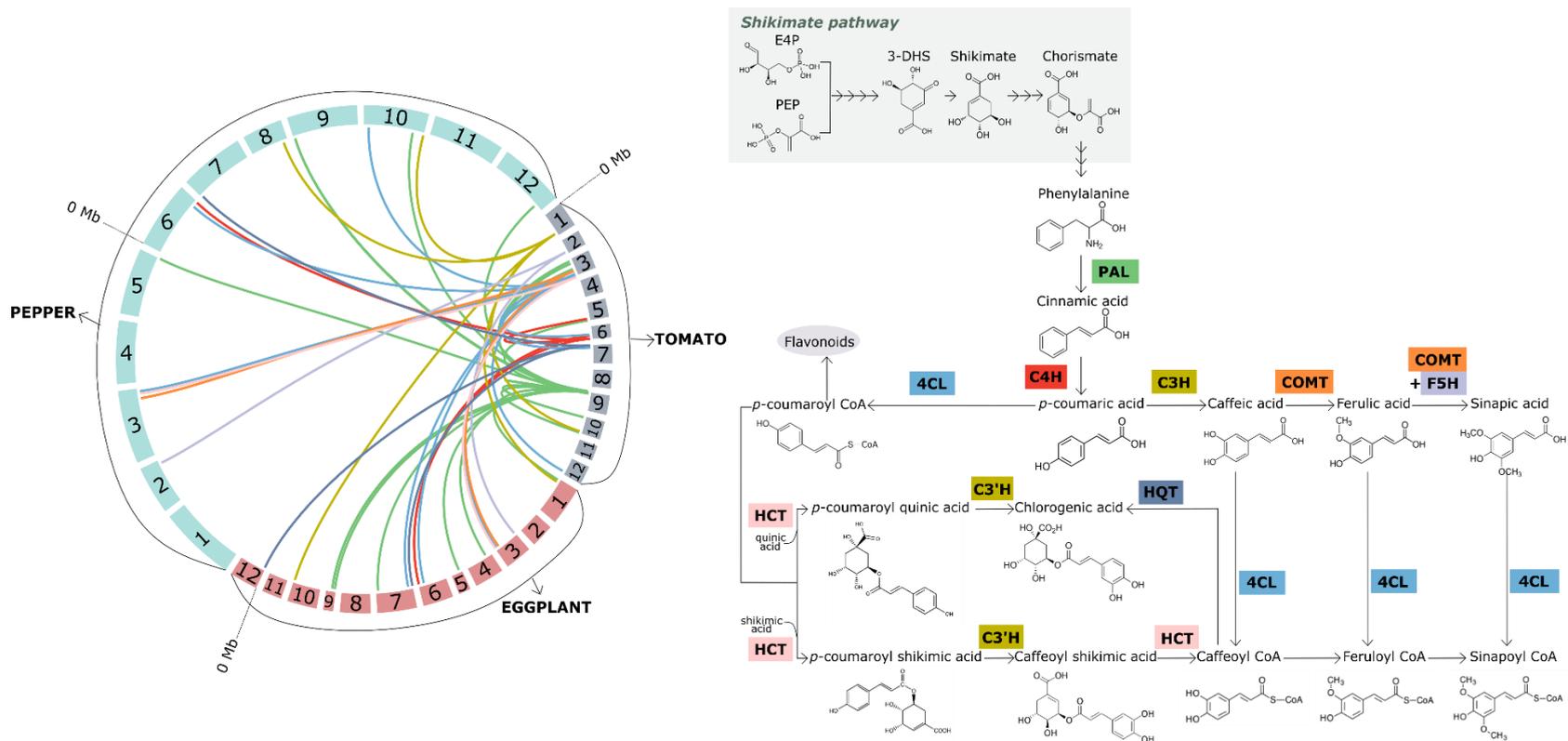


Figure 2. Macro-synteny among the tomato, eggplant and pepper genomes for candidate genes encoding the key enzymes involved in the synthesis of hydroxycinnamic acids (left), and overview of the aforementioned synthesis pathway (right). The 12 chromosomes of each of the three genomes represented, which are arranged to form the circle on the left, are coloured differently according to the species. The chromosomes are scaled according to their size (Mb). Links with different colours inside the circle were used to connect the physical position of homologous genes within and among the genomes. Same colour links represent genes encoding the same key enzyme of the pathway. The acronym of each key enzyme is indicated along the synthesis pathway (right), and framed in the same colour as the corresponding link. PAL: phenylalanine ammonia-lyase; C4H: cinnamate 4-hydroxylase; 4CL: 4-coumaroyl-CoA ligase; C3H: 4-coumarate 3-hydroxylase; C3'H: *p*-coumaroyl ester 3'-hydroxylase; HCT: hydroxycinnamoyl-CoA:shikimate/quinic acid hydroxycinnamoyl transferase; HQT: hydroxycinnamoyl-CoA:quinic acid hydroxycinnamoyl transferase; COMT: caffeate *O*-methyltransferase; F5H: ferulate 5-hydroxylase (F5H). The genes used for creating the links were the result of a BLASTP against current protein databases of tomato, eggplant and pepper at the Solgenomics platform (<http://www.solgenomics.net>). The hits with the lowest e-value, higher sequence size and higher sequence identity (>70%) were considered.

2.1.2. QTLs and candidate genes

Tomato

Many identified QTLs encompass the generalist trait of total phenolics content and total antioxidant capacity. Although several classes of compounds may be included in this category, we believe that it is relevant to include them in this review. Because we cannot classify them in specific categories, we dedicated a separate section to them (see section 2.3). Table 1 summarizes all the QTLs found in the literature associated with phenolic compounds in tomato, eggplant and pepper, and classified according to the different phenolic groups analyzed in this review.

Regarding the accumulation of phenolic acids in tomato fruits, the interspecific tomato mapping population consisting of introgression lines (ILs) of *Solanum pennellii* (LA0716) in the genetic background of the *S. lycopersicum* cv. ‘M82’ (LA3475) (Eshed and Zamir, 1995) has been exhaustively studied, yielding an array of QTLs. Using this population, Alseekh et al. (2015) identified 33 robust metabolic QTLs (mQTLs) controlling hydroxycinnamates accumulation in the fruit pericarp (Table 1). In addition, authors reported that in an experiment in two separate growing years, the heritability in a pool of 23 identified different hydroxycinnamates was high for eleven of those compounds, intermediate for four, and low for eight (Alseekh et al., 2015).

An interspecific inbred backcross line (IBL) population derived from the cross between *S. lycopersicum* cv. ‘Tueza’ and *S. pimpinellifolium* (LA1589) composed of 94 individuals was used in a recent study to perform QTL mapping regarding a wide range of fruit secondary metabolites (Çolak et al., 2020). Tested metabolites included both hydroxybenzoic (gallic, vanillic, syringic and hydroxybenzoic acid) and hydroxycinnamic acids (cinnamic, coumaric, ferulic, caffeic, sinapic, and chlorogenic acid). Both parental lines showed distinct phenolic acids profiles. In this way, cultivar ‘Tueza’ was significantly richer in gallic, vanillic, hydroxybenzoic and chlorogenic acid, whereas the wild parent had higher coumaric, ferulic and syringic acid levels. Six QTLs were identified associated to contents in hydroxybenzoic acids, while 19 were linked to hydroxycinnamic acids (Table 1). Thus, gallic and cinnamic acid were linked to one QTL each, vanillic, hydroxybenzoic, ferulic and chlorogenic acid to two QTLs each, coumaric and caffeic acid to three different QTLs each, and sinapic and syringic acid to four different QTLs each (Çolak et al., 2020). The QTLs detected for syringic acid (*sy8.1*), gallic acid (*ga8.1*) and coumaric acid (*coa8.1*), located at the IL8-2, were also in agreement with previous reports by Rousseaux et al. (2005), Alseekh et al. (2015) and Ballester et al. (2016). Likewise, QTLs for vanillic acid (*va7.1*) and sinapic acid (*sa7.1*) coincided with the locations provided by Rousseaux et al. (2005). Finally, candidate genes were identified within the QTL regions controlling vanillic acid (SolyC06g043130.1 and SolyC06g043120.1), sinapic acid (SolyC03g031470.2) and chlorogenic acid accumulation (SolyC12g005350.1). Interestingly, in the case of

chlorogenic acid the authors found three MYB-related transcription factors (Solyc12g005640.1, Solyc12g005800.1 and Solyc12g005890.1) around the QTL on chromosome 12 (*chla12.1*) (Çolak et al., 2020) (Table 1).

An IL population derived from a cross between *Solanum chmielewskii* (LA1840) and *S. lycopersicum* cv. ‘Moneyberg’ has also provided important insights into the phenolic acids accumulation in tomato fruits. Through liquid chromatography quadrupole time of flight-mass spectrometry coupled with physical mapping of the introgressions, Ballester et al. (2016) identified 126 different compounds from several classes, including phenolic acids, 56 of which significantly increased/decreased compared to the cultivated parent (Table 1). Thus, an introgression in chromosome 7 (IL7d) was linked to significant increases of di- and tri-caffeoylquinic acid and chlorogenic acid. Interestingly, within this region the coding gene for hydroxycinnamoyl-CoA:quinic acid hydroxycinnamoyl transferase (*HQT*), which is responsible for CGA synthesis, is located. IL4d was linked to an increase of tri-caffeoylquinic acid, chlorogenic acid and 4-caffeoylquinic acid. Contrastingly, the introgression in chromosome 12 (IL12d) showed almost a three-fold loss in di- and tri-caffeoylquinic acid compared to the IL cultivated parent (Ballester et al., 2016).

Eggplant

Being the most abundant phenolic acid in eggplant fruits, molecular and genetic studies of the phenolic profile have naturally focused on chlorogenic acid synthesis. In that way, an interspecific anchored linkage map, based on a first backcross generation (BC₁) of *S. melongena* × *Solanum incanum*, was developed by Gramazio et al. (2014) and exploited for locating genes involved in the CGA synthesis pathway. Based on synteny with the Tomato EXPEN-2000 genetic linkage map, the authors mapped candidate genes encoding each of the six key enzymes involved in the core CGA synthesis pathway in eggplant (*PAL*, *C4H*, *4CL*, *HCT*, *C3'H*, *HQT*). They were located on linkage groups E09, E06, E03, E03, E01 and E07, respectively, and showed collinearity with the corresponding gene in the tomato genetic map. Interestingly, these genes were not linked, except the ones encoding 4CL and HCT (Gramazio et al., 2014).

To our knowledge, only one QTL approach for deciphering the genetic basis of chlorogenic acid accumulation in fruits has been used on a *S. melongena* F₂ population derived from an intraspecific crossing (‘305E40’ × ‘67/3’). The donor parent was also used for the eggplant reference genome assembly (Barchi et al., 2019). The authors evaluated the chlorogenic acid content of the fruit flesh, among other quality traits, on plants cultivated under two environments. They identified two conserved QTLs for chlorogenic acid content, which were located in linkage groups E04 (*CGAE04*) and E06 (*CGAE06*). In both cases the positive allele came from the ‘305E40’ parent. No candidate genes could be identified within the QTLs regions. However, according to the authors, *CGAE04* and *CGAE06* could be related to quinic acid, since they were syntenic

to two tomato ILs containing QTLs for that moiety of chlorogenic acid (Schauer et al., 2006) (Table 1).

Pepper

Only one study aimed at the identification of QTLs associated to phenolic compounds was found for pepper at the time of writing. In this study, metabolomic and expression QTLs (mQTLs and eQTLs, respectively) associated to the occurrence of phenolic compounds in ripe fruits of pepper, as well as associated candidate genes, were detected. Authors used a genetic linkage map constructed from an interspecific F₂ population from the cross between *C. annuum* AC1979 (no. 19) and *Capsicum chinense* No. 4661 Selection (no. 18) to locate the identified QTLs and genes (Wahyuni et al., 2014). They previously performed a comprehensive untargeted metabolic profiling, which led to the identification of more than 500 semi-polar compounds including phenolics. The authors then employed both the QTL and candidate gene approaches for the identification of genomic regions associated to the detected metabolites, leading to the identification of one mQTL for the phenolic acid derivative ferulic acid-hexose, and its location within the linkage group P11 (Wahyuni et al., 2014) (Table 1).

2.2. Flavonoids

Flavonoids are the most diverse class of phenolics, comprising more than 8000 different metabolites (Ku et al., 2020). All flavonoids share a C6-C3-C6 carbon backbone formed by a benzo- γ -pyrone structure (ring A) and a phenyl ring (ring B) bind by an oxygen-containing γ -pyrone ring (ring C) (Figure 3).

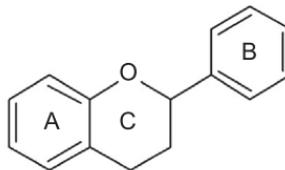


Figure 3. Basic structure of flavonoids.

Based on the position at which the B ring is attached to the C ring, as well as the oxidation and saturation degree of the heterocyclic C ring, flavonoid molecules are classified into flavones, flavonols, flavanones, flavanonols, anthocyanidins, flavanols or isoflavones (Ku et al., 2020; Vogt, 2010). Structural variations among different flavonoids and the ability to be modified enzymatically by a wide range of processes, such as hydroxylation, glycosylation, methylation, prenylation, acetylation and sulphation, are the main reasons contributing to their unique properties and broad functional diversity (Ku et al., 2020; Vogt, 2010).

2.2.1. Biosynthesis and genes encoding structural enzymes

The genetic basis behind the biosynthesis of flavonoids has been extensively studied over the last decades; hence, most of the key elements have been identified, especially in tomato (Bovy et al., 2007; Ku et al., 2020; Tohge et al., 2017). Several clusters of homologous genes encoding key enzymes of the synthesis of flavonoids have been identified in tomato, pepper and eggplant genomes and their location throughout the genomes is displayed in Figure 4. Briefly, naringenin chalcone (flavanone) is synthesised from *p*-coumaroyl-CoA and malonyl-CoA by chalcone synthase (CHS) and represents the main intermediate of flavonol biosynthesis. Naringenin chalcone is catalysed by chalcone isomerase (CHI) to the flavanone naringenin, which is a branching point for the formation of several groups of flavonoids. For example, naringenin can be converted to flavonols through the action of four key enzymes. First, flavanone-3-hydroxylase (F3H) converts naringenin into dihydrokaempferol (DHK), which is then converted into dihydroquercetin (DHQ) through the action of flavanone-3'-hydroxylase (F3'H). Finally, dihydroquercetin is catalysed by flavonol synthase (FLS) in order to produce the flavonol quercetin. Likewise, the flavonol kaempferol is immediately synthesized from dihydrokaempferol through FLS catalysis, skipping hydroxylation at the C-3' position (Figure 4).

Gene expression data of ripening tomato peel showed significantly high levels of transcripts of most of the enzymes involved in the flavonoid biosynthesis (CHS, F3H and FLS) except for chalcone isomerase (CHI), whose levels were very low or even below the limit of detection. This result confirmed that the complete flavonoid pathway is present in the fruit peel but the regulation mechanism of chalcone isomerase (CHI) expression is rate-limiting the flavonol biosynthesis, probably due to a mutation in the chalcone isomerase promotor sequence. In contrast to peel, cultivated tomato flesh showed no detectable levels of any of the above-mentioned transcripts, indicating that the flavonoid biosynthesis pathway is inactive in this tissue (Bovy et al., 2002; Muir et al., 2001; Willits et al., 2005).

Furthermore, dihydrokaempferol can be hydroxylated at the 5' positions of the B-ring in addition to the 3' position by the flavonoid-3',5'-hydroxylase (F3'5'H), converting DHK into dihydromyricetin (DHM), which is in turn transformed into the flavonol myricetin by FLS. DHM also acts as the first substrate leading to the anthocyanin delphinidin, a purple-coloured pigment. In fact, both dihydroquercetin and dihydrokaempferol can also be converted into cyanidin and pelargonidin, respectively, the other two main anthocyanins present in *Solanaceae* fruits. For the synthesis of these three anthocyanins, DHM, DHQ and DHK, the enzymes dihydroflavonol 4-reductase (DFR) and subsequently anthocyanidin synthase (ANS) are required instead of flavonol synthase (Figure 4). The *Solanaceae* anthocyanin biosynthetic pathway has been thoroughly analysed in a recent review, hence, it is not discussed herein (Liu et al., 2018). Moreover, the first step for cyanidin formation, catalysed by the enzyme DFR,

converts DHQ into a flavandioid intermediate, also called leucocyanidin. Both cyanidin and its intermediate leucocyanidin can act as precursors for the formation of another subclass of flavonoids, the flavan-3-ols, mainly represented by catechin and epicatechin. In this way, the enzyme leucoanthocyanidin reductase (LAR) would catalyse the synthesis of catechin from leucocyanidin, and the anthocyanidin reductase (ANR), the synthesis of epicatechin from cyanidin (Figure 4).

Naringenin can also act as a precursor for the other two main subclasses of flavonoids: flavones and isoflavones. The latter are mainly found in legumes, nuts and cereals but are less relevant in tomato, eggplant and pepper, thus they are not discussed herein. Naringenin can be converted to the flavones apigenin and luteolin through the action of flavone synthases FNS-I and FNS-II, which have been characterized as a 2-oxoglutarate-dependent dioxygenase and a cytochrome P450 monooxygenase from the CYP93 family, respectively. Both enzymes catalyse the addition of a double bond between C-2 and C-3 in the heterocycle of naringenin (Scossa et al., 2019) (Figure 4).

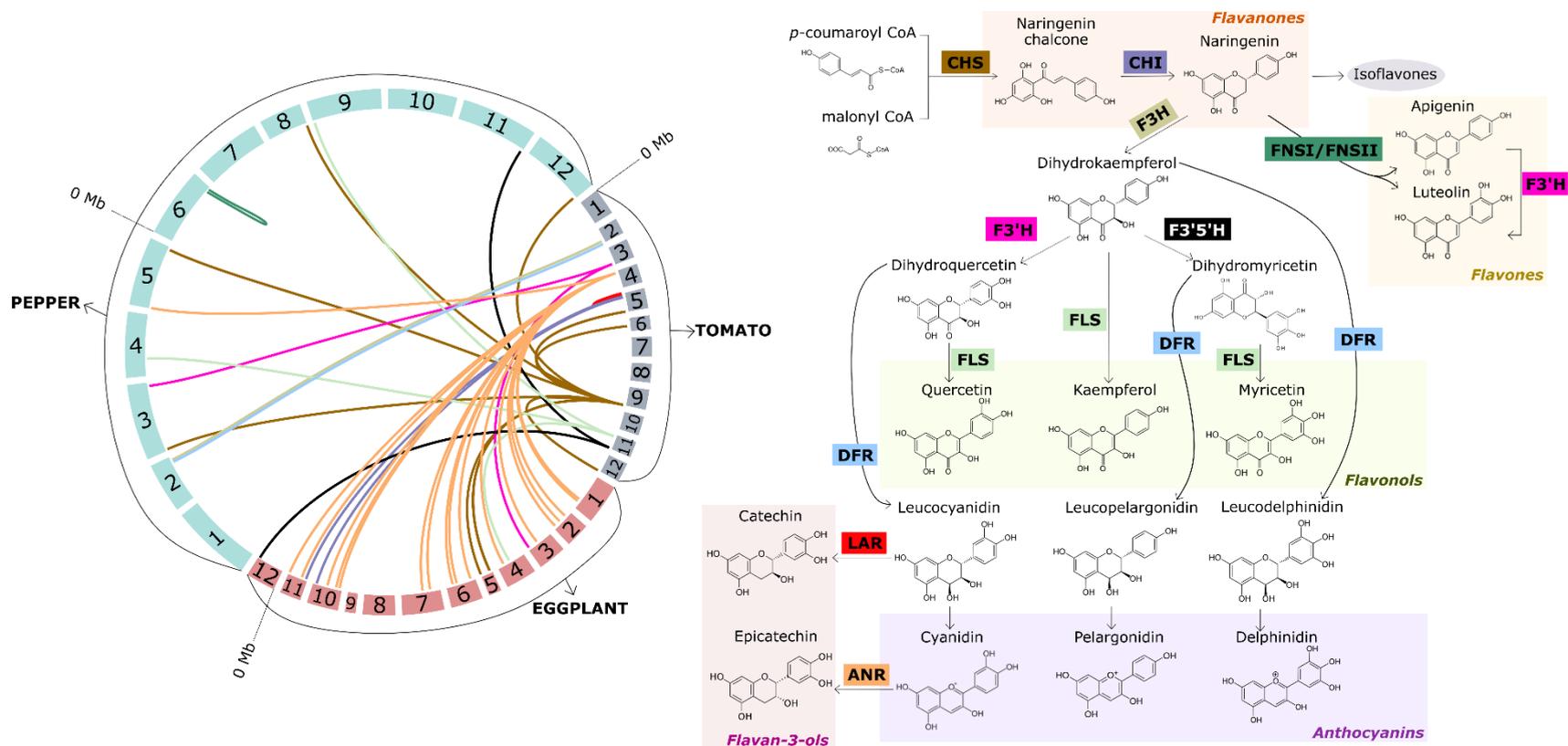


Figure 4. Macro-synteny among the tomato, eggplant and pepper genomes for candidate genes encoding the key enzymes involved in the synthesis of flavonoids (left), and overview of the aforementioned synthesis pathway (right). The 12 chromosomes of each of the three genomes represented, which are arranged to form the circle on the left, are coloured differently according to the species. The chromosomes are scaled according to their size (Mb). Links with different colours inside the circle were used to connect the physical position of homologous genes within and among the genomes. Same colour links represent genes encoding the same key enzyme of the pathway. The acronym of each key enzyme is indicated along the synthesis pathway (right), and framed in the same colour as the corresponding link. CHS: chalcone synthase; CHI: chalcone isomerase; F3H: flavanone-3-hydroxylase; F3'H: flavanone-3'-hydroxylase; F3'5'H: flavanone-3',5'-hydroxylase; FLS: flavonol synthase; DFR: dihydroflavonol 4-reductase; LAR: leucoanthocyanidin reductase; ANR: anthocyanidin reductase; FNSI/FNSII: flavone synthase I/II. The genes used for creating the links were the result of a BLASTP against current protein databases of tomato, eggplant and pepper at the Solgenomics platform (<http://www.solgenomics.net>). The hits with the lowest e-value, higher sequence size and higher sequence identity (>70%) were considered.

2.2.2. QTLs and candidate genes

Tomato

Several interspecific tomato mapping populations have been used throughout the years to study the genetics of flavonoid accumulation, yielding an array of promising QTLs and genes (Tohge et al., 2017). The most studied introgression lines (ILs) population is probably the one of *S. pennellii* (LA0716) in the genetic background of the *S. lycopersicum* cv. ‘M82’ (LA3475) (Eshed and Zamir, 1995). Alseekh et al. (2015) detected several different flavonols in the fruit pericarp and identified 26 robust metabolic QTLs controlling flavonoid accumulation. (Table 1). Authors reported high heritability for most of these compounds, especially naringenin chalcone, and a similar proportion between the dominant and additive modes of inheritance (Alseekh et al., 2015).

Likewise, ILs population derived from a cross between *S. chmielewskii* (LA1840) and *S. lycopersicum* cv. ‘Moneyberg’ has been used to study flavonoids accumulation in tomato fruits (Table 1). Combining genomics and metabolomics, Ballester et al. (2016) identified an introgression in chromosome 5 (IL5b) responsible for the significant increases of kaempferol and quercetin glycosides in the fruit peel. This region proved to harbour 511 genes, although only 17 were upregulated three-fold or higher, one encoding chalcone isomerase (CHI), which is responsible for directing the flux of the pathway towards the synthesis of flavonol glycosides (Muir et al., 2001). Similarly, an introgression in chromosome 4 (IL4d) showed interesting potential to increase kaempferol and quercetin glycosides, although in a more modest way (between 3- and 5-fold compared to controls). Other introgressions showed a positive effect on one or two metabolites, like IL2b for quercetin feruloyl deoxyhexose-dihexose or IL6e for quercetin coumaroyl hexose-deoxyhexose-hexoside. Contrarily ILs 8a, 9d and 12d showed a significant negative impact on the concentration of quercetin and naringenin glycosides (Ballester et al., 2016).

Seven ILs derived from a cross between *S. habrochaites* (LA1777) and *S. lycopersicum* cv. ‘E6203’ (Monforte and Tanksley, 2000) were selected from the original population to be further analysed regarding their chemical profiles (Hanson et al., 2014). Introgression line LA3984, encompassing a small fragment of *S. habrochaites* in chromosome 5, showed high levels of rutin in ripe fruits. The QTL proved to be harbouring 38 genes, including an annotated chalcone-flavonone isomerase gene. The introgression of this *S. habrochaites* QTL increased the expression of chalcone isomerase and restored the flavonol synthesis pathway, resulting in elevated rutin content (Hanson et al., 2014) (Table 1).

Using a BC₂F₂ population derived from the cross between *S. habrochaites* (LA1223) and *S. lycopersicum* ‘TA1166’, Ökmen et al. (2011) reported four QTLs involved in the flavonoid accumulation in tomato fruits, one with a positive effect, located at

chromosome 11, and three with negative effect, located at chromosomes 2, 3 and 5 (Table 1). Despite the negative effect of most of the wild alleles, QTL located at chromosome 11 increased by 24% the flavonoid content.

Nine different flavonoids were quantified in the interspecific inbred backcross line (IBL) population derived from the cross between *S. lycopersicum* cv. 'Tueza' and *S. pimpinellifolium* (LA1589) (Çolak et al., 2020), namely quercetin, myricetin, chrysin, apigenin, luteolin, flavan-3-ols, catechin, epicatechin, and epigallocatechin. Strikingly, neither kaempferol nor naringenin were detected among the IBLs and parental lines. Variation was high among individuals with several of them showing impressive levels of chrysin, apigenin and catechin as a result of transgressive segregation. Authors reported 18 QTLs linked to flavonoid fraction of the fruit metabolomic profile (Table 1). Hence, apigenin was linked to four different QTLs, quercetin, catechin, epicatechin and epigallocatechin contents were linked to three QTLs each, and chrysin to two different QTLs. Commonly, QTLs appeared scattered across several chromosomes and depending on the compound and in some cases the QTL in question, both the cultivated and the wild allele contributed to improving the flavonoid content (Çolak et al., 2020). Many of the QTLs had also been identified in other works. For instance, two QTLs for catechin (ctn6.1 and ctn7.1) had been previously identified by Rousseaux et al. (2005). Furthermore, researchers were able to identify candidate genes to several of those QTLs, such as for apigenin (SolyC09g066310.2), catechin (SolyC02g089770.2) and epicatechin (SolyC01g087640.2 and SolyC11g044830.1) (Çolak et al., 2020) (Table 1).

Tomato mutants, carrying natural or induced mutations, may also represent a source of valuable alleles regarding metabolic regulation of different pathways (Bovy et al., 2010). In that regard, *high pigment* (*hp*) tomatoes comprise a particularly interesting source of variability since their phenotype is the result of exaggerated accumulation of many antioxidants and photoprotective metabolites, including flavonoids, in their fruits (Azari et al., 2010; Levin et al., 2006). The different tomato *hp* mutants contain mutations in either the *DE-ETIOLATED1* (*DET1*) gene (*hp-2*, *hp-2^l*, and *hp-2^{dg}*), a negative regulator of photomorphogenesis, or the *UV DAMAGED DNA BINDING* protein 1 (*DDB1*) gene (*hp-1* and *hp-1^w*) which interacts with the *DET1* gene (Azari et al., 2010; Levin et al., 2006). Several studies reported the metabolic profiles of the different *hp* mutants and showed that these mutations not only led to the accumulation of high levels of flavonoids, but also to increase levels of other bioactive compounds, such as carotenoids (Bino et al., 2005; Levin et al., 2006; Long et al., 2006; Rutley et al., 2021). *hp* mutations have been extensively introgressed into elite cultivars, mainly to increase the lycopene content in processing tomatoes (Levin et al., 2006). Likewise, different combinations of *hp* mutations have been stacked in the same genetic background to shed light on to their genetic control and their impact on metabolite content. The homozygote double-mutants carrying *Anthocyanin fruit* (*Aft*) with *hp-1^w* and *Aft* with *hp-2^l* showed a significant increase in the accumulation of both

anthocyanins and rutin compared to the single-mutation lines (van Tuinen et al., 2006). In addition, the stacking of *Aft* with *hp-1* showed promising results by synergistically increasing the production of delphinidin-, petunidin- and malvidin-type anthocyanins and quercetin- and kaempferol-type flavonols in the fruit (Sapir et al., 2008).

Eggplant

Studies about the flavonol accumulation other than anthocyanins in eggplant are scarce. This may be due to their rare occurrence in the eggplant fruit flesh and peel compared to other important phenolic compounds such as chlorogenic acid. Among the flavonol derivatives that have been quantified in the fruit peel, genetic studies have been focused mainly on rutin. In this regard, Dong et al. (2020) evaluated the inheritance model of the rutin accumulation in three eggplant F₂ populations from crossings between parents with contrasting rutin content. The authors found the rutin content to be controlled by one or two major gene(s). In addition, a recent metabolic study of a F₆ recombinant inbred line (RIL) population (Sulli et al., 2021), derived from the eggplant lines ‘305E40’ and ‘67/3’, identified one mQTL on chromosome 10 associated to the level of rutin (P-RUT.10.1). Two other mQTLs were associated to the level of the flavonol glycoside kaempferol 3-*O*-beta-D-sophoroside. One of them was also located on chromosome 10 (P-KSOPH.10.1) near P-RUT.10.1, while the other was located on chromosome 7 (P-KSOPH.7.1) (Table 1). Several candidate genes for these QTLs were also suggested by the authors, encompassing a *PYL4* abscisic acid receptor (SMEL_010g352880), which is known to positively regulate flavonoid/anthocyanin biosynthesis in tomato (Diretto et al., 2020), peroxidase-encoding genes and other transcription factors (Sulli et al., 2021) (Table 1).

Pepper

As we have already mentioned, a single study has been performed for pepper phenolic compounds, which used the interspecific F₂ population from *C. annuum* × *C. chinense* (Wahyuni et al., 2014). In terms of flavonoids, this study identified 12 mQTLs for different flavonoid glycosides encompassing quercetin, apigenin, luteolin, flavanone derivatives, and naringenin chalcone, scattered over the linkage groups P02, P06, P07, P09 and P10. Likewise, four structural candidate genes, namely a chalcone synthase (*CHS-2*) and a chalcone isomerase (*CHI-2*) on P05, and another chalcone isomerase (*CHI-4*) and a flavone synthase (*FS-2*) on P06, were identified. Moreover, 15 eQTLs for flavonoid pathway genes were identified: (i) eQTLs for chalcone synthase (*CHS-1*, *CHS-2*), chalcone isomerase (*CHI-2*) and flavonol synthase (*FLS*) colocalised on P01 and overlapped with a mQTL for naringenin chalcone, eQTLs for *CHS-1*, *CHS-2*, *CHI-1*, *CHI-2* and *FS-2* colocalised with a naringenin chalcone mQTL on P09, (iii) eQTLs for genes encoding flavanone-3'-hydroxylase (*F3'H-1* and *F3'H-4*), and a gene encoding a flavanone-3-hydroxylase (*F3H*) were detected on P03, P04 and P08, respectively (Wahyuni et al., 2014) (Table 1).

In a recent study aimed at identifying QTLs associated to α -glucosidase inhibitory activity of flavonoids and other compounds present in pepper leaves and fruits (Park et al., 2020), the authors found a QTL on chromosome 9, which co-located with the cluster of mQTLs and eQTLs identified in the previous mentioned work (Wahyuni et al., 2014).

2.3. Total phenolics content

In several studies, QTLs have been reported for total phenolics content. We considered relevant to include them in this review, due to the coincidence, in many cases, with QTLs reported for specific metabolites in other studies. Using the tomato IL population derived from *S. pennellii* (LA0716) \times *S. lycopersicum* cv. 'M82' (LA3475), firstly, Rousseaux et al. (2005), and then Di Matteo et al. (2010), identified 10 robust metabolic QTLs (mQTLs) controlling phenolics accumulation in the fruit pericarp (Table 1). Sacco et al. (2013) further explored the location and inheritance model of the QTL for increasing total phenolics content previously identified in the IL7-3 of this population, and located the QTL at 39-64 cM in chromosome 7 and confirmed its recessive mode of inheritance (Di Matteo et al., 2010).

Using the BC₂F₂ population from *S. habrochaites* (LA1223) \times *S. lycopersicum* 'TA1166', Ökmen et al. (2011) reported five QTLs, scattered across chromosomes 1, 6, 7, 9 and 12, involved in the total phenolics fraction of the tomato fruits derived from this cross (Table 1). All QTLs showed a positive effect over this trait and two coincided with the locations previously identified by Rousseaux et al. (2005) at chromosome 7 and 9. Wild allele QTLs increased the phenolics content between 8 and 17%. The QTL located at chromosome 7 showed the biggest increase in phenolics content (Ökmen et al., 2011).

A GWAS approach was carried out by Ruggieri et al. (2014) for the detection of QTLs associated to fruit nutritional and quality traits in a collection of 96 cultivated tomato genotypes and using the SNP-based SolCAP array for genotyping. Two significant QTLs were identified for total phenolics content, located in chromosomes 8 and 11 (Table 1). In both cases, the minor alleles had a positive effect on the trait. In addition, four candidate genes co-localized with the marker at the beginning of chromosome 11, encoding for 14-3-3, ABC-2 and MATE transporter proteins, which have been reported to be involved in enhancing the vacuolar compartmentalization of phenolic compounds (Di Matteo et al., 2013).

More recently, the study of RILs, ILs and SubILs derived from a cross between *S. pimpinellifolium* (TO-937) and *S. lycopersicum* cv. 'Moneymaker' identified nine QTLs involved in the accumulation of phenolics across six different chromosomes (Barraj Barraj et al., 2021) (Table 1). Two wild alleles located at chromosomes 4 and 12 were linked to an increase of 20% and 30% in ripe fruits, respectively, while two other wild alleles located at chromosome 5 showed increased phenolics during immature and mature green stages. The other five QTLs, carrying the cultivated allele, showed a

negative effect on the accumulation of phenolics in the fruit peel. The cultivated QTL located at the chromosome 12 had a particularly high negative impact, leading to a 40% decrease of phenolics. An epistatic relationship between a region in chromosome 11 and the wild QTL found in chromosome 12 was detected and, although the region of the chromosome 11 had no significant effect over the phenolics content, it increased the effect of the QTL on chromosome 12 (Barraj Barraj et al., 2021).

Table 1. Summarized information of the studies in which QTLs associated with phenolic acids, flavonoids and total phenolics content were identified for tomato, eggplant and pepper.

Species	Mapping population	Type of phenolic metabolites affected	Number of QTLs identified	QTLs location (chrs.)	Candidate genes	Reference
Tomato	BC2F2 <i>S. habrochaites</i> (LA1223) × <i>S. lycopersicum</i> (TA1166)	Flavonoids	4	2, 3, 5, 11	-	Ökmen et al. (2011)
		Total phenolics content	5	1, 6, 7, 9, 12	-	
	GWAS (96 cultivated genotypes)	Total phenolics content	2	8, 11	-	Ruggieri et al. (2014)
		ILs <i>S. chmielewskii</i> (LA1840) × <i>S. lycopersicum</i> cv. Moneyberg	Flavonoids	10	2, [4-9], 12	
	ILs <i>S. habrochaites</i> (LA1777) × <i>S. lycopersicum</i> cv. E6203	Phenolic acids	5	3, 4, 7, 10, 12	-	Hanson et al. 2014
		Flavonoids (rutin)	1	5	-	
	ILs <i>S. pennellii</i> (LA0716) × <i>S. lycopersicum</i> cv. M82	Flavonoids	26	1, 2, [4-11]	-	Alseekh et al. 2015
		Phenolic acids	33	[1-12]	-	
	RILs, ILs and SubILs <i>S. pimpinellifolium</i> (TO-937) × <i>S. lycopersicum</i> cv. Moneymaker	Total phenolics content	9	3, [5-9]	-	Rousseaux et al. (2005)
		Total phenolics content	1	7	-	
	RILs, ILs and SubILs <i>S. pimpinellifolium</i> (TO-937) × <i>S. lycopersicum</i> cv. Moneymaker	Total phenolics content	9	1, 4, 5, 7, 8, 11, 12	-	Di Matteo et al. (2013)
		ILs <i>S. lycopersicum</i> cv. Moneymaker	Flavonoids	18	[1-4], [6-9], 11, 12	
RILs, ILs and SubILs <i>S. pimpinellifolium</i> (TO-937) × <i>S. lycopersicum</i> cv. Moneymaker	<i>S. lycopersicum</i> cv. Tuezua × <i>S. pimpinellifolium</i> (LA1589)	Flavonoids	18	[1-4], [6-9], 11, 12	SolyC06g043130.1 and SolyC06g043120.1 (vanillic acid), SolyC04g076380.2 (cinnamic acid), SolyC03g031470.2 and SolyC03g032220.2 (sinapic acid), SolyC12g005350.1; SolyC12g005640.1; SolyC12g005800.1 and SolyC12g005890.1 (CGA)	Çolak et al. (2020)
		Phenolic acids	25	[1-8], 10, 12	SolyC11g044820.1 (epicatechin)	
Eggplant	F2 population from <i>S. melongena</i> 305E40 × <i>S. melongena</i> 67/3	Phenolic acids (CGA)	2	4, 6	-	Toppino et al. (2016)
		Flavonoids	3	7, 10	SMEL_007g288740.1, SMEL_007g288700.1, SMEL_007g288720.1, SMEL_007g288660.1, SMEL_007g288690.1, SMEL_007g288730.1, SMEL_007g288920.1, SMEL_010g353210.1, SMEL_010g352840.1, SMEL_010g352880, SMEL_010g352930.1, SMEL_010g353090.1, SMEL_010g353110.1, SMEL_010g353170.1 and SMEL_010g353190.1 (kaempferol 3-O-beta-D-sophoroside), SMEL_010g352880 (rutin)	
Pepper	F2 from <i>C. annum</i> AC1979 (no. 19) × <i>C. chinense</i> No. 4661 selection (no. 18)	Flavonoids	27	[1-10]	<i>CaMYB12</i> (naringenin chalcone, FLS, CHI, CHS)	Wahyuni et al. (2014)
		Phenolic acids (ferulic acid-hexose)	1	11	-	

2.4. Regulation of the phenylpropanoid biosynthesis pathway

Although the phenolics biosynthetic pathway is conserved across these species, the accumulation profile of these metabolites in tomato, eggplant and pepper differs significantly (Rosa-Martínez et al., 2021). Moreover, studies have proven that flavonoids accumulate in a tissue-specific and developmental-dependent ways (Bovy et al., 2002; Muir et al., 2001). This implies that, in addition to the structural genes, the flavonoid pathway is tightly controlled by regulatory complexes. Transcription factors of the R2R3-type MYB, bHLH, MADS, WRKY and WD40 families have been reported to regulate the phenylpropanoid metabolism in plants, including the Solanaceae (Ku et al., 2020; Tohge et al., 2017, 2015). Among these, the R2R3-type MYB transcription factors are the most relevant group, as well as the most extensively studied in tomato, eggplant and pepper.

MYB12 role in the phenylpropanoid metabolism has been thoroughly characterised in several studies using introgression lines (ILs) and genetic engineering techniques, mostly in tomato, in the last few years. *AtMYB12* was identified as a flavonol-specific transcriptional activator in *Arabidopsis thaliana*, however, it has been shown to positively affect also the synthesis of chlorogenic acid and its derivatives when expressed in tomato and tobacco (Luo et al., 2008). In parallel, the study of the metabolomic and molecular profiles of pink-coloured fruits in the two sets of ILs with wild introgressions from *S. pennellii* (Adato et al., 2009) and *S. chmielewskii* (Ballester et al., 2010) specifically linked *SIMYB12* to the genomic region harbouring the *colourless fruit epidermis* (*y*) mutation on chromosome 1, and to the regulation of naringenin chalcone accumulation in the fruit peel. This effect was demonstrated by downregulation (Adato et al., 2009), overexpression (Wang et al., 2018) and by studying mutations in the sequence of the gene encoding *SIMYB12* in tomato (Fernandez-Moreno et al., 2016; Jung et al., 2017). These studies have also determined that *SIMYB12* controls the expression of the structural genes *SIPAL*, *SI4CL*, *SICH5*, *SICHI*, *SIF3H*, *SIF3'H* and *SIFLS*, being the main activator of the flavonol biosynthetic branch (Adato et al., 2009; Ballester et al., 2010; Jung et al., 2017; Luo et al., 2008; Wang et al., 2018). In addition, Zhang et al. (2015) demonstrated, in transformed tomato plants with *AtMYB12* linked to the fruit-specific E8 promoter, that *AtMYB12* is not only able to bind to specific phenylpropanoid structural genes, but also to genes associated to the primary metabolism, such as the genes encoding 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (*DAHPS*) and plastidial enolase (*ENO*), and thus it exerts its activity at several metabolic points. This complex regulatory action of *SIMYB12* was also pointed out by Fernandez-Moreno et al. (2016) through transcriptome analysis. In this way, besides increasing the demand for flavonoid synthesis, MYB12 would increase the flow of carbon entering the shikimate pathway and the formation of aromatic amino acids that serve as substrates for the synthesis of phenolic compounds (Y. Zhang et al., 2015). Among the QTLs for phenolic acids and flavonoids identified in tomato in the

previously mentioned studies, one was linked to both *SlMYB12* and *DE-ETIOLATED1* (*DET1*) genes and located on chromosome 1 using a set of RILs, ILs and SubILs derived from *S. pimpinellifolium* (TO-937) × *S. lycopersicum* cv. ‘Moneymaker’ (Barraj Barraj et al., 2021).

In pepper, using sequence homology with the tomato *SlMYB12*, Wahyuni et al. (2014) located a candidate gene for *CaMYB12* in chromosome 1 in the F₂ population from *C. annuum* × *C. chinense* genetic linkage map. In addition, through metabolic and gene expression analyses in ripe fruits of this population, the authors identified a mQTL for naringenin chalcone and eQTLs for *CaMYB12* and other genes encoding specific enzymes in the flavonoid pathway (*CaCHS-1*, *CaCHS-2*, *CaCHI-1* and *CaFLS*), which colocalised at the *CaMYB12* locus on chromosome 1 (Wahyuni et al., 2014). This suggests that a similar regulatory action of MYB12 transcription factor may control the phenylpropanoid metabolism in pepper and tomato.

Not only *SlMYB12*, but other MYB-type transcription factors have been reported to act as regulators at different levels of both primary and phenylpropanoid metabolism in tomato. R2R3-type MYB transcription factor was targeted in Cin et al. (2011). The authors studied the regulation of the phenylpropanoid pathway starting point in tomato fruits through the ectopic expression of *ODORANT1* (*ODO1*), which regulates the synthesis of phenylalanine and volatile derivatives in flowers of *Petunia hybrida*. Besides the establishment of phenylalanine synthesis pathway via arogenate with the discovery of an *ODO1*-induced novel enzyme prephenate aminotransferase in tomato, the results indicated that *ODO1* overexpression induced a limited set of genes associated to specific branches of the phenylpropanoid pathway. In this way, several feruloyl esters were significantly increased in fruits of all transgenic lines, in some cases 2-fold more than caffeic acid, the most common phenylpropanoid in the wild-type. On the other hand, *ODO1* expression did not significantly affect other pathway-derived compounds such as flavonoids, and even lower levels of some compounds such as phenylalanine, naringenin and quercetin were found in transgenic tomato fruits (Cin et al., 2011). Wu et al. (2020) reported a significant impact of the *SlMYB72* on several vital metabolic pathways, including the phenylpropanoid pathway, increasing the total flavonoids, as well as individual rutin, quercetin, and kaempferol, gallic acid and chlorogenic acid contents, when *SlMYB72*-was downregulated, whereas *SlMYB72*-upregulated plants showed no significant differences (Wu et al., 2020). The *SlMYB72* was also reported as a significant player by directly targeting 4-coumarate-CoA ligase (*4CL*) and chalcone synthase (*CHS1* and *CHS2*) genes (Wu et al., 2020).

In eggplant, efforts in this area so far have resulted in the identification of more than 100 R2R3-MYB transcription factors (Li et al., 2021), categorized into 20 subgroups (SGs), of which four (SG4 to SG7) were reported to modulate the phenylpropanoid pathway, mainly anthocyanin and flavonol biosynthesis. Within these SGs, ten genes were identified by the authors. One of these genes, *SmMYB1* (SG6), was isolated from

an accidental eggplant variety with the purpose of characterising its expression level in different tissues and its regulation mechanism (Docimo et al., 2016). *SmMYB1* is an orthologous gene to *SIANT1* and the locus *A* in *C. annuum*, widely characterised in tomato and pepper, respectively, as main regulators of anthocyanin synthesis. The authors found that *SmMYB1* was responsible for differentially regulating both the chlorogenic acid and anthocyanin biosynthesis in eggplant. Through *Nicotiana benthamiana* transient expression of the *SmMYB1* and a C-terminal truncated form of the protein, the authors demonstrated the essential role of the C-terminal region in anthocyanin biosynthesis, while its deletion does not limit the capability of *SmMYB1* to regulate chlorogenic acid accumulation. These results pointed out that transcription factors involved in the phenylpropanoid pathway might contain several action domains that can bind to different substrates and exert their regulatory action at different points along the pathway (Docimo et al., 2016).

In addition to key enzymes involved in the synthesis of phenylpropanoids, the regulation of phenolics accumulation in tomato, pepper and eggplant fruits also occurs at the level of cellular transporters. In this way, Di Matteo et al. (2013) used the IL7-3 (LA4066) of the tomato IL population with introgressions from *S. pennellii*, which harbours a stable positive QTL for phenolics content in chromosome 7, to characterise the mechanisms controlling the accumulation of flavonoids in the fruit vacuoles. The authors identified and characterised an ethylene responsive factor (*ERF1*) in chromosome 5 which coordinates the transport and accumulation of flavonoids in the fruits (Di Matteo et al., 2013).

3. Breeding strategies for improving phenolic acid and flavonoid content in tomato, eggplant and pepper

The current knowledge about the genetics of the biosynthesis and accumulation of phenolic acids and flavonoids in fruits of tomato, eggplant and pepper, including QTLs, structural and regulatory genes, as well as the characterization of the available germplasm, provides enough tools to improve phenolics levels and thus, the health-promoting function of three of the most consumed vegetables in the world. Several works have proved the effectiveness of combining molecular tools and variability through both conventional and genetic engineering strategies.

3.1. Conventional breeding

Great diversity has been found among different cultivated varieties of tomato, eggplant and pepper with respect to phenolic acid and flavonoid concentration in fruit. For instance, in tomato, rutin values ranged from 2 to 231 mg kg⁻¹ of fresh weight (FW) in a collection of commercial materials and between 22 to 80 mg kg⁻¹ FW in a collection traditional varieties (Bovy et al., 2010; Rosa-Martínez et al., 2021). Stommel and Whitaker (2003) found differences of nearly 20-fold for total hydroxycinnamic acid content in a collection of 101 cultivated eggplant accessions from the USDA-ARS core

collection. Mori et al. (2013) also found varietal variations in CGA content between 0.1 and 2.5 mg g⁻¹ FW among 34 eggplant cultivars and lines with diverse growth habits, fruit shapes, sizes, and colours. Huge variation has been reported for most of the phenolic acids and flavonoids present in different pepper varieties, such as vanillic acid (3.1–13.3 mg kg⁻¹ FW), ferulic acid (2.2–12.5 mg kg⁻¹ FW), quercetin (3.3–783.8 mg kg⁻¹ FW) or luteolin (0.2–103.5 mg kg⁻¹ FW) (Lemos et al., 2019).

In addition, many elite materials come from mapping populations. These are incredibly useful genetic resources as they carry favourable alleles for the accumulation of metabolites, usually introgressed from wild accessions into a cultivated genetic background, that can be readily incorporated into breeding programs (Prohens et al., 2017). Breeders have long relied on the wild germplasm as sources of resistance or tolerance to stresses and for their higher content in health-promoting metabolites. However, the application of wild materials is not always an easy way to improve cultivated varieties since many materials show strong incompatibility barriers and agronomically undesirable traits. Furthermore, the genomic fragments introgressed from wild relatives usually has a significant linkage drag associated, due to the low recombination, which makes it extremely difficult to dissect the genetic region responsible for the trait of interest (Prohens et al., 2017). In this way, introgression lines (ILs) are a useful and powerful resource to introgress traits of interest from wild relatives, since each line carries only a small percentage of the introgressed donor genome (Lippman et al., 2007). Furthermore, ILs can be directly introduced in breeding pipelines as they present a cultivated genetic background (Prohens et al., 2017).

Therefore, the main aspects of conventional breeding should be cataloguing, selecting and hybridising these elite materials. In addition, to successfully employ the available genetic resources in large-scale conventional breeding pipelines for phenolics content, the identification of QTLs and the underlying genes is of paramount importance. The application of fine-mapping tools and the development of functional markers could have unprecedented impact in the development of high-phenolics varieties without the negative impact of linkage drag (Salgotra and Neal Stewart, 2020).

As outlined before, the flavonoid pathway is disabled in the tomato fruit flesh of the cultivated germplasm, rendering it impossible to restore just by selecting and cross-pollinating the best materials in the primary genepool, including the tomato ancestor *S. lycopersicum* var. *cerasiforme* (Willits et al., 2005). Fortunately, within the wild genepool, there are materials that could address both the restoration of the flavonoid pathway in the fruit flesh and the increase of levels of flavonols in the fruit peel. Willits et al. (2005) reported several wild accessions able to express the complete flavonoid pathway in both the fruit peel and flesh. The LA1926 (*S. pennellii*), expressing the chalcone isomerase in the peel and the complete pathway in the flesh, was successfully hybridised with a cultivated variety, lacking expression of chalcone isomerase in both the peel and flesh. The resulting F₁ progeny accumulated high levels of quercetin in the

fruit peel and flesh, increasing by 11-fold the amount accumulated in the cultivated variety (Willits et al., 2005). Hanson et al. (2014) developed a stable high-rutin-producing line derived from a cross between the IL LA3984, encompassing a small fragment of *S. habrochaites* in chromosome 5, and the AVRDC line CLN2498E. The improved line accumulated 12-fold more rutin than fresh market and processing tomato cultivars in a controlled experiment spanning two years and two seasons (Hanson et al., 2014).

Marker-assisted selection and pyramiding of genes and QTLs has been proven to be an effective strategy for improving several traits simultaneously or a single quantitative trait with polygenic control (Rigano et al., 2014; Sacco et al., 2013; Salgotra and Neal Stewart, 2020). Numerous QTLs and candidate genes associated to key enzymes, as well as transcription factors, involved in the phenylpropanoid pathway are scattered throughout the genome. The high recombination frequencies among these regions would make it easier to combine several favourable alleles in one line.

In tomato, this breeding strategy has been successfully used for pyramiding QTLs for total phenolics, ascorbic acid and soluble solids in four genotypes of a F₃ population from the crossing of two ILs (7-3 and 12-4) of *S. pennellii* harbouring positive alleles for five QTLs related to these traits (Sacco et al., 2013). In a subsequent study, the four selected genotypes showed an increase in total phenolics content between 59% and 108% compared to the *S. lycopersicum* IL parent 'M82' (Rigano et al., 2014). They also performed a targeted metabolite analysis, which served to demonstrate that introgressed alleles of *S. pennellii* on chromosomes 7 and 12 had a synergistic effect on increasing phenolic compounds, and that this effect occurred specifically on phenolic acids, mainly gallic acid and ferulic acid, while flavonoids were not affected (Rigano et al., 2014).

In eggplant, higher levels of antioxidant activity, total phenolics and chlorogenic acid have been reported in the eggplant wild relative *S. incanum* compared to *S. melongena* (Kaur et al., 2014; Stommel and Whitaker, 2003). Prohens et al. (2013) evaluated the parents, F₁, F₂, and first backcross population from the cross *S. incanum* (MM577) × *S. melongena* (ANS26), for total phenolics and hydroxycinnamic acids profile. They found considerable variation and a number of plants from the backcross population with a good combination of phenolic acids content and fruit weight or flesh browning. Therefore, the ILs developed from this population (Gramazio et al., 2017), which have been genotyped, could constitute a powerful resource for identifying QTLs associated with higher phenolic acids content and be introduced into breeding pipelines.

2.3. Genetic engineering

Genetic engineering tools have enabled a great deal of important discoveries regarding the tomato phenylpropanoid pathway. In several cases, it led to outstanding results, unlikely to be achieved through the conventional techniques (Colliver et al., 2002; Schijlen et al., 2006). However, the commercialization of genetically modified fruits, is

not yet legally accepted in some parts of the globe, such as Europe. Even the precise gene-editing technology CRISPR-Cas9 has been considered a genetically-modifying technique and is subjected to the same limitations (Biswas et al., 2021).

Breeding for enhanced flavonoid content in tomato can be approached in two ways: increasing the amount that is stored in the peel or activating the flavonoid pathway in the fruit flesh. Since the flavonols quercetin and kaempferol have higher bioactive properties, the main goal should be to increase their content. A comprehensive amount of work has been performed towards those ends (Tohge et al., 2017). Through transgenesis, Muir et al. (2001) expressed in tomato plants a *P. hybrida* gene encoding for chalcone isomerase (*CHI*), which led to an increase of peel flavonol content up to 78-fold. These plants had higher levels of both quercetin and kaempferol glycosides in the fruit peel, whereas the naringenin chalcone levels had significantly been depleted. Despite that, flesh flavonoid content remained unaltered Muir et al. (2001). On the other hand, the simultaneous ectopic expression of four *P. hybrida* genes (*CHI*, *CHS*, *F3H* and *FLS*) was able to produce high flavonol content in the peel and modest levels in the flesh (Colliver et al., 2002; Verhoeyen et al., 2002). Further experiments with transformed tomato plants carrying isolated or different combinations of *P. hybrida* genes led to increased accumulation of naringenin glycosides when chalcone synthase (*CHS*) was ectopically expressed and that those glycosides are converted to flavonol glycosides when chalcone synthase and flavonol synthase (*FLS*) are stacked in the same background (Colliver et al., 2002; Verhoeyen et al., 2002). In another approach to restore the flavonoid pathway in the tomato flesh, Bovy et al. (2002) developed transgenic plants expressing the maize transcription factors *Lc* (*leaf Color*; MYC-type) and *C1* (*colorless-1*; MYB-type) genes, simultaneously, under the fruit-specific E8 promoter control. Authors reported a strong induction of flavonoid biosynthesis and increases up to 60-fold of kaempferol glycosides as well as significant increases of other naringenin glycosides in the fruit flesh (Bovy et al., 2002).

Modulation of regulatory genes has been a primary target towards improving the fruit content in flavonoids. Davuluri et al. (2005) used RNA interference (RNAi) to suppress the accumulation of *DE-ETIOLATED1* (*DETI*) transcripts in transgenic fruits in order to increase fruit bioactive compounds content while avoiding the negative impact on plant development. Mutations in the *DETI* gene sequence are known to lead to exaggerated photoresponsiveness and to elevated accumulation of both flavonoids and carotenoids, resulting in deeply pigmented fruits. This resulted in significant increases of naringenin chalcone, flavonol glycosides, chlorogenic acid, and carotenoids with no significant impact on other quality and growth parameters (Davuluri et al., 2005). Lim and Li (2017) proposed ectopic co-expression of two transcription factors, *Del* (*Delila*; bHLH-type) and *Ros1* (*Roseal1*; MYB-type) from *Antirrhinum majus*, in combination with the *Allium cepa* chalcone isomerase (*CHI*) gene in order to produce transgenic *S. lycopersicum* cv. 'Rubion' tomatoes with high levels of both anthocyanins and

flavonols. The strategy aimed at alleviating the major rate-limiting factor of the tomato flavonoid pathway by upregulating the expression of chalcone isomerase followed by redirection of the pathway towards the production of anthocyanins. Authors reported a significant increase of both anthocyanins and flavonols in the fruit peel and flesh, reaching up to 200-fold the total flavonol content of wild-type tomatoes (Lim and Li, 2017a). In a different work, Lim and Li (2017b) transformed the same cultivar to express the *A. cepa* chalcone isomerase (*CHI*) gene in combination with the *A. thaliana* production anthocyanin pigment 1 (*PAP1*) gene. The combination of structural and regulatory genes led to a 130-fold increase in rutin, along with a 30-fold increase of total anthocyanins, compared to the wild-type peel (Lim and Li, 2017b). Also worth mentioning is that the *A. cepa* flavonol synthase (*FLS*) gene was able to increase rutin content by as much as 3.5-fold in *S. lycopersicum* cv. ‘Rubion’ transgenic tomatoes, in contrast to what was observed with the *Petunia hybrida* *FLS* gene, which was only effective when present in combination with the chalcone isomerase gene (Lim and Li, 2017b; Verhoeven et al., 2002). Regarding phenolic acids, the contents of chlorogenic acid, caffeic acid, and coumaric acid were significantly higher in the *PAP1*-only lines compared to the wild type, and only modest increases were observed in the stacked lines (Lim and Li, 2017b).

Besides the above-mentioned *AtPAP1* and *AmROS1*, other MYB-type transcription factor genes have been successfully used to increase the flavonoid content in tomato. Luo et al. (2008) developed transgenic *S. lycopersicum* cv. ‘MicroTom’ and cv. ‘Moneymaker’ plants expressing the *A. thaliana* transcription factor *AtMYB12* and reported strikingly-high amounts of rutin, kaempferol-glycosides, as well as high levels of caffeoylquinic acid and chlorogenic acid, in both fruit peel and flesh. Authors reported that flavonoid content made up to 10% of the fruit dry weight and that it had no significant effect on the carotenoid accumulation of the transformed tomatoes (Luo et al., 2008). Likewise, the expression of the *AtMYB12* homolog *AtMYB11* in the genetic background of *S. lycopersicum* var. ‘CSL’ led to an increase of both flavonoid and caffeoylquinic acids content (Li et al., 2015). Alterations of the flavonol chemical profile were greater in the fruit peel, and transformed plants accumulated up to 18- and 33-fold the amount of rutin and kaempferol-glycosides, respectively, compared to the controls. In the flesh, flavonols accumulation was modest, showing a 3-fold increase of kaempferol compared to the controls (Li et al., 2015).

The down-regulation of *SlMYB12* by artificial microRNA targeting in ‘MicroTom’ plants led to *colourless fruit epidermis* (y) and lower accumulation of flavonols, whereas the overexpression of *SlMYB12* led to a higher accumulation of naringenin chalcone and to yellow-coloured peel (Adato et al., 2009). Wang et al. (2018) developed *SlMYB12*-overexpressing transgenic tomato plants using the *S. lycopersicum* cultivars ‘MicroTom’, ‘CS109-03’ and ‘Sheng Nv-Guo’. They reported a significant positive correlation between *SlMYB12* expression levels and the accumulation of flavonols in

the fruits. Thus, overexpression of this transcription factor led to a five-fold increase of naringenin chalcone, 15-fold increase of rutin and five-fold increase of kaempferol, on average (Wang et al., 2018). Recently, Wu et al. (2020) developed both downregulated and upregulated transgenic lines of *S. lycopersicum* cv. 'MicroTom' to study the function of another member of the tomato R2R3-MYB family, the *SlMYB72*. Authors reported significant impact on several vital metabolic pathways, including the phenylpropanoid pathway. Thus, the total flavonoid as well as individual rutin, quercetin, and kaempferol, as well as gallic acid and chlorogenic acid contents increased in the *SlMYB72*-downregulated plants, whereas *SlMYB72*-upregulated plants showed no significant differences (Wu et al., 2020).

It is worth mentioning that overexpression of phenylpropanoid regulatory genes may have negative implications on other important traits associated to fruit quality. For instance, *SlMYB12* has been reported to act upon genes related to primary metabolism in addition to phenylpropanoid-specific structural genes in tomato (Fernandez-Moreno et al., 2016; Y. Zhang et al., 2015). In this way, the flow of carbon is channelled towards the phenylpropanoid pathway, which indirectly decreased the sugars accumulated in the fruit, which could affect the flavour. Therefore, additional metabolic profiling is necessary in transformed plants to ensure that the fruit quality is conserved.

The gene editing technology CRISPR/Cas9 has been successfully applied in many crops, including tomato and the phenylpropanoid pathway. Concretely, researchers used CRISPR/Cas9 to successfully generate pink-fruited tomatoes via knockout of the *SlMYB12* transcription factor gene in the genetic background of four different red-fruited inbred lines. This led to transparent peels and reduced transcription of the chalcone synthase genes (Deng et al., 2018). By doing so, the authors showed that it is an efficient way to engineer fruit colour and the metabolic pathway in tomato without jeopardising other traits while reducing breeding programs duration (Deng et al., 2018).

Both eggplant and pepper are recalcitrant species to *in vitro* organogenesis and therefore the successful application of genetic engineering techniques has been difficult and has lagged behind compared to tomato (Gammoudi et al., 2017; García-Forteza et al., 2021). In eggplant, a recent work transformed plants for increasing phenolics. In that study, the authors used agroinfiltration for the transient overexpression of *SmHQT*, encoding the main enzyme for chlorogenic acid synthesis, in eggplant fruits and the results showed a 2-fold increased chlorogenic acid content in those fruits (Kaushik et al., 2020).

Finally, in pepper, no work was available at the time of writing of this article that used transformation for increasing the metabolites considered herein. However, successful results have been reported when applying genetic engineering, specifically using the virus-induced gene silencing (VIGS) approach targeting MYB and WD40 transcription factors (Aguilar-Barragán and Ochoa-Alejo, 2014; Z. Zhang et al., 2015). Similar to the tomato works, these reported that overexpression of transcription factors induced the

expression of several structural genes along the phenylpropanoid pathway (*PAL*, *C4H*, *4CL*, *CHS*, *CHI*, *F3H*, *F3'5'H*, *DFR*, *ANS*, among others), which means that the content of more phenolic metabolites could be affected, although more analyses are needed. This paves the way for the utilization metabolic engineering techniques with the aim of improving other flavonoid and phenolic acid accumulation in fruits of pepper.

4. Concluding remarks

We present herein a comprehensive review of the literature on the genetics of the biosynthesis of phenolic compounds in tomato, eggplant and pepper, as well as on the breeding strategies available to increase their content in the fruits of these species. We compiled all the QTLs identified for phenolic acids, flavonoids and total phenolics content in the three *Solanaceae* species, which are an essential tool for marker-assisted selection and breeding. The identification and location of the annotated putative orthologue genes encoding for key enzymes involved in the phenylpropanoid pathway highlighted the usefulness of synteny and QTL orthology studies among tomato, eggplant and pepper for exploiting genomic resources and closing the knowledge gap in eggplant and pepper, which still lags far behind that of tomato. Finally, we have pointed out the importance of the regulatory elements of the phenolic compound synthesis pathway, reviewing also the associated genes and QTLs in the literature. We hope that this review may facilitate the development of new tomato, eggplant and pepper varieties with an improved phenolic profile and greater functional potential.

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OBJECTIVES

In the present doctoral thesis, we aimed at characterizing the diversity found within the tomato, eggplant and pepper germplasm, in order to search for new potential sources of variation for the improvement of fruit quality, as well as for their adaptation to a more sustainable agriculture with low inputs. Particularly, we aimed at enhancing the use of introgression lines and local varieties as useful genetic resources for these purposes, through the evaluation of their response to low inputs, and the development of powerful genomic tools for breeding.

Therefore, we proposed the following specific main objectives:

1. Assessment of the diversity and comparison of the profile of fruit internal quality, encompassing organoleptic, nutritional and bioactive compounds, among tomato, eggplant and pepper varieties grown under the same conditions and organic farming practices.
 - 1.1. Identification of potential materials for the improvement of quality of fruits of tomato, eggplant and pepper.
2. Evaluation of compounds and parameters related to the organoleptic, nutritional and functional quality in fruits of a set of eggplant introgression lines carrying genome fragments of its wild relative *S. incanum*, along with the recipient and donor parents and the F₁ hybrid in two environments.
 - 2.1. Determination of the safety for consumption of fruits produced by the population through the analysis of glycoalkaloid contents.
 - 2.2. Evaluation of the environment effect and the genotype × environment interaction occurrence for the traits evaluated.
 - 2.3. Identification of potential materials for the improvement of fruit quality in eggplant.
 - 2.4. Identification of genomic regions associated with the traits evaluated. Linking the phenotype and the genotype of the lines will allow the detection of stable QTLs and candidate genes for the traits evaluated.
3. Evaluation of morpho-agronomic and fruit composition traits in a set of eggplant introgression lines carrying genome fragments of its wild relative *S. incanum*, together with the recipient parent, grown under high and low N fertilization conditions.
 - 3.1. Evaluation of the effect of minimizing N inputs on yield, plant growth, fruit morphology, fruit size and fruit composition parameters, as well as genotype × N treatment interactions.

- 3.2. Identification of potential materials for eggplant breeding under low N inputs.
 - 3.3. Identification of genomic regions associated with plant nitrogen use efficiency and the traits evaluated.
4. Characterization of a collection of local and commercial varieties of 'de penjar' long shelf-life tomato, grown under high and low N fertilization levels, for agronomic and fruit quality traits.
 - 4.1. Evaluation of the effect of the reduction of N inputs on yield and fruit quality and composition, as well as genotype \times N treatment interactions.
 - 4.2. Comparison of local varieties vs. commercial materials in terms of fruit quality and adaptation to low N inputs.
 - 4.3. Identification of potential materials for tomato breeding under low N inputs.

RESULTS

**Chapter I - Diversity for fruit
internal composition and
comparison of quality profiles
among *Solanaceae* vegetable crops**

Research article

Fruit composition profile of pepper, tomato and eggplant varieties grown under uniform conditions

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Ph.D. candidate contribution

E. R.-M. had a main role in the following activities: performing the experiments, data collection, data analysis, data visualization, drafting manuscript, manuscript review and editing.

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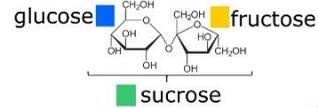
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Supplementary data can be found at:

<https://www.sciencedirect.com/science/article/pii/S0963996921004300#s0130>

Graphical abstract

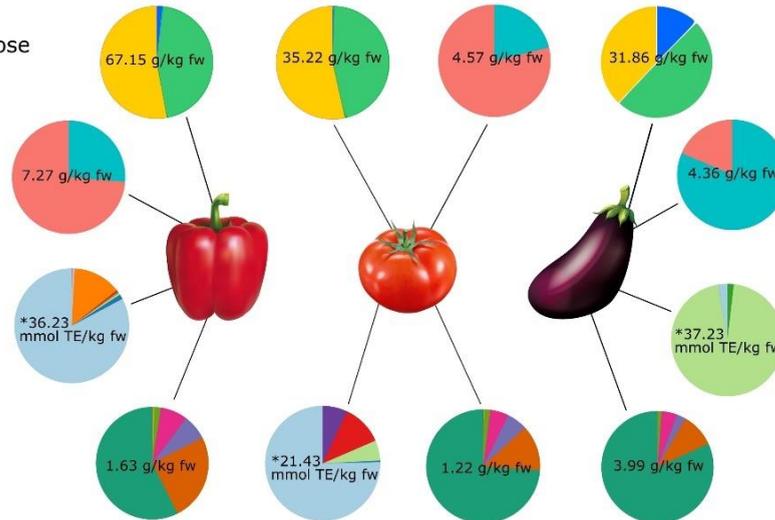
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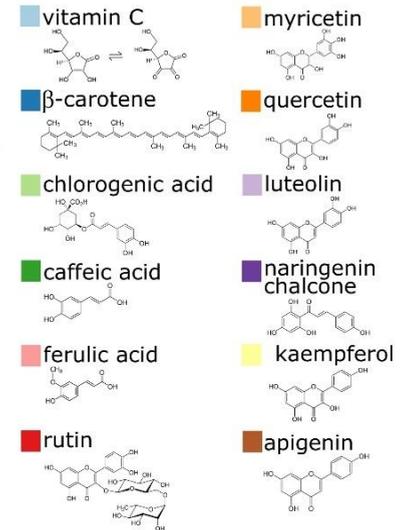


Minerals



Antioxidants

(*total antioxidant activity)



Highlights

- Peppers, tomatoes and eggplants are highly variable for fruit composition profile.
- Peppers had the highest content in total sugars, acids, β -carotene, vitamin C and Fe.
- Eggplants were higher in protein, sucrose, malic, chlorogenic acid, K, Mg and Cu.
- A diverse array of antioxidants was found, the most abundant being vitamin C, chlorogenic acid, rutin and quercetin.

Abstract

The study of the diversity within and between major *Solanaceae* crops (pepper, tomato, eggplant) is of interest for the selection and development of balanced diets. We have measured thirty-six major fruit composition traits, encompassing sugars, organic acids, antioxidants and minerals, in a set of 10 accessions per crop for pepper, tomato and eggplant, grown under the same cultivation conditions. The aim was to evaluate the diversity within species and to provide an accurate comparison of fruit composition among species by reducing to a minimum the environmental effect. Pepper, tomato and eggplant had a clearly distinct composition profile. Pepper showed the highest average content in total sugars and organic acids. Fructose and glucose were the major sugar compounds in the three species, although in pepper and tomato sucrose was present only in trace amounts. Citric acid was the major organic acid in pepper and tomato, while in eggplant it was malic acid. Pepper and eggplant had the highest total antioxidant activity. Vitamin C content was much higher in pepper than in tomato and eggplant, while eggplant accumulated high concentrations of chlorogenic acid. Furthermore, eggplant was the species with higher content in most minerals, particularly for K, Mg and Cu, while pepper was the richest in Fe. Due to their complementary nutritional profiles, a combined regular consumption of the three vegetables would supply more than 20% of the Dietary Reference Intake of several of the analysed phytochemicals. The large diversity within each species is of interest for selecting varieties with better nutritional and organoleptic profiles, as well as for breeding new cultivars.

Keywords: sugar-acid balance, composition profiles, Dietary Reference Intake, nutritional quality, organoleptic quality

1. Introduction

The *Solanaceae* family includes some of the world's most economically important berry-producing vegetables such as pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.) (Olmstead et al., 2008). In 2019, they ranked seventh ($38 \cdot 10^6$ t), first ($181 \cdot 10^6$ t) and fifth ($55 \cdot 10^6$ t), respectively, among vegetables for world total production (FAOSTAT, 2019). The fruits of pepper, tomato and eggplant are part of the cuisine of the whole world, although the predominant form of consumption varies among different cultures. Fruits of eggplant are harvested and consumed physiologically immature, while fruits of tomato are usually consumed at physiological maturity, and fruits of pepper can be consumed at both maturity stages. Both peppers and tomatoes are consumed fresh, cooked or dried; while eggplants are generally cooked. A daily dietary intake of these and other vegetables is highly recommended since they represent low-calorie nutrient-dense foods, constituting an important source of fibre, vitamins, minerals and a diverse array of other health-promoting phytochemicals like phenolics and flavonoids (Bursać Kovačević et al., 2020; Yahia et al., 2019).

Many studies on chemical composition of several cultivars of pepper, tomato and eggplant have been carried out over the last years. These studies reveal that peppers represent an outstanding source of vitamin C, flavonols (quercetin, kaempferol) and flavones (luteolin, apigenin) (Chassy et al., 2006; Fratianni et al., 2020; Lemos et al., 2019; Mennella et al., 2018; Wahyuni et al., 2013), and contain more than 20 different carotenoids (Giuffrida et al., 2013). Based on the published Dietary Reference Intake (DRI) values (Institute of Medicine, 2006), a portion of 100 g of pepper usually contributes over 100% to the daily intake of vitamin C and tocopherol, and provides 5-10% of provitamin A intake (Wahyuni et al., 2013). Tomatoes appear as the main source of lycopene in diet, but they also constitute an important source of vitamin C, E and flavonoids naringenin and quercetin-3-*O*-rutinoside (rutin) (Martí et al., 2018; Siddiqui et al., 2015; Slimestad et al., 2008). A serving size (200g) of fresh tomatoes has been reported to contribute mainly to the intakes of fibre and antioxidant compounds, providing 30 to 36% of the Recommended Dietary Allowance (RDA) for vitamin C; but it also contributes in a moderate proportion to the reference intake values of minerals, providing 10% of the Adequate Intake (AI) for K and 5-10% of the RDA for P and Mg (Casiraghi et al., 2013; Frusciante et al., 2007; Mohammed et al., 2020). Eggplants stand out for their high content in phenolics, mostly anthocyanins in the purple-coloured peel, and chlorogenic acid in the flesh (Docimo et al., 2016; García-Salas et al., 2014; Singh et al., 2009; Whitaker and Stommel, 2003), but they are also known for being a good source of minerals (Ayaz et al., 2015; Raigón et al., 2010). In that way, in a previous comprehensive fruit quality study of 31 varieties of eggplant, a portion of 100 g of fruit was reported to provide 7.7–13.5% of the estimated daily intake for phenolics; but it

also contributed 2.8–6.2% to P RDA, 3.3–5.9% to K RDA, and 4.3–9.7% to Cu RDA (Raigón et al., 2008).

In the last decade, secondary metabolites such as phenolic acids and flavonoids have garnered increasing attention in both food research and plant breeding due to their bioactive role and health-promoting function (Pott et al., 2019). Those compounds have shown antioxidant, antitumoral, anti-inflammatory and/or anti-microbial activity in several assays (Frutos et al., 2019; Granger and Eck, 2018; Kelkel et al., 2011; Kleemann et al., 2011; Sato et al., 2011), which contribute to the health benefits associated to the consumption of peppers, tomatoes and eggplants. Most of those benefits are related to protective effects, lowering the risk of cardiovascular, neurodegenerative and chronic diseases, including metabolic disorders and different types of cancer (Yahia et al., 2019). Furthermore, breeding for high content in bioactive compounds would lead to higher resilience to abiotic and biotic stresses, since those metabolites are involved in plant defence mechanisms (Yang et al., 2018).

Chemical composition of fruits is strongly dependent on the environmental and cultivation conditions (light, temperature, humidity, cultural practices, etc.). In this way, Tripodi et al. (2018) found the environment as an important source of variation, accounting for more than 30%, for carotenoids, vitamin C and tocopherols accumulation in fruits of hot pepper cultivated in two contrasting locations. Lower carotenoids in fruit, but higher concentrations of glucose and antioxidant activity were reported in the Mediterranean long shelf-life tomato under greenhouse conditions compared to open field cultivation by Figàs et al. (2018). Stommel et al. (2015) also found a highly significant genotype \times environment effect for content in phenolics in several varieties of eggplant, with two- to four-fold differences between mean values in two different cultivation sites. In addition, some studies aiming at assessing the genotype effect on composition traits reported a wide diversity among varieties for each of the three crops. In this way, Fratianni et al. (2020) found different polyphenol profiles among 14 traditional pepper varieties, Casals et al. (2019) found considerable variation for taste-related compounds among tomato varieties within two different cultivar groups, and San José et al. (2013) reported a wide diversity among seven eggplant cultivar groups for fibre, protein, carbohydrates, phenolics and vitamin C. Hence, environmental and genetic differences make it difficult to compare composition profiles from different studies. To our knowledge, no evaluation and comparison of fruit composition has been performed for a diverse set of these three crops grown under the same conditions.

In the present work, we have measured thirty-six major relevant compounds and parameters related to nutritional and organoleptic quality, including sugars, organic acids, β -carotene, vitamin C, phenolic acids, flavonoids and minerals, in fresh fruits at commercial ripeness of ten varieties for each of pepper, tomato and eggplant, grown under the same environmental conditions and organic agricultural practices. We have also evaluated the contribution of each crop to the available RDA or AI for several

compounds analysed. The main objective was to provide insight into the variation of the nutritional and organoleptic quality profile of these three solanaceous crops.

2. Materials and methods

2.1. Plant material and cultivation conditions

A total of 30 varieties of pepper ($n = 10$; P_ codes), tomato ($n = 10$; T_ codes) and eggplant ($n = 10$; E_ codes) were evaluated in the present study. The varieties selected were representative of the diversity of each species. A representative picture of fruits of the varieties used, including accession names, is displayed in Figure 1. Five plants per accession were grown in open field, in the spring-summer of 2017. For each species, the plants were distributed in a completely randomized design in a plot located in Burriana (Valencian Region, Mediterranean coast of Spain). The cultivation was carried out under organic farming following the standard horticultural management practices used in the area. The same conditions were applied to the three crops, since they have similar ranges of nutrition, temperature, soil pH requirements for open field cultivation during the spring-summer season (Rubatzky and Yamaguchi, 1997). In addition, we did not observe that any of the three crops had suboptimal growth or production. The plants were staked with canes and trained, and spaced at 0.50-m and 1.5-m intervals within and between rows, respectively. Flood irrigation was provided as needed to avoid water deficit stress and to compensate the crop evapotranspiration. Fertilization was provided by a single organic basal dressing consisting of chicken manure (N-P-K composition of 5-2-1) at a dose of 500 g m⁻². Soil texture was clay loam, which represents a well-balanced soil with intermediate compaction degree, avoiding rapid loss of nutrients and water and allowing proper aeration (Tracy et al., 2013). Average temperature during the cultivation period was 23.9 °C, and varied between 17.0 °C and 29.9 °C. Relative humidity was 64.4% in average, and ranged between 39.3% and 86.7%. May and June were the months with the highest mean radiation, with 27.2 MJ m⁻² and 28.0 MJ m⁻², respectively, and this parameter declined during the following months, to an average of 17.4 MJ m⁻² in September. Pluviometry was scarce and mostly concentrated on May 18th (8.5 mm), June 4th (20.4 mm) and August 29th (13.8 mm).

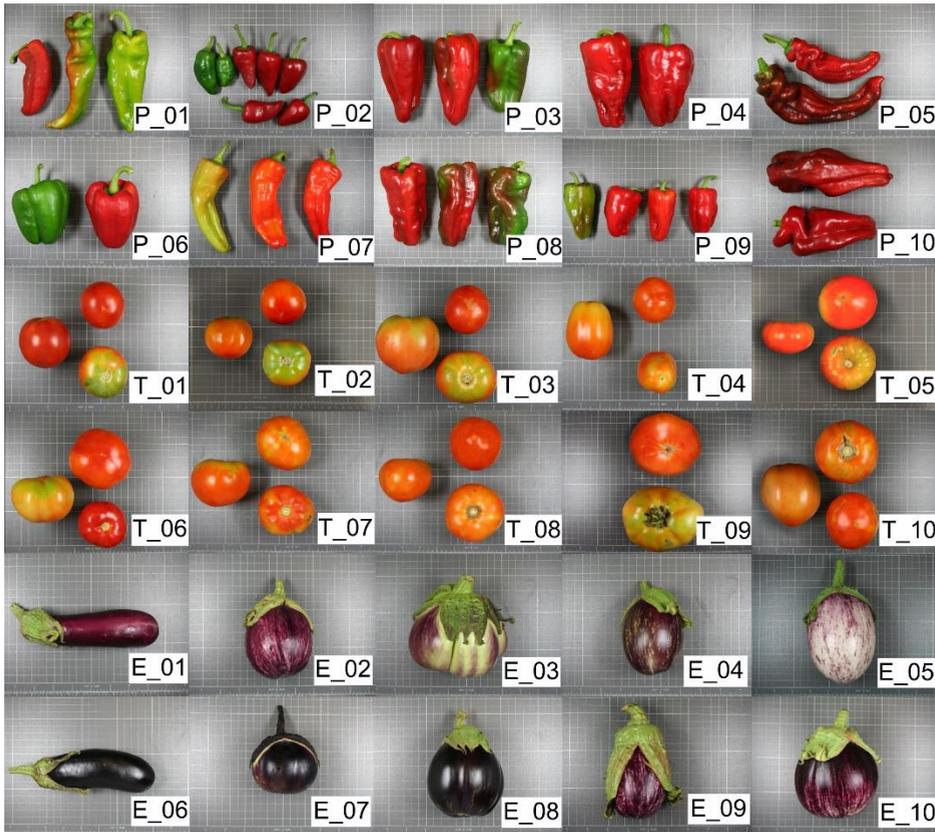


Figure 1. Representative fruits of the 10 varieties of pepper (P_ codes), tomato (T_ codes) and eggplant (E_ codes) used for the evaluation of chemical composition in the present work. The grid cells in the pictures measure 1 x 1 cm.

2.2. Sample preparation and metabolites analyses

2.2.1. Sample preparation

Fruits of pepper and tomato accessions were harvested at the red stage of maturity, which determines both commercial and physiological ripeness (Grierson and Kader, 1986; Harel et al., 2020). On the other hand, fruits of eggplant accessions were collected at the commercial ripeness stage, i.e., when fruits reached full size but yet physiologically immature. Three replicates per accession were taken, each one corresponding to 3-5 fruits from different plants. The fruits collected were washed, cut in pieces and seeds were eliminated. For each replicate, three differently processed samples were obtained for subsequent chemical analyses: a) liquid extract was collected using a HR 1832/45 domestic juice extractor (Philips, Eindhoven, Netherlands); b) freeze-dried and homogenized material was obtained using a VirTis Genesis lyophilizer (SP Scientific, Warminster, PA, USA) and a 908503000 domestic grinder (Taurus,

Oliana, Spain); and, c) dried material in a drying oven Digit DOD-20 (Raypa, Terrassa, Spain) at 70 °C up to constant weight was collected and powdered. Every trait analysed, with abbreviations used in Tables and Figures and units in which they are expressed, are listed in Table 1.

Table 1. Fruit composition traits evaluated in the pepper, tomato and eggplant varieties, abbreviations used and units in which they are expressed.

Fruit composition trait	Abbreviation	Units
Dry matter	dm	g kg ⁻¹ fw ^a
Soluble solids content	ssc	%
Fruit crude protein content	prot	g kg ⁻¹ fw
Citric acid content	cit	g kg ⁻¹ fw
Malic acid content	mal	g kg ⁻¹ fw
Total acid (citric + malic) content	tacid	g kg ⁻¹ fw
Citric:malic acid ratio	citmalr	-
Fructose content	fru	g kg ⁻¹ fw
Glucose content	glu	g kg ⁻¹ fw
Sucrose content	suc	g kg ⁻¹ fw
Total sugar (fructose + glucose + sucrose) content	tsug	g kg ⁻¹ fw
Total sweetness index	tsi	-
Total sugars:total acids ratio	tsugtacidr	-
Vitamin C (ascorbic + dehydroascorbic acid) content	vitc	g kg ⁻¹ fw
β-carotene content	bcar	mg kg ⁻¹ fw
Total phenolics content	tpc	g GAE ^b kg ⁻¹ fw
Total antioxidant activity	taa	mmol TE ^c kg ⁻¹ fw
Chlorogenic acid	cga	mg kg ⁻¹ fw
Caffeic acid	caf	mg kg ⁻¹ fw
Coumaric acid	coum	mg kg ⁻¹ fw
Ferulic acid	fer	mg kg ⁻¹ fw
Rutin	rut	mg kg ⁻¹ fw
Myricetin	myr	mg kg ⁻¹ fw
Quercetin	quer	mg kg ⁻¹ fw
Lutein	lut	mg kg ⁻¹ fw
Naringenin chalcone	chal	mg kg ⁻¹ fw
Kaempferol	kaemp	mg kg ⁻¹ fw
Apigenin	api	mg kg ⁻¹ fw
Minerals (potassium, phosphorus, calcium, magnesium, sodium, iron, copper, zinc)	K, P, Ca, Mg, Na, Fe, Cu, Zn	mg kg ⁻¹ fw

^afw: fresh weight

^bGAE: gallic acid equivalents

^cTE: trolox equivalents

2.2.2. Proximate traits

Dry matter was calculated as $100 \times (\text{dry weight/fresh weight})$. Crude protein content in fruit was estimated as $6.25 \times \text{total nitrogen}$, which was measured from 0.4 g of oven-dried powdered samples, through the Kjeldahl method (AOAC International, 2016), using a Kjeltec 2100 Distillation Unit (Foss Tecator, Högamäs, Sweden). Soluble solids content was measured using liquid extracts and a HI 96801 digital refractometer (HANNA instruments, Padua, Italy).

2.2.3. Sugars and organic acids

Sugars (fructose, glucose, sucrose) and organic acids (citric, malic) were identified by HPLC using a 1220 Infinity LC System (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump, an automatic injector and a UV detector. Quantification was based on calibration curves performed using known concentrations of standard references of each compound (Sigma-Aldrich, Saint Louis, MO, USA). For those analyses, aliquots of liquid extract were centrifuged for 5 min at 10 000 rpm and the supernatant diluted with water by $\frac{1}{4}$ and $\frac{1}{2}$ only for pepper and tomato samples, respectively. The diluted samples were filtered through 0.22 μm PVDF MILLEX-GV filters (Merck Millipore, Burlington, MA, USA). The same sample was used to perform the analysis of sugars and organic acids. Fructose, glucose and sucrose were separated using a Luna® Omega SUGAR column (3 μm ; 150 \times 4.6 mm; Phenomenex, Torrance, CA, USA). An isocratic gradient of the mobile phase 75% solvent A (acetonitrile): 25% solvent B (HPLC-grade water) was applied. The flow rate was 1 mL min⁻¹. Sugars were subsequently detected by refractive index using a 350 RI detector (Varian, Palo Alto, CA, USA) coupled to the HPLC system. Citric and malic acids were analysed by HPLC-UV at 210 nm using a Rezex™ ROA-Organic Acid H+ (8%) column (150 \times 7.8 mm; Phenomenex). The mobile phase consisted of an isocratic gradient of 100% 1 mM H₂SO₄ and the flow rate was 0.5 mL min⁻¹. Total sugar and total acid content were calculated from concentrations of individual compounds as fructose + glucose + sucrose and citric acid + malic acid, respectively. Ratios of contents in citric to malic acid and total sugars to total acids were also calculated. In addition, total sweetness index (tsi) was determined according to Beckles (2012), using the formula $\text{tsi} = (1.50 \times [\text{fructose}]) + (0.76 \times [\text{glucose}]) + (1.00 \times [\text{sucrose}])$.

2.2.4. Vitamin C

Content of vitamin C was evaluated from liquid extract preserved with 3% metaphosphoric acid (1:1, v/v). Detection and quantification of vitamin C was performed by HPLC-UV at 254 nm using the 1220 Infinity LC System (Agilent Technologies) and a Brisa “LC2” C18 column (3 μm ; 150 \times 4.6 mm; Teknokroma,

Barcelona, Spain), as the summation of ascorbic and dehydroascorbic acids, following the method described in Chebrolu et al. (2012).

2.2.5. β -carotene

β -carotene was extracted using 0.03 g (for pepper and tomato) and 0.1 g (for eggplant) of freeze-dried powder in ethanol:hexane 4:3 (v/v), in a shaker at 200 rpm for 1 h, keeping the samples in the dark. The hexane phase was subsequently separated, and β -carotene was quantified from absorbance values of this fraction measured at 452 nm and 510 nm using a 'UviLine 9400' UV-VIS spectrophotometer (Schott Instruments, Mainz, Germany) (Zscheile and Porter, 1947).

2.2.6. Phenolic compounds

Standards of the major free phenolic compounds (Sigma-Aldrich) in fruits of pepper, tomato and eggplant, according to literature (García-Salas et al., 2014; Lemos et al., 2019; Slimestad et al., 2008), were used for their identification and quantification in our collection. Compounds analysed are listed in Table 1. Phenolics were extracted using 0.1 g of freeze-dried homogenate, as described in Plazas et al. (2014). One aliquot of this extract was separated to carry out a hydrolysis by adding 3 M HCl (2:1, v/v) for 1 h at 95 °C, in order to free the aglycones (i.e., phenolic skeleton) from their attached sugar chain(s) in flavonoids. Samples were then analysed in the 1220 Infinity LC System (Agilent Technologies) using a Brisa "LC²" C18 column (3 μ m; 150 \times 4.6 mm; Teknokroma). Two gradient elution programs were used for analysis of phenolic acids (EP1) and aglycones (EP2), as described in Guijarro-Real et al. (2019). The same mobile phase, consisting of solvent A, 0.1% formic acid in ultrapure water, and solvent B, methanol, was used for both elution programs. Peaks were detected at a fixed wavelength of 320 nm (EP1) and 360 nm (EP2). A tentative identification and quantification of the compounds was conducted by overlapping the retention times of the sample peaks with those of the standards and with data from bibliography. Only compound peaks that exceeded the detection limit (LOD), given by a signal to noise ratio of 3:1 (International Conference on Harmonization, 2014), were considered for quantification. Due to overlapping signal in the EP2 of the major flavonol (quercetin) and flavanone (naringenin) in tomato samples, the standards of the most common quercetin glycoside in tomato, rutin (quercetin-3-*O*-rutinoside), and naringenin chalcone (Sigma-Aldrich) were used to quantify those compounds in the three crops, in addition to those already mentioned, using the EP1 elution program. Thus, tomato samples were not subjected to hydrolysis.

2.2.7. Total phenolics and total antioxidant activity

Total phenolics were extracted using 0.125 g of freeze-dried powder and spectrophotometrically determined according to the Folin-Ciocalteu procedure

(Singleton and Rossi, 1965), as indicated in Plazas et al. (2014). Gallic acid was used as standard reference (Sigma-Aldrich).

Total antioxidant activity was evaluated using the colourimetric assay of DPPH• (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capacity, according to Brand-Williams et al. (1995), with slight modifications. Subsamples of 0.150 g freeze-dried powder were incubated in a shaker with 5 mL of methanol:hydrochloric acid (99:1, v/v) in darkness for 1 h. Subsequently, 0.1 mL of diluted extract with methanol (1:10, v/v) was added to 3.9 mL DPPH• solution (0.025 g L⁻¹) and incubated in darkness for 1 h. Total antioxidant activity was determined from absorbance values of the solution at 515 nm, and using the antioxidant Trolox (Scharlab S.L., Barcelona, Spain) as standard.

2.2.8. Minerals

Extraction of the mineral fraction was performed on 2 g of oven-dried powder, as described in Raigón et al. (2010). Subsequently, content of the minerals (K, P, Ca, Mg, Na, Fe, Cu, Zn) was determined following the MAPA (1994) procedures.

2.3. Data analysis

For each species, the average and its standard error, range and coefficient of variation was calculated for all traits. Data were subjected to analysis of variance using species as factor, and to Student-Newman-Keuls post-hoc multiple range test at $p < 0.05$ for assessing significant differences among species means. Data sets of sucrose, vitamin C, β -carotene and chlorogenic acid content were log-transformed due to wide ranges of values among species and association of means and standard deviation values (Bartlett, 1947). In addition, differences among species for traits that did not have a normal distribution were evaluated using the Kruskal-Wallis non-parametric test (Sheskin, 2020). The relative abundance of the different compounds with respect to the total amount of metabolites within the same category (sugars, acids, antioxidants and minerals) was calculated using average contents for each of the three species. For total antioxidant value, the sum of contents in individual compounds contributing to it (i.e., vitamin C, β -carotene, individual phenolic acids and flavonoids) was considered. Principal components analysis (PCA) was performed using pairwise Euclidean distances among accession means for all the traits. Prediction ellipses for each species with a 95% level of confidence were added to the PCA score plot. A heatmap was constructed in order to cluster the accessions based on fruit composition profile. The software ClustVis was used for that purpose (Metsalu and Vilo, 2015). Original values were $\ln(x + 1)$ -transformed, unit variance scaling was applied to all traits and both accessions and traits were clustered using correlation distance and average linkage. The compounds unable to be quantified by HPLC, being below detection limit, were considered in data analysis as 0.

3. Results

3.1. Differences among species for fruit composition profile

The analysis of variance revealed significant differences among species for all traits evaluated, except for total sugars to total acids ratio. In this respect, the three species were significantly different from each other for average contents in dry matter, crude protein, fructose, citric and malic acid, citric to malic acid ratio, vitamin C, caffeic acid, rutin, Mg, Fe and Cu (Tables 2, 3 and 4). Regarding traits related to primary metabolism, pepper had the highest average content in fructose by 1.9-fold and 2.9-fold compared to tomato and eggplant, respectively. The same happened for citric acid content, which was 1.5-fold and 6.6-fold greater, respectively. In addition, pepper had around 2-fold higher average content in soluble solids, glucose, total sugars, total acids and total sweetness index, compared to tomato and eggplant (Table 2). The sugar profile was very similar between pepper and tomato, with fructose content contributing around 50% to the total sugars, and sucrose representing less than 2% (Figure 2). Although sucrose was the less abundant sugar for the three species, average levels of it in eggplant were more than 3-fold and 30-fold higher than those of pepper and tomato, respectively (Table 2). Thus, sucrose contributed to the eggplant sweetness profile to a larger extent (12%), at the expense of a reduced percentage in fructose content (38%) (Figure 2). Eggplant also stood out for its higher content in crude protein and malic acid, which were, respectively, 1.3-fold and 1.9-fold higher than in pepper; and 3.7-fold higher than in tomato, in both cases (Table 2). Therefore, malic was the acid contributing the most to the total acid content in eggplant, accounting for 82%; whereas the opposite was found for pepper and tomato, in which citric acid accounted for 74% and 79%, respectively (Figure 2).

With respect to secondary metabolites, no significant differences were found between pepper and eggplant for average total antioxidant activity, and both values were 1.7-fold higher than for tomato. Both vitamin C and β -carotene contents had mean values significantly higher, by a large extent, in pepper than in tomato or eggplant (Table 3). Among the antioxidant compounds evaluated, vitamin C represented the highest percentage in pepper (83%) and tomato (75%), while in eggplant, it represented less than 3% (Figure 2). Although no significant differences between pepper and eggplant were found for total phenolics content, diverse profiles of individual phenolic compounds were identified among the three species. Eggplant showed an outstandingly high average content of chlorogenic acid (1813.9 mg kg⁻¹, expressed on a fresh weight basis (fw)), contributing with 96% to the total content in antioxidants for that species; while pepper and tomato accumulated less than 25.0 mg kg⁻¹ fw of chlorogenic acid. The flavonoid quercetin was the most important phenolic compound in pepper (384.5 mg kg⁻¹ fw on average), which contributed 14% to the content of total antioxidants, followed by luteolin (17.7 mg kg⁻¹ fw on average). Rutin constituted the most relevant phenolic compound for tomato (41.2 mg kg⁻¹ fw on average), which accounted for 12% of the total content of antioxidants (Table 3, Figure 2).

Regarding mineral composition, eggplant stood out for its high average content in K (3266.4 mg kg⁻¹ fw), which was around 3.5-fold higher than in both pepper and tomato. Pepper displayed the highest average content in Fe (5.77 mg kg⁻¹ fw), which was 1.6 and 3.2-fold higher than in eggplant and tomato, respectively. Tomato, in general, had the lowest concentrations of minerals among the three species (Table 4). Despite those differences, the relative contribution of each mineral compound to the total content of those analysed was similar for the three species, although proportions changed slightly among them. Thus, K represented the highest percentage of the total (between 57% in pepper and 82% in eggplant); whereas Fe, Cu and Zn represented less than 1% of the total, for the three species (Figure 2).

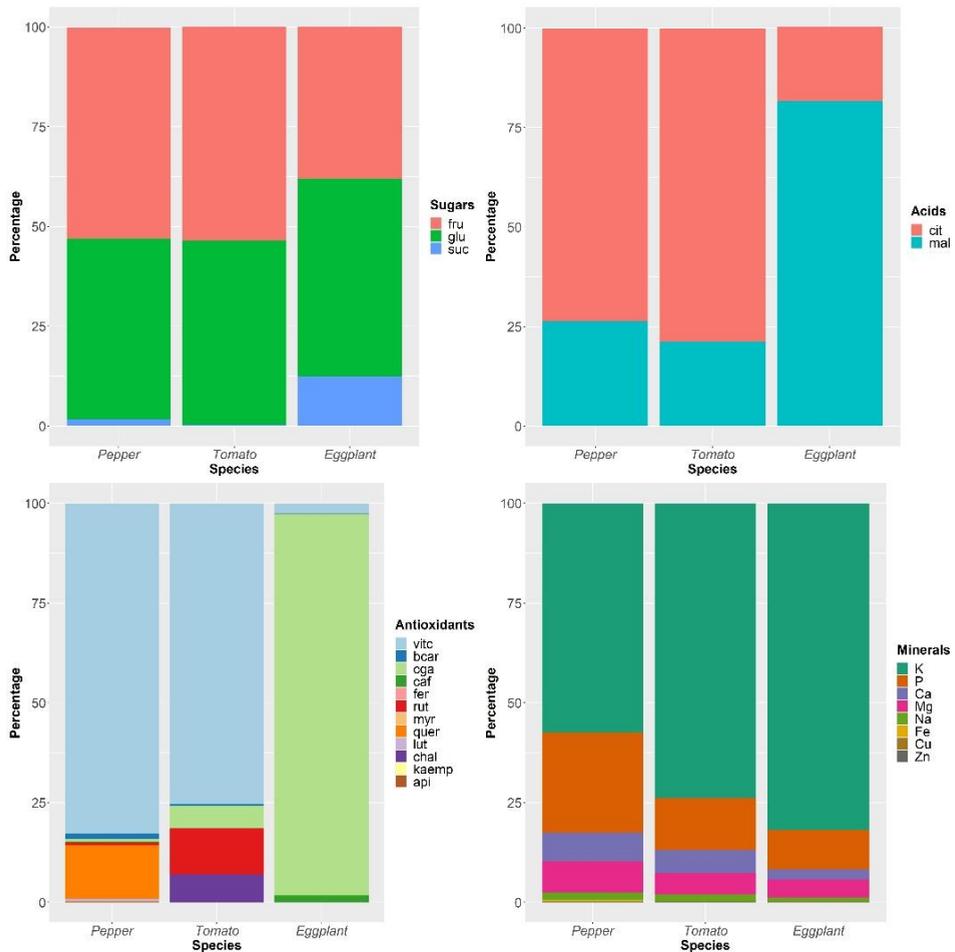


Figure 2. Stacked bar plots showing relative abundances (%) of average contents in different sugar, acid, antioxidant and mineral compounds over the total amount of each category present in each of the three species evaluated (pepper, tomato and eggplant). The names of the compounds are shown as the abbreviation used in the present work and listed in Table 1.

Considerable variation was found among the accessions regarding primary and secondary metabolism traits and minerals (Tables 2, 3, and 4). In this way, the coefficient of variation (CV) exceeded 100% in the case of sucrose for the three species, and also for chlorogenic acid and myricetin for pepper. Apart from sucrose, the highest CV in tomato was found for the content of naringenin chalcone (93.9%), and in eggplant, for total carotenoid content (81.4%). On the other side, the traits that displayed the lowest CV were total antioxidant activity and P content for pepper (around 22%), content in K and fructose for tomato (around 14%), and glucose and soluble solids contents for eggplant (15.4% and 17.4%, respectively) (Tables 2, 3, and 4). Individual data for each variety of the collection and the differences among them within species is included as Supplementary file S1.

Table 2. Mean \pm standard error (SE), range and coefficient of variation (CV) for species of traits related to primary metabolism analysed in the collection. Species means with different letters are significantly different at $p < 0.05$, while means with ^{ns} are not significantly different. The full name of each trait abbreviation in the first column can be found in Table 1.

Traits	Pepper			Tomato			Eggplant		
	Mean \pm SE	CV (%)	Range	Mean \pm SE	CV (%)	Range	Mean \pm SE	CV (%)	Range
dm (g kg ⁻¹ fw)	123.0 ^c \pm 28.1	22.9	76.2-159.1	54.5 ^a \pm 8.5	15.7	40.7-66.1	99.3 ^b \pm 29.8	30.0	63.3-157.2
prot (g kg ⁻¹ fw)	11.3 ^b \pm 3.4	30.6	6.5-17.0	4.0 ^a \pm 0.8	20.0	2.8-5.7	14.9 ^c \pm 3.7	24.7	8.1-20.8
ssc (%)	11.0 ^b \pm 3.1	27.9	5.8-17.1	5.7 ^a \pm 1.4	24.1	4.3-9.2	6.0 ^a \pm 1.0	17.4	4.6-8.1
fru (g kg ⁻¹ fw)	35.5 ^c \pm 10.1	28.4	16.0-48.0	18.9 ^b \pm 2.8	14.7	15.3-23.5	12.1 ^a \pm 3.1	25.4	8.5-17.6
glu (g kg ⁻¹ fw)	30.4 ^b \pm 9.5	31.0	16.6-50.8	16.3 ^a \pm 3.5	21.4	12.2-22.3	15.8 ^a \pm 2.4	15.4	10.2-18.4
suc (g kg ⁻¹ fw)	1.2 ^{ab} \pm 1.3	106.9	0.0-3.3	0.1 ^a \pm 0.2	229.0	0.0-0.7	3.9 ^b \pm 4.1	104.7	0.6-14.7
tsug (g kg ⁻¹ fw)	67.2 ^b \pm 18.3	27.3	35.8-95.2	35.2 ^a \pm 6.0	17.0	28.1-45.9	31.9 ^a \pm 8.3	25.9	19.5-50.3
tsi	77.6 ^b \pm 21.1	27.2	39.8-105.0	40.7 ^a \pm 6.5	16.0	32.9-52.3	34.1 ^a \pm 9.3	27.2	21.3-54.8
cit (g kg ⁻¹ fw)	5.4 ^c \pm 1.7	32.3	2.8-7.7	3.6 ^b \pm 0.9	25.1	2.5-5.4	0.8 ^a \pm 0.3	39.9	0.3-1.0
mal (g kg ⁻¹ fw)	1.9 ^b \pm 0.6	30.9	0.7-2.8	1.0 ^a \pm 0.2	21.2	0.6-1.4	3.6 ^c \pm 1.2	32.9	2.3-6.3
tacid (g kg ⁻¹ fw)	7.3 ^b \pm 2.2	30.5	3.5-10.3	4.6 ^a \pm 1.0	21.8	3.4-6.7	4.4 ^a \pm 1.4	32.5	2.6-7.5
citmalr	2.9 ^b \pm 0.7	23.4	2.0-4.0	3.8 ^c \pm 0.8	21.9	2.0-4.6	0.2 ^a \pm 0.1	34.1	0.1-0.4
tsugtacidr	9.7 ^{ns} \pm 2.7	27.2	5.8-14.0	7.9 ^{ns} \pm 1.8	23.1	5.8-11.6	7.6 ^{ns} \pm 1.6	21.0	5.5-9.9

Table 3. Mean \pm standard error (SE), range and coefficient of variation (CV) for species of traits related to secondary metabolism (antioxidants) analysed in the collection. Species means with different letters are significantly different at $p < 0.05$. The full name of each trait abbreviation in the first column can be found in Table 1.

Traits	Pepper			Tomato			Eggplant		
	Mean \pm SE	CV (%)	Range	Mean \pm SE	CV (%)	Range	Mean \pm SE	CV (%)	Range
vitc (g kg ⁻¹ fw)	2.36 ^c \pm 0.66	27.8	1.56-3.75	0.27 ^b \pm 0.05	18.6	0.20-0.37	0.05 ^a \pm 0.01	22.8	0.03-0.06
bcar (mg kg ⁻¹ fw)	39.15 ^b \pm 16.93	43.2	6.82-63.44	1.60 ^a \pm 1.04	64.8	0.66-3.77	0.48 ^a \pm 0.52	107.3	0.01-1.38
cga (mg kg ⁻¹ fw)	15.8 ^a \pm 18.6	117.8	6.0-67.5	20.3 ^a \pm 14.8	73.1	8.1-56.0	1813.9 ^b \pm 580.6	32.0	1278.9-2956.2
caf (mg kg ⁻¹ fw)	6.99 ^a \pm 2.78	39.7	0.88-10.51	BDL ¹			34.55 ^b \pm 12.55	36.3	16.26-51.84
fer (mg kg ⁻¹ fw)	4.08 \pm 3.63	89.1	0.00-9.50	BDL			BDL		
coum (mg kg ⁻¹ fw)	BDL			BDL			BDL		
rut (mg kg ⁻¹ fw)	15.4 ^a \pm 9.3	60.3	0.0-24.3	41.2 ^b \pm 20.9	50.9	22.5-80.2	BDL		
myr (mg kg ⁻¹ fw)	0.93 \pm 1.01	107.9	0.00-3.08	BDL			BDL		
quer (mg kg ⁻¹ fw)	384.5 \pm 158.0	41.1	167.6-707.6	BDL			BDL		
lut (mg kg ⁻¹ fw)	17.7 \pm 8.0	45.3	7.1-32.9	BDL			BDL		
chal (mg kg ⁻¹ fw)	BDL			25.1 \pm 23.6	93.9	0.0-70.6	BDL		
kaemp (mg kg ⁻¹ fw)	3.05 \pm 1.11	36.3	0.98-4.91	BDL			BDL		
api (mg kg ⁻¹ fw)	3.36 \pm 1.42	42.1	0.60-4.85	BDL			BDL		
tpc (g GAE kg ⁻¹ fw)	1.11 ^b \pm 0.52	46.9	0.62-2.41	0.38 ^a \pm 0.09	23.8	0.22-0.49	2.70 ^b \pm 0.72	26.7	1.92-3.76
taa (mmol TE kg ⁻¹ fw)	36.2 ^b \pm 8.1	22.4	23.0-47.4	21.4 ^a \pm 3.6	17.0	15.7-26.7	37.2 ^b \pm 7.5	20.1	22.5-51.2

¹ BDL: below detection limit

Table 4. Mean \pm standard error (SE), range and coefficient of variation (CV) for species of minerals analysed in the collection. Species means with different letters are significantly different at $p < 0.05$. The full name of each trait abbreviation in the first column can be found in Table 1.

Traits	Pepper			Tomato			Eggplant		
	Mean \pm SE	CV (%)	Range	Mean \pm SE	CV (%)	Range	Mean \pm SE	CV (%)	Range
K (mg kg ⁻¹ fw)	935.0 ^a \pm 266.8	28.5	615.8-1364.4	900.1 ^a \pm 128.0	14.2	683.6-1176.9	3266.4 ^b \pm 567.1	17.4	2421.4-4465.5
P (mg kg ⁻¹ fw)	408.7 ^b \pm 92.4	22.6	311.0-583.9	158.9 ^a \pm 24.3	15.3	124.4-211.6	388.9 ^b \pm 77.9	20.0	297.2-534.3
Ca (mg kg ⁻¹ fw)	117.4 ^b \pm 31.6	26.9	74.4-167.3	70.8 ^a \pm 17.8	25.1	59.7-113.5	102.9 ^b \pm 23.1	22.4	81.0-145.5
Mg (mg kg ⁻¹ fw)	126.3 ^b \pm 34.0	26.9	78.5-189.1	64.5 ^a \pm 15.7	24.3	40.0-95.5	179.3 ^c \pm 46.6	26.0	106.8-249.7
Na (mg kg ⁻¹ fw)	31.0 ^{ab} \pm 13.0	41.9	14.2-53.0	20.4 ^a \pm 3.8	18.8	14.4-26.7	41.0 ^b \pm 15.2	37.0	24.0-67.2
Fe (mg kg ⁻¹ fw)	5.77 ^c \pm 1.75	30.3	2.91-7.95	1.81 ^a \pm 0.51	28.3	1.11-2.39	3.53 ^b \pm 1.11	31.3	2.07-5.25
Cu (mg kg ⁻¹ fw)	0.86 ^b \pm 0.37	42.9	0.35-1.40	0.39 ^a \pm 0.10	26.6	0.23-0.58	1.21 ^c \pm 0.31	26.0	0.83-1.77
Zn (mg kg ⁻¹ fw)	2.71 ^b \pm 0.77	28.4	1.39-3.93	1.34 ^a \pm 0.29	21.4	0.81-1.82	3.07 ^b \pm 0.81	26.3	1.81-4.77

3.2. Principal components and cluster analyses

The first two principal components (PCs) of the PCA explained 75.1% of the total variation observed, with PC1 and PC2 accounting for 41.0% and 34.1% of the total variation, respectively (Figure 3A, 3B). When considering traits with correlations above 0.2 with PC1 or PC2 it was found that dry matter, contents in fructose, glucose, total sugars and acids, β -carotene, vitamin C, flavonoids (except rutin, myricetin and naringenin chalcone), and Fe, were negatively correlated to PC1. Besides, contents in protein, malic acid, total phenolics content, phenolic acids (except ferulic acid), and minerals (except P, Ca and Fe), were positively correlated to PC2, while citric:malic acid ratio and rutin were negatively correlated to PC2 (Figure 3A).

The PCA clearly separated the thirty varieties evaluated into three groups that matched the corresponding species (Figure 3B). All eggplant varieties clustered together in the upper right quadrant of the PCA score plot (Figure 3B), corresponding to positive values for both PC1 and PC2; all tomato varieties were grouped together in the lower right quadrant, showing positive values for PC1 but negative for PC2; finally, most of the pepper varieties plotted in the lower left quadrant, with negative values for both PC1 and PC2, except for one accession that showed positive values for PC1 and one accession that showed low positive values for PC2 (Figure 3B). The more widespread distribution of pepper accessions, as opposed to the narrower distribution of the tomato and eggplant accessions, indicated a larger variability; while the lowest variation was found within tomato. Confidence ellipses of pepper and tomato overlapped, although to a very small extent, due to the pepper variety P_07, whose composition profile was more similar to the one of tomato (Figure 3B).

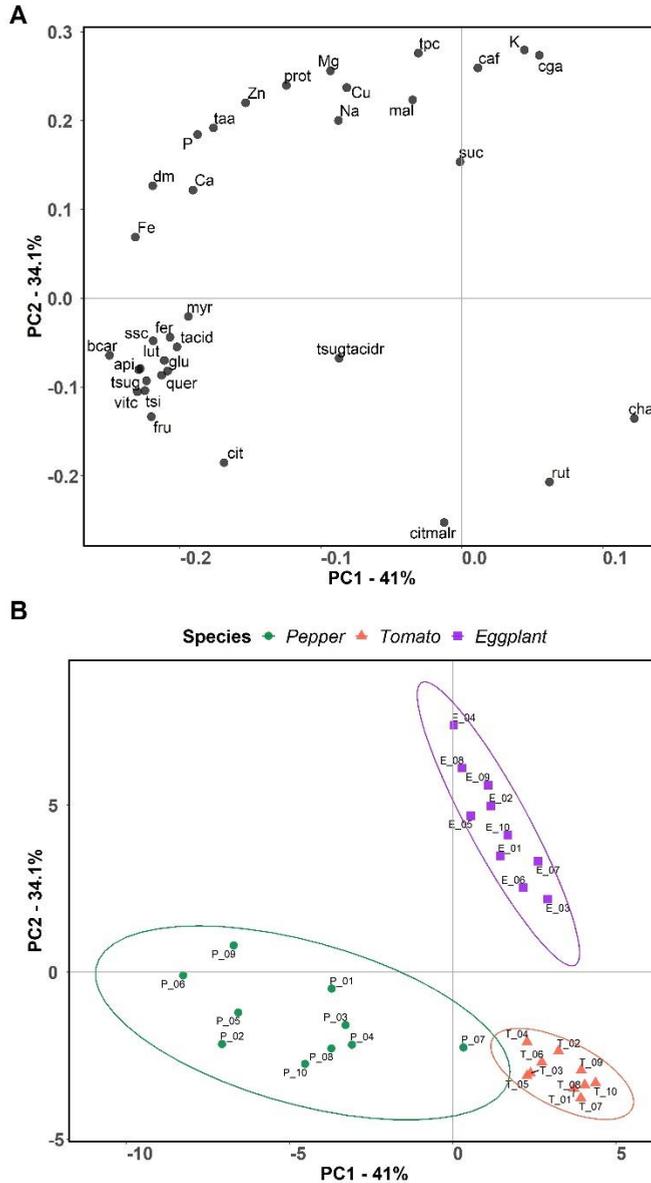


Figure 3. PCA loading plot (A) and score plot (B) evaluated in the present study. based on the two first principal components of PCA. First and second components account for 41.0% and 34.1% of the total variation, respectively. The accessions are represented by different symbols and colour according to the species: green circle for pepper, orange triangle for tomato and purple square for eggplant. Ellipses grouped the accessions of each species with a 95% confidence level. The full name of each trait abbreviation can be found in Table 1.

The multivariate cluster heatmap in Figure 4 revealed three major clusters for the varieties, each of which corresponded to each of the species studied, indicating considerable differences in the composition profile of fruits of pepper, tomato and eggplant. In addition, the analysis separated the compounds and parameters analysed in two large clusters. The upper cluster grouped all the mineral compounds together and the phenolic acids, except ferulic acid. On the other side, all the flavonoids appeared in the lower cluster, along with vitamin C, β -carotene, and all the parameters related to sweetness, acidity and the balance between them, except sucrose and malic acid (Figure 4). Within the diversity found for each species, eggplant variety E_05 showed the highest average content in sucrose and malic acid, while E_04 had the best profile in total polyphenols and antioxidant activity, phenolic acids (chlorogenic and caffeic) and minerals. In addition, pepper varieties P_06 and P_09 stood out for having the greatest content in myricetin, and high values for mineral composition; P_04 showed the best content in major flavonoids in pepper: quercetin, luteolin and apigenin; and P_10 and P_02 accumulated more sugars in their fruits, having the best sweetness profile, as well as an outstanding content in vitamin C. Lastly, the tomato variety T_07 had the highest contents in flavonoids naringenin chalcone and rutin (Figure 4).

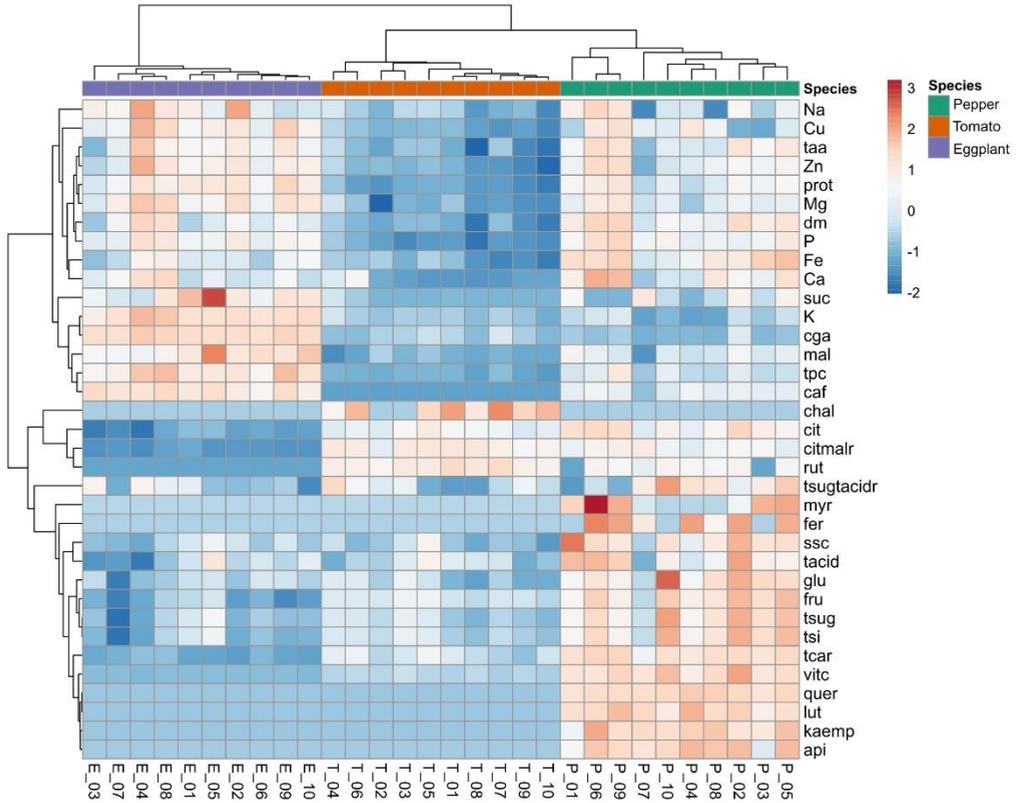


Figure 4. Heatmap representing the hierarchical clustering of the 30 accessions studied based on their fruit composition profiles. Columns represent the accessions and rows represent the traits evaluated. Unit variance scaling was applied to rows. Both rows and columns are clustered using correlation distance and average linkage. The scale of the colour intensity is shown in the top right corner and it represents a proportional value of the compound content. The full name of each trait abbreviation can be found in Table 1.

3.3. Contribution to RDA/AI

Based on data of the nutrients included in the daily Recommended Dietary Allowance (RDA) and the Adequate Intake (AI) reports (Institute of Medicine, 2006), and using average data for species, consuming daily 100 g portion of red sweet pepper, tomato or eggplant, could contribute to the RDA/AI in a low (< 3%) or moderate (3-6%) level for most of the compounds evaluated (Table 5). Nevertheless, a 100 g portion of pepper largely exceeded the daily RDA for vitamin C, with an outstanding average contribution of 262% and 314% for males and females, respectively. In addition, there is a relevant (> 6%) contribution to the daily RDA of 100 g of pepper and eggplant for Cu (9.6% and 13.4%, in average, respectively). Pepper also stood out for its mean contribution to daily RDA for Fe (7.2% for adult males, and females above 51 years old). Tomato, in a 100 g portion basis, had lower percentages of contribution to the RDA than pepper and eggplant in all cases; however, among the compounds evaluated, tomato had an average contribution to the vitamin C daily RDA of 30% and 36% for males and females, respectively (Table 5).

Regarding relative ranges of variation of 100 g portion contributions to daily RDA/AI, the largest values for pepper and tomato were found for their contributions to the Cu daily RDA (4-fold and 2.5-fold) and for eggplant, to the Na daily AI (2.8 to 2.9-fold). On the other hand, the lowest values of the same parameter for eggplant and pepper were observed for their contribution to the P daily RDA (1.8 and 1.9-fold), and in the case of tomato, to the daily RDA for carbohydrates (estimated as total sugars) (1.6-fold) (Table 5).

Table 5. Contribution of 100 g of the pepper, tomato and eggplant fruits to daily Recommended Dietary Allowances (RDA) or Adequate Intake (AI) (Institute of Medicine, 2006) for protein, carbohydrates (estimated as total sugars), vitamin C and all minerals studied, considering average values and range (in parentheses) per species of those traits. RDA or AI considered corresponded to adult males and females ≥ 18 years old (yo).

Nutrient	Daily RDA/AI		Contribution of 100 g to daily RDA/AI (%)					
			Pepper		Tomato		Eggplant	
	Male	Female	Male	Female	Male	Female	Male	Female
Protein (g ^a)	56	46	2.0 (1.2-3.0)	2.4 (1.4-3.7)	0.7 (0.5-1.0)	0.9 (0.6-1.2)	2.7 (1.4-3.7)	3.2 (1.8-4.5)
Carbohydrates (g)	130	130	5.2 (2.8-7.3)	5.2 (2.8-7.3)	2.7 (2.2-3.5)	2.7 (2.1-3.5)	2.5 (1.5-3.9)	2.5 (1.5-3.9)
Vitamin C (g)	0.090	0.075	262 (173-417)	314 (208-500)	30.0 (22.2-41.1)	36.0 (26.7-49.3)	5.6 (3.8-7.0)	6.7 (4.5-8.4)
Ca (mg)	1000	1000	1.2 (0.7-1.7)	1.2 (0.7-1.7)	0.7 (0.6-1.1)	0.7 (0.6-1.1)	1.0 (0.8-1.5)	1.0 (0.8-1.5)
	1200 (≥ 51 yo)	1200 (≥ 51 yo)	1.0 (0.6-1.4)	1.0 (0.6-1.4)	0.6 (0.5-1.0)	0.6 (0.5-1.0)	0.9 (0.7-1.2)	0.9 (0.7-1.2)
Cu (mg)	0.9	0.9	9.6 (3.9-15.6)	9.6 (3.9-15.6)	4.3 (2.6-6.4)	4.3 (2.6-6.4)	13.4 (9.2-19.7)	13.4 (9.2-19.7)
Fe (mg)	8	8	7.2 (3.6-9.9)	7.2 (3.6-9.9)	2.3 (1.4-3.0)	2.3 (1.4-3.0)	4.4 (2.6-6.6)	4.4 (2.6-6.6)
		18 (19-50 yo)		3.2 (1.6-4.4)		1.0 (0.6-1.3)		2.0 (1.2-2.9)
Mg (mg)	400 (19-30 yo)	310 (19-30 yo)	3.2 (2.0-4.7)	4.1 (2.5-6.1)	1.6 (1.0-2.4)	2.1 (1.3-3.1)	4.5 (2.7-6.2)	5.8 (3.5-8.1)
	420 (≥ 31 yo)	320 (≥ 31 yo)	3.0 (1.9-4.5)	3.9 (2.5-5.9)	1.5 (1.0-2.3)	2.0 (1.3-3.0)	4.3 (2.5-5.6)	5.6 (3.3-7.8)
P (mg)	700	700	5.8 (4.4-8.3)	5.8 (4.4-8.3)	2.3 (1.8-3.0)	2.3 (1.8-3.0)	5.6 (4.3-7.6)	5.6 (4.3-7.6)
K (mg)	4700	4700	2.0 (1.3-2.9)	2.0 (1.3-2.9)	1.9 (1.5-2.5)	1.9 (1.5-2.5)	6.9 (5.2-9.5)	6.9 (5.2-9.5)
Na (mg)	1500	1500	0.21 (0.09-0.35)	0.21 (0.09-0.35)	0.14 (0.10-0.18)	0.14 (0.10-0.18)	0.27 (0.16-0.45)	0.27 (0.16-0.45)
	1300 (51-70 yo)	1300 (51-70 yo)	0.24 (0.11-0.41)	0.24 (0.11-0.41)	0.16 (0.11-0.21)	0.16 (0.11-0.21)	0.32 (0.18-0.52)	0.32 (0.18-0.52)
	1200 (≥ 70 yo)	1200 (≥ 70 yo)	0.26 (0.12-0.44)	0.26 (0.12-0.44)	0.17 (0.12-0.22)	0.17 (0.12-0.22)	0.34 (0.20-0.56)	0.34 (0.20-0.56)
Zn (mg)	11	8	2.5 (1.3-3.6)	3.4 (1.7-4.9)	1.2 (0.7-1.7)	1.7 (1.0-2.3)	2.8 (1.7-4.3)	3.8 (2.3-6.0)

^aBased on g protein per kg of body weight for the Reference Body Weight (70 kg for adult males and 57 kg for adult females) (Institute of Medicine, 2006).

4. Discussion

The present study provides a thorough comparison of the nutritional and organoleptic value of three major vegetables from the highly important family *Solanaceae*. The experiment was performed under the same environmental conditions, which is of considerable interest due to the polygenic control of fruit composition traits and the strong influence of the environmental conditions on the final phenotype (Cebolla-Cornejo et al., 2011; Figàs et al., 2018; Stommel et al., 2015; Tripodi et al., 2018). Results showed considerably different quality profiles among the three species. This is in agreement with studies in comparative genomics that have already reported the large diversity within the *Solanaceae* family, more specifically within the genus *Solanum* (Arnoux et al., 2021).

Regarding all traits except phenolic compounds, compared to USDA National Nutrient Database (U.S. Department of Agriculture, 2021) and data from other works, our results showed similar or higher average and higher maximum values for most of the traits evaluated in pepper (Chassy et al., 2006; Eggink et al., 2012; Fratianni et al., 2020; Guilherme et al., 2020; Lo Scalzo et al., 2020; Mennella et al., 2018), tomato (Casals et al., 2019; Cebolla-Cornejo et al., 2011; Chassy et al., 2006; Hallmann, 2012; Martí et al., 2018; Martínez-Valverde et al., 2002), and eggplant (Ayaz et al., 2015; Hanson et al., 2006; Luthria et al., 2010; Raigón et al., 2010; San José et al., 2013). Furthermore, ranges of values between our experiment and the others overlapped to a greater or lesser extent; e.g., β -carotene content values for pepper in Mennella et al. (2018), dry matter results for tomato in Martínez-Valverde et al. (2002) or Mg contents for eggplant in Ayaz et al. (2015). Contrarily, higher average contents were found for a few compounds in other works than ours, including total phenolics and K for pepper in the USDA National Nutrient Database, and K in Guilherme et al. (2020); protein, β -carotene, total phenolics and all mineral compounds for tomato in the USDA National Nutrient Database, all minerals except K in Fernández-Ruiz et al. (2011), and total phenolics in Hallmann (2012); finally, for eggplant, vitamin C in Ayaz et al. (2015), and Na in Raigón et al. (2010).

Regarding phenolic compounds, a wide range with considerable variation in concentration have been described in several cultivars of pepper (Chassy et al., 2006; Fratianni et al., 2020; Lemos et al., 2019; Ribes-Moya et al., 2020), tomato (Chassy et al., 2006; Hallmann, 2012; Martí et al., 2018; Slimestad et al., 2008) and eggplant (García-Salas et al., 2014; Singh et al., 2009; Whitaker and Stommel, 2003), especially for the former. Based on the previous literature, the most abundant compounds in each of the three species were selected to analyse their concentration in fruits of our collection. Thus, more compounds were identified and quantified in pepper (9 out of 11) than in tomato (3 out of 11) and eggplant (2 out of 11). The widest range of variation among studies was found for pepper. Although lower average values for some compounds were found in our study compared to others, ranges of values overlapped to

a large extent; except for lutein and myricetin contents in Ribes-Moya et al. (2020), chlorogenic acid and myricetin in Fratianni et al. (2020) and kaempferol in Chassy et al. (2006). For tomato, higher average and large but overlapping ranges were observed for chlorogenic acid and rutin as compared to the data obtained by Hallmann (2012) and Martí et al. (2018). In addition, similar range of values to those observed by Slimestad et al. (2008) was found for naringenin chalcone in tomato. Contrarily, trace amounts of caffeic, *p*-coumaric and ferulic acid were detected in tomato by Martí et al. (2018), while none exceeded the detection limit in our study.

It should be noted that, in the literature mentioned, only cultivated varieties with similar way of consumption to those used by us, and in which fruits were analysed raw at the same ripening stage as in our experiment, were taken into account. The differences observed among studies may be due to the cultivars selected, analytical methods used and the different environment and cultivation practices under which plants were grown. Furthermore, more differences and larger ranges of variation among studies were found for secondary metabolites compared to the primary metabolites and mineral contents, which may be the result of domestication (Pott et al., 2019). Those data confirm the strong environmental influence on the synthesis and accumulation of compounds related to nutritional and organoleptic quality, especially for secondary metabolites, since their synthesis is usually triggered by a stressful condition (Yang et al., 2018).

Based on our results, a selection of varieties with better profiles could be of interest for future breeding programs and for cultivation under organic conditions. Among the varieties evaluated in the present study, the pepper P_10 would represent a good source for improving sweetness and vitamin C content. Besides, P_09 and P_06 showed high nutritional properties for their mineral and carotenoid contents, and total antioxidant activity. P_04 was another variety to consider for improving content in major flavonoids (quercetin, luteolin and apigenin) in pepper. The best tomato varieties to consider for future breeding programs were T_04, for its content in minerals, protein and sugar-acid balance, T_05 for its content in carotenoids and T_07 for its content in major flavonoids. In the case of eggplant varieties, E_04 and E_08 would represent the best source for improving mineral content and antioxidant properties in this species. In addition, these varieties were among the best regarding sugar-acid balance. On the other hand, E_05 could be a good resource for the improvement of sweetness in general and sucrose content in particular.

4.1. Pepper, tomato and eggplant differences in primary metabolites

In the present study, significant differences among pepper, tomato and eggplant, for sugar and acid profiles were detected, which would contribute to the differences in perception of their taste. Pepper showed the highest average values of total sugars, total sweetness index, and total acid content; while tomato and eggplant showed no significant differences in terms of total sugars and acids. In addition, results revealed

that major sugars in fruit for the three species were fructose and glucose, while sucrose accumulation was marginal. Nevertheless, sucrose accumulation in eggplant was significantly higher than in the other two species, representing a higher percentage of total sugars at the expense of fructose. On the other hand, citric was the major organic acid in pepper and tomato, while in eggplant was malic. The same profile of sugars and acids was described for pepper (Lo Scalzo et al., 2020), tomato (Quinet et al., 2019) and eggplant (Ayaz et al., 2015; San José et al., 2013). Although the sugar-acid balance showed no significant differences among the three species, the differences in their sugar and acid profiles would give a sweeter or more sour taste to the fruit, since malic acid has twice the acidic potential of citric acid and the sweetness potential of sugars follows this order: fructose > sucrose > glucose (Beckles, 2012).

Our results suggest a similar pattern, among the three species, of the mechanisms involved in the accumulation of sugars in the fruit. The marginal accumulation of sucrose could be explained by the limited activity of the sucrose-phosphate-synthase compared to a significant increase in the invertase activity during fruit ripening (Quinet et al., 2019). In addition, it has been reported that, during tomato fruit ripening, an important part of malic acid is converted to simple sugars via gluconeogenesis (Schouten et al., 2016). In this way, the higher proportion of sucrose found in eggplant with respect to tomato and pepper in the present study, as well as the predominance of malic over citric acid, might be due to the fact that the eggplant is harvested and consumed at physiological immaturity.

4.2. Pepper, tomato and eggplant differences in secondary metabolites

Considerable differences among the three solanaceous fruits were detected for the profile of secondary metabolites. Unlike for primary metabolites, data of total antioxidant activity are difficult to compare with other reports because of the lack of a standard official procedure (López-Alarcón and Denicola, 2013). In our experiment, no significant differences were found between pepper and eggplant for antioxidant activity and both had higher average values than tomato. In Morales-Soto et al. (2014), who evaluated 44 fruits and vegetables for total antioxidant activity, different profiles, depending on the methodology used, were found. However, pepper and eggplant had higher maximum values than tomato in most of the cases. Interestingly, pepper and eggplant were among the five best fruits and vegetables out of the 44 evaluated by these authors.

Major compounds contributing to the fruit antioxidant activity were considerably different among the three species. In addition, each antioxidant compound would contribute differently to the total antioxidant activity depending on their free radical scavenging capacity. According to Kim and Lee (2004), the ranking of compounds analysed in the present study for their scavenging capacity relative to vitamin C, would be as follows: myricetin > quercetin > luteolin > ferulic acid > naringenin > kaempferol

> caffeic acid > vitamin C > apigenin > chlorogenic acid > rutin > carotenoids. Furthermore, some differences in this ranking could be found depending on the antioxidant assay used for the evaluation. For instance, carotenoids are not capable of scavenge the DPPH• (Müller et al., 2011), and thus, did not contribute to the total antioxidant activity in this study.

In pepper, the main antioxidant compound of those evaluated was vitamin C, representing 82% of the total content in antioxidants evaluated, followed by the flavonoid quercetin (13%) and β -carotene (1.4%). This is in agreement with other studies (Chassy et al., 2006; Fratianni et al., 2020). Others reported luteolin as the predominant phenolic, ahead of quercetin (Ribes-Moya et al., 2020). In tomato, vitamin C (74%) was also the main antioxidant compound, followed by the flavonoids rutin (quercetin-3-*O*-rutinoside) (11%) and naringenin chalcone (7%). This is in agreement with previous studies (Chassy et al., 2006; Hallmann, 2012; Martí et al., 2018; Slimestad et al., 2008). Eggplant showed a more distinct profile of antioxidant compounds than pepper and tomato. While vitamin C content only represented 3% of the total antioxidants, chlorogenic acid was identified as the major antioxidant in eggplant (96%), as observed in previous research (Luthria et al., 2010; Whitaker and Stommel, 2003). Fewer characterizations of vitamin C content in eggplant have been carried out compared to pepper and tomato. However, the coefficient of variation for that compound indicated that there is room for improvement in this species. Selecting high-vitamin C varieties in eggplant would not only imply a health benefit related to consumption, but it would also be beneficial for the fruit organoleptic quality, as it may reduce browning, a non-desirable trait for consumers, caused by the oxidation of polyphenols (San José et al., 2013).

A wide range of natural pigments within the group of carotenoids exists among vegetables (Young and Lowe, 2018). Although these compounds are synthesised from the same metabolic pathway, the differential expression of genes associated with enzymes involved in it results in different carotenoids accumulating to a greater or lesser extent in fruits of peppers, tomatoes and eggplants (Barchi et al., 2019; Paran and Van Der Knaap, 2007). We have focused on β -carotene due to its important role in human health. Besides its function as free radical quencher, and thus its protective role against oxidative stress, its role as vitamin A precursor has been widely studied (Fiedor and Burda, 2014). Our results showed a higher content of β -carotene in pepper than in tomato or eggplant by around 40-fold. Results in most of other works showed considerably lower amounts of β -carotene in pepper (Fratianni et al., 2020; Marín et al., 2004; U.S. Department of Agriculture, 2021), indicating a lower difference with tomato. This may be due to the presence of other yellow-orange carotenoids in pepper, as lutein and zeaxanthin, absent in tomato and eggplant, that had absorption maxima at similar wavelengths, potentially overestimating β -carotene content estimates in our study (Ribes-Moya et al., 2018). Although it would be more accurate to quantify carotenoids

by more specific and sensitive methods such as HPLC, using spectrophotometry is a reliable, easy, fast and cost-effective method for comparison among different samples in the same study.

Selecting and breeding for higher content in phenolic compounds, which includes both phenolic acids and flavonoids, is of utmost interest due to the health benefits associated to them (Cory et al., 2018; Rodriguez-Mateos et al., 2014). In the present study, results showed significant differences among the three solanaceous species for total phenolics content in fruits. Thus, eggplant showed the highest average values and the lowest were found for tomato, while pepper had intermediate values. Regarding phenolic acids, chlorogenic acid was detected in the three species, although in eggplant the average content was around 100-fold higher than in the others; caffeic acid was quantified in pepper and eggplant; ferulic acid only in pepper; and *p*-coumaric acid was detected in none of them. In other studies, higher resolution analyses using LC-MS technology identified caffeic acid and *p*-coumaric acid and its derivatives in tomato landraces (Pinela et al., 2019). With respect to flavonoids, most were detected only in pepper fruits: quercetin, luteolin, apigenin, kaempferol and myricetin, in decreasing order of relevance. The detection of a certain amount of rutin (quercetin-3-*O*-rutinoside) in peppers indicates that a small proportion of the quantified quercetin (4% on average) would appear as that glycoside. The presence of rutin in pepper has also been reported in other works such as Fratianni et al. (2020). For tomato, there is evidence in literature that most of quercetin content appear as rutin (Slimestad et al., 2008). We were able to detect and quantify rutin as the major flavonoid of those evaluated in tomato, followed by naringenin chalcone. Those results are in agreement with Slimestad et al. (2008) and Martí et al. (2018). Other studies were able to quantify kaempferol and other flavonoids in tomato, although in trace amounts (Chassy et al., 2006; Hallmann, 2012; Pinela et al., 2019). Both rutin and free quercetin have been reported to show health benefits at some extent, reducing risks of neurodegenerative disorders, cancer, atherosclerosis and other cardiovascular diseases (Frutos et al., 2019; Jeong et al., 2009; Kleemann et al., 2011). On the other hand, in the present study, no flavonoids were detected in eggplant fruits. Although some studies have been able to identify and quantify flavonoids in eggplant flesh, those compounds were found in trace amounts, thus most of the studies in this area focused their efforts in identify isomers and derivatives of chlorogenic and caffeic acid (García-Salas et al., 2014; Singh et al., 2009).

4.3. Pepper, tomato and eggplant differences in mineral composition

The present study suggests the same pattern of accumulation of mineral compounds in pepper, tomato and eggplant. In addition, the major mineral compound in the three species was K, followed by P and Ca or Mg. This is in agreement with other works (Ayaz et al., 2015; Fernández-Ruiz et al., 2011; Guilherme et al., 2020; Raigón et al., 2010; U.S. Department of Agriculture, 2021), although individual contents may vary among studies, probably due to their high dependence on the soil composition and

minerals availability (Raigón et al., 2010). High variation coefficients were found, mainly among pepper varieties, which facilitate the selection of better genotypes for its cultivation under organic conditions. Although studies have been done to elucidate the genetic control of mineral accumulation in tomato fruit (Capel et al., 2017), detailed analysis of the regulation of fruit mineral contents, as well as their characterization in different populations of pepper, tomato and eggplant, is still lacking.

4.4. Relevance of pepper, tomato and eggplant on a balanced diet

According to the contributions of 100 g portion to the daily RDA/AI, pepper, tomato and eggplant are, as most vegetables (Yahia et al., 2019), poor sources of protein, sugars, Na and Ca. Except for Ca, this promotes a healthy diet, since an excess of sugars and salt increases the risks of diseases such as Type II diabetes and hypertension. Furthermore, these three vegetables are rich sources of antioxidant compounds and other minerals. As it is already known (García-Closas et al., 2004; Yahia et al., 2019), pepper and tomato would be two of the best sources for dietary vitamin C intake, making outstanding contributions to the daily RDA (288% and 33% in average, respectively). In agreement with our results, other studies reported pepper (100 g serving) contributions to the vitamin C RDA over 100% (Howard et al., 2000; Wahyuni et al., 2013) with similar ranges of values. In fact, the range of mean values for vitamin C content in the pepper varieties in the present study was higher than the USDA standard reference values for this trait in vegetables such as broccoli and cauliflower, and in fruits such as kiwi and oranges (U.S. Department of Agriculture, 2021). In tomato (100 g serving), we have found higher contribution percentages to the vitamin C RDA in our data compared to other studies (Casiraghi et al., 2013). On the contrary, a serving of tomato in our study showed lower contribution percentages to AI or RDA values for minerals regarding K, P and Mg than in other works (Casiraghi et al., 2013; Hernández Suárez et al., 2007; Mohammed et al., 2020). Eggplant, on the other hand, stood out for being the best source of mineral compounds among the three species, specifically for Cu, Mg, K, Na and Zn. Compared with data in Raigón et al. (2008), higher average contribution to the K AI was found herein, while for P RDA the contribution percentages were within the same range. In any case, the complementary profile of fruit composition observed for pepper, tomato and eggplant would make it advisable to combine the consumption of those three vegetables in the diet.

5. Conclusions

The comprehensive characterization of fruit composition profile performed herein provides insight into the different regulation patterns of metabolite accumulation among pepper, tomato and eggplant, which were grown under the same organic cultivation conditions. Results allowed to describe the existing diversity within and among the three species for fruit quality. Given the results, the combination of pepper, tomato and eggplant consumption would constitute an advantageous option for a well-balanced diet

due to their complementary nutritional and functional profile. In this way, pepper stood out for its high content in vitamin C, so that a 100 g serving would cover the recommended daily intake of this essential nutrient. Besides being an important source of vitamin C, tomatoes are also rich in the health-promoting flavonoid rutin. Eggplant, on the other side, represented the best source of minerals and phenolic acids, mainly chlorogenic acid, which contributes to its high antioxidant activity. The contribution of a 100 g serving of pepper, tomato and eggplant to the RDA or AI values for several compounds analysed is also provided, which may be of interest for nutrition programs guidance. The wide variability found among the varieties evaluated indicates that it would be inaccurate to assign absolute values when describing the nutritional content of these three vegetables, but that ranges of values should be given instead. In addition, some varieties of pepper, tomato and eggplant were highlighted for its use and conservation due to their higher content in one or more traits analysed. This diversity would provide researchers relevant information for selection of varieties with better nutritional and organoleptic properties as well as for potential utilization in breeding programs.

Conflict of interest: The authors declare that the research was conducted in the absence of conflict of interest.

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**Chapter II - Eggplant interspecific
introgression lines for breeding for
fruit quality and adaptation to low N
inputs**

Communication

Fruit composition of eggplant lines with introgressions from the wild relative *S. incanum*: interest for breeding and safety for consumption

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Ph.D. candidate contribution

E. R.-M. had a main role in the following activities: performing the experiments, data collection, data analysis, data visualization, drafting manuscript, manuscript review and editing.

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Abstract

The wild species *Solanum incanum* has been used as donor parent for the development of a set of eggplant introgression lines (ILs), which are of interest for breeding for stress tolerances and relevant morpho-agronomic traits, but could also be useful for breeding for fruit quality, due to the generally higher content in health-promoting compounds of *S. incanum*. The use of eggplant ILs with introgressions from *S. incanum* requires ensuring that glycoalkaloids levels are below safety limits. We evaluated 25 fruit composition traits, including proximate composition, sugars, acids, phenolics, glycoalkaloids and minerals in a set of 16 eggplant ILs with *S. incanum*, both parents and the F₁, grown under two environments (open field and greenhouse). The results demonstrated that the parents were significantly different regarding most fruit composition traits. Large variation was found among the 16 ILs for all traits analysed and a strong influence of the environment accounted for the variation of 17 out of the 25 traits evaluated. Although the *S. incanum* parent produced fruits with high levels of glycoalkaloids, the 16 ILs showed mean values of total glycoalkaloids below the currently accepted safety limit for human consumption (200 mg kg⁻¹ fresh weight). Overall, the ILs produced fruits that are safe for consumption, with nutritional and functional quality similar to the recipient parent. Furthermore, six putative QTLs were detected spread over chromosomes 3 for crude protein, 5 for malic and total acids, and 7 for chlorogenic acid and solamargine, and potential candidate genes were spotted for most of them, which provide new relevant information for eggplant breeding.

Keywords: *Solanum melongena*, nutritional quality, bioactive compounds, QTLs, pre-breeding materials, glycoalkaloids

1. Introduction

Eggplant (*Solanum melongena* L.) fruits represent an important source of dietary fiber, minerals and antioxidants (Gürbüz et al., 2018). Their functional properties are linked to an outstanding content in phenolic compounds, mainly anthocyanins in the peel and chlorogenic acid in the flesh (Azuma et al., 2008; Plazas et al., 2013). Several nutritional and bioactive compounds have been identified and quantified in eggplant and its wild relatives, revealing the interest of crop wild relatives for improving eggplant fruit composition (Mennella et al., 2012; Prohens et al., 2013; Stommel and Whitaker, 2003). However, utilization of crop wild relatives in breeding is challenging (Prohens et al., 2017).

Introgression lines (ILs) are useful resources for breeding, as they are elite materials with a mostly cultivated genetic background, and can be directly incorporated by breeders in their breeding pipelines (Prohens et al., 2017). Furthermore, ILs are powerful tools for the elucidation of complex genetic traits, as they have the advantage over other mapping populations such as F₂, double haploids or RILs of minimizing the linkage drag (Lippman et al., 2007; Pratap et al., 2021). So far, only one collection of eggplant ILs covering a significant proportion of the donor genome is available in eggplant (Gramazio et al., 2017). This ILs set was developed using the wild relative *S. incanum* L. as a donor parent and it has been characterized for morphological and agronomic traits, including a detailed characterization of fruit shape (Mangino et al., 2021, 2020). These latter studies revealed the interest of this set of ILs for the genetic improvement of eggplant for important morpho-agronomic traits.

Solanum incanum is a wild species of interest for eggplant breeding due to its tolerance to drought and several diseases (Knapp et al., 2013; Mishra et al., 2021), but could also be of interest for breeding for composition traits. In this way, higher levels of antioxidant activity, total phenolics and chlorogenic acid have been reported in *S. incanum* compared with *S. melongena* (Kaur et al., 2014; Prohens et al., 2013; Stommel and Whitaker, 2003). Also, because eggplant wild relatives often have high concentrations of glycoalkaloids (Mennella et al., 2012; Sánchez-Mata et al., 2010), frequently above 200 mg kg⁻¹ of fresh weight, which is the internationally accepted safety limit (OECD, 2020), the use of ILs in breeding requires ensuring their safety in terms of glycoalkaloids content.

In this work, we performed a detailed evaluation of 25 composition traits, including proximate composition, sugars, acids, phenolics, glycoalkaloids and minerals in the set of eggplant ILs with *S. incanum*, both parents and the hybrid in two environments (open field and greenhouse). The results will provide information on the interest of *S. incanum* and their derived introgression lines for eggplant breeding for composition traits as well as on their consumption safety. Thanks to a previous high-throughput genotyping of the ILs set (Gramazio et al., 2017), the detection of stable QTLs for the

traits evaluated will be possible, providing relevant information for eggplant breeding for fruit quality traits.

2. Materials and methods

2.1. Plant material and growing conditions

A total of 16 introgression lines (ILs) from the set of ILs developed in the eggplant background (*S. melongena*; accession AN-S-26) carrying fragments of the genome of a wild relative (*S. incanum*; accession MM577) (Gramazio et al., 2017) were used for fruit composition evaluation. Details about the genetic and phenotypic characteristics of the parents and the ILs selected are available in Gramazio et al. (2017) and Mangino et al. (2021, 2020).

Five plants of each of the two parents (AN-S-26 and MM577), the F₁ hybrid and each of the 16 ILs were grown in a randomized block design under each of two environments (open field and greenhouse), and were distributed in five blocks per environment; i.e., five plants per genotype were tested in the open field ($n = 5$) and five plants per genotype in the greenhouse ($n = 5$). Each plant was considered a replicate. The two environments were located in the campus of the Universitat Politècnica de València (GPS coordinates: latitude, 39° 28' 55" N; longitude, 0° 20' 11" W; 7 m a.s.l.) (Figure 1). The same standard crop management practices and drip fertigation were applied to both environments. In addition, manual weeding and phytosanitary treatments were performed when necessary.

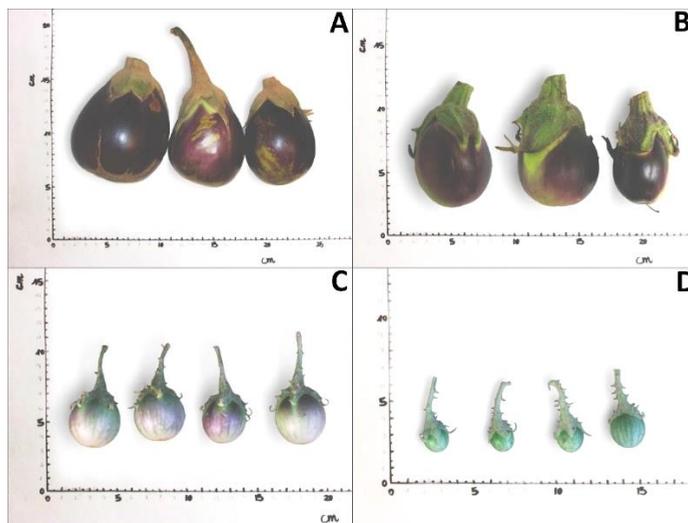


Figure 1. Representative fruits of the parents of the IL population and the hybrid: A) fruits of the recipient parent AN-S-26 (*S. melongena*) harvested under open field; B) fruits of AN-S-26 under greenhouse; C) fruits of the F₁ hybrid under open field; D) fruits of the donor parent MM577 (*S. incanum*) under open field. The scale is in cm.

2.2. Fruit processing and chemical analyses

At least three fruits per replicate were harvested at the commercial ripeness stage, then washed, peeled and cut into pieces. The peel was frozen in liquid N and subsequently freeze-dried for anthocyanin and chlorophyll quantification. One fraction of the flesh pieces was also freeze-dried and homogenized using a domestic grinder for content determination of sugars, acids, chlorogenic acid, total phenolics, total antioxidant activity and glycoalkaloids. The other fraction was dried in an oven at 70 °C up to constant weight and powdered for subsequent quantification of crude protein and minerals. Dry matter was calculated for each accession as the average of $100 \times [\text{dry weight (dw)}/\text{fresh weight (fw)}]$ and expressed as g kg^{-1} fw. Units of the rest of the traits are expressed on a dw basis.

Anthocyanins (mg cm^{-2} dw) were extracted from the part of the peel with a darker colour, and quantified from absorbance values of the extract at 530 nm as cyanidin-3-galactoside equivalents (Giusti and Wrolstad, 2001). Total chlorophylls in peel (mg g^{-1} dw) were also measured spectrophotometrically, as described in Herraiz et al. (2016). Sugars and organic acids were determined by High Performance Liquid Chromatography (HPLC) with a 1220 Infinity LC System (Agilent Technologies, Santa Clara, CA, USA) and quantified using external standard curves. Fructose (FRU; mg g^{-1} dw), glucose (GLU; mg g^{-1} dw) and sucrose (SUC; mg g^{-1} dw) were then detected by refractive index using a 350 RI detector (Varian, Palo Alto, CA, USA), whereas malic (MAL; mg g^{-1} dw) and citric (CIT; mg g^{-1} dw) acids were detected by UV at 210 nm. Contents in total sugars (mg g^{-1} dw) and total acids (mg g^{-1} dw) were calculated from concentrations of individual compounds as FRU+GLU+SUC and CIT+MAL, respectively. Chlorogenic acid and total phenolics were extracted and measured according to the methods described in Plazas et al. (2014). While chlorogenic acid content (mg g^{-1} dw) was determined by reversed phase (RP) HPLC-UV at 325 nm, total phenolics content (mg g^{-1} dw), expressed as chlorogenic acid equivalents, was estimated spectrophotometrically according to the Folin-Ciocalteu procedure optimized to carry out the redox reaction in a 96-well plate. Total antioxidant activity was evaluated following the colourimetric assay of DPPH• (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capacity (Brand-Williams et al., 1995), and results were expressed as $\mu\text{mol Trolox equivalents (TE) g}^{-1}$ dw. The glycoalkaloids solamargine (SM; mg g^{-1} dw) and solasonine (SS; mg g^{-1} dw) were extracted using 95% ethanol and quantified by RP-HPLC-UV at 205 nm, according to Mennella et al. (2012), and total glycoalkaloids (mg g^{-1} dw) were calculated as SM + SS. Crude protein content (mg g^{-1} dw) in fruit was estimated as $6.25 \times$ total N, which was measured following the Kjeldahl method (AOAC International, 2016). Also, mineralized samples were obtained for subsequent determination of minerals (Fe, Cu, Zn, Na, Mg, Ca, K, P) following the MAPA procedures (MAPA, 1994), as described in Raigón et al. (2010), and contents

were expressed as mg g⁻¹ dw. Detailed information on the methods of fruit composition analysis is provided in Supplementary file S1.

2.3. Data analysis

For each of the traits analysed, the mean and its standard error (SE) were calculated for the recipient parent (AN-S-26) in each of the two environments (open field and screenhouse). For the donor parent (MM577) and the F₁ hybrid, no data was obtained in the screenhouse because they did not set fruit under these conditions. Thus, in these cases, mean and SE were calculated for all traits only under open field conditions. Normality of data within each of the two parents and the F₁ was checked with a Shapiro–Wilk test. Statistical differences among the means of the four groups (i.e., AN-S-26 in open field, AN-S-26 in screenhouse, MM577 in open field and the F₁ in open field) were assessed with an analysis of variance (ANOVA) and a Student-Newman-Keuls post-hoc multiple range test at $p < 0.05$ for all traits. Mid-parent heterosis was also calculated for all traits only under open field conditions and its significance was evaluated with a Student's *t*-test at $p < 0.05$.

Data of the 16 ILs along with AN-S-26 for all traits were subjected to a bifactorial ANOVA for the evaluation of differences among the accessions (G, 17 levels), between environments (E, 2 levels) and for the occurrence of G × E interactions (Gomez and Gomez, 1984). Normality of data within each of the ILs was checked with a Shapiro–Wilk test. Furthermore, mean, range values and phenotypic coefficient of variation (CV_P) of the ILs, together with the recipient parent, were calculated under each environment (open field and screenhouse).

A principal component analysis (PCA) was performed using pairwise Euclidean distances among means of the ILs and AN-S-26 for all the traits for each environment, in order to globally evaluate the variation of the ILs compared to the recurrent parent based on the traits evaluated. The *ggplot2* (Wickham, 2016) and *stats* packages of the R statistical software v4.0.2 (R Core Team, 2016, 2013) were used for this purpose.

Given that each IL harboured only one introgressed fragment from the donor wild parent on a single chromosome within the cultivated genetic background, the existence of a significant difference between the mean of one IL and the cultivated parent was assumed to indicate the presence of a QTL for a particular trait within the introgressed fragment. In order to detect significant QTLs, the mean of the replicates for each trait, IL, and environment was compared with the recipient parent AN-S-26 using a Dunnett's test at $p < 0.05$ (Dunnett, 1955), as described by Mangino et al. (2021). A stable QTL was reported when the difference between the IL and the recipient parent AN-S-26 was consistently significant in both environments. For each putative QTL detected, the relative increase over the recipient parent and the allelic effect were calculated in each of the environments.

3. Results and Discussion

The ANOVA performed among the recipient parent AN-S-26 in each of the two environments, the donor parent MM577 and the F₁ revealed significant differences for all traits evaluated except sucrose, Fe, Mg and K (Table 1). The results demonstrated that the parents were considerably different regarding fruit composition. In this way, significant differences were observed for 18 out of the 25 traits evaluated. Among these, on average, fruits of AN-S-26 accumulated more anthocyanins and had 3-fold higher chlorophyll content in the peel. As well, AN-S-26 had half less dry matter content, accumulated 3.5-fold more total sugars, reflected only in glucose and fructose but not in sucrose, and showed 1.9-fold lower organic acid content than MM577 (Table 1). In addition, although for both parents the malic acid was the major organic acid, the proportion of citric acid to the total acids was much lower in AN-S-26 (6.2%) than in MM577 (35.8%). Regarding minerals, AN-S-26 accumulated, on average, 1.7- and 1.9-fold lower Na and Ca, respectively, than MM577 but higher Cu, Zn and P by 2.3-, 1.5- and 2.7-fold, respectively. As for major secondary metabolites in fruit flesh, AN-S-26 showed lower mean values of chlorogenic acid (CGA) content by 1.2-fold (Table 1), suggesting that there is scope for improving the content of this compound in cultivated eggplant using the wild species *S. incanum*.

CGA is known to be the predominant phenolic acid and antioxidant in eggplant and *S. incanum* (Prohens et al., 2013) and the current interest for this molecule resides in its health-promoting properties such as free radical scavenger, anti-inflammatory and antimicrobial, among others (Gürbüz et al., 2018). In agreement with our data, *S. incanum* has shown contents in CGA above those of cultivated varieties (Prohens et al., 2013; Stommel and Whitaker, 2003). However, no differences were detected for total antioxidant activity and total phenolics between the two parents (Table 1). This could be due to the presence of other compounds in AN-S-26 with greater antioxidant capacity even at low concentrations (Kim and Lee, 2004). The largest differences between the parents were found for total and individual glycoalkaloids. In this way, fruits of AN-S-26 had much lower contents of solamargine and solasonine with an average of 10.7-fold less total glycoalkaloids compared to the wild parent MM577 (Table 1). Thus, total glycoalkaloid content for the latter was, on average, above the safety limit for human consumption (OECD, 2020). These findings are in agreement with other works on *S. incanum* as well as on other eggplant wild relatives (Eltayeb et al., 1997; Mennella et al., 2012; Sánchez-Mata et al., 2010), and underlines the potential problem of using eggplant wild species for breeding due to the linkage drag of undesirable traits (Mennella et al., 2010; Prohens et al., 2017). The differences found for fruit composition between the cultivated and the wild parents show the result of selection events for more palatable non-toxic fruits during domestication (Page et al., 2019). In this way, changes in the regulation of invertase and other enzymes activity related to carbohydrate metabolism could explain the differences found for taste-related compounds (Kortstee

et al., 2007). Similarly, the early selection of five major loci during tomato domestication has been demonstrated to be responsible for the dramatic reduction of glycoalkaloids accumulation in fruits (Zhu et al., 2018). Also, leaving aside the selection for bioactive compounds and stress tolerance during domestication likely resulted in the elimination of alleles that contribute to the high content in phenolics (Meyer et al., 2015).

Mean values of fruit composition traits for F_1 were intermediate between the two parents for contents in dry matter, glucose, total sugars and solasonine. On the other hand, fruits of F_1 showed anthocyanins in peel like the recipient parent AN-S-26 (Table 1). The genetic dominance of the presence over non-presence of anthocyanins in peel in interspecific hybrids has already been reported in other works (Doganlar et al., 2002; Prohens et al., 2013). Similarly, F_1 fruits were phenotypically more similar to AN-S-26 for average contents in total chlorophylls, total phenolics, CGA, Cu, Zn and P; and similar to the wild parent MM577 for average contents in fructose, organic acids, solamargine, total glycoalkaloids and Ca. The mid parent heterosis only was significantly positive for content in malic acid and negative for fructose and CGA (Table 1). Our results differed from those of Prohens et al. (2013), who found the interspecific hybrid showing intermediate values of phenolics content. However, these authors evaluated groups of phenolics conjugates instead of CGA individually. Besides, values of heterosis for biochemical compounds have been reported to be highly variable, strongly dependent on the environment (San José et al., 2014). On the other hand, our results are in agreement with previous studies that evaluated CGA and/or total phenolics content in different inter- and intraspecific hybrids (Mennella et al., 2010; Prohens et al., 2012; Toppino et al., 2016), which showed lower CGA content than the mid-parent value or even lower than the parent with the lowest value.

Table 1. Mean values and standard error (SE) of each trait analysed for the recipient parent (*S. melongena*; AN-S-26) under open field (OF) ($n = 5$) and greenhouse (SH) ($n = 5$), and for the wild parent (*S. incanum*; MM577; $n = 5$) and the F_1 ($n = 5$) under OF. Units are expressed on a dry weight (dw) basis except for dry matter. The hybrid mid-parent heterosis (HMP) calculated under OF with its significance is also shown.

Trait (units)	<i>S. melongena</i> AN-S-26 ($n = 5$ in each environment)		<i>S. incanum</i> MM577 ($n = 5$)		F_1 ($n = 5$)
	Mean \pm SE SH	Mean \pm SE OF	Mean \pm SE OF	Mean \pm SE OF	H _{MP} (%) OF ^b
Dry matter (g kg ⁻¹ fw) ^a	86.0 ^a \pm 3.0	90.0 ^a \pm 2.7	178.2 ^c \pm 8.8	126.8 ^b \pm 7.0	-5.3
Anthocyanins in peel (mg cm ⁻² dw)	0.030 ^b \pm 0.003	0.038 ^b \pm 0.009	0.000 ^a \pm 0.000	0.021 ^b \pm 0.004	41.5
Total chlorophylls in peel (mg g ⁻¹ dw)	0.47 ^b \pm 0.06	0.49 ^b \pm 0.10	0.16 ^a \pm 0.04	0.43 ^b \pm 0.04	26.2
Fructose (mg g ⁻¹ dw)	117.2 ^b \pm 2.8	116.9 ^b \pm 7.7	35.2 ^a \pm 4.2	32.7 ^a \pm 2.9	-55.9***
Glucose (mg g ⁻¹ dw)	124.4 ^c \pm 4.9	114.4 ^c \pm 5.8	22.1 ^a \pm 2.7	81.3 ^b \pm 2.9	20.6
Sucrose (mg g ⁻¹ dw)	14.0 ^{ns} \pm 2.5	17.9 ^{ns} \pm 3.1	12.7 ^{ns} \pm 3.2	16.2 ^{ns} \pm 3.2	7.7
Total sugars (mg g ⁻¹ dw)	255.5 ^c \pm 7.7	249.2 ^c \pm 14.8	70.0 ^a \pm 7.8	130.1 ^b \pm 7.5	-17.0
Malic acid (mg g ⁻¹ dw)	12.34 ^a \pm 1.47	14.55 ^a \pm 2.12	19.29 ^b \pm 0.47	20.42 ^b \pm 0.88	21.6**
Citric acid (mg g ⁻¹ dw)	1.29 ^a \pm 0.22	0.96 ^a \pm 0.13	10.78 ^b \pm 2.50	4.86 ^b \pm 0.91	5.7
Total acids (mg g ⁻¹ dw)	13.63 ^a \pm 1.28	15.52 ^a \pm 2.12	30.07 ^b \pm 2.39	25.28 ^b \pm 1.10	13.5
Protein (mg g ⁻¹ dw)	91.9 ^a \pm 3.0	107.3 ^b \pm 5.8	116.5 ^b \pm 2.5	108.0 ^b \pm 4.0	-2.5
Total phenolics content (mg g ⁻¹ dw)	22.31 ^{ab} \pm 1.72	20.78 ^{ab} \pm 0.89	24.64 ^b \pm 1.57	18.19 ^a \pm 0.84	-18.6
Total antioxidant activity (μ mol ET g ⁻¹ dw)	240.6 ^a \pm 4.8	307.6 ^b \pm 9.4	314.2 ^b \pm 4.5	301.2 ^b \pm 3.9	-3.0
Chlorogenic acid (mg g ⁻¹ dw)	6.27 ^a \pm 0.14	6.70 ^a \pm 0.14	7.69 ^b \pm 0.15	6.32 ^a \pm 0.16	-12.0*
Solasonine (mg g ⁻¹ dw)	0.32 ^a \pm 0.06	0.33 ^a \pm 0.03	5.37 ^c \pm 1.33	3.25 ^b \pm 0.33	28.9
Solamargine (mg g ⁻¹ dw)	0.50 ^a \pm 0.08	0.83 ^a \pm 0.10	6.47 ^b \pm 2.91	4.27 ^b \pm 1.59	35.3
Total glycoalkaloids (mg g ⁻¹ dw)	0.82 ^a \pm 0.10	1.15 ^a \pm 0.09	12.35 ^b \pm 3.58	7.52 ^b \pm 1.69	28.6
Fe (mg g ⁻¹ dw)	0.017 ^{ns} \pm 0.005	0.016 ^{ns} \pm 0.001	0.024 ^{ns} \pm 0.000	0.025 ^{ns} \pm 0.002	38.6
Cu (mg g ⁻¹ dw)	0.010 ^a \pm 0.000	0.018 ^b \pm 0.002	0.008 ^a \pm 0.000	0.018 ^b \pm 0.002	54.2
Zn (mg g ⁻¹ dw)	0.023 ^b \pm 0.001	0.027 ^b \pm 0.001	0.018 ^a \pm 0.001	0.023 ^b \pm 0.001	2.7
Na (mg g ⁻¹ dw)	0.23 ^a \pm 0.05	0.37 ^b \pm 0.02	0.64 ^c \pm 0.04	0.17 ^a \pm 0.04	-59.6
Mg (mg g ⁻¹ dw)	2.21 ^{ns} \pm 0.11	2.48 ^{ns} \pm 0.30	2.65 ^{ns} \pm 0.08	2.58 ^{ns} \pm 0.26	10.5
Ca (mg g ⁻¹ dw)	3.01 ^b \pm 0.64	1.54 ^a \pm 0.04	2.90 ^b \pm 0.18	3.17 ^b \pm 0.16	46.2
K (mg g ⁻¹ dw)	26.00 ^{ns} \pm 0.81	30.72 ^{ns} \pm 1.63	30.21 ^{ns} \pm 3.42	26.75 ^{ns} \pm 0.71	-6.0
P (mg g ⁻¹ dw)	2.68 ^a \pm 0.63	4.54 ^b \pm 0.42	1.67 ^a \pm 0.16	4.96 ^b \pm 0.10	66.6

^a For each trait, where significant differences have been found, means separated by different letters are significant at according to the Student-Newman-Keuls test at a significance level of $p < 0.05$, while means with ^{ns} are not significantly different.

^b ***, **, * indicate significant at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

The results of the ANOVA performed to evaluate the significant effects of the genotype (G), environment (E) and $G \times E$ among the 16 ILs and AN-S-26 are shown in Table 2. Significant differences among genotypes were observed for all traits evaluated except total antioxidant activity (TAA), Fe, Mg and Ca. A significant environment (E) effect was detected for eleven traits, with average values of dry matter, total chlorophylls, protein, TAA, CGA, Cu and K being higher under open field (OF) conditions, and of citric acid, total acids, Fe and Ca being higher under greenhouse (SH) conditions. For those 11 traits, F-ratio values for E were much greater than those of G factor, with TAA showing the highest value. Combining significant E and $G \times E$ interaction effects, a strong influence of the environment accounted for the variation of 17 out of 25 traits evaluated, which makes harder the identification of stable QTLs, but could represent an advantage for selection and breeding for specific environmental conditions (Diouf et al., 2018). Significant seasonal (Stommel et al., 2015), environmental (San José et al., 2014) and cultivation practices (Luthria et al., 2010; Raigón et al., 2010) effects have also been reported within and among cultivated varieties for several fruit composition traits. Relative ranges of variation (maximum mean value/minimum mean value) were higher under SH, except for malic and total acids, solamargine, Fe, Cu and Zn. The lowest phenotypic coefficient of variation (CV_P) was observed for total antioxidant activity under both OF and SH (4.1% and 3.6%, respectively), while the highest CV_P was observed for solasonine and citric acid content under OF (93.4% and 76.2%, respectively) and SH (126.3% and 179.7%, respectively) (Table 2). Despite the large variation found within the ILs set, the wild parent MM577 had glycoalkaloids levels significantly higher than those of the ILs and recipient parent, and mean values of total glycoalkaloids for each of the 16 ILs were below the internationally accepted safety limit for human consumption (OECD, 2020) (Table 2). This is in agreement with a previous characterization of glycoalkaloids in a set of advanced backcrosses derived from three eggplant allied species (Mennella et al., 2010). This is a result of special interest for the development and release of new eggplant varieties using the set of ILs of *S. incanum*, since glycoalkaloids are the main undesirable compounds that can accumulate in eggplant and related species in high doses such as to cause harm to human health (Mennella et al., 2010; Sánchez-Mata et al., 2010). Another relevant undesirable trait in eggplant are steroidal saponins, which are not considered lethally toxic but may cause gastrointestinal irritation, and, like glycoalkaloids, produce the bitter taste of the fruit (Oleszek and Oleszek, 2020). However, the accumulation of glycoalkaloids at high concentrations is more of a concern when releasing new varieties to the market since they are very stable to cooking processes, in contrast to saponins (Lo Scalzo et al., 2016).

Table 2. F-ratio values for genotype (G; 16 ILs plus the recipient parent AN-S-26), environment (E; open field and greenhouse) and genotype per environment interaction ($G \times E$), obtained from the bifactorial ANOVA, and significance of each effect for each trait. Mean, range and phenotypic coefficient of variation (CV_P) of the ILs, together with the recipient parent, under both open field and greenhouse are also shown. Units are expressed on a dry weight (dw) basis except for dry matter.

Trait (units)	Factors ^a			Mean	Open Field		Greenhouse		
	G	E	$G \times E$		Range	CV_P	Mean	Range	CV_P
Dry matter (g kg ⁻¹ fw)	3.40***	13.11**	1.21	82.5	75.0 - 97.1	11.5	77.3	67.6 - 87.9	11.1
Anthocyanins in peel (mg cm ⁻² dw)	4.23***	2.48	1.87*	0.034	0.020 - 0.060	56.6	0.028	0.006 - 0.077	47.5
Total chlorophylls in peel (mg g ⁻¹ dw)	6.45***	13.68**	0.79	0.38	0.16 - 0.64	51.8	0.21	0.07 - 0.47	41.9
Fructose (mg g ⁻¹ dw)	7.84***	3.63	3.30***	124.4	97.9 - 146.0	15.3	118.2	80.8 - 170.8	19.4
Glucose (mg g ⁻¹ dw)	6.26***	3.34	1.61	115.4	85.4 - 147.8	19.5	120.1	77.5 - 153.9	22.5
Sucrose (mg g ⁻¹ dw)	3.89***	0.95	1.13	22.1	9.6 - 29.1	42.1	24.5	13.4 - 50.0	55.3
Total sugars (mg g ⁻¹ dw)	6.59***	0.00	1.86*	262.0	211.0 - 318.2	15.5	262.1	199.0 - 343.6	16.9
Malic acid (mg g ⁻¹ dw)	9.31***	3.21	1.32	15.60	9.72 - 28.30	44.4	17.68	10.70 - 30.45	47.5
Citric acid (mg g ⁻¹ dw)	3.61***	3.75***	21.30**	1.43	0.88 - 4.00	76.2	2.68	0.86 - 9.19	179.7
Total acids (mg g ⁻¹ dw)	9.49***	8.93*	1.67	16.93	10.80 - 29.57	41.7	20.53	13.03 - 34.76	46.5
Protein (mg g ⁻¹ dw)	5.52***	32.93***	1.18	113.1	97.4 - 130.6	11.5	102.2	82.1 - 117.8	9.8
Total phenolics content (mg g ⁻¹ dw)	6.12***	0.36	0.90	20.14	15.57 - 28.10	21.1	19.74	10.99 - 29.04	27.0
Total antioxidant activity (μmol ET g ⁻¹ dw)	1.34	887.40***	0.39	298.0	289.0 - 308.5	4.1	215.2	203.9 - 240.6	3.6
Chlorogenic acid (mg g ⁻¹ dw)	4.25***	24.56**	1.15	5.70	4.72 - 6.70	15.9	4.75	3.68 - 6.27	16.2
Solasonine (mg g ⁻¹ dw)	5.07***	4.22	1.73	0.35	0.11 - 0.98	93.4	0.57	0.08 - 1.12	126.3
Solamargine (mg g ⁻¹ dw)	5.03***	3.49	1.99*	0.46	0.03 - 0.83	65.7	0.30	0.05 - 0.52	45.0
Total glycoalkaloids (mg g ⁻¹ dw)	3.50***	0.14	2.23**	0.77	0.46 - 1.38	50.7	0.83	0.27 - 1.40	69.0
Fe (mg g ⁻¹ dw)	0.90	8.62*	1.51	0.016	0.013 - 0.019	18.5	0.018	0.014 - 0.023	32.3
Cu (mg g ⁻¹ dw)	2.79***	41.91***	2.04*	0.019	0.012 - 0.025	23.4	0.010	0.007 - 0.013	16.4
Zn (mg g ⁻¹ dw)	2.77***	1.52	2.33**	0.024	0.018 - 0.029	17.6	0.025	0.022 - 0.030	12.9
Na (mg g ⁻¹ dw)	1.98*	1.55	1.34	0.28	0.20 - 0.37	29.9	0.33	0.22 - 0.59	69.9
Mg (mg g ⁻¹ dw)	0.67	0.00	0.82	2.38	1.94 - 2.76	23.6	2.38	1.95 - 2.92	22.3
Ca (mg g ⁻¹ dw)	0.86	28.72***	1.01	1.70	1.40 - 1.99	19.3	2.74	1.99 - 3.29	48.1
K (mg g ⁻¹ dw)	2.07*	27.86***	1.31	31.35	28.02 - 36.31	11.5	26.53	22.77 - 29.74	8.0
P (mg g ⁻¹ dw)	1.82*	5.03	1.11	4.65	3.21 - 5.42	20.1	3.31	2.48 - 5.09	26.2

^a***, **, * indicate significant differences at $p < 0.001$, < 0.01 , or < 0.001 , respectively.

The PCA also reflected the strong environment effect that influenced fruit composition of the ILs and AN-S-26 (Figure 2). The two first principal components (PCs) of the PCA accounted for 44.5% of the total variation observed. Contents in Ca, total acids and solasonine were the traits displaying the highest positive correlation ($r > 0.2$) with PC1, while total antioxidant activity, CGA, K, Cu, P, protein and total chlorophylls were the traits with the highest absolute negative correlation with PC1. On the other hand, dry matter, citric acid and Fe were positively correlated with PC2, while malic acid and sugars except for sucrose displayed the highest absolute negative values for the correlation with PC2. All the accessions under OF, except SMI_7.1, clustered together with negative values of PC1, whereas all accessions under SH were grouped together with positive values of PC2. In addition, minimal overlapping between the 95% significance ellipses of each of the two environments was observed in the PCA score plot, and the more widespread distribution of accessions under SH indicated a larger variability under those conditions. It is noteworthy that ILs SMI_7.1 and SMI_7.2, which overlap for most of the wild genome fragment they contain, plotted close to each other in both environments in the PCA score plot. Furthermore, ILs SMI_12.6, SMI_5.1, SMI_7.1 and SMI_7.2 were the farthest and thus the most different from AN-S-26 under both environments (Figure 2).

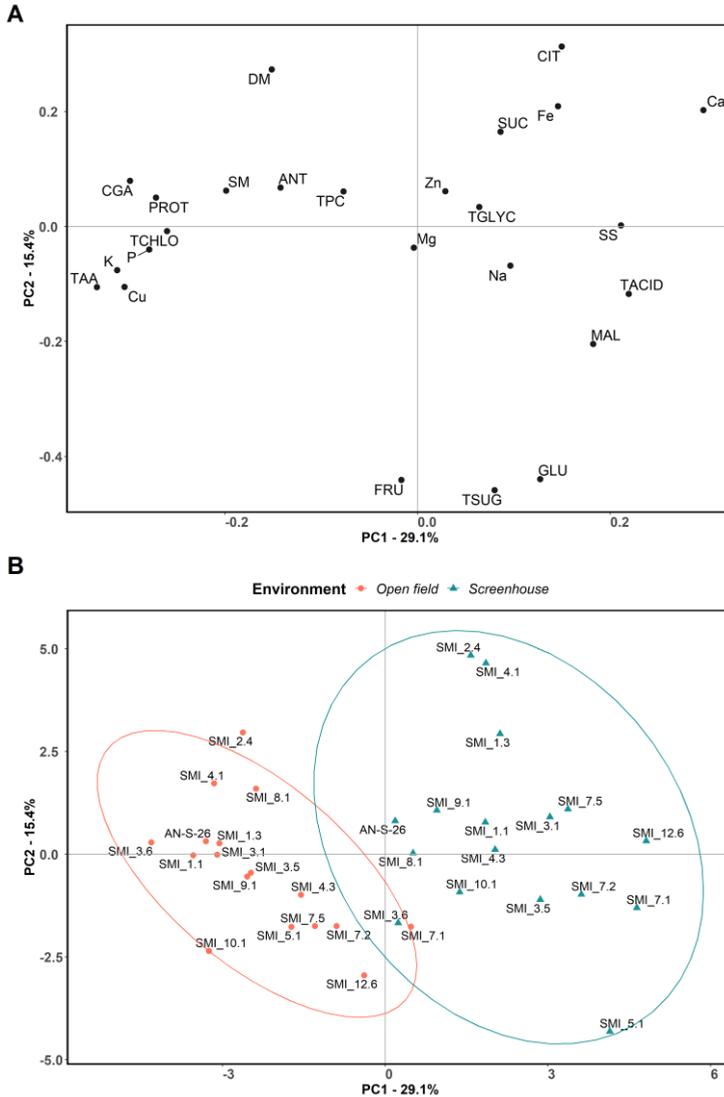


Figure 2. PCA loading plot (A) and score plot (B) based on the two first principal components for 16 introgression lines with *S. incanum* (SMI names) and the recipient *S. melongena* parent (AN-S-26) for 25 composition traits. First and second components account for 29.1% and 15.4% of the total variation, respectively. The accessions are represented by different symbols and colour according to the environment in which they were grown: orange circle for open field, and blue triangle for screenhouse. Ellipses grouped the accessions for each treatment with a 95% confidence level. Acronyms used in the loading plot and their meaning are ANT: anthocyanins; TCHLO: total chlorophylls; FRU: fructose; GLU: glucose; SUC: sucrose; TSUG: total sugars; MAL: malic acid; CIT: citric acid; TACID: total acids; PROT: crude protein; TPC: total phenolics; TAA: total antioxidant activity; CGA: chlorogenic acid; SS: solasonine; SM: solamargine; TGLYC: total glycoalkaloids.

The assessment of the differences among each of the ILs and the recipient parent AN-S-26 resulted in the detection of six stable and novel QTLs in five ILs carrying introgressed fragments of three out of twelve *S. incanum* chromosomes (Table 3). However, in most cases, the wild alleles had a negative effect on the fruit organoleptic and functional quality compared to the cultivated eggplant. One QTL was found for content in malic acid (*ma5*), which mapped in the same position than the QTL identified for total acids (*ac5*) at the end of chromosome 5 (35-43 Mbp) and accounted for a considerable increase of each trait mean value over AN-S-26 (Table 3).

Table 3. List of stable (i.e., detected in both environments) putative QTLs detected in the eggplant IL population. The allelic effect is expressed as mg g⁻¹ on a dry weight basis (dw).

Trait	QTL	Chr	Position (Mb)	Open field			Screenhouse		
				Increase over AN-S-26 (%)	Allelic effect	<i>p</i> -value	Increase over AN-S-26 (%)	Allelic effect	<i>p</i> -value
Malic acid	<i>ma5</i>	5	35-43	94.5	6.87	<0.001	124.8	7.70	0.0048
Total acids	<i>ac5</i>	5	35-43	90.6	7.03	<0.001	109.8	7.48	0.0121
Protein	<i>pro3</i>	3	93-96	21.8	11.67	0.0185	16.9	7.76	0.0238
Chlorogenic acid	<i>cga7</i>	7	129-135	-19.2	-0.64	0.0031	-33.5	-1.05	<0.001
Solamargine	<i>sm7.1</i>	7	129-135	-46.8	-0.18	0.0026	-68.5	-0.17	<0.001
Solamargine	<i>sm7.2</i>	7	135-139	45.5	0.18	0.0054	51.2	0.13	0.0113

A search throughout the ‘67/3’ eggplant reference genome assembly (V3 version) (Barchi et al., 2019) using the Sol Genomics Network database (<http://www.solgenomics.net>) identified two potential candidate genes that mapped to the region of the detected QTLs. The genes encode a phosphoenolpyruvate carboxykinase (SMEL_005g236230.1), which catalyses a reversible reaction involved in gluconeogenesis derived from malic acid, and a peroxisomal acetate/butyrate-CoA ligase (SMEL_005g239840.1) that is probably involved in the activation of exogenous acetate for entry into the glyoxylate cycle. One QTL was detected for crude protein content (*pro3*), at the end of chromosome 3 (93-96 Mbp), which also increased this trait mean value over AN-S-26 (Table 3). Another QTL was detected for CGA content (*cga7*) and its location was narrowed down between 129 to 135 Mbp of chromosome 7. In this case, the introgressed wild allele led to a reduction in CGA (Table 3). An orthologous gene (SMEL_007g290860.1) of the tomato Solyc09g007920, which encodes for phenylalanine ammonia-lyase 1 (*SIPALI*), a core enzyme in CGA biosynthesis pathway, was identified within the eggplant genome region of *cga7* and might be a potential candidate for this association. Interestingly, the gene coding the enzyme HQT, which catalyzes the synthesis of CGA from its precursor, quinic acid, was located in the upper part of chromosome 7 in a previous linkage map (Gramazio et al., 2014). Furthermore, we found a cluster of three orthologous genes to AT1G05260.1 (*Arabidopsis thaliana*) encoding a peroxidase, which catalyses the oxidation of phenolic compounds, that is situated within this region (SMEL_007g288660.1.01, SMEL_007g288680.1.01,

SMEL_007g288690.1.01). Lastly, two putative QTLs with opposite effect were detected for solamargine content (*sm7.1* and *sm7.2*). The QTL *sm7.1* was identified between 129 and 135 Mbp on chromosome 7 and led to a decrease of the solamargine average content over AN-S-26, while *sm7.2* was found downstream (135-139 Mbp) and led to an increase of the trait, but of slightly lesser extent than the reducing effect of *sm7.1* (Table 3). The *GAME* (*GLYCOALKALOID METABOLISM*) genes have been widely studied in tomato and potato, and a cluster of these genes have been located in a region of chromosome 7 in tomato (Itkin et al., 2013). However, orthologous genes in eggplant were located in the same chromosome but upstream the region of *sm7.1* and *sm7.2* (Barchi et al., 2019). Among the genes annotated in the eggplant genome within those regions, we were able to identify nine coding for the 72A subfamily of cytochrome P450-like proteins (Barchi et al., 2019). Some proteins of this subfamily have already been associated to the glycoalkaloid metabolism in tomato (Itkin et al., 2013), and may be related with the solamargine QTLs identified in this work.

4. Conclusions

The characterization performed revealed that the set of 16 eggplant introgression lines carrying fragments of the wild relative *S. incanum* genome generally exhibits a nutritional and functional quality similar to that of the recipient parent. This demonstrates the potential of ILs as pre-breeding material and their safety for human consumption, since linkage drag of undesirable quality traits such as glycoalkaloids is avoided. The ILs evaluated produce fruits safe for consumption with good quality characteristics, which could be used in the future in breeding programs aimed at improving other interesting traits, such as tolerance to drought and several diseases. In addition, the QTLs detected provide new relevant information for eggplant breeding.

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Research article

Characterization and QTL identification of morpho-agronomic and composition traits in a set of eggplant interspecific introgression lines under two N fertilization levels

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E. R.-M. had a main role in the following activities: performing the experiments, data collection, data analysis, data visualization, drafting manuscript, manuscript review and editing.

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Abstract

Lowering nitrogen (N) inputs is a major goal for sustainable agriculture. In the present study, a set of 10 *Solanum melongena* introgression lines (ILs) developed using *Solanum incanum* as exotic donor parent were grown under two N fertilization doses supplied with the irrigation system. The two N doses corresponded to 8.25 mM NH₄NO₃ added to the intake water, which was considered as a high N treatment (HN), and no external N supply to the intake water, which was considered as a low N treatment (LN). Twenty traits, including plant growth and yield parameters, fruit size and morphology, N and C distribution in leaf and fruit, and phenolics content in fruit, were evaluated. No significant differences were observed between the soil characteristics of the high and low N treatments, except for N and Fe contents, which were slightly lower in the high N treatment, probably as a consequence of higher nutrient removal from soil by plants in the latter. The analysis of variance showed that lowering N inputs significantly decreased the average chlorophyll content in leaves, plant height and biomass, early yield, total number of fruits, fruit dry matter, N and C content in leaf and N content in fruit, while it did not significantly affect final yield, fruit morphology, size, and phenolics content. The assessment of the differences between each IL and the recipient parent resulted in the identification of 36 QTLs associated with most of the traits analysed, which were scattered across 9 out of the 10 chromosomes explored. Among these, 12 QTLs were specific to the HN treatment, 17 QTLs were specific to the LN treatment and 7 QTLs were stable across the two N treatments. These findings provide useful information and tools for the utilization of *S. incanum* in the improvement of eggplant under lower N fertilization inputs.

Keywords: *Solanum melongena*, *Solanum incanum*, crop wild relatives, N inputs, abiotic stress, QTLs

1. Introduction

The high yields achieved through the development of modern varieties during the second half of the 20th century have strongly relied on the application of inorganic nitrogen (N)-enriched fertilizers (Lammerts Van Bueren et al., 2011). However, there is a growing awareness of the harmful effects that over-fertilization is causing on the environment (Stevens, 2019), which has led to a shift in production policies and new challenges in breeding programs towards a more sustainable agriculture (Fess et al., 2011; Zhang et al., 2015). Therefore, one of the main objectives in the development of new varieties for a sustainable agriculture is their adaptation to low input conditions and with greater efficiency in the use of nutrients and water. Since N is an essential nutrient for plant growth and development, and also the main contributor to the environmental impact of agriculture, it is necessary to investigate the effects of lowering N inputs on plant performance and to screen the existing diversity for potential materials with high nitrogen use efficiency (NUE). In this way, N rate has been reported to significantly affect plant height, leaf area and shoot dry weight in wheat seedlings (Wang et al., 2019), yield and fruit load in processing tomato (Elia and Conversa, 2012), plant growth and chlorophyll content in a local variety of pepper (Stagnari et al., 2021), but also grain quality in rice (Zhou et al., 2018), content in flavour-related compounds (Wang et al., 2007) and antioxidants (vitamin C, carotenoids, phenolics) in different varietal groups of tomato (Hernández et al., 2020).

Crop wild relatives (CWRs) constitute a valuable source of variation for tolerance to abiotic stresses including low inputs, since they naturally grow in areas with harsh environmental conditions (Dempewolf et al., 2017; Prohens et al., 2017). CWRs can also be an advantageous material for the improvement of other traits such as nutritional content or the introduction of new characteristics of fruit such as shapes, sizes and colours (Ebert, 2020; Leiva-Brondo et al., 2012). In addition, using CWRs in breeding broadens the genetic base of the cultivated species, making new genetic diversity potentially available to the breeders. However, the direct use of CWRs in breeding pipelines is often difficult (Dempewolf et al., 2017). In this way, interspecific introgression lines (ILs) have the advantage of carrying a single fragment of a wild genome on a mostly cultivated genetic background, facilitating their use in breeding (Pratap et al., 2021; Prohens et al., 2017). In addition, ILs are a powerful tool for the detection of Quantitative Trait Loci (QTLs) associated with complex traits.

Eggplant (*Solanum melongena* L.) is the fifth most economically important vegetable worldwide (FAOSTAT, 2019). However, there is little information on the diversity in eggplant for nitrogen use efficiency and performance under reduced N-inputs (Mauceri et al., 2021, 2019; Villanueva et al., 2021). Furthermore, genetic research and development of genomic tools in eggplant have lagged behind other important vegetables and crops such as tomato, potato and pepper (Gramazio et al., 2021, 2018). Among eggplant CWRs (Knapp et al., 2013), *Solanum incanum* L. is a cross-compatible

CWR of eggplant from desertic and semi-desertic areas from East Africa and the Middle East (Vorontsova and Knapp, 2016), which has tolerance to drought and resistance to biotic stresses such as *Fusarium* and bacterial wilts (Knapp et al., 2013; Mishra et al., 2021; Yamakawa and Mochizuki, 1979). Given that it naturally grows in areas with poor soils, we hypothesize that it could also be a source of variation for resilience to low N inputs. In addition, it has been reported to exhibit a high content in bioactive phenolics of interest for human health (Kaur et al., 2014; Prohens et al., 2013). A set of ILs carrying fragments of *S. incanum* genome in an *S. melongena* cultivated genetic background has been developed recently (Gramazio et al., 2017). This IL collection has already proven its interest in eggplant breeding for important agronomic and fruit morphology traits in previous studies (Mangino et al., 2021, 2020). In addition, it has been characterized in terms of fruit quality in a recent study (Rosa-Martínez et al., 2022), which demonstrated the safety for consumption of their fruits, since they accumulated levels of glycoalkaloids below the internationally accepted limit (200 mg kg⁻¹ of fresh weight) (OECD, 2020).

In the present work, a set of eggplant lines with introgressions of *S. incanum* (Gramazio et al., 2017), along with their *S. melongena* recipient parent (AN-S-26), were evaluated for morpho-agronomic and composition traits under two N fertigation conditions. The aim was to provide insight into the ILs performance to identify potential materials for eggplant breeding under low N inputs. In addition, the combination of phenotyping data with the available genotyping (Gramazio et al., 2017) of the ILs allowed the detection of QTLs associated with traits of interest for eggplant breeding.

2. Materials and methods

2.1. Plant material

A set of ten introgression lines (ILs) of *S. melongena* AN-S-26 with introgressed fragments of the wild relative *S. incanum* (MM577) were used for the present study (Gramazio et al., 2017). Each IL carried a single fragment of one donor chromosome, corresponding to chromosomes 1 to 5, 7 to 9, and 11. Altogether, the set of ILs covered 64.6% of the *S. incanum* genome, with introgressions ranging from 1.9% for chromosome 10 to 90.1% for chromosome 7 (Figure 1). Detailed information about the ILs population and the corresponding parents can be found in Gramazio et al. (2017) and Mangino et al. (2020, 2021).



Figure 1. Graphical genotypes of the eggplant introgression lines (ILs) with *S. incanum* introgressions evaluated in the present work. Each row corresponds to the genotype of each IL. The first column indicates the IL codes and the top row indicates the eggplant chromosomes and respective length (Mb). Homozygous introgressions of *S. incanum* (MM577) are coloured in green, while the genetic background of *S. melongena* (AN-S-26) is coloured in pink. The start and end position of the introgressed fragment for each chromosome is displayed at the bottom of the figure.

2.2. Cultivation conditions

Plants were grown in an open-air field located in the campus of Universitat Politècnica de València (GPS coordinates: latitude, 39° 28' 55" N; longitude, 0° 20' 11" W; 7 m a.s.l.) during the late summer season of 2019 (August 21st to November 28th). Within the field, the plants were distributed in two plots located 3 m apart, each submitted to different nitrogen (N) fertigation dosage provided with a drip irrigation system.

Physicochemical analysis of the soil was performed before cultivation following the same procedure described in Rosa-Martínez et al. (2021). Intake water was also analyzed (Moliner and Masaguer, 1996). Based on soil and water analyses and the nutrient requirements for eggplant cultivation (Baixauli and Aguilar, 2002), one plot was submitted to a fertigation solution of 8.25 mM NH₄NO₃ (Antonio Tarazona SL., Valencia, Spain) plus 2.3 mM K₂SO₄ (Antonio Tarazona SL., Valencia, Spain), which were added to the irrigation system. This was considered as the high N treatment (HN) in the present work. The other plot was submitted to a N-reduced fertigation solution prepared by adding 2.3 mM K₂SO₄ to the irrigation system, thus without providing any N other than that already supplied by the intake water (Table 1). This was considered as the low N treatment (LN). In addition, both HN and LN solutions were supplemented with 0.025 L m⁻³ of a microelements Welgro Hydroponic fertilizer mix (Química Massó S.A., Barcelona, Spain), which contained copper (Cu-EDTA; 0.17% p/v), iron (Fe-DTPA; 3.00% p/v), boron (BO₃³⁻; 0.65% p/v), manganese (Mn-EDTA, 1.87% p/v), zinc (Zn-EDTA; 1.25% p/v) and molybdenum (MoO₄²⁻; 0.15% p/v). The pH of both irrigation solutions was finally adjusted to 5.5-5.8 with 20% HCl. Another chemical analysis of the irrigation water flowing out of the drip irrigation emitters was performed for each of the two N treatments after the fertigation solutions were prepared. The compositional characteristics of the intake water and the final HN and LN treatment fertigation solutions are displayed in Table 1. The intake water was slightly basic, had a

low content of nitrates, ammonium, phosphates, carbonates and potassium (K^+), moderate content of sulphates and magnesium (Mg^{2+}), and high content of calcium (Ca^{2+}) (Table 1). After preparing the fertigation solutions, in addition to the expected difference in nitrate and ammonium content between the HN and LN solutions, the electrical conductivity increased, mainly for HN, as well as the content in sulphates, magnesium and potassium, while bicarbonates were reduced (Table 1). Another soil physicochemical analysis was also carried out after the cultivation period in each of the two field plots (HN and LN) separately.

Table 1. Irrigation water chemical composition before (intake water) and after preparing the fertilizer solution for each N treatment (HN solution and LN solution).

Water characteristics	Intake water	HN solution	LN solution
pH	7.5	6.2	6.1
Electrical conductivity ($mS\ cm^{-1}$)	1.6	2.8	1.9
Nitrates ($mmol\ L^{-1}$)	0.60	10.00	0.79
Ammonium ($mmol\ L^{-1}$)	0.06	0.23	0.09
Phosphates ($mmol\ L^{-1}$)	0.00	0.00	0.00
Sulphates ($mmol\ L^{-1}$)	3.7	6.3	6.6
Carbonates ($mmol\ L^{-1}$)	0.00	0.00	0.00
Bicarbonates ($mmol\ L^{-1}$)	6.00	0.80	1.40
Ca^{2+} ($mmol\ L^{-1}$)	5.4	5.4	5.3
Mg^{2+} ($mmol\ L^{-1}$)	1.9	2.1	2.6
K^+ ($mmol\ L^{-1}$)	0.15	3.86	3.80

Five plants per IL, along with 15 plants of the recipient parent *S. melongena* AN-S-26, were distributed in each plot following a completely randomized design. The plants in each plot were separated 1.5 m and 0.7 m between and within rows, respectively. Similar crop management practices were applied to both field plots, which included no pruning, manual weeding and phytosanitary treatments against whiteflies and spider mites when necessary. For the drip irrigation, the same irrigation timings were applied in both HN and LN field plots during the entire cultivation period, making it to a total of 57.5 L of fertigation solution supplied per plant. Considering this, a total amount of 13.81 g N $plant^{-1}$ and 0.53 g N $plant^{-1}$ was supplied with the irrigation to the HN and the LN field plots, respectively.

2.3. Morpho-agronomic traits evaluated

A total of 13 agronomic and fruit morphology-related traits were evaluated for both high and low N treatments (Table 2). Regarding agronomic traits, the SPAD value was measured per plant using a chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan) as the mean of ten readings distributed in five fully expanded leaves. The SPAD measures were taken on the same day for all the plants, 38 days after the transplant (September 27th), during the plant vegetative growth period, and fruits were harvested when commercially ripe and weighed. At the end of the cultivation (November 28th),

the maximum height of each plant was measured and the stem diameter was measured using a digital caliper. Subsequently, they were cut at ground level, and immediately, the plant aerial biomass was weighed using a Sauter FK-250 dynamometer (Sauter, Balingen, Germany). Yield was calculated as the total fruit biomass produced per plant, while early yield was calculated as the fruit biomass produced per plant on October 7th. Nitrogen Use Efficiency (NUE) was calculated per plant as the ratio between fruit yield, expressed on a dry weight basis, and the amount of N supplied per plant (Mauceri et al., 2019; Moll et al., 1982). In addition, the total number of fruits per plant was counted and the fruit mean weight was calculated as the ratio between yield and fruit number. Regarding fruit morphological traits, the pedicel length, calyx length, fruit length and fruit width were measured in three representative commercially mature fruits per plant and the mean was calculated for each trait. Units in which the traits analysed are expressed in the present work and the abbreviations used in Tables and Figures are shown in Table 2.

2.4. Leaf and fruit processing and composition analyses

At least five fully expanded leaves per plant were collected on November 7th, frozen in liquid nitrogen and stored at -80 °C for subsequent freeze-drying in a VirTis Genesis lyophilizer (SP Scientific, Warminster, Pennsylvania, USA). In addition, during the harvest period, at least three commercially mature fruits per plant were harvested, cleaned, peeled and cut into pieces, which were subjected to the same freezing and freeze-drying steps as the leaves. Subsequently, freeze-dried leaves and fruits were homogenized using a domestic grinder. Fruit dry matter was calculated as $100 \times (\text{freeze-dried weight/fresh weight})$.

Total carbon (C) and nitrogen (N) contents were determined in fruit and leaf using 0.5 g of the homogenized materials in a TruSpec CN elemental analyser (Leco, Michigan, USA). The analysis was based on complete combustion of the sample at 950 °C in the presence of oxygen. C and N were measured as CO₂ and N₂ gases, respectively. An infrared detector was used for C determination, while N was detected in a thermal conductivity cell (Gazulla et al., 2012).

Total phenolics content was determined in fruit by spectrophotometry using 0.125 g of homogenized fruit material according to the Folin-Ciocalteu method (Singleton and Rossi, 1965), while chlorogenic acid content was evaluated in 0.1 g of homogenized fruit material by high-performance liquid chromatography (HPLC) using a 1220 Infinity LC System (Agilent 196 Technologies, CA, USA) equipped with a binary pump, an automatic injector and a UV detector. The extraction and analysis procedure followed for each trait is described in detail in Plazas et al. (2014). Units in which the traits analysed are expressed in the present work and the abbreviations used in Tables and Figures are shown in Table 2.

Table 2. Description of agronomic, fruit and composition traits evaluated in the set of ILs, abbreviations used in Tables and Figures, and units in which they are expressed in the present work.

Trait	Abbreviation	Units
<i>Morpho-agronomic traits</i>		
SPAD	SPAD	-
Plant height	P_Height	cm
Aerial biomass	P_Biomass	kg fw ^a
Stem diameter	P_Diam	mm
Early yield	E_yield	g fw plant ⁻¹
Yield	Yield	g fw plant ⁻¹
Nitrogen Use Efficiency	NUE	-
Total number of fruits per plant	F_Number	-
Fruit mean weight	F_Weight	g fw
Fruit pedicel length	F_PedLength	mm
Fruit calyx length	F_CaLength	mm
Fruit length	F_Length	mm
Fruit width	F_Width	mm
<i>Composition traits</i>		
Fruit dry matter	F_dm	%
Nitrogen content in leaf	N_Leaf	g kg ⁻¹ dw ^b
Carbon content in leaf	C_Leaf	g kg ⁻¹ dw
Nitrogen content in fruit	N_Fruit	g kg ⁻¹ dw
Carbon content in fruit	C_Fruit	g kg ⁻¹ dw
Total phenolics content	TPC	g kg ⁻¹ dw
Chlorogenic acid content	CGA	g kg ⁻¹ dw

^a fw: fresh weight

^b dw: dry weight

2.5. Data analysis

Physicochemical data of the soil before cultivation, after cultivation in the HN field plot, and after cultivation in the LN field plot were subjected to a one-factor analysis of variance (ANOVA) and to a post-hoc Student-Newman-Keuls multiple range test to evaluate significant differences among the three conditions for each trait ($p < 0.05$).

Regarding morpho-agronomic and compositional data of the set of selected ILs and the recipient parent (*S. melongena* AN-S-26), a bifactorial ANOVA was performed for every trait evaluated in order to assess the effect of genotype (G), N treatment (N) and G×N interaction (Gomez and Gomez, 1984). Mean value and its standard error (SE) were calculated for every trait analysed from the genotypes' means for each N treatment. In addition, the paired difference between the average value of each trait under HN and LN was calculated only for the traits which showed a significant effect of the N treatment.

Mean values per genotype of the traits evaluated were subjected to a principal component analysis (PCA) for each N treatment using the package *stats* (R Core Team,

2016) of the R software (R Core Team, 2013). PCA score plot was drawn using R package *ggplot2* (Wickham, 2016). In addition, Pearson correlation coefficients were calculated between means of all traits and their significance was evaluated at $p < 0.01$, using the Statgraphics Centurion XVIII software (Statpoint Technologies, Warrenton, Virginia, USA).

2.6. QTL detection and candidate gene identification

Detection of significant QTLs was carried out in both N treatments separately for all traits by means of a Dunnett's test at $p < 0.05$ (Dunnett, 1955), which compares the mean value of each IL with the mean value of the recipient parent AN-S-26. The R package *multcomp* (Hothorn et al., 2021) was used for the analysis. For each putative QTL detected, the relative increase over AN-S-26 and the allelic effect were calculated.

In order to identify potential candidate genes within each QTL region, a search throughout the '67/3' eggplant reference genome assembly (V3 version) (Barchi et al., 2019) was conducted, using the Sol Genomics Network database (<http://www.solgenomics.net>). In addition, syntenic regions of the tomato genome (Wu et al., 2009) were accessed to identify candidate genes co-localizing with the eggplant QTLs, using the comparative map viewer tool of the Sol Genomics Network database (https://maps.solgenomics.net/Map/view_comp).

3. Results

3.1. Soil characteristics

Soil texture for both fields was sandy, with a composition of 86% sand, 11% clay, and 3% silt (Soil Science Division Staff, 2017). The analysis of variance of the soil characteristics revealed no significant differences among the three soil stages (before cultivation, after cultivation in the HN field plot and after cultivation in the LN field plot) for pH in water, C:N ratio and content in carbonates, organic matter, phosphorus and zinc (Table 3). Significant differences were found between soils before and after cultivation for pH in KCl and contents in calcium, magnesium, potassium and copper. For the first three traits, the soils after cultivation showed higher values by 1.04-fold, 1.5-fold and 1.2-fold, respectively. Contrarily, contents in potassium and copper were significantly higher in the soil before cultivation by 1.5-fold and 1.2-fold. The electrical conductivity increased after the cultivation period, although it was only significant in the HN plot soil (1.6-fold increase). A slightly but significantly lower average N content (0.56 g kg^{-1} on a dry weight (dw) basis) was found in the HN plot soil after cultivation compared to both the LN plot soil ($0.63 \text{ g kg}^{-1} \text{ dw}$) and the field soil before cultivation ($0.69 \text{ g kg}^{-1} \text{ dw}$). Lastly, average Fe content decreased significantly in soil after the cultivation period, the reduction being more pronounced in the case of HN plot soil (1.6-fold decrease) (Table 3).

Table 3. Mean values and standard error (SE) of the soil chemical composition before transplant and after the cultivation period in each N treatment separately. For each trait, means with different letters are significantly different at $p < 0.05$ according to the Student-Newman-Keuls multiple range test, while means with ^{ns} are not significantly different.

Soil characteristics	Before cultivation	After the cultivation period	
	Mean \pm SE	HN Mean \pm SE	LN Mean \pm SE
pH in water	8.12 ^{ns} \pm 0.05	8.29 ^{ns} \pm 0.07	8.16 ^{ns} \pm 0.02
pH in KCl	7.60 ^a \pm 0.03	7.92 ^b \pm 0.03	7.87 ^b \pm 0.05
Electrical conductivity (μ S cm ⁻¹)	213.2 ^a \pm 26.7	332.2 ^b \pm 45.1	283.8 ^{ab} \pm 13.7
Total N (g kg ⁻¹ dw)	0.69 ^b \pm 0.03	0.56 ^a \pm 0.02	0.63 ^b \pm 0.02
Carbonates (g kg ⁻¹ dw)	271.6 ^{ns} \pm 12.5	274.4 ^{ns} \pm 2.12	279.0 ^{ns} \pm 2.48
Organic matter (g kg ⁻¹ dw)	14.6 ^{ns} \pm 2.2	13.7 ^{ns} \pm 0.4	12.6 ^{ns} \pm 0.2
C:N ratio	12.50 ^{ns} \pm 1.9	14.4 ^{ns} \pm 0.7	11.7 ^{ns} \pm 0.3
P (mg kg ⁻¹ dw)	121.1 ^{ns} \pm 10.1	113.6 ^{ns} \pm 4.6	122.4 ^{ns} \pm 4.9
K (mg kg ⁻¹ dw)	176.1 ^b \pm 12.8	113.9 ^a \pm 12.8	112.5 ^a \pm 7.5
Ca ²⁺ (cmol kg ⁻¹ dw)	159.6 ^a \pm 2.1	242.1 ^b \pm 8.4	239.4 ^b \pm 4.7
Mg ²⁺ (cmol kg ⁻¹ dw)	4.80 ^a \pm 0.12	5.77 ^b \pm 0.22	5.57 ^b \pm 0.11
Fe (mg kg ⁻¹ dw)	5.28 ^c \pm 0.12	3.45 ^a \pm 0.09	3.85 ^b \pm 0.22
Zn (mg kg ⁻¹ dw)	8.99 ^{ns} \pm 0.53	8.73 ^{ns} \pm 0.12	9.35 ^{ns} \pm 0.21
Cu (mg kg ⁻¹ dw)	5.92 ^b \pm 0.09	4.77 ^a \pm 0.25	5.15 ^a \pm 0.20

3.2. Characterization of the ILs and the recipient parent under high and low N treatments

According to the bi-factorial ANOVA performed for data of the set of ten ILs plus the recipient parent, significant differences among genotypes were found for all traits evaluated except C content in fruit (C_Fruit) (Table 4). Representative fruits produced by each IL and the recipient parent AN-S-26 are pictured in Figure 2. In addition, the ANOVA revealed a significant effect of the N treatment over ten out of the twenty traits evaluated (Table 4). Except for NUE, the other nine traits presented higher values under high N treatment (HN). In this way, the agronomic and vegetative traits SPAD, plant height (P_Height), plant biomass (P_Biomass), early yield (E_yield) and fruit number (F_Number) were reduced by 8.6%, 13.0%, 33.3%, 20.6% and 16.5%, respectively, under the low N treatment (LN) compared to HN. Furthermore, the composition traits fruit dry matter (F_dm), N content in leaf (N_Leaf), C content in leaf (C_Leaf) and N content in fruit (N_Fruit) were also reduced under LN by 4.7%, 10.2%, 1.1% and 11.0%, respectively (Table 4). Among the traits that showed significant differences between N treatments, the F-ratio of the N treatment factor was higher than the genotype factor in all cases except for F_dm (Table 4). Only NUE and C_Leaf showed a significant genotype \times N treatment interaction, although the F-ratio of this factor was lower than the F-ratio of the genotype or N treatment effects.



Figure 2. Characteristics of fruits from *S. melongena* (AN-S-26) and introgression lines of the latter with *S. incanum* MM577. Fruits of the *S. incanum* donor parent are also included. The scale is in cm.

Table 4. F-ratio values for genotype (G), N treatment (N), and genotype per N treatment interaction (G×N) of each trait evaluated in the present study, obtained from the bifactorial ANOVA. Mean values ± standard error (SE) for each trait and N treatment are also shown. Only for the traits with a significant effect of the N treatment, the paired difference HN-LN was calculated, based on the genotype means. ***, **, * indicate significant differences at $p < 0.001$, < 0.01 , or < 0.001 , respectively.

Trait	Genotype (G)	N treatment (N)	G×N	Mean ± SE HN	Mean ± SE LN	Paired HN-LN ^a
<i>Morpho-agronomic traits</i>						
SPAD	2.3*	51.7***	0.85	55.9 ± 0.7	51.1 ± 0.6	4.8
P_Height (cm)	5.5***	19.5***	0.62	74.4 ± 2.4	64.7 ± 2.6	9.7
P_Biomass (kg fw)	4.06***	25.1***	0.30	0.54 ± 0.15	0.36 ± 0.03	0.18
P_Diam (mm)	2.66**	1.61	0.42	16.5 ± 0.5	15.8 ± 0.6	
E_yield (g fw plant ⁻¹)	4.66***	4.98*	0.60	376.2 ± 42.4	299.0 ± 39.2	77.3
Yield (g fw plant ⁻¹)	4.87***	3.45	0.86	1007.4 ± 90.3	894.4 ± 91.3	
NUE	2.20*	237.5***	1.94*	6.20 ± 0.56	137.3 ± 12.5	-131.1
F_Number	2.76**	5.25*	1.05	13.3 ± 0.9	11.1 ± 1.0	2.2
F_Weight (g fw)	5.21***	0.47	0.75	75.4 ± 3.2	78.0 ± 5.6	
F_PedLength (mm)	8.53***	0.09	1.52	53.0 ± 1.9	53.4 ± 2.5	
F_CaLength (mm)	3.22**	0.02	1.01	51.3 ± 1.4	51.5 ± 1.7	
F_Length (mm)	4.24***	0.09	0.95	77.1 ± 2.3	77.7 ± 2.6	
F_Width (mm)	5.69***	2.26	1.06	54.5 ± 1.7	56.9 ± 2.0	
F_dm (%)	8.49***	5.78*	1.20	8.65 ± 0.23	8.24 ± 0.31	0.41
<i>Composition traits</i>						
N_Leaf (g kg ⁻¹ dw)	3.00**	48.4***	1.53	55.0 ± 0.6	49.4 ± 1.0	5.6
C_Leaf (g kg ⁻¹ dw)	3.93***	7.00**	3.81***	433.6 ± 1.9	429.0 ± 2.8	4.6
N_Fruit (g kg ⁻¹ dw)	4.46***	21.4***	1.01	31.9 ± 0.7	28.5 ± 0.9	3.5
C_Fruit (g kg ⁻¹ dw)	0.95	0.05	0.88	420.2 ± 1.3	420.4 ± 0.8	
TPC (g kg ⁻¹ dw)	5.27***	0.40	1.09	24.5 ± 1.2	23.9 ± 1.1	
CGA (g kg ⁻¹ dw)	7.89***	0.07	1.80	16.2 ± 1.0	16.0 ± 1.2	

^aCalculated only for traits with significant difference between HN and LN

3.3. Multivariate principal components analysis

The two first components (PCs) of the principal components analysis (PCA) for all the traits evaluated accounted for 37.2% and 18.6% of the variation, respectively. However, as NUE was highly correlated (0.459) with the second component and basically separated the HN and LN treatments (Supplementary Figure S1) a new PCA was performed excluding NUE. In this new PCA excluding NUE, the first two components accounted for 56.0% of the total variation observed, with PC1 and PC2 accounting for 39.2% and 16.8% of the variation, respectively. Among the traits with the highest correlation coefficient (≥ 0.2), those related to yield and fruit morphology, except for SPAD, P_Height and F_Width, were negatively correlated to PC1, while fruit dry matter (F_dm) was positively correlated to the same component (Table 5). On the other hand, the composition traits N in fruit (N_Fruit), total phenolics (TPC) and chlorogenic acid (CGA), as well as P_Height, were highly positively correlated to PC2, whereas F_Width was negatively correlated to PC2 (Table 5).

The distribution of the genotypes in the PCA score plot showed a trend towards higher PC1 and lower PC2 values for genotypes subjected to the low N treatment compared to those of the high N treatment (Figure 3). This change is associated with a higher dry matter of the fruit, C content in leaf and fruit width and weight, but to lower yields, fruit number, plant height and biomass, stem diameter and N content in fruit. The exception to this trend was the IL SMI_12.6, which for the LN treatment had PC1 values slightly than those of the HN treatment (Figure 3). The most distant ILs from the recipient parent in the score plot, and therefore most different in terms of the traits represented in the analysis, were SMI_2.9 and SMI_12.6 in the case of HN, and SMI_2.9, SMI_3.1 and SMI_4.1 for LN (Figure 3). Some of the ILs performed similarly under both N treatments, as they appear in close proximity according to PC1 and PC2. This is the case of SMI_5.1, SMI_12.6 and SMI_9.5 (Figure 3).

Table 5. Correlation coefficients between all traits evaluated except NUE and the two first principal components (PC1 and PC2) of the PCA. Those correlations with absolute values ≥ 0.2 are highlighted in bold.

Traits	PC1	PC2
<i>Morpho-agronomic traits</i>		
SPAD	-0.0818	0.1769
P_Height	-0.1991	0.4172
P_Biomass	-0.2894	0.0566
P_Diam	-0.3236	0.0276
E_yield	-0.2887	0.0574
Yield	-0.3384	0.0271
F_Number	-0.2786	0.1510
F_Weight	-0.2739	-0.1904
F_PedLength	-0.2881	0.0225
F_CaLength	-0.2518	0.0656
F_Length	-0.3004	-0.0915
F_Width	-0.1989	-0.2510
<i>Composition traits</i>		
F_dm	0.2482	0.0029
N_Leaf	-0.2031	0.0545
C_Leaf	-0.0897	-0.1544
N_Fruit	-0.0361	0.4599
C_Fruit	0.0781	0.0822
TPC	0.1020	0.4467
CGA	0.0994	0.4548

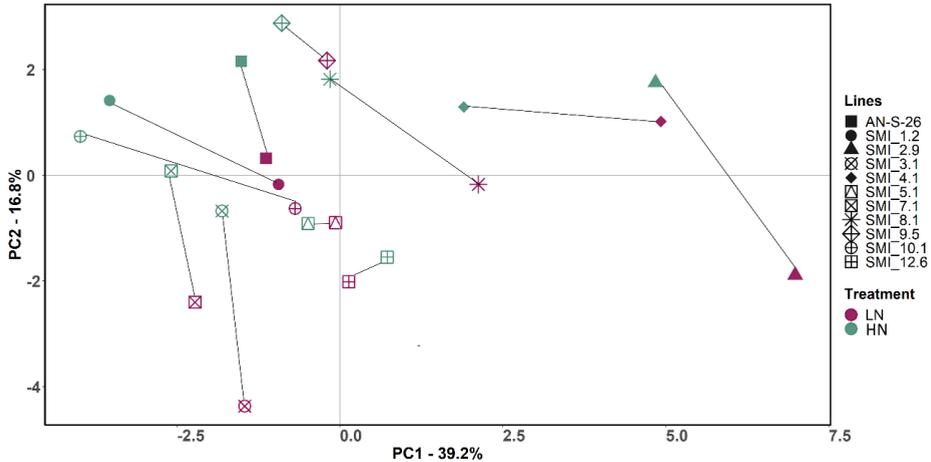


Figure 3. PCA score plot based on the two principal components (PC1 and PC2) of the analysis performed for all traits except NUE. PC1 and PC2 accounted for 39.2% and 16.8% of the total variation, respectively. The ILs (SMI_ codes) and the *S. melongena* recipient parent (AN-S-26) are represented by different symbols and different colour according to the N treatment in which they were grown, as displayed in the figure legend. Black lines connect the same genotype under each of the two N treatments.

3.4. Analysis of correlations

Significant correlations between traits at $p < 0.01$ were found for 16 out of the 20 traits evaluated considering the HN and/or the LN treatments (Table 6). High positive (>0.70) correlations of similar values were found among most agronomic traits, as well as between total phenolics (TPC) and chlorogenic acid content (CGA), for both LN and HN (Table 6). Among these, the correlation coefficient (r) between NUE and yield was the highest (0.98 for HN and 0.97 for LN). In addition, yield and NUE were positively correlated with early yield (E_yield) and the total number of fruits (F_Number) under both N treatments, as well as yield with traits related to plant and fruit sizes (P_Biomass, P_Diam and F_Length) (Table 6). More significant positive correlations were found for each N treatment separately. In this way, plant size-related traits (P_Biomass and P_Diam) were correlated with N content in leaf (N_Leaf) under the HN treatment, as well as yield-related traits (E_yield and Yield) to fruit weight (F_Weight) and fruit pedicel length (F_PedLength) (Table 6). NUE was correlated with P_Biomass and F_Length only under HN, while it was correlated with P_Diam only under LN (Table 6). Furthermore, correlations with high positive r were found among traits related to fruit size for LN (i.e., F_Weight with F_Width or F_Length with F_PedLength and F_CaLength). Also for LN, N and C content in leaf (N_Leaf, C_Leaf) were correlated to each other (Table 6). Finally, only three highly negative (< -0.75) correlations were found among traits, which corresponded to fruit dry matter (F_dm) with P_Diam under HN, and F_dm with F_PedLength and F_Length under LN (Table 6).

Table 6. Pearson linear correlation coefficients (r) between accession mean values for the traits evaluated in the present work. Correlations under the high N treatment (HN) are shown above the diagonal; correlations under the low N treatment (LN) below the diagonal. Only significant correlations at $p < 0.01$ are displayed. Traits for which no significant correlation was obtained have been excluded from the table. The colour scale from red to green represents the scale of r values from 1.0 (the highest positive correlation) to -1.0 (the highest negative correlation), respectively.

	P_Bio mass	P_Diam	E_yield	Yield	NUE	F_Num ber	F_Weight	F_Ped Length	F_Ca Length	F_Length	F_Width	F_dm	N_Leaf	C_Leaf	TPC	CGA
P_Biomass	1.000	0.753		0.788	0.747								0.795			
P_Diam	0.886	1.000		0.753		0.760						-0.788	0.779			
E_yield			1.000	0.826	0.806		0.773	0.788								
Yield	0.742	0.835	0.861	1.000	0.977	0.903	0.751	0.771		0.752						
NUE		0.785	0.893	0.965	1.000	0.906				0.766						
F_Number			0.804	0.855	0.853	1.000										
F_Weight		0.824					1.000	0.847								
F_PedLength								1.000								
F_CaLength									1.000							
F_Length				0.790				0.743	0.736	1.000						
F_Width							0.830				1.000					
F_dm								-0.852		-0.883		1.000				
N_Leaf													1.000			
C_Leaf													0.739	1.000		
TPC															1.000	0.866
CGA															0.859	1.000

3.5. Detection of putative QTLs

The results of the Dunnett's test for the comparison of each IL with the *S. melongena* parent (AN-S-26) allowed the detection of 19 putative QTLs for 10 traits evaluated under the high N (HN) treatment, as well as 24 putative QTLs for 15 traits under the low N (LN) treatment (Table 7).

Under the HN treatment, the total number of QTLs detected were scattered over 6 of the 10 chromosomes represented in the IL population. The highest number of QTLs under this N treatment was identified on the chromosome (chr.) 12 (Table 7). In this way, one QTL for each of the following traits: plant height (P_Height), fruit pedicel length (F_PedLength), fruit N content (N_Fruit) total phenolics (TPC) and chlorogenic acid content (CGA) colocalised on the introgressed fragment of chr. 12 (3-96 Mb), all of them accounting for a significant decrease of the trait mean value compared to AN-S-26 (Table 7). Besides those, eight QTLs specific to the HN treatment were identified associated with both morpho-agronomic and composition traits. In this regard, QTLs were identified for P_Height on chr. 3 and chr. 5, for CGA on chr. 5 and chr. 7, and one QTL was identified for each of the following traits: early yield (E_yield) and F_PedLength, which colocalized on chr. 2, fruit length (F_Length) on chr. 10, N content in leaf (N_Leaf) on chr. 4, and C content in leaf (C_Leaf) on chr. 5 (Table 7). Except for the QTL for F_Length, the wild introgression had a reducing effect on the final phenotype compared to the recipient parent (Table 7).

On the other hand, the QTLs detected under the LN were scattered over 8 of the 10 chromosomes represented in the IL population, with chr. 2 harbouring the highest number of QTLs (Table 7). In this way, ten QTLs identified for several traits related to plant growth (P_Height, P_Biom, P_Diam), yield (Yield) and fruit size and morphology (F_Weight, F_PedLength, F_CaLength, F_Length, F_Width) colocalised on chr. 2 (0-78 Mb) and the *S. incanum* alleles accounted for a considerable reduction of the trait mean value compared to AN-S-26 (Table 7). Besides those, eight identified QTLs were specific to the LN treatment, which were mostly associated with fruit morphology and leaf composition. In this way, four QTLs were linked to F_Width and located on chr. 1, 4, 9 and 12, and one QTL was detected for each of the following traits: F_Length on chr. 4, F_PedLength and N_leaf, which collocated on chr. 9, and C_Leaf on chr. 10 (Table 7). Except for the QTL for F_PedLength, the wild introgression had a reducing effect on the final phenotype compared to the recipient parent (Table 7).

Seven identified QTLs for five traits (P_Height, F_PedLength, F_dm, N_Fruit and CGA) were consistent under both HN and LN treatments, having the same allelic effect. They were detected in four ILs carrying fragments of the *S. incanum* chromosomes 2, 3, 4 and 12 (Table 7). QTLs for plant height (P_Height) for each HN (*ph2.HN*) and LN (*ph2.LN*) were located on chr. 2. Two QTLs for each N treatment were identified for fruit pedicel length (F_PedLength) on chromosomes 2 (*fped2.HN*, *fped2.LN*) and 4

(*fped4.HN*, *fped4.LN*). On the same two chromosomes, two QTLs were detected for fruit dry matter (F_dm), both in HN (*fdm2.HN*, *fdm4.HN*), and LN (*fdm2.LN*, *fdm4.LN*). One QTL was identified for N content in fruit (N_Fruit) under both N treatments (*fnit12.HN*, *fnit12.LN*) and mapped on chr. 12. Lastly, one QTL was detected for chlorogenic acid content (CGA) and located on chr. 3 for HN (*cga3.HN*) and LN (*cga3.LN*) (Table 7). For all of those putative QTLs except F_dm, the introgressed fragment of *S. incanum* accounted for a considerable reduction of each trait mean value compared to AN-S-26 (Table 7).

3.6. Identification of candidate genes

The search throughout the ‘67/3’ eggplant reference genome assembly (V3 version) (Barchi et al., 2019) identified potential candidate genes that could be associated with some of the putative QTLs detected in our study. Three candidate genes for QTLs associated with TPC and CGA were detected. One candidate gene encoding 4-hydroxycinnamoyl-CoA ligase 2 (4CL2), located on chr. 3 at 80.8 Mb, was related to the stable QTL for CGA (*cga3.HN*, *cga3.LN*) and the QTL for TPC (*tpc3.LN*). Furthermore, another homologous 4CL gene (4CL5) and a phenylalanine ammonia-lyase (PAL)-encoding gene, located at 17.7 Mb and 134.8 Mb, respectively, on chr. 7, were putative candidates to be associated with the QTL for CGA identified under HN (*cga7.HN*). The search through the genome assembly failed to highlight candidate genes on the introgressed regions of chr. 5 and 12. However, genes encoding a PAL and a cinnamate 4-hydroxylase (C4H) were also located at the beginning of chr. 5 in the 67/3’ eggplant reference genome assembly (V3 version) (Barchi et al., 2019).

Table 7. List of putative QTLs detected in the ILs population under both high and low N treatments separately.

Trait	Chr.	High Nitrogen (HN)					Low Nitrogen (LN)				
		QTL	Position (Mb)	Increase over MEL	Allelic effect (units)	<i>p</i> -value	QTL	Position (Mb)	Increase over MEL	Allelic effect (units)	<i>p</i> -value
P_Height (cm)	2	<i>ph2.HN</i>	0-78	-21.77	-9.29	0.0302	<i>ph2.LN</i>	0-78	-34.95	-13.00	0.0020
	3	<i>ph3.HN</i>	7-86	-22.65	-9.67	0.0093					
	5	<i>ph5.HN</i>	35-43	-25.23	-10.77	0.0028					
	12	<i>ph12.HN</i>	3-96	-21.01	-8.97	0.0194					
P_Biomass (kg fw)	2					<i>pbio2.LN</i>	0-78	-69.23	-0.14	0.0259	
P_Diam (mm)	2					<i>pdiam2.LN</i>	0-78	-36.42	-3.31	0.0036	
E_yield (g fw plant ⁻¹)	2	<i>ey2.HN</i>	0-78	-73.98	-158.33	0.0211					
Yield (g fw plant ⁻¹)	2					<i>y2.LN</i>	0-78	-73.67	-343.07	0.0240	
F_Weight (g fw)	2					<i>fw2.LN</i>	0-78	-47.49	-21.81	0.0032	
F_PedLength (mm)	2	<i>fped2.HN</i>	0-78	-22.82	-6.63	0.0179	<i>fped2.LN</i>	0-78	-23.08	-6.23	0.0144
	4	<i>fped4.HN</i>	5-106	-21.58	-6.27	0.0294	<i>fped4.LN</i>	5-106	-21.93	-5.92	0.0230
	9						<i>fped9.LN</i>	4-36	20.43	5.51	0.0419
	12	<i>fped12.HN</i>	3-96	-24.21	-7.03	0.0039					
F_CaLength (mm)	2					<i>fcald2.LN</i>	0-78	-27.13	-7.49	0.0079	
F_Length (mm)	2					<i>fl2.LN</i>	0-78	-20.74	-8.23	0.0480	
	4					<i>fl4.LN</i>	5-106	-25.39	-10.07	0.0077	
	10	<i>fl10.HN</i>	0-2	25.82	9.44	0.0224					
F_Width (mm)	1					<i>fwid1.LN</i>	26-134	-19.95	-6.69	0.0141	
	2					<i>fwid2.LN</i>	0-78	-31.08	-10.42	< 1e-04	
	4					<i>fwid4.LN</i>	5-106	-24.92	-8.36	0.0011	
	9					<i>fwid9.LN</i>	4-36	-24.90	-8.35	0.0011	
	12					<i>fwid12.LN</i>	3-96	-17.32	-5.81	0.0476	
F_dm (%)	2	<i>fdm2.HN</i>	0-78	26.03	1.04	0.0014	<i>fdm2.LN</i>	0-78	34.43	1.31	<1e-04
	4	<i>fdm4.HN</i>	5-106	24.91	1.00	0.0382	<i>fdm4.LN</i>	5-106	24.97	0.95	<1e-04
	9						<i>leafnit9.LN</i>	4-36	-15.67	-4.15	0.0189
N_Leaf (g kg ⁻¹ dw)	4	<i>leafnit4.HN</i>	5-106	-9.85	-2.73	0.0359					
C_Leaf (g kg ⁻¹ dw)	1						<i>leafC1.LN</i>	26-134	-3.94	-8.53	0.0053
	5	<i>leafC5.HN</i>	35-43	-3.68	-8.10	0.0191					
	10						<i>leafC10.LN</i>	0-2	-3.81	-8.23	0.0078
N_Fruit (g kg ⁻¹ dw)	12	<i>fnit12.HN</i>	3-96	-18.36	-3.24	0.0156	<i>fnit12.LN</i>	3-96	-24.32	-3.81	0.0095
TPC (g kg ⁻¹ dw)	3						<i>tpc3.LN</i>	7-86	-33.69	-4.66	0.0091
CGA (g kg ⁻¹ dw)	12	<i>tpc12.HN</i>	3-96	-38.21	-5.14	0.0018					
	3	<i>cga3.HN</i>	7-86	-33.01	-3.36	0.0012	<i>cga3.LN</i>	7-86	-65.98	-6.28	<1e-04
	5	<i>cga5.HN</i>	35-43	-42.77	-4.35	< 1e-04					
	7	<i>cga7.HN</i>	9-137	-34.83	-3.54	0.0006					
	12	<i>cga12.HN</i>	3-96	-43.33	-4.40	< 1e-04					

4. Discussion

4.1. Changes in soil with different N fertilization

Interactions occur among the different components of soil and inorganic fertilizers during cultivation that significantly affect soil properties, as well as nutrient absorption capacity of the plant. Soil analysis would be therefore an essential task when planning the most appropriate fertilization for a sustainable agriculture model, although it has been usually not taken into account (Soto et al., 2015).

In the present work, a soil analysis was performed before and after the cultivation period for both high N treatment (HN) and low N treatment (LN). The only difference between the fertigation solutions prepared for each treatment was the addition of N as ammonium nitrate in a similar concentration to the optimal for eggplant cultivation (Baixauli and Aguilar, 2002) under the HN, while no N fertilizer was supplied for LN. However, after the cultivation period, the N status in soil was lower for HN compared to the initial soil condition and the LN treatment. A similar trend towards decreasing available N in soil was observed for banana cultivation under high N fertilization doses above a certain optimal level (Sun et al., 2020). The authors found that the highest N rates significantly decreased soil enzymatic activity, which plays a key role in assimilable N and P regeneration in soil. Long-term N fertilization supply can also affect soil microorganisms and organic matter (SOM), which are the most important factors for soil fertility (Zeng et al., 2016). However, in our study, after a short-time cultivation period, no differences were found for SOM associated with the two fertilization solutions applied, which is in agreement with other studies (Nascente et al., 2013; Sun et al., 2020). In addition, according to the Spanish standard classifications (Yáñez Jiménez, 1989), the average SOM was low in our study. Furthermore, Sun et al. (2020) found that higher yields achieved with higher N fertilization rates led to a higher nutrient removal from soil by plants, thus reducing soil available nutrients. In this respect, our results showed that, although not significantly different, the collection reached a higher average yield on a fresh weight basis at the end of the cultivation period under HN compared to LN. Also, a significantly higher yield after one month from the first harvest (early yield) was achieved under HN, suggesting increased N extraction from the soil by plants at least during the first harvesting period.

The decrease in soil potassium, as well as the micronutrients iron and copper, observed after cultivation for both HN and LN was probably also associated with their removal from the soil by plants through root absorption and also by leaching. The significant increase of calcium and magnesium in soil after cultivation may be due to the high concentration of these cations in the irrigation intake water. The slight increase in soil pH with fertilization may have also influenced it, insofar as a sandy, alkaline soil favours phosphorus precipitation in the form of insoluble phosphates by binding cations such as calcium and magnesium (Miller et al., 1970).

In brief, a combination of conditions, including the interaction of N fertilization rate with soil enzymatic activity, plant yield and nutrient uptake, the rapid leaching nature of nitrates in soil (Wang and Li, 2019), and having a sandy soil texture which is weak in nutrient and water retention (Tracy et al., 2013), might explain our results.

4.2. Effect of N treatment on the ILs and AN-S-26, and potential materials for breeding

In the present study, the N treatment significantly affected traits related to plant growth (plant height and aerial biomass) and chlorophyll content (SPAD), as well as N content in both leaves and fruits, all of which were reduced under the low N rate. This is in agreement with Mauceri et al. (2019), which evaluated chlorophyll content index and N content in fruits and leaves in several eggplant accessions with different origins. To our knowledge, no other studies evaluating plant growth, yield parameters, fruit morphology and antioxidant compounds in eggplant as a response to different N fertilization doses have been reported so far. However, our results were also congruent with studies in other species (Agegnehu et al., 2016; Bénard et al., 2009; Han et al., 2014; Liu and Wiatrak, 2011; Si et al., 2020; Truffault et al., 2019). In this way, N is a major component of chlorophyll, as well as proteins and nucleic acids, thus it is the main macronutrient on which plant growth and development depend. The reduction of chlorophyll content in leaves, the photosynthesis rate, leaf area, plant height or biomass allocation by promoting the root system are part of how plants adapt to N deficiency (Stagnari et al., 2021; Yan et al., 2019). Sugars can be transported from leaves to roots where they act as signalling molecules for biomass allocation and changes in root architecture in response to N and P deficiencies (Hermans et al., 2006). In addition, sugars can also act as repressors of photosynthetic activity in leaves under N shortage (Paul and Driscoll, 1997). Transport of sugars as signalling compounds, the reduction of photosynthetic rate and sink strength may explain the lower average content of C in leaves under LN compared to HN, while no significant differences were found for C content in fruit. Furthermore, in both leaves and fruits, C:N ratio was higher under LN, which has been reported as the driving factor for sugars to act as photosynthesis repressors (Paul and Driscoll, 1997). Plants under high N fertilization doses tend to grow vegetatively rather than reproductively (Elia and Conversa, 2012), thus the contrary seemed to be happening in our collection under the LN treatment.

Regarding yield parameters, early yield and total fruit number were significantly reduced under LN, while the final yield after the cultivation period was not significantly affected by the N treatment. These results are in agreement with Hernández et al. (2020), which found that N fertilization affected tomato yield in terms of fruit load instead of fruit weight. This suggests that minimizing N inputs promoted a more efficient N uptake and utilization in our collection, after a first period of recession and adaptation. In this sense, much higher NUE values were obtained under the LN treatment compared to HN. However, early yield is a trait of utmost relevance for farmers as it implies a much earlier

market release of the product. Identifying the best lines for this trait, as well as studying an optimal N fertilization level between the HN and LN doses for eggplant sustainable production in our conditions would be effective strategies for eggplant breeding for NUE in early stages of the harvest period.

In the present study, genotype per N treatment (G×N) interactions were non-significant for most of the traits, which implies that the lines would show a similar trend in the phenotype in response to lowering N inputs. The distribution of the lines according to the two principal components of the PCA also reflected this similar trend, except for the IL SMI_12.6, which could be an interesting material for adaptation to low N inputs, as it performed better in terms of morpho-agronomic traits under the LN compared to the HN treatment. Besides SMI_12.6, the ILs SMI_5.1 and SMI_9.5 could also be potential materials for breeding, as they performed similarly under the two N treatments, since the same line under each HN and LN appeared very close to each other according to the first two principal components.

Correlations among traits can assist the breeder in predicting the phenotype for some traits by determining the phenotype of only a few. Highly positive correlations (> 0.7) were found among the traits related to plant growth, N and C in leaves and those related to yield and NUE, which is in agreement with other studies (Dinh et al., 2017; Frary et al., 2003; Getahun et al., 2020; Villanueva et al., 2021). Interestingly, these correlations were not N-treatment dependent, since they were very similar under both HN and LN. The same happened among traits related to fruit morphology, also in agreement with several studies (Frary et al., 2003; Mangino et al., 2021; Portis et al., 2015).

Furthermore, minimizing N inputs slightly reduced the average dry matter of fruits, which is in agreement with other studies reporting an increased dry matter content in fruits of diverse crops with increasing N fertilization (Ronga et al., 2019; Sharifi et al., 2007). Different correlations were found for fruit dry matter under HN and LN. For the latter, fruit dry matter was negatively correlated to fruit and pedicel length, suggesting that under N shortage, bigger fruits tend to have a higher water proportion, which might be related to a reduced photoassimilates production, as well as low dry matter allocation to fruit, under LN.

Regarding antioxidant compounds, N treatment did not affect significantly total phenolics (TPC) or chlorogenic acid content (CGA) in our eggplant ILs, although studies have reported an increased expression of the phenylpropanoid pathway in response to abiotic stresses, including N shortage (Galièni et al., 2015; Hernández et al., 2019). TPC and CGA were highly and positively intercorrelated under both N treatments. This is in agreement with numerous studies reporting CGA as the major phenolic compound, by far, in the fruit flesh of *S. melongena* and *S. incanum*, usually accounting for more than 70% of TPC (Luthria et al., 2010; Prohens et al., 2013; Stommel and Whitaker, 2003). This could facilitate eggplant breeding for the

improvement of antioxidant capacity for a more sustainable agriculture, as we could expect a similar response of the phenotype under two contrasting N inputs.

4.3. Putative QTLs and candidate genes identified

The assessment of the differences among each of the ILs and the recipient parent AN-S-26 resulted in the identification of seven putative QTLs stable across the two N treatments, which could be very useful for breeding assistance under different conditions. Other 12 putative QTLs under the HN treatment and 17 putative QTLs under the LN treatment were also detected. This supports the strong effect of N levels in the changes of a considerable number of the traits analysed. Nevertheless, identifying QTLs for each N treatment separately could represent an advantage for selection and breeding for specific conditions (Diouf et al., 2018).

For most of the putative QTLs, the wild alleles had a negative effect compared to the cultivated eggplant. The significant differences found between SMI_2.9 and AN-S-26 allowed the detection of a cluster of putative QTLs for several morpho-agronomic traits, which colocalized on the introgressed fragment of chromosome (chr.) 2. Related traits are expected to share common QTLs (Causse et al., 2002). Therefore, the collocations on chr. 2, compared with the significant inter-trait correlations found among plant growth, yield and fruit size parameters could indicate the presence of genetic linkage or a pleiotropic locus (Causse et al., 2002; Fulton et al., 1997; Portis et al., 2014). Our results are in agreement with the QTL cluster on chr. 2 for early and total yield, as well as fruit size traits, identified in an eggplant intraspecific F₂ population obtained by crossing the breeding lines “305E40” × “67/3” (Portis et al., 2014). In addition, QTLs for fruit length and fruit weight were also detected on chr. 2 in an eggplant interspecific F₂ population with *S. linnaeanum* (Doganlar et al., 2002; Frary et al., 2014). Interestingly, the QTL for stem diameter (*pdiam2.LN*), for fruit weight (*fw2.LN*) and fruit pedicel length (*fped4.LN*, *fped4.HN* and *fped12.HN*) were coincident with QTLs identified in a previous two-environment evaluation of a set of the eggplant lines with introgressions from *S. incanum* (Mangino et al., 2020). For all of them, the introgressed fragment had the same negative effect on the average trait value, except for the QTL for stem diameter on chr. 2, which had the opposite effect. This could be explained by the significantly larger introgressed fragment carried by the line SMI_2.9 in our study than the fragment in Mangino et al. (2020). Thus, the QTLs in each of the studies may have different locations on the chromosome or there may be interactions with other regions of the fragment that modify the QTL effect. Fruit weight is a trait of utmost interest for farmers, which genetic basis has been extensively investigated for decades in *Solanaceae*, especially in tomato and pepper, and major QTLs have been mapped on chromosome 2 in several studies (Cambiaso et al., 2019; Grandillo and Tanksley, 1996; Illa-Berenguer et al., 2015; Paran and Van Der Knaap, 2007; Zygier et al., 2005). This supports the conservation of gene function among the *Solanaceae*, as well as the

importance of synteny studies for genetic inferences in less studied species (Doganlar et al., 2002; Rinaldi et al., 2016; Wu et al., 2009).

QTLs with a positive effect of the introgression were detected for fruit pedicel length on chr. 9 (*fped9.LN*), for fruit length on chr. 10 (*fl10.HN*), and for fruit dry matter on chr. 2 (*fdm2.HN*, *fdm2.LN*) and chr. 4 (*fdm4.HN*, *fdm4.LN*), which accounted for a significant increased average value of each trait. These are novel QTLs in eggplant, except for a QTL for fruit dry matter, which was also detected on chr. 2 on the eggplant “305E40” × “67/3” intraspecific F₂ population (Toppino et al., 2016). In addition, QTLs for dry matter were also identified on syntenic fragments of chr. 2 and 4 in tomato (Bertin et al., 2009; Causse et al., 2002; Lecomte et al., 2004; Prudent et al., 2009).

The search for candidate genes that underlay the identified QTLs in our study was challenging because the introgressed fragments covered almost 100% of the chromosomes harbouring the QTL, thus a massive number of genes. Obtaining sub-ILs, with shorter and overlapping introgression fragments, would be a future strategy to fine-mapping the QTLs. A relatively short introgressed fragment was obtained at the beginning of chromosome 10 (0-2 Mb). Within this fragment, a QTL for carbon content in leaf was identified under the LN treatment, which could be associated with N shortage response. No other QTLs were identified in eggplant for C metabolism, but a recent study using an IL population of *S. pennellii* into a cultivated tomato background reported the identification of a vast number of QTLs related to photosynthesis and primary metabolism, which were scattered over all chromosomes (Silva et al., 2018). The set of QTLs from that study included some associated with photosynthesis, respiration rates and starch turnover in a region of chromosome 5 syntenic to the beginning of chromosome 10 (Silva et al., 2018).

Finally, QTLs for total phenolics (TPC) and chlorogenic acid content (CGA) in fruit colocalized on chr. 3 and 12, which probably indicate the presence of a common QTL associated with both traits on each chromosome, due to the highly positive correlation between these under the two N treatments. In addition, two other QTLs were detected only for CGA on chr. 5 and 7. The introgressed fragment for every QTL had a negative effect on the mean trait value, which was unexpected because a higher content in these antioxidant compounds was reported on *S. incanum* accessions compared to cultivated varieties (Kaur et al., 2014; Prohens et al., 2013). A possible explanation might be the occurrence of epistatic effects between loci of the wild introgressed fragment and the cultivated genetic background (von Korff et al., 2010). Interestingly, the identified QTL on chr. 7 (*cga7.HN*) colocalized with another QTL for reduced CGA, which was detected at the end of the chromosome (129-135 Mb) in a recent study using the set of ILs from *S. melongena* × *S. incanum* (Rosa-Martínez et al., 2022). The major structural genes encoding the key enzymes involved in the CGA synthesis pathway are also well known and have previously been located in a *S. melongena* × *S. incanum* linkage map (Gramazio et al., 2014). In addition, thanks to synteny to other important *Solanaceae*

and the availability of various eggplant reference genome assemblies (Barchi et al., 2019; Hirakawa et al., 2014; Li et al., 2021; Wei et al., 2020), several orthologs of those core genes of the CGA synthesis pathway as well as transcription factors involved in its regulation have been positioned on the eggplant physical map. Three candidate genes encoding key enzymes of the chlorogenic acid synthesis pathway were spotted within the regions where the QTLs associated with TPC and CGA were located on chr. 3 and 7. Although no candidate genes were identified for the QTLs on chr. 5 and 12, interestingly, another QTL for increased CGA was recently detected at the beginning of chr. 5 in an advanced backcross population of eggplant with the wild relative *S. elaeagnifolium* (Villanueva et al., 2021). Also, genes encoding a PAL and a cinnamate 4-hydroxylase (C4H) were located at the beginning of chr. 5 in the ‘67/3’ eggplant reference genome assembly (V3 version) (Barchi et al., 2019). A better knowledge of the regulation of CGA synthesis pathway and its genetic determinants will be necessary to unravel all these QTLs effects.

5. Conclusions

We reported herein the first morpho-agronomic and composition characterization of the response to N fertilization shortage on a set of eggplant introgression lines (ILs) and the recipient parent AN-S-26, from the first interspecific IL collection obtained in eggplant carrying fragments of the wild relative *S. incanum*. In addition, the effect of the two different N fertigation doses on soil composition was evaluated.

In general, minimizing N inputs significantly affected traits related to plant growth, early yield, N and C distribution in plant and fruit dry matter, while it did not significantly affect final yield, fruit morphology and size, or the content in phenolic compounds in the fruit flesh. An optimal fertilization level could be investigated to maximise plant yields without causing damage to the environment. Among the ILs evaluated, some were identified as potential materials for breeding eggplant for adaptation to low N inputs. Finally, thanks to the available genotyping of the ILs, several QTLs were identified to be associated with most of the traits analysed under each of the N treatments, including seven stable QTLs between the two N treatments, which provides a useful tool for marker-assisted breeding in eggplant.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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7. Supplementary material

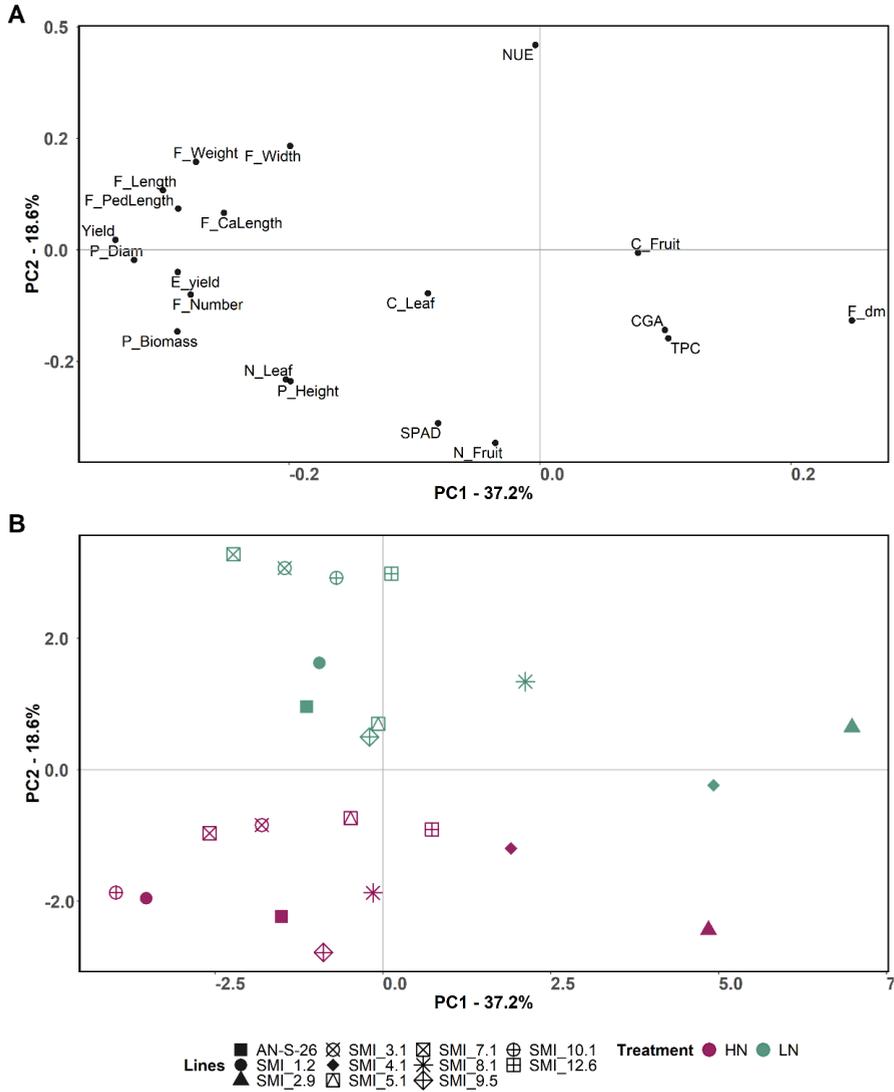


Figure S1. Principal component analysis loading plot (A) and score plot (B) performed in the present study for all traits, based on the two first principal components of PCA. The accessions are represented by different symbols, which are coloured differently according to the N treatment in which they were grown. The full name of each trait abbreviation is found in Table 2.

Chapter III - Local varieties of 'de penjar' tomato for breeding for fruit quality and adaptation to low N inputs

Research article

Variation for composition and quality in a collection of the resilient Mediterranean ‘de penjar’ long shelf-life tomato under high and low N fertilization levels

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Ph.D. candidate contribution

E. R.-M. had a main role in the following activities: performing the experiments, data collection, data analysis, data visualization, drafting manuscript, manuscript review and editing.

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Abstract

The ‘de penjar’ tomato (*Solanum lycopersicum* L.) is a group of local varieties from the Spanish Mediterranean region carrying the *alc* mutation, which provides long shelf-life. Their evolution under low input management practices has led to the selection of resilient genotypes to adverse conditions. Here we present the first evaluation on nutritional fruit composition of a collection of 44 varieties of ‘de penjar’ tomato under two nitrogen (N) fertilization levels, provided by doses of manure equivalent to 162 kg N ha⁻¹ in the high N treatment and 49 kg N ha⁻¹ in the low N treatment. Twenty-seven fruit composition and quality traits, as well as plant yield and SPAD value, were evaluated. A large variation was observed, with lycopene being the composition trait with the highest relative range of variation (over 4-fold) under both N treatments, and significant differences among varieties were detected for all traits. While yield and most quality traits were not affected by the reduction in N fertilization, fruits from the low N treatment had, on average, higher values for hue (5.9%) and lower for fructose (-11.5%), glucose (-15.8%) and total sweetness index (-12.9%). In addition, lycopene and β -carotene presented a strongly significant genotype \times N input interaction. Local varieties had higher values than commercial varieties for traits related to the ratio of sweetness to acidity and for vitamin C, which reinforces the appreciation for their organoleptic and nutritional quality. Highest yielding varieties under both conditions displayed wide variation in the composition and quality profiles, which may allow the selection of specific ideotypes with high quality under low N conditions. These results revealed the potential of ‘de penjar’ varieties as a genetic resource in breeding for low N inputs and improving the organoleptic and nutritional tomato fruit quality.

Keywords: *Solanum lycopersicum*, plant breeding, local varieties, metabolites, taste, nutritional quality.

1. Introduction

Use of nitrogen (N) enriched fertilizers has sharply escalated since the Green Revolution and has allowed dramatic increases in crop yields. However, severe impacts of over-fertilization on the environment forced governments to implement sustainable agriculture policies (Zhang et al., 2015). The current environmental situation has prompted research studies to understand the effects of decreasing N inputs on different crops and the development of new varieties with improved N use efficiency. In general terms, N shortage is associated with a limitation of plant growth, photosynthesis rate, and synthesis and accumulation of bioactive compounds in fruits.

Tomato (*Solanum lycopersicum* L.) is the second vegetable in acreage after onions (FAOSTAT, 2019). Official recommendation of N inputs for intensive tomato cultivation vary between 200 and 240 kg N ha⁻¹ in open field, and between 380 and 410 kg N ha⁻¹ in greenhouse (Ramos and Pomares, 2010). However, in the last decades, growers have been supplying nitrogen fertilizers well above those requirements (Thompson et al., 2007). Studies about the N supply effects on tomato showed that yield increased with N fertilization until a certain level, above which N had no longer a positive effect on yield, and even decreased it (Djidonou et al., 2013; Elia and Conversa, 2012). On the other hand, controversial responses to low N inputs are reported regarding tomato fruit quality (De Pascale et al., 2016; Hernández et al., 2020; Truffault et al., 2019).

Within the broad array of diversity of tomato types, there is a group of varieties (known as ‘de penjar’) which could potentially constitute a genetic resource in breeding for low N inputs. The ‘de penjar’ tomato is distinctively characterized by the presence of the *alc* mutation, which is associated to long shelf-life (LSL) of fruits, up to 6-12 months after harvest (Casals et al., 2012). The ‘de penjar’ (literally meaning “for hanging”) type of tomato is mainly composed of local varieties preserved by generations of small farmers. Plants of ‘de penjar’ tomato generally produce round to flat, medium sized fruits (30-90 g) with higher acidity and soluble solids content than standard tomato; however, the ‘de penjar’ varieties display a great variability in morphoagronomic and fruit quality characteristics, according to their traditional area of cultivation and to their uses (Casals et al., 2015; Cebolla-Cornejo et al., 2013; Figàs et al., 2015). Historically, ‘de penjar’ tomatoes have been cultivated in open field and under rain-fed, low-input conditions (Conesa et al., 2020). This has led to the selection for resilient varieties. The drought tolerance of LSL varieties is well documented and has been studied in recent years (Fullana-Pericàs et al., 2019). However, to our knowledge, scarce information is available, and no study has been carried out on nitrogen fertilization for ‘de penjar’ nor other LSL tomato cultivation. Some conducted trials indicate that N needs of ‘de penjar’ tomato are around 170-180 kg ha⁻¹ (Seda and Muñoz, 2011), far below the N fertilization levels required for intensive standard tomato cultivation. Considering that ‘de penjar’ tomato has evolved under low input management practices and their already reported

drought tolerance, we hypothesize that it could also show resilience against other abiotic stresses, such as low nitrogen supply.

In the present work, we evaluated a collection of 39 local varieties and 5 commercial varieties of ‘de penjar’ tomato, grown under two N levels, for agronomic and nutritional fruit composition traits. The aim was to provide information on the existing variability and behaviour of these materials under different N supply conditions, and to draw conclusions on the effect of the reduction of N inputs on their fruit quality and composition.

2. Materials and methods

2.1. Plant material and cultivation conditions

A collection of 44 varieties of ‘de penjar’ tomato from different origins throughout the Valencian Region, located on the Spanish eastern coast, were evaluated under two N fertilization conditions. The collection was composed of 39 local varieties and 5 commercial varieties. Passport data on each of the accessions used is included as Supplementary Table S1.

Plants were grown during the spring-summer season of 2019 in two neighbouring open field plots located in Alcossebre (Castelló, Valencian Region; GPS coordinates of the field plots: 40°13’21” N 0°15’51” E). Both field plots were certified for organic farming and had followed the same agricultural practices for the last five years. Similar crop management practices and fertilization were applied to both field plots, except for the N fertilization level. One field plot was submitted to a N fertilization dose of 162 kg N ha⁻¹, equivalent to the levels commonly provided in the cultivation of ‘de penjar’ tomato (Seda and Muñoz, 2011). This dose has been considered in the present work as high N fertilization treatment (HN). For the other field plot, a dose of 49 kg of N ha⁻¹ (i.e., 30.2% of the HN) was applied. This dose has been considered in the present work as low N fertilization treatment (LN). According to the organic farming practices that have been followed in the experiment, certified organic fertilizers were used. An organic basal dressing consisting of sheep manure (Organia, Fertinagro, Teruel, Spain) was applied in both field plots, shortly before cultivation, to provide the desired levels of N fertilization. Besides, the LN was supplemented with P (Fosfoser ECO GR, Mapryser, Barcelona, Spain) and K (Summum Líquido Quality 0-0-15, Fertinagro, Teruel, Spain), to equal the quantities of P and K present in the manure of the HN treatment (60 kg P₂O₅ ha⁻¹ and 174 kg K₂O ha⁻¹). Both P and K fertilizers in the LN were supplied with the irrigation system. Since fertilization in the form of manure is characterized by a slow release of nutrients, the total P and K fertigation was distributed on a fortnight basis. Plantlets were transplanted at the five true-leaves stage on May 8th 2019. Plants in both field plots were irrigated throughout the entire cultivation period using an exudation irrigation system (16 mm; Poritex, Barcelona, Spain), for a total volume of 127 L plant⁻¹, so that the water would not be limiting in the evaluation, as confirmed by the lack of

phenotypic symptoms of water stress and by the calculation of crop evapotranspiration (ETc) for ‘de penjar’ tomato. Immediately after the transplant, a watering of 5 L plant⁻¹ was applied. Subsequently, 4 L plant⁻¹ were applied weekly during the next three weeks, distributed in two days per week; from week 4 to 12, 6 L plant⁻¹ were applied weekly, distributed in three days per week; from week 13 to 16, 8 L plant⁻¹ were applied weekly, distributed in four days per week; finally, from week 17 to 20, plants were irrigated with 6 L plant⁻¹ on a two days per week basis.

A total of six replicates per accession (i.e., three replicates per accession and N fertilization treatment, with two plants per replicate) were distributed in a completely randomized block design. Standard local practices for the ‘de penjar’ tomato were used during the experiment. Plants were stacked with canes that were inclined so that a triangular structure was formed enabling a double row distribution, with 3.00 and 0.80 m between wide and narrow rows, respectively. Plants were spaced at 0.35-m intervals within rows. Crop management included no pruning and manual weeding.

Climate conditions on the entire cultivation period are included as Supplementary Figure S1. Average temperature during the cultivation period varied between 15.6 and 28.1 °C. The month with the highest mean radiation was June, with 24.8 MJ m⁻², and this parameter declined during the following months, to an average of 16.6 MJ m⁻² in September. Pluviometry was mostly concentrated on May 24th (12.1 mm), June 5th (14.3 mm) and at the end of the cultivation period, on August 20th (23.7 mm), September 11th (21.3 mm), 13th (14.2 mm) and 21st (36.2 mm).

2.2. Soil analysis

A soil physicochemical and composition analysis was performed in both field plots before the transplant and before applying the fertilization. Five samples consisting of five randomly selected spots per each of the two field plots were considered for soil analysis ($n = 5 \times 2 = 10$). For each sample, five fractions of soil surrounding the selected spot, between 10 and 30 cm deep, were extracted, homogenized and left to dry at room temperature. A portion of 500 g of each dried homogenate was used for the analysis. Physical and chemical parameters were evaluated following the procedures described in Reeuwijk (2002): particle size analysis, pH in water and pH in potassium chloride, electrical conductivity, contents in total nitrogen, carbonates and organic matter, carbon:nitrogen ratio and mineral contents of available phosphorus, potassium, calcium, magnesium, iron, zinc and copper. Soil texture for both fields was clay-loam, with a composition of 41% sand, 36% clay and 23% silt (Soil Science Division Staff, 2017). There were no significant differences between the two field plots for any of the soil physicochemical and composition parameters analysed. According to the Spanish interpretation scales for each of the different elements evaluated (Yáñez Jiménez, 1989), the soil was slightly saline, had normal contents of total nitrogen and carbonates, high

presence of organic matter and moderately high carbon:nitrogen ratio. Data of soil characteristics is included as Supplementary Table S2.

2.3. Vegetative and plant traits evaluation

A chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan) was used to take SPAD values from upper fully expanded leaves. Four readings per replicate were taken. Readings were done according to manufacturer's instructions. Total number of fruits (fn) produced per replicate was counted during the harvest period (July 15th - September 30th). Fruits at the red stage of ripeness in both N fertilization treatments were harvested once a week during this period, so that the same number of harvests were made in the two treatments. Yield was calculated from the previous data and the average fruit weight (frw) per replicate as $fn \times frw$. In addition, a resilience index, for each accession of the 44 evaluated and trait, was calculated as the ratio between the mean value under the low N treatment and the mean value under the high N treatment (LN/HN).

2.4. Fruit traits analysis

Five to ten fruits per replicate were brought to the laboratory to be processed for chemical composition analysis. The fruits were collected from the second to fifth truss at the red stage of ripeness. Before processing, five representative fruits per replicate were weighed in a digital scale in order to calculate the average fruit weight (frw). Units in which vegetative and fruit traits are expressed are included in Table 1.

2.4.1. Sample preparation

Fruits were longitudinally cut in half and seeds were eliminated. One half of cut tomatoes were bulked and squeezed with a domestic juice extractor for subsequent analysis of pH, titratable acidity, contents in soluble solids, reducing sugars (glucose, fructose), organic acids (citric, malic) and amino acids (glutamic, aspartic). For vitamin C analysis, 3% metaphosphoric acid (MPA) was added to the tomato liquid extract (1:1), in order to lower the pH for preventing degradation of ascorbic acid (Chebrolu et al., 2012). The other tomato halves were homogenized in liquid nitrogen using a domestic grinder, for subsequent freeze-drying. Homogenized tomato powder was used for analysis of contents in lycopene, β -carotene, total carotenoids, total nitrogen and total carbon.

2.4.2. Soluble solids content, pH and titratable acidity

Soluble solids content was measured using 0.5 mL of liquid extract with a HI 96801 digital refractometer (HANNA instruments, Padua, Italy). Titratable acidity and pH were measured with a PH-Matic 23 analyser (Crison instruments, Barcelona, Spain), from 10% (w/v) aqueous tomato extract, using NaOH 0.1 M as titrating reagent. From values of soluble solids content and titratable acidity, the ratio between those was calculated.

Table 1. Vegetative and fruit traits evaluated in the ‘de penjar’ tomato collection, abbreviations used in tables and figures and units in which they are expressed in the present work.

Trait	Abbreviation	Units
<i>Vegetative and plant traits</i>		
SPAD value	spad	-
Yield	y	kg plant ⁻¹
<i>Fruit traits</i>		
Fruit mean weight	frw	g
Lightness (colour coordinates)	L	-
Chroma (colour coordinates)	chroma	-
Hue (colour coordinates)	hue	°
Fruit nitrogen content	Nf	g kg ⁻¹ dw ^a
Fruit carbon content	Cf	g kg ⁻¹ dw
pH	pH	-
Soluble solids content	ssc	%
Titrateable acidity	ta	%
Citric acid content	cit	g kg ⁻¹ fw ^b
Malic acid content	mal	g kg ⁻¹ fw
Total acid (citric + malic) content	tacid	g kg ⁻¹ fw
Citric:malic acid ratio	citmalr	-
Fructose content	fru	g kg ⁻¹ fw
Glucose content	glu	g kg ⁻¹ fw
Total sugar (fructose + glucose) content	tsug	g kg ⁻¹ fw
Fructose:glucose ratio	fruglur	-
Total sweetness index	tsi	-
Soluble solids content:titrateable acidity ratio	ssctar	-
Total sugars:total acids ratio	tsugtacidr	-
Total sweetness index:titrateable acidity ratio	tsitar	-
Vitamin C (ascorbic + dehydroascorbic) content	vitc	g kg ⁻¹ fw
Lycopene content	lyc	mg kg ⁻¹ fw
β-carotene content	bcar	mg kg ⁻¹ fw
Total carotenoid content	tcar	mg kg ⁻¹ fw
Glutamic acid content	gluta	g kg ⁻¹ fw
Aspartic acid content	aspa	g kg ⁻¹ fw

^adw: dry weight^bfw: fresh weight

2.4.3. Sugars, organic acids, vitamin C and amino acids

Contents in reducing sugars, organic acids, vitamin C and amino acids were measured by High Performance Liquid Chromatography (HPLC) using a 1220 Infinity LC System (Agilent Technologies, CA, USA) equipped with a binary pump, an automatic injector and a UV detector. One aliquot of liquid extract per replicate was centrifuged for 5 min at 10 000 rpm, diluted with Milli-Q® water (1:1) and filtered through 0.22 µm PVDF MILLEX-GV filters (Merck Millipore, MA, USA). The same sample was used to perform the analysis of sugars and organic acids, following the method indicated in Fernández-Ruiz et al. (2004), with slight modifications. Glucose and fructose separations were performed using a Luna® Omega SUGAR column (3 µm; 150 × 4.6

mm; Phenomenex, CA, USA) and a refractive index detector (350 RI detector, Varian, CA, USA) coupled to the HPLC system. The mobile phase consisted of solvent A (ACN 100%) and solvent B (water). The gradient was isocratic 75% A: 25% B and the flow rate, 1 mL min⁻¹. Citric and malic acids were separated using a Rezex™ ROA-Organic Acid H+ (8%) column (150 × 7.8 mm; Phenomenex) and detected by HPLC-UV at 210 nm. The mobile phase consisted of an isocratic gradient of 100% 1 mM H₂SO₄ and the flow rate was 0.5 mL min⁻¹.

From the values of contents in fructose, glucose, citric and malic, total sugar content and total acid content were calculated as fructose + glucose and citric acid + malic acid, respectively. In addition, the ratios fructose:glucose, citric:malic and total sugars:total acids were determined. Total sweetness index was also calculated as (Beckles, 2012) (0.76 × [glucose]) + (1.50 × [fructose]), and used for also determining the ratio between total sweetness index and titratable acidity.

For extraction of vitamin C, one aliquot of liquid tomato extract in 3% MPA per replicate was cold centrifuged and filtered through 0.22 μm PVDF filters. In order to quantify vitamin C as the sum of ascorbic (AA) and dehydroascorbic (DHA) acids, the DHA present in each sample was reduced to AA adding 5 mM Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) in 1:1 proportion. AA peak was subsequently detected and quantified by HPLC-UV at 254 nm using a Brisa “LC²” C18 column (3 μm; 150 × 4.6 mm; Teknokroma, Barcelona, Spain), following the method described in Chebroli et al. (2012).

Determination of glutamic and aspartic acid contents was carried out using liquid tomato homogenate. After a previous derivatisation with 30 mM 2,4-dinitro-1-fluorobenzene (DNFB) reagent at moderate basic pH and 60 °C, peaks detection and analysis by reversed-phase HPLC-UV at 363 nm were performed as described by Agius et al. (2018).

2.4.4. Carotenoids

Lycopene, β-carotene and total carotenoids were extracted using 30 mg of freeze-dried powdered material per replicate, which was incubated with ethanol:hexane 4:3 (v/v), in darkness and shaking at 200 rpm for 1 h. Subsequently, carotenoids were quantified from UV/V spectrophotometry absorbance values at 452, 485 and 510 nm of the previously separated hexane phase, using the following equations (Zscheile and Porter, 1947):

$$\text{lycopene} = \frac{\text{Abs}_{510} \times 537 \times 2.7}{\text{weight} \times 172} \times 100$$

$$\beta - \text{carotene} = \frac{[\text{Abs}_{452} - (\text{Abs}_{510} \times 0.9285)] \times 533.85 \times 2.7}{\text{weight} \times 139} \times 100$$

$$\text{total carotenoids} = \frac{\text{Abs}_{485} \times 2.7}{\text{weight} \times 181} \times 100$$

2.4.5. Total nitrogen and carbon

A sample of 0.5 g of freeze-dried and powdered material per replicate was used for N and C determination in fruit. The analysis was based on a complete combustion of the sample at 950 °C in the presence of oxygen, using a TruSpec CN elemental analyser (Leco, MI, USA) (Gazulla et al., 2012).

2.5. Data analysis

A bifactorial (genotype and N treatment) analysis of variance was performed for every trait studied for the evaluation of differences among the accessions (genotypes, G), between N treatments (N) and for the occurrence of G×N interactions (Gomez and Gomez, 1984). In the ANOVA, two levels were established for the N treatment factor, corresponding to HN and LN; whereas levels of the genotype factor were the 44 accessions of the collection. Data from the six replicates per accession were used, making a total number of cases, $n = 264$. The block effect due to the experimental design was removed to evaluate the effect of the two factors and the interaction (Gomez and Gomez, 1984). Mean values and range for all traits were calculated from accession means for both LN ($n = 44$) and HN ($n = 44$). Comparisons of average differences between the sets of local ($n = 39$) and commercial ($n = 5$) varieties were assessed with a *t*-student test at $p < 0.05$ using R statistical software v4.0.2 (R Core Team, 2013). Only traits with significant differences between both groups in the two environments were considered as displaying a stable significant difference.

A principal component analysis (PCA) was performed using pairwise Euclidean distances among accession means for each N treatment and for all the traits. PCA resulting loading and score plots were drawn using R package *ggplot2* (Wickham, 2016). Prediction ellipses for LN and HN with a 95% level of confidence were added to the PCA score plot. Phenotypic and environmental correlations among traits were calculated from accession means and residuals, respectively, using the R packages *psych* (Revelle, 2018) and *corrplot* (Wei et al., 2017). Pearson linear coefficients of correlation (r) were calculated between pairs of traits and significance of correlations was evaluated with the Bonferroni test at $p < 0.05$ (Hochberg, 1988).

2.5.1. Genetic parameters

The genotypic (σ^2_G) and phenotypic variance ($\sigma^2_P = \sigma^2_G + \sigma^2_N + \sigma^2_{G \times N}$) of each trait were obtained from the mean squares (MS) of the genotype, G×N interaction and residuals of the ANOVA performed, in order to estimate broad-sense heritability (H^2) using the formula $H^2 = \sigma^2_G / \sigma^2_P$ (Wricke and Weber, 2010). Standard errors (SE) of the heritabilities were calculated by the Delta method, using the following formulas (Nyquist, 1991):

$$SE(H^2) = H^2 \times \sqrt{\frac{\sigma_a^2}{A^2} + \frac{\sigma_b^2}{B^2} - 2 \times \frac{\text{cov}(A, B)}{A \times B}}$$

$$A = \frac{M_1 - M_2}{\delta \times r}$$

$$B = \frac{M_1 - M_2}{\delta \times r} + \frac{M_2 - M_3}{r} + M_3$$

$$\sigma_a^2 = \left(\frac{1}{\delta \times r}\right)^2 \times \left[\frac{2 \times M_1^2}{df_{M_1} + 2} + \frac{2 \times M_2^2}{df_{M_2} + 2} \right]$$

$$\sigma_b^2 = \left(\frac{1}{\delta \times r}\right)^2 \times \frac{2 \times M_1^2}{df_{M_1} + 2} + \left(\frac{\delta - 1}{\delta \times r}\right) \times \frac{2 \times M_2^2}{df_{M_2} + 2} + \left(1 - \frac{1}{r}\right)^2 \times \frac{2 \times M_3^2}{df_{M_3} + 2}$$

where M_1 , M_2 and M_3 are the MS for genotype, G×N interaction and residuals, respectively; df_{M_1} , df_{M_2} and df_{M_3} , the degrees of freedom on which M_1 , M_2 and M_3 were calculated, δ the number of treatments and r the number of replications.

The coefficients of genetic (CV_G) and phenotypic (CV_P) variation were estimated from the corresponding variance components (σ^2_G and σ^2_P) and the mean value of the trait (μ) as (Wricke and Weber, 2010):

$$CV = \frac{\sqrt{\sigma^2}}{\mu} \times 100$$

and their SE were calculated as:

$$SE(CV) = \frac{CV \times \sqrt{1 + 2 \times (CV/100)^2}}{\sqrt{2 \times N}}$$

where N is the total number of individuals used in the CV estimation.

3. Results

3.1. Analysis of variance

Analysis of variance (ANOVA) revealed a significant effect of the genotype for all traits. For N treatment, no significant effect was observed, except for hue, contents of

fructose, glucose, total sugars, and total sweetness index (Table 2). For these five traits, F-ratio values were much greater than those of the genotype factor. However, no significant effect of the interaction G×N was detected for any of those traits. On the other hand, a significant G×N interaction was observed for lightness of colour, fructose:glucose ratio, contents in lycopene, β-carotene, total carotenoids, and total carbon (Table 2). Except for lycopene and total carotenoid content, F-ratio values of the G×N interaction were lower than those of the genotype effect. In the cases of traits with a significant interaction G×N, it is worth mentioning the particular accessions that showed a significant difference between HN and LN, and how the latter affected those traits. Therefore, for lightness of colour, only three out of 44 accessions showed a significantly increased mean value under LN (MO1, MO2, TE1), among which MO2 had the largest increase (20%). For fructose:glucose ratio, the same effect under LN was observed on three accessions (MO2, SN2, TO1), while AC1 and FA2 showed a significantly lower average fructose:glucose ratio. SN2 was the accession with the greatest difference between N treatments for fructose:glucose ratio, which showed an increased mean value by 36% under LN. In the case of lycopene content, as well as for total carotenoids, LN significantly increased mean values of three accessions (BL1, MT1, TO1), while had the opposite effect on six accessions (AY1, LA2, LL2, TA1, VH2, VI1). For both lycopene and total carotenoid content, the largest difference between N treatments was found for BL1 (63% and 60% of increase under LN, respectively). Likewise, AC1, BL1, MT1 and TO1 showed higher average β-carotene content under LN, and FI1, LA2 and VI1 had lower mean values of this trait under the same treatment. TO1 had the greatest percentage of increase for average β-carotene content under LN (45%), while VI1 had the highest percentage of decrease under the same conditions (47%). Finally, total carbon content was, in average, significantly higher under LN in four out of the 44 accessions evaluated (C4, C5, CO1, TO1). In this case, the commercial variety C5 had the highest percentage of increase (5%). Data of average values per accession and N treatment for each trait analysed are included as Supplementary Table S3. For none of the traits where G×N interaction was significant, a significant effect of N treatment was observed (Table 2).

Table 2. F-ratio values for genotype (G), nitrogen treatment (N; low or high) and genotype per nitrogen treatment interaction (G×N) of each trait evaluated in the present study, obtained from the bifactorial ANOVA, which considered the 44 ‘de penjar’ accessions of the collection with three replicates per N treatment ($n = 44 \times 2 \times 3 = 264$). The full name of each trait abbreviation in the first column can be found in Table 1. Significant differences are indicated with * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

Traits	Genotype (G)	N treatment (N)	G×N interaction
<i>Vegetative and plant traits</i>			
spad	4.15***	0.15	1.23
y	3.64***	0.66	0.83
<i>Fruit traits</i>			
frw	19.04***	2.13	0.82
L	4.16***	1.04	1.64*
chroma	5.80***	0.00	1.17
hue	4.70***	16.86*	1.11
Nf	3.25***	3.77	0.94
Cf	3.32***	4.87	1.54*
pH	2.27***	0.42	1.32
ssc	4.68***	0.90	0.91
ta	5.04***	0.02	0.91
cit	5.37***	0.54	1.28
mal	10.44***	0.96	1.33
tacid	4.51***	0.45	1.33
citmalr	11.31***	1.40	1.10
fru	3.83***	53.25**	1.25
glu	4.40***	10.90*	1.35
tsug	4.19***	23.70**	1.27
fruglur	3.93***	1.39	2.19***
tsi	4.09***	32.16**	1.25
ssctar	4.64***	0.01	0.93
tsugtacidr	3.78***	2.40	0.88
tsitar	4.83***	1.19	0.96
vitc	2.39***	0.91	0.91
lyc	1.80**	0.40	2.23***
bcar	2.69***	2.40	2.04***
tcar	1.92**	0.71	2.25***
gluta	2.29***	0.23	1.22
aspa	2.66***	0.78	1.03

Mean values with their standard difference and range, as well as the paired difference between mean values in high N and low N treatment, of the whole ‘de penjar’ tomato collection ($n = 44$) under each N treatment, are shown in Table 3. Average yield and fruit weight were, respectively, 2.20 kg plant⁻¹ and 72.7 g (LN) and 1.98 kg plant⁻¹ and 70.4 g (HN), while for total N and C, mean values were 21.1 g kg⁻¹ of dry weight (dw)

and 408.0 g kg⁻¹ dw (LN), and 19.9 g kg⁻¹ dw and 405.8 g kg⁻¹ dw (HN), respectively (Table 3). Average soluble solids content was 5.52% (LN) and 5.69% (HN), while pH and titratable acidity mean values were 4.13 and 0.57% (LN) and 4.11 and 0.58% (HN), respectively. Average values for total organic acids and reducing sugars were, respectively, 8.03 g kg⁻¹ of fresh weight (fw) and 34.30 g kg⁻¹ fw (LN) and 7.62 g kg⁻¹ fw and 39.65 g kg⁻¹ fw (HN). Mean values of fructose:glucose ratio were slightly above 1.0 under both treatments (1.26 for LN and 1.19 for HN), while the citric:malic acid ratio was over 5.0. Regarding ratios between sugar and acid parameters (soluble solids content:titratable acidity, total sugars:total acids and total sweetness index:titratable acidity), mean values were a great deal above 1.0 in both treatments with values of 10.54, 4.64 and 7.68, respectively, for LN and 10.63, 5.57 and 8.63, respectively, for HN (Table 3).

When comparing significantly different average values between the N treatments, taking high N conditions as a reference, hue was significantly higher (5.6%) under low N conditions, while it was significantly lower for contents in fructose (-11.5%), glucose (-15.8%), total sugars (-13.5%), and total sweetness index (-12.9%).

Considerable variation was found among the 44 varieties under both conditions for the traits evaluated. Representative fruits of the ‘de penjar’ collection studied are pictured in Figure 1. In this way, the traits with a larger value for the relative range of variation (maximum/minimum values) for LN conditions were fruit weight (4.57-fold), lycopene content (4.36-fold) and glutamic acid content (4.05-fold) for LN. Under HN, fruit weight (4.59-fold) and lycopene content (4.34-fold) were also the traits with highest relative range of variation, followed by total carotenoid content (3.83-fold). On the other side, the traits with lowest values for the relative range of variation were total C content (1.04-fold for LN and 1.07-fold for HN), pH (1.21-fold for LN and 1.18-fold for HN), and lightness of colour (1.35-fold for LN and 1.29-fold for HN). Noteworthy, for all traits, the ranges of variation between both N conditions overlapped to a large extent (Table 3).

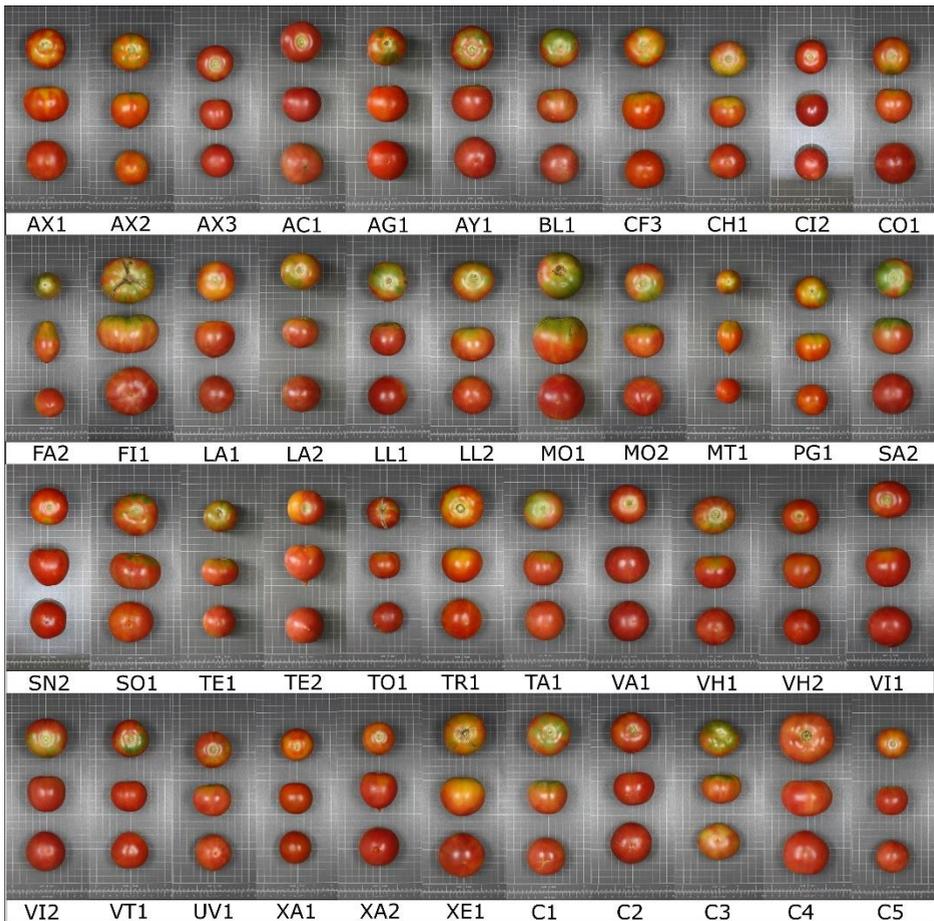


Figure 1. Representative fruits of the 39 ‘de penjar’ tomato local varieties and the five commercial varieties (accession name for each code can be found in Table S1) used for agronomic and composition characterization under two levels of nitrogen inputs. The grid cells in the pictures measure 1 x 1 cm.

Table 3. Mean \pm standard deviation (SD) and range, based on the accession averages, for the traits measured in the ‘de penjar’ tomato collection used in the present study in low (LN) and high nitrogen (HN) treatment ($n = 44 \times 2 = 88$); and mean of the paired difference HN – LN, based on the accession averages ($n = 44$). The full name of each trait abbreviation in the first column can be found in Table 1. Significant differences between LN and HN treatments are based on the results of the ANOVA ($n = 264$) and indicated with * ($p < 0.05$) and ** ($p < 0.01$).

Traits	Low nitrogen ($n = 44$)		High nitrogen ($n = 44$)		Paired HN – LN ($n = 44$)
	Mean \pm SD	Range	Mean \pm SD	Range	Mean
<i>Vegetative and plant traits</i>					
spad	49.64 \pm 3.54	40.67-56.25	48.98 \pm 5.41	36.38-60.90	-0.67
y (kg plant ⁻¹)	2.20 \pm 0.47	0.96-3.18	1.98 \pm 0.46	1.23-3.90	-0.22
<i>Fruit traits</i>					
frw (g)	72.7 \pm 19.9	29.0-132.5	70.4 \pm 20.3	29.3-134.6	-2.3
L	36.57 \pm 2.95	32.01-43.29	35.50 \pm 2.09	31.23-40.42	-1.07
chroma	21.50 \pm 3.45	14.69-32.28	21.58 \pm 3.28	15.30-33.23	0.08
Hue (°)	40.00 \pm 5.91	30.53-51.32	37.78 \pm 4.19	29.27-46.11	-2.22*
Nf (g kg ⁻¹ dw)	21.1 \pm 1.9	17.2-25.4	19.9 \pm 2.2	14.6-26.6	-1.2
Cf (g kg ⁻¹ dw)	408.0 \pm 3.1	402.3-416.3	405.8 \pm 4.6	390.5-416.7	-2.26
pH	4.13 \pm 0.17	3.83-4.63	4.11 \pm 0.18	3.83-4.50	-0.02
ssc (%)	5.52 \pm 0.57	4.28-6.58	5.69 \pm 0.70	4.02-6.87	0.16
ta (%)	0.57 \pm 0.13	0.34-0.96	0.58 \pm 0.12	0.37-0.85	0.01
cit (g kg ⁻¹ fw)	6.73 \pm 1.77	4.22-12.28	6.30 \pm 1.62	4.31-12.66	-0.43
mal (g kg ⁻¹ fw)	1.30 \pm 0.36	0.73-2.00	1.33 \pm 0.33	0.83-2.36	0.03
tacid (g kg ⁻¹ fw)	8.03 \pm 1.78	5.22-13.60	7.62 \pm 1.60	5.55-13.98	-0.41
citmalr	5.71 \pm 2.25	2.69-10.02	5.14 \pm 1.99	2.75-9.44	-0.57
fru (g kg ⁻¹ fw)	18.83 \pm 2.41	13.69-22.77	21.28 \pm 2.46	16.31-27.50	2.45**
glu (g kg ⁻¹ fw)	15.47 \pm 3.02	9.57-21.52	18.37 \pm 3.06	10.99-24.93	2.90*
tsug (g kg ⁻¹ fw)	34.30 \pm 5.17	24.27-42.87	39.65 \pm 5.29	28.96-52.07	5.35**
fruglur	1.26 \pm 0.18	1.01-1.88	1.19 \pm 0.15	1.02-1.93	-0.06
tsi	4.00 \pm 0.56	2.86-4.94	4.59 \pm 0.58	3.43-5.97	0.59**
ssctar	10.54 \pm 2.65	5.74-19.27	10.63 \pm 2.38	6.84-16.41	0.09
tsugtacidr	4.64 \pm 1.21	2.29-7.79	5.57 \pm 1.15	3.11-8.70	0.92
tsitar	7.68 \pm 2.14	4.14-14.40	8.63 \pm 2.10	5.21-13.65	0.95
vitc (g kg ⁻¹ fw)	0.27 \pm 0.04	0.15-0.39	0.29 \pm 0.04	0.21-0.38	0.02
lyc (mg kg ⁻¹ fw)	7.99 \pm 2.22	3.64-15.87	7.64 \pm 2.84	3.34-14.50	-0.34
bcar (mg kg ⁻¹ fw)	3.45 \pm 0.84	2.00-5.72	3.17 \pm 0.80	1.96-5.15	-0.28
tecar (mg kg ⁻¹ fw)	15.41 \pm 4.15	7.51-29.71	14.58 \pm 5.04	6.79-26.01	-0.83
gluta (g kg ⁻¹ fw)	3.42 \pm 1.08	1.73-7.01	3.83 \pm 0.78	2.34-5.61	0.41
aspa (g kg ⁻¹ fw)	0.76 \pm 0.21	0.33-1.33	0.90 \pm 0.19	0.51-1.40	0.14

3.2. Differences between varietal types

The groups of local varieties ($n = 39$) on one side and commercial varieties ($n = 5$) on the other, did not display significant differences ($p < 0.05$) between them under both LN and HN conditions for most of the traits analysed (Table 4). However, significant

differences were found for ratios of both soluble solids content and total sweetness index with titratable acidity, and vitamin C content. For each of these traits, local varieties presented higher means than commercial varieties in both conditions. Thus, average ratio between soluble solids content and titratable acidity for local varieties was 18.4% higher under LN and 30.0% under HN. The same occurred for average ratio between total sweetness index and titratable acidity, with values being 22.0% (LN) and 28.8% (HN) higher in local varieties over commercial ones, as well as for vitamin C content, with the former displaying higher mean values by 25.0% (LN) and 17.2% (HN) (Table 4).

Table 4. Mean \pm standard error (SE) and *t* value of the traits that showed a significant difference between local (L; *n* = 39) and commercial (C; *n* = 5) varieties used in the present study, for both nitrogen treatments. The full name of each trait abbreviation in the first column can be found in Table 1. Significant differences between varietal type are indicated with * (*p* < 0.05), ** (*p* < 0.01), *** (*p* < 0.001).

Traits	Low nitrogen			High nitrogen		
	Mean \pm SE		<i>t</i> value	Mean \pm SE		<i>t</i> value
	L (<i>n</i> = 39)	C (<i>n</i> = 5)		L (<i>n</i> = 39)	C (<i>n</i> = 5)	
ssctar	10.76 \pm 0.44	8.78 \pm 0.25	3.96***	11.00 \pm 0.36	7.70 \pm 0.26	7.37***
tsitar	7.87 \pm 0.35	6.14 \pm 0.48	2.92*	8.92 \pm 0.33	6.35 \pm 0.24	6.36***
vitc (g kg ⁻¹ fw)	0.28 \pm 0.01	0.21 \pm 0.02	2.78*	0.29 \pm 0.01	0.24 \pm 0.02	3.06*

3.3. Genetic parameters

Heritability in a broad sense (H^2), genetic and phenotypic coefficients of variation (CV_G and CV_P) for the traits studied are presented in Table 5. Considering both treatments and their interaction, fruit weight had the greatest H^2 with 0.76. Apart from that, only malic acid and citric:malic ratio showed a broad-sense heritability higher than 0.50. On the other hand, lycopene, β -carotene and total carotenoid content had the lowest H^2 . As expected, the coefficient of phenotypic variance was higher than the coefficient of genetic variance for all traits studied. The lowest percentages of CV_G were obtained for lycopene and total carotenoids; which, at the same time, had the highest values of CV_P , together with citric:malic acid ratio. The greatest values of CV_G were found for citric:malic acid ratio, fruit weight and malic acid content. Finally, carbon content in fruit, pH and lightness of colour had the lowest values of CV_P .

Table 5. Broad sense heritability estimates (H^2), genotypic and phenotypic variance coefficient (CV_G and CV_P , respectively) and their standard errors of the traits analysed in our ‘de penjar’ tomato collection having into account the two nitrogen conditions. The full name of each trait abbreviation in the first column can be found in Table 1.

Traits	$H^2 \pm SE$	$CV_G (\%) \pm SE$	$CV_P (\%) \pm SE$
<i>Vegetative and plant traits</i>			
spad	0.31 ± 0.08	6.82 ± 0.73	12.23 ± 1.32
y	0.33 ± 0.07	17.70 ± 1.95	30.77 ± 3.58
<i>Fruit traits</i>			
frw	0.76 ± 0.04	26.90 ± 3.07	30.78 ± 3.58
L	0.26 ± 0.08	4.68 ± 0.50	9.23 ± 0.99
chroma	0.42 ± 0.07	12.74 ± 1.38	19.60 ± 2.17
hue	0.37 ± 0.07	10.36 ± 1.12	17.12 ± 1.88
Nf	0.30 ± 0.07	7.51 ± 0.80	14.14 ± 1.54
Cf	0.40 ± 0.15	0.58 ± 0.06	1.29 ± 0.14
pH	0.12 ± 0.07	2.22 ± 0.24	6.28 ± 0.67
ssc	0.39 ± 0.07	9.36 ± 1.01	14.94 ± 1.63
ta	0.41 ± 0.07	18.21 ± 2.00	28.27 ± 3.25
cit	0.38 ± 0.08	20.44 ± 2.27	32.99 ± 3.88
mal	0.58 ± 0.07	23.19 ± 2.60	30.52 ± 3.54
tacid	0.32 ± 0.08	15.96 ± 1.74	28.08 ± 3.22
citmalr	0.62 ± 0.06	35.53 ± 4.24	45.05 ± 5.69
fru	0.28 ± 0.07	8.60 ± 0.92	16.13 ± 1.76
glu	0.31 ± 0.08	13.09 ± 1.42	23.39 ± 2.63
tsug	0.31 ± 0.08	10.36 ± 1.12	18.64 ± 2.05
fruglur	0.17 ± 0.09	7.24 ± 0.78	17.46 ± 1.92
tsi	0.28 ± 0.07	9.69 ± 1.04	17.59 ± 1.93
ssctar	0.39 ± 0.07	19.43 ± 2.15	31.20 ± 3.64
tsugtacidr	0.33 ± 0.07	18.24 ± 2.01	31.54 ± 3.68
tsitar	0.20 ± 0.04	21.29 ± 2.37	33.85 ± 4.00
vitc	0.20 ± 0.06	9.48 ± 1.02	21.10 ± 2.35
lyc	0.00 ± 0.07	0.00 ± 0.00	46.20 ± 5.88
bcar	0.07 ± 0.08	9.05 ± 0.97	33.22 ± 3.91
tcar	0.00 ± 0.08	0.00 ± 0.00	43.08 ± 5.38
gluta	0.14 ± 0.07	14.34 ± 1.56	37.97 ± 4.59
aspa	0.21 ± 0.07	16.26 ± 1.78	35.31 ± 4.21

3.4. Correlation among traits

Few significant ($p < 0.05$) phenotypic correlations were detected (Figure 2). In addition, the analysis did not reveal any significant correlation between SPAD, yield or fruit weight on one side, and fruit composition traits on the other. As for phenotypic correlations, fructose and glucose contents were positively and significantly correlated to each other ($r = 0.84$) and to total sugar content ($r = 0.95$ and 0.97 , respectively), total sweetness index ($r = 0.98$ and 0.94 , respectively), and, with lower correlation coefficient

values, to soluble solids content ($r = 0.73$ and 0.82 , respectively). The same pattern was observed for correlations between citric acid content and citric:malic ratio ($r = 0.77$), total acid content ($r = 0.98$) or titratable acidity ($r = 0.80$). Citric acid content was also negatively correlated to total sugars:total acids ratio ($r = -0.75$). On the contrary, malic acid content was not significantly correlated to any of these traits. Different carotenoid contents were positively correlated to each other, with $r = 0.76$. Lycopene displayed higher correlation coefficient values than β -carotene to total carotenoids ($r = 0.99$ and 0.84 , respectively) (Figure 2).

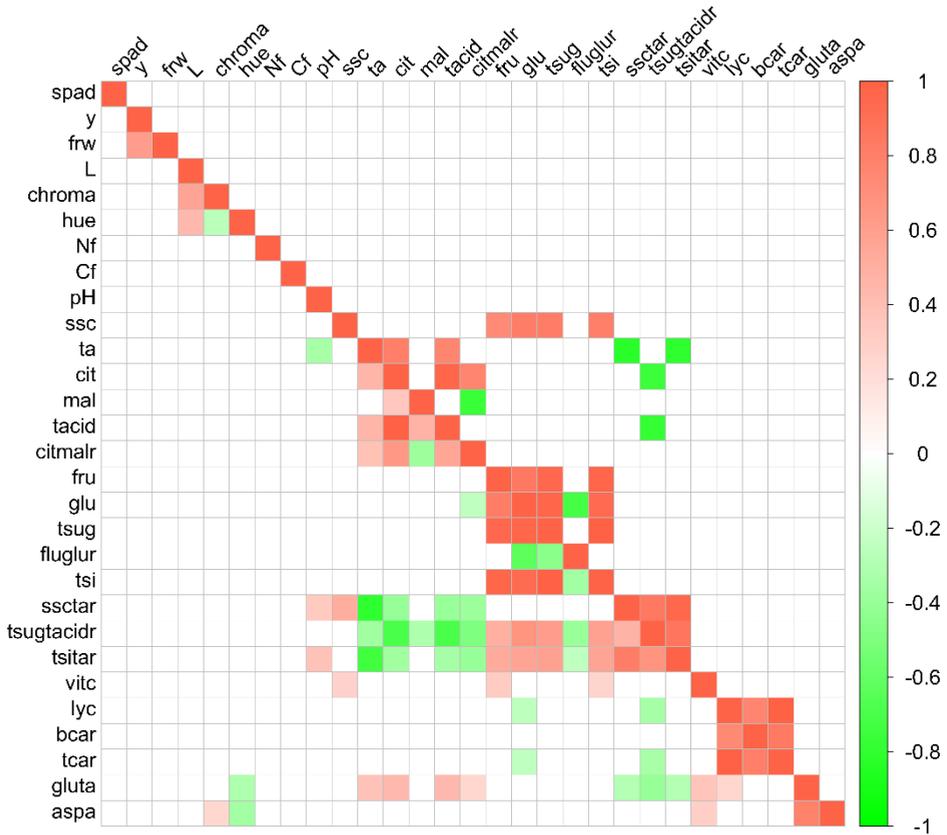


Figure 2. Correlogram among traits evaluated in the ‘de penjar’ tomato collection. Phenotypic correlations are shown above the diagonal, and environmental correlations below the diagonal. Only significant correlations at $p < 0.05$ according to the Bonferroni test are displayed. Green and red colours correspond to negative and positive correlations, respectively. The full name of each trait abbreviation can be found in Table 1.

In the case of environmental correlations, the number of significant ones was higher than for phenotypic correlations (Figure 2). The same strong correlations found among traits in the phenotypic analysis were detected. Fruit weight and yield were environmentally correlated with $r = 0.61$. In the same way, a significant but slightly

lower correlation was detected between vitamin C and traits related to sugar content, with r values between 0.26 and 0.32. Vitamin C also had a significant and positive environmental correlation to aspartic and glutamic acid contents ($r = 0.29$ and 0.37 , respectively). With respect to these amino acids, they were environmentally intercorrelated, with $r = 0.79$. Aspartic acid also presented a negative significant correlation to colour parameters chroma ($r = 0.26$) and hue ($r = -0.35$), and positive, to vitamin C content ($r = 0.29$). Glutamic acid displayed significant positive environmental correlations to acidity and related traits, with r between 0.25 and 0.44, to vitamin C ($r = 0.37$) and lycopene contents ($r = 0.24$); but negative significant correlations to sugars:acids ratio related traits ($r = -0.28$ to -0.40). Glucose content and total sugars:total acids ratio also showed negative environmental correlations to lycopene ($r = -0.25$ and -0.35 , respectively) and total carotenoid content ($r = -0.25$ and -0.34 , respectively) (Figure 2).

3.5. Principal Components Analysis

The first two principal components (PCs) of the PCA explained 40.5% of the total variation observed, with PC1 and PC2 accounting for 25.7% and 14.8% of the total variation, respectively. Total sugars:total acids ratio, total sweetness index:titratable acidity ratio and glucose content were the traits displaying the highest positive correlation with PC1, while fructose:glucose ratio, citric acid and total acid contents were the ones with highest absolute negative values with PC1. On the other hand, titratable acidity, citric acid and total acid contents also displayed high positive correlations with PC2, whereas pH, soluble solids content:titratable acidity ratio and yield were highly negatively correlated with PC2 (Figure 3).

The principal component analysis did not clearly separate the HN and LN treatments, although for the same variety, compared to the control, the projection corresponding to the LN treatment tended to display lower values for PC1 and PC2, which is associated to higher content of carotenoids, fructose:glucose ratio, hue and slightly higher fruit carbon and nitrogen contents and yield; and to lower contents of sugars and slightly lower vitamin C (Figure 3).

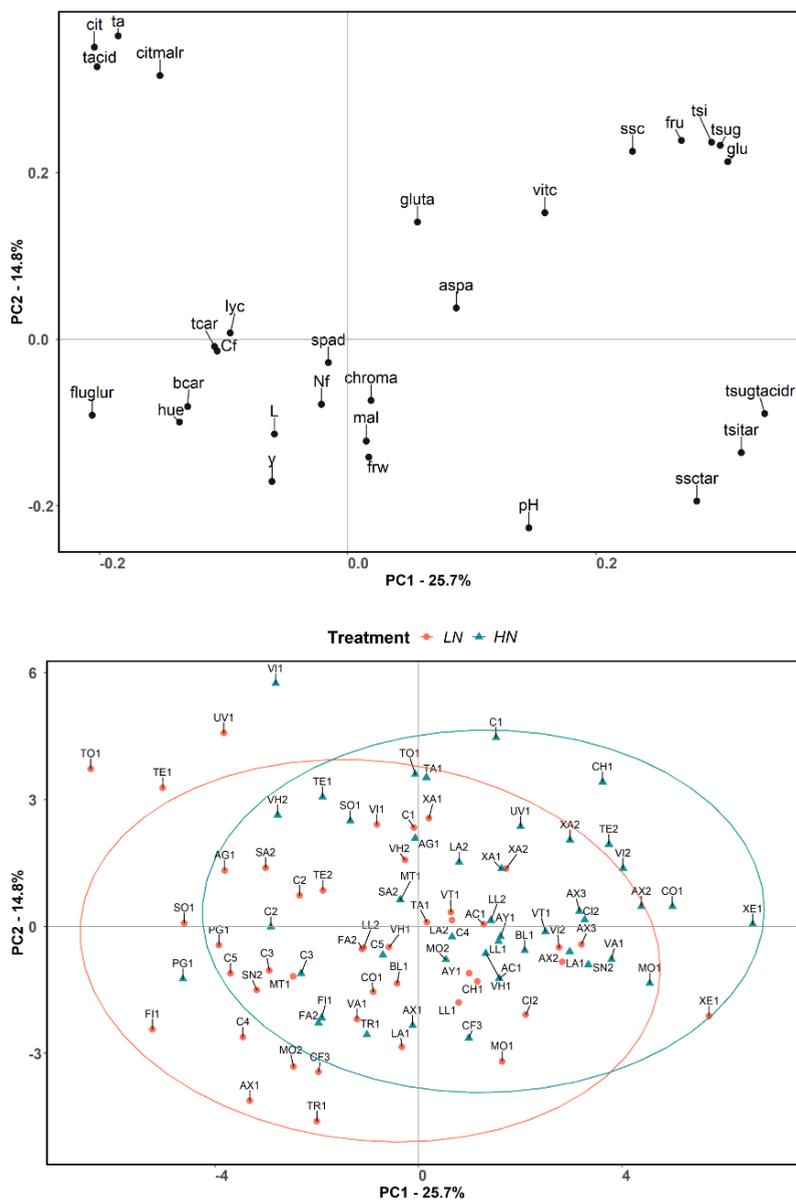


Figure 3. PCA loading plot (above) and score plot (below) evaluated in the present study, based on the two first principal components of PCA. First and second components account for 25.7% and 14.8% of the total variation, respectively. The accessions are represented by different symbols and colour according to the treatment in which they were grown: circle and orange for low nitrogen, and triangle and blue for high nitrogen. Ellipses grouped the accessions for each treatment with a 95% confidence level. The full name of each trait abbreviation can be found in Table 1. Accession name for each code can be found in Table S1.

It is noteworthy that a group of accessions under HN conditions (CH1, UV1, XA2, TE2, AX2, CO1, VI2, C1) plotted in the second quadrant of the PCA (positive values for PC1 and PC2), being associated to higher total sweetness index, total and individual contents of sugars, soluble solids and their ratios with total acids and titratable acidity, vitamin C, glutamic and aspartic acid contents. Another group to highlight is that of the accessions AX1, FI1, TR1, C4, MO2 and CF3 under LN conditions, which plotted in the third quadrant (negative values of PC1 and PC2), which are associated to brighter fruits with external colour tending to orange, higher fructose:glucose ratio and β -carotene content, in addition to high yield. Besides that, there are some accessions that appear outside the 95% confidence ellipses. In this respect, UV1, TE1 and TO1 under LN conditions and VI1 under HN produced the most acidic tomatoes. XE1 under LN conditions also remained outside the ellipse, presenting high sugar contents, resulting in greater ratios of total sugars:total acids, soluble solids content:titratable acidity and total sweetness index:titratable acidity. Finally, some accessions (AX3, AC1, TR1, XA1, XA2, XE1 and C3) seemed to perform similarly under LN and HN conditions as they appeared very close to each other according to PC1 and PC2 (Figure 3).

3.6. Characteristics of highest yielding varieties

Table 6 shows the ranking of the 10 best ‘de penjar’ tomato accessions evaluated in the present work for yield, with their performance and ranking position within the entire collection for the most important composition traits, under both treatments separately. The ranking of the top 10 ‘de penjar’ tomato accessions of the collection for resilience in yield, with their resilience index and ranking position within the entire collection for the most important composition traits, is displayed in Table 7.

Several local varieties showed comparable or even better performance in yield and other composition traits than commercial varieties, both in LN and HN. As far as commercial varieties are concerned, in LN conditions, C2, C1 and C3 ranked fifth (2.89 kg plant⁻¹), sixth (2.65 kg plant⁻¹) and ninth (2.59 kg plant⁻¹) in yield, while only C3 and C4 were among the 10 best yielding varieties in HN with 2.50 kg plant⁻¹ (5th place) and 2.24 kg plant⁻¹ (8th place) (Table 6). It is worth noting that, in addition, C2 and C1 ranked second (1.55) and fourth (1.44), respectively, in terms of yield resilience (Table 7). Regarding local varieties, TR1, MT1 and FI1 stood out as the three best yielding varieties in LN (3.18, 3.13 and 3.06 kg plant⁻¹, respectively), while FI1, MO1 and AX1, were the highest yielding in HN (3.90, 2.89 and 2.77 kg plant⁻¹, respectively) (Table 6). None of these accessions were among the top 10 in resilience; although MO2 and CO1, which appeared among the 10 best accessions for yield in LN, ranked third (1.45) and tenth (1.29), respectively, for yield resilience (Table 7). When considering the rest of traits, there is a wide variation among the two sets of 10 highest yielding accessions. Among them, FI1 ranked first in both yield and fruit weight (134.6 g) in HN. Contrarily, MT1, with the lowest fruit mean weight in the whole collection (29.0 g in LN and 29.3 g in HN), appeared as the second and sixth best yielding variety in LN (3.13 kg plant⁻¹) and

HN (2.48 kg plant⁻¹), respectively (Table 6). Taking into account average content of N in fruit, the local variety AX1 ranked third under both LN (23.9 g kg⁻¹ dw) and HN (22.5 g kg⁻¹ dw) (Table 6). Regarding sweetness, it is worth mentioning the local variety XE1, which presented an outstanding average in total sugar content (52.07 g kg⁻¹ fw), ranking first, under HN. Furthermore it also showed the highest average total sugars:total acids ratio under the same conditions (8.70). On the other hand, MO2, FI1 and C3 were among the top 10 varieties in fructose:glucose ratio under LN with mean values of 1.61, 1.50 and 1.38, respectively; while AC1 and again FI1 and C3 had the highest mean values in HN (1.50, 1.33 and 1.31, respectively) (Table 6). In general, the 10 best yielding varieties, under LN and HN, ranked in intermediate or low positions for both average total acid content and citric:malic ratio (Table 6). Regarding main antioxidants evaluated, MO1 was the accession with the greatest average content of vitamin C (0.38 g kg⁻¹ fw) under HN, followed by XE1 (5th place; 0.33 g kg⁻¹ fw). MT1 stood out for accumulating, in average, the highest concentrations of β -carotene (5.72 mg kg⁻¹ fw) and the second highest of total carotenoids (25.39 mg kg⁻¹ fw) in their fruits, under LN; while under HN, FI1 ranked first in β -carotene content (5.15 mg kg⁻¹ fw) (Table 6). As for glutamic and aspartic acid contents, any of the local varieties in both sets of best yielding varieties ranked above commercial varieties C1 under LN and C4 under HN. However, MT1 was placed within the top 10 positions for glutamic under HN (6th; 4.71 g kg⁻¹ fw). Regarding aspartic, the same happened for AX1 (10th; 0.89 g kg⁻¹ fw) and AC1 (6th; 1.10 g kg⁻¹ fw) under LN and HN, respectively (Table 6).

When considering average resilience indexes of the other traits for the top 10 accessions in yield resilience, it is worth mentioning some accessions of both local and commercial varieties. As for local varieties, MO2 ranked third for fructose:glucose resilience (1.29); FA2 stood out for having the highest resilience in total sugar content (1.20) and ranked third for total sugars:total acids resilience (1.11); XA1, which ranked first for yield resilience (1.79), had a remarkable resilience in glutamic and aspartic acid (1.25 and 1.13, respectively), ranking seventh for both of them (Table 7). With respect to commercial varieties, C2 stood out for its resilience in vitamin C (1.12) and total carotenoid content (1.82), ranking fourth and seventh, respectively; while C1 highlighted for its resilience in β -carotene content (1.53), ranking seventh in the collection (Table 7).

Table 6. Ranking of the 10 best ‘de penjar’ tomato accessions evaluated in the present work for yield, with their performance and ranking position (in parentheses) within the entire collection for the most important composition traits, in both treatments separately. The full name of each trait abbreviation in the first row can be found in Table 1.

	y (kg plant ⁻¹)	frw (g)	Nf (g kg ⁻¹ dw)	tsug (g kg ⁻¹ fw)	fruglur	tacid (g kg ⁻¹ fw)	citmalr	tsugtacidr	vitc (g kg ⁻¹ fw)	tcar (mg kg ⁻¹ fw)	bcar (mg kg ⁻¹ fw)	gluta (g kg ⁻¹ fw)	aspa (g kg ⁻¹ fw)
<i>Low nitrogen</i>													
TR1	3.18 (1)	84.5 (10)	20.4 (31)	29.32 (36)	1.24 (22)	6.09 (40)	3.58 (37)	5.05 (17)	0.24 (38)	16.88 (14)	4.59 (4)	2.08 (41)	0.56 (36)
MT1	3.13 (2)	29.0 (44)	19.8 (33)	37.09 (15)	1.25 (20)	7.38 (30)	3.30 (40)	5.14 (14)	0.25 (29)	25.39 (2)	5.72 (1)	2.95 (28)	0.58 (34)
FII	3.06 (3)	132.5 (1)	20.7 (28)	25.66 (41)	1.50 (4)	10.32 (4)	4.24 (27)	2.49 (43)	0.29 (16)	14.36 (28)	3.33 (27)	2.54 (36)	0.33 (44)
MO2	2.90 (4)	86.5 (7)	18.2 (42)	31.35 (33)	1.61 (3)	7.41 (28)	3.17 (41)	4.31 (28)	0.27 (18)	17.96 (10)	3.82 (14)	3.24 (22)	0.71 (26)
C2	2.89 (5)	83.7 (12)	19.4 (35)	34.22 (24)	1.19 (26)	8.85 (11)	7.36 (13)	3.97 (32)	0.24 (32)	14.37 (27)	3.41 (24)	2.33 (38)	0.64 (30)
C1	2.65 (6)	85.1 (8)	20.4 (30)	42.68 (3)	1.14 (32)	8.49 (15)	7.53 (12)	5.07 (15)	0.24 (36)	15.52 (18)	3.56 (19)	3.98 (12)	0.89 (9)
MO1	2.62 (7)	117.8 (2)	22.9 (8)	35.47 (17)	1.01 (44)	5.73 (42)	6.07 (20)	6.20 (6)	0.25 (30)	12.58 (34)	3.44 (23)	3.53 (15)	0.75 (23)
CO1	2.62 (8)	76.7 (20)	21.1 (23)	33.79 (25)	1.10 (38)	6.87 (34)	6.14 (19)	5.03 (18)	0.24 (39)	12.64 (33)	2.62 (36)	2.87 (30)	0.85 (15)
C3	2.59 (9)	64.8 (29)	18.2 (41)	32.49 (30)	1.38 (7)	10.26 (5)	4.67 (22)	3.32 (37)	0.26 (28)	14.08 (32)	4.08 (8)	2.32 (39)	0.50 (40)
AX1	2.58 (10)	83.2 (13)	23.9 (3)	24.27 (44)	1.31 (12)	6.47 (36)	4.14 (32)	4.06 (31)	0.23 (40)	17.42 (12)	3.90 (13)	3.50 (16)	0.89 (10)
<i>High nitrogen</i>													
FII	3.90 (1)	134.6 (1)	18.6 (33)	33.91 (37)	1.33 (5)	8.41 (12)	4.66 (20)	4.41 (37)	0.29 (24)	19.54 (8)	5.15 (1)	3.81 (19)	0.78 (33)
MO1	2.89 (2)	126.9 (2)	20.0 (21)	42.80 (12)	1.13 (29)	7.37 (20)	4.11 (25)	6.25 (12)	0.38 (1)	15.94 (17)	3.59 (16)	3.73 (25)	0.81 (30)
AX1	2.77 (3)	102.2 (3)	22.5 (3)	33.23 (38)	1.21 (18)	5.95 (40)	3.84 (27)	5.73 (21)	0.29 (22)	12.77 (28)	2.86 (28)	3.80 (20)	1.00 (12)
TR1	2.69 (4)	79.0 (12)	19.8 (23)	32.02 (40)	1.23 (13)	5.55 (44)	3.48 (34)	5.84 (18)	0.27 (32)	15.72 (18)	3.69 (14)	2.40 (43)	0.60 (41)
C3	2.50 (5)	59.1 (34)	14.6 (44)	31.99 (41)	1.31 (6)	7.94 (14)	4.18 (23)	4.29 (39)	0.21 (43)	11.29 (31)	2.97 (24)	2.43 (42)	0.58 (42)
MT1	2.48 (6)	29.3 (44)	16.6 (42)	38.86 (25)	1.18 (19)	7.03 (26)	4.34 (22)	5.57 (22)	0.21 (44)	12.48 (29)	3.24 (18)	4.71 (6)	0.93 (20)
XE1	2.35 (7)	97.7 (5)	21.2 (11)	52.07 (1)	1.08 (35)	5.93 (41)	3.66 (31)	8.70 (1)	0.33 (5)	17.56 (12)	3.88 (11)	3.74 (24)	0.84 (24)
C4	2.24 (8)	100.0 (4)	26.6 (1)	37.97 (27)	1.14 (26)	6.69 (31)	3.99 (26)	5.90 (17)	0.25 (38)	11.76 (30)	2.87 (27)	5.16 (3)	1.30 (2)
CF3	2.21 (9)	84.8 (8)	19.7 (24)	36.49 (35)	1.21 (16)	5.86 (42)	3.34 (37)	6.30 (11)	0.27 (34)	19.60 (7)	4.05 (6)	3.43 (29)	0.75 (36)
AC1	2.19 (10)	80.2 (11)	19.3 (29)	36.92 (32)	1.50 (2)	6.09 (39)	5.08 (19)	6.08 (14)	0.27 (33)	17.14 (13)	3.10 (21)	3.93 (18)	1.10 (6)

Table 7. Ranking of the 10 best ‘de penjar’ tomato accessions evaluated in the present work for resilience in yield, calculated as the ratio between mean value under low N treatment and mean value under high N treatment. Resilience and ranking position (in parentheses) within the entire collection of these 10 varieties for the most important composition traits, are also shown. The full name of each trait abbreviation in the first row can be found in Table 1.

	y	frw	Nf	tsug	fruglur	tacid	citmalr	tsugtacidr	vite	tcar	bcar	gluta	aspa
<i>Resilience index</i>													
XA1	1.79 (1)	1.02 (24)	1.17 (7)	1.00 (8)	1.01 (31)	1.34 (3)	1.54 (2)	0.72 (31)	0.91 (26)	0.71 (37)	0.75 (37)	1.25 (7)	1.13 (7)
C2	1.55 (2)	1.19 (5)	0.93 (42)	1.12 (2)	1.02 (28)	1.13 (16)	1.02 (30)	0.95 (15)	1.12 (4)	1.82 (7)	1.40 (10)	0.89 (22)	0.75 (31)
MO2	1.45 (3)	1.23 (3)	0.96 (37)	0.83 (25)	1.29 (3)	1.05 (25)	1.02 (31)	0.80 (26)	0.97 (20)	1.16 (22)	0.96 (29)	0.78 (31)	0.86 (21)
C1	1.44 (4)	1.27 (2)	1.16 (12)	0.94 (12)	1.09 (16)	0.94 (34)	1.09 (23)	0.98 (12)	0.81 (35)	1.82 (8)	1.53 (7)	0.92 (20)	0.90 (19)
PG1	1.36 (5)	0.88 (41)	0.95 (40)	1.09 (3)	1.01 (30)	0.98 (33)	0.98 (33)	1.06 (5)	1.07 (10)	0.77 (35)	0.95 (31)	0.81 (29)	0.81 (27)
SO1	1.32 (6)	1.17 (6)	0.94 (41)	0.72 (41)	1.14 (9)	0.99 (31)	1.12 (17)	0.76 (30)	0.92 (25)	1.70 (9)	1.13 (23)	0.88 (24)	0.95 (13)
VI2	1.32 (7)	1.10 (13)	1.06 (23)	0.92 (15)	1.09 (17)	1.00 (30)	1.29 (7)	0.86 (22)	0.82 (34)	1.07 (25)	1.16 (22)	0.50 (43)	0.44 (43)
FA2	1.29 (8)	1.03 (23)	1.00 (30)	1.20 (1)	0.65 (44)	1.17 (15)	1.03 (28)	1.11 (3)	1.12 (5)	1.05 (26)	0.94 (33)	0.59 (40)	0.65 (38)
VT1	1.29 (9)	1.11 (11)	1.12 (16)	1.03 (5)	0.95 (38)	1.25 (8)	0.79 (41)	0.81 (23)	0.87 (32)	1.19 (20)	1.33 (17)	1.06 (11)	0.81 (25)
CO1	1.29 (10)	1.19 (4)	1.04 (24)	0.74 (39)	0.95 (39)	1.06 (22)	1.07 (27)	0.71 (34)	0.77 (38)	1.56 (11)	1.20 (20)	0.85 (26)	0.80 (28)

4. Discussion

The present work constitutes the first study of 'de penjar' tomato involving different doses of N fertilization. A diverse array of 39 local varieties together with five commercial varieties were characterized in open field under two nitrogen fertilization levels, one corresponding to similar values of their traditional cultivation (162 kg ha^{-1} ; high N) (Seda and Muñoz, 2011) and the other to less than one third of the first dosage (49 kg ha^{-1} ; low N). The large variability existing among 'de penjar' collection for morphological, agronomic and quality traits observed in this study paves the way for selection and breeding of this overlooked type of tomato. In terms of yield, average production in our collection (around 2 kg plant^{-1}) stands on intermediate values reported for other LSL varieties from the eastern Spain ('de penjar') and Balearic Islands ('de ramellet') (Casals et al., 2012; Cebolla-Cornejo et al., 2013; Figàs et al., 2015, 2018b; Fullana-Pericàs et al., 2019). Our data about fruit mean weight are in agreement with Conesa et al. (2020). Regarding fruit quality, soluble solids content in the collection studied ranged around 4-7%, with average of 5.5%, similar to values found in Fullana-Pericàs et al. (2019), but slightly lower to the values found in Figàs et al. (2015, 2018b). Similarly, when looking at individual concentrations of reducing sugars, mean values of fructose and glucose under both low and high N treatment fitted within the range of values obtained in Casals et al. (2015), which evaluated the sugar and acid profile of 25 accessions of 'de penjar' tomato. However, the ranges obtained by Casals et al. (2015) were wider, and showed higher maximum average values by 1.26 and 1.19-fold, respectively, than the collection of the present study. Average titratable acidity in the present collection was also similar to those found for other 'de penjar' and 'de ramellet' tomato varieties (Cebolla-Cornejo et al., 2013; Figàs et al., 2015; Figàs et al., 2018b). However, none of the varieties showed means of titratable acidity higher than 1.0%, while this commonly happens in 'de ramellet' tomato (Fullana-Pericàs et al., 2019). This might be due to genetic differences or to differences in the ripening stage at the time of harvesting. In any case, our work supports previous information indicating that LSL varieties from Spain generally are slightly more acidic than the varieties from Italy, while the latter accumulate more soluble solids (Conesa et al., 2020). On the other side, as opposed to fructose and glucose contents, the 'de penjar' tomato collection of the present work showed mean values of individual citric and malic acid content slightly higher than in the collection evaluated in Casals et al. (2015), with larger ranges of variation among accessions. In addition, the maximum average values found in the present collection for contents in citric and malic acid were $12.66 \text{ g kg}^{-1} \text{ fw}$ and $2.36 \text{ g kg}^{-1} \text{ fw}$, respectively, while the maximum values in Casals et al. (2015) were 1.78 and 1.50-fold lower, respectively. Ranges for content in glutamic acid in the 'de penjar' collection of this study showed wider variation than in other works (Casals et al., 2015), although both glutamic and aspartic acids are compounds still barely studied in 'de penjar' tomatoes.

Compared with USDA standard nutritional references of cultivated tomato (Haytowitz et al., 2011), the ‘de penjar’ collection studied contained in average around 1.5-fold more glucose and fructose and 2-fold more vitamin C. However, lycopene and β -carotene contents were 3.3 and 1.4-fold, respectively, lower in ‘de penjar’ fruits, probably due to the pleiotropic effect of the *alc* mutation (Kumar et al., 2018). The same trend was observed for glutamic and aspartic acids, with mean contents of 1.2 and 1.6-fold lower in our collection compared to the USDA standard nutritional references (Haytowitz et al., 2011). This confirms that ‘de penjar’ LSL varieties are very different in composition terms to standard tomato varieties (Cebolla-Cornejo et al., 2013; Figàs et al., 2015).

4.1. Importance of soil conditions and cultivation practices

Some recent studies have addressed the impact of organic farming alone and together with low N inputs on agronomic and quality traits of tomato. De Pascale et al. (2016) suggested that organic cultivation practices might be a better approach than conventional methods for improving yield and nutritional quality of tomato under limiting N and water conditions, although it depends on cultivar and soil type. In this study, ‘de penjar’ tomato was cultivated following organic farming practices. In this respect, ‘de penjar’ tomato have been traditionally cultivated in open field under rain-fed, low-input conditions, which makes this tomato crop ideal for adaptation to organic farming.

The relationship between soil characteristics and fertilization is often overlooked and there are interactions between different elements of its composition that heavily affect the efficiency of nutrient absorption by the roots (Jones Jr, 2012). In this respect, the imbalanced N-P-K fertilization in the low N treatment was taken into account before setting the fertilization program by evaluating the possible impaired plant availability of other macronutrients. However, only synergistic or zero interactions have been identified in literature between nitrogen and phosphorus or potassium. Antagonistic effects are mostly found between divalent cations (Rietra et al., 2017). In addition, the soil texture influences the development of the roots and their degree of absorption (De Pascale et al., 2016). A clay-loam soil, as in the present work, would enhance root efficiency in exploring the soil for nutrients as it represents a well-balanced soil with intermediate compaction degree, which avoids rapid loss of nutrients and water as well as allows proper aeration (Tracy et al., 2013). Our experiment was carried out in an officially recognised area for ‘de penjar’ tomato cultivation. In this work, soil analysis showed slight salinity, which is optimal for ‘de penjar’ type cultivation (Conesa et al., 2020), and high organic matter concentration, which is known to have a positive impact on nutrients availability and reducing soil compaction (Metzger and Yaron, 1987). No nutrient deficiencies were found in the soil of study. Contrarily, concentrations of K, Ca^{2+} and Mg^{2+} were excessive, which could result in salts formation causing antagonism between ions (e.g. Na^+ vs. K^+ , Cl^- vs. NO_3^-) and mineral imbalance with negative impact

on plant growth (Jones Jr, 2012). In addition, very high P concentrations were found in soil, which will eventually be washed out through the soil, contributing to pollution and eutrophication of waters. These data prove that there is scope for reducing, at a large extent, the supply of these nutrients in fertilization of ‘de penjar’ tomato.

4.2. Variation observed

Considerable phenotypic variation was observed in our collection for most of the traits evaluated, which is in agreement with the large genetic diversity described for ‘de penjar’ tomato in other works (Casals et al., 2012; Cebolla-Cornejo et al., 2013; Esposito et al., 2020). Genotyping data of our collection would have been relevant to confirm at the genetic level the high diversity we have found. However, since we mostly evaluated quantitative traits with polygenic control, a larger number of accessions would have been needed for a robust ‘Genome Wide Association Study’ (GWAS) (Korte and Farlow, 2013). The specific effect of the *alc* mutation in the traits investigated could be studied by means of crosses between parents carrying the *alc* mutation and its corresponding wild allele. The PCA confirmed the wide variation observed, visually represented with the different accessions studied scattered all over the score plot. This supports the definition of ‘de penjar’ tomato by Conesa et al. (2020) as a “population of landraces”, in which the *alc* mutation is introgressed into different genetic backgrounds (Casals et al., 2012), maintaining high heterogeneity within the ‘de penjar’ type.

The higher value of phenotypic than genotypic variation for every trait analysed shows an important environmental effect, especially for fruit bioactive and quality compounds. This is in agreement with previous studies that reported tomato quality traits being highly polygenic, strongly influenced by environmental conditions and showing low heritability (Causse et al., 2003). Fruit mean weight showed the highest broad-sense heritability estimate (H^2), followed by malic acid content and citric:malic acid ratio, which was in line with them showing the highest genotypic variation coefficient (CV_G). In this terms, similar results were reported in other works. Morphology traits in tomato, such as fruit weight and skin colour, are known to have higher values of heritability than fruit quality traits or yield (Figàs et al., 2018a). Panthee et al. (2012) also observed that acid traits showed higher heritability than sugars and soluble solids content. Contrarily, lycopene, total carotenoid and β -carotene contents had the lowest H^2 and the highest phenotypic variation coefficient (CV_P) in the collection of the present study. Both H^2 and CV_P estimates for lycopene and total carotenoid content showed values of 0. We attribute this phenomenon not to the absence of genetic variation in our collection, but to a strong interaction $G \times N$, meaning that different trends in the response to increasing or decreasing N inputs were observed among genotypes. Panthee et al. (2012) also found a similar interaction genotype \times environment effect resulting in low heritability for lycopene in tomato. Both heritability and a strong interaction $G \times N$ constitute relevant information for breeding and selection (Figàs et al., 2018b; Panthee et al., 2012). While high heritability estimates would make more efficient the selection of genotypes

expecting the same performance under different N supply conditions, having a strong interaction G×N would allow breeders to select varieties with the best response under certain conditions, in our case, low N.

4.3. Effects of low nitrogen inputs on traits evaluated

Yield is one the most valuable traits for growers, and it is directly correlated with N availability for plants (Zhang et al., 2015). In the present work, no significant differences were found between the two N treatments regarding average yields for any of the varieties evaluated. Our results suggested that, although N supplied in the high N treatment could not be considered excessive in a detrimental way to yield, the N availability in the low N treatment was suitable for an optimal plant growth and for obtaining similar yields. More plant growth parameters would have been needed for better support of our statement. Most of the studies testing tomato cultivars in soil with different rates of N fertilization found that yield increased linearly with N input but reached a plateau where it became insensitive to more N fertilization levels (Djidonou et al., 2013; Elia and Conversa, 2012). However, among those studies, the minimum N fertilization level maximizing tomato yield was at least 168 kg ha⁻¹ in open field (Djidonou et al., 2013), while for the 'de penjar' collection studied, we found that even with only 49 kg ha⁻¹ of N fertilization, no differences were obtained compared to the standard fertilization. In addition, data of SPAD were similar to those of the plant yield. More data about plant morphological changes would be appropriate to robustly evaluate the effect of the LN treatment on plants, like plant biomass, plant height, leaf size or root morphology (Freschet et al., 2018; Hermans et al., 2006). In particular, it would have been of interest to have plant biomass data to evaluate nitrogen use efficiency parameters. However, SPAD values have been widely used for evaluating plant N status in crop management, as leaf chlorophyll content is closely related to leaf N content, and a reduction in these values have been already reported for tomato under N fertilization stress (Dunn et al., 2018; Ling et al., 2011; Padilla et al., 2015). Thus, we decided to use this parameter to estimate plant N status for being a simple, non-destructive and relatively quick measure to take. Fruit mean weight was also not influenced by the N rate in the present study. In fact, Elia and Conversa (2012) and Hernández et al. (2020) suggested that the effects of N inputs on tomato yield were due to changes in fruit load more than fruit mean weight. External colour of fruit was evaluated through lightness, chroma and hue values, but only the collection average for hue was significantly affected by the N treatment, increasing when low N was applied. According to the HCL colour space, this would represent a slight change of colour towards orange under low N inputs, possibly due to a slightly higher content of β -carotene under these conditions.

Taste and flavour of 'de penjar' tomato is one of the attributes most appreciated by the local consumer, and it is associated with its traditional use (Conesa et al., 2020). Therefore, we considered important to evaluate the effects of lowering the dose of N fertilization on those characteristics. Our results showed that high N rate did not affect

significantly either the N or the C contents in fruit, probably due to a N redistribution between leaves and fruits in response to an exceeded N supply. In fact, Elia and Conversa (2012) found that increasing N inputs of tomato from 200 to 300 kg ha⁻¹ increased leaf N, while N storage in fruits decreased. This was explained by a plant tendency to grow vegetatively rather than reproductively when N availability increases over the demand.

Citric, malic, total acid contents and titratable acidity in fruits showed no significant differences between N treatments. Our results differed to the ones reported in recent studies for tomato, both under hydroponic and soil cultivation (De Pascale et al., 2016; Truffault et al., 2019), in which higher acidity was found related to an increasing N rate. In addition, our results suggest that citric acid is likely the highest contributor to the fruit acidity in 'de penjar' collection, as its content in fruits exceeds by 2.7 to 10.0-fold in average that of malic acid. This is in agreement with the results obtained in the collection evaluated by Casals et al. (2015), which showed nearly the same range of citric:malic ratio (2.4 to 9.3).

Fruit contents in fructose, glucose, total sugar and total sweetness index were the only composition traits evaluated in this study that showed a significant effect of the N treatment. All of them suffered an average reduction of 10-20% in response to low N inputs. Although the ANOVA did not show significant differences for these traits between N treatments for each accession separately, the existence of a significant effect of the N treatment indicates a tendency towards the reduction of sugar content under low N in the 'de penjar' tomato. Results about the N effect on carbohydrates and total soluble solids in fruits are the most controversial among other works in tomato crop. Our results are in agreement with some of them (De Pascale et al., 2016). Others reported the opposite effect (Hernández et al., 2020), while others even found no significant effect of N rate on sugar content (Truffault et al., 2019). According to Hermans et al. (2006), N deficiency in plants may cause an accumulation of starch and sugars in leaves, consequently decreasing their content in fruits, and regulating photosynthesis by negative feed-back. This could explain our results. Level of sweetness is also related to different proportion of main sugars, with fructose being the sweetest, followed by sucrose and finally glucose. Fructose and glucose are also known to be usually in the same proportion (1:1) in tomato pericarp (Beckles, 2012). However, our results showed mean values of fructose:glucose ratio over 1.0 for every accession and was not affected significantly by N inputs. Furthermore, a highly significant interaction G×N was observed, which gives scope to select accessions with higher fructose:glucose ratio under low N inputs for breeding purposes.

Since the end of 20th century, flavour was increasingly understood as a complex parameter not only due to main sugars and organic acids, but also to their ratio, texture and volatile compounds. Although studies including the different elements of flavour are emerging, it is still a difficult parameter to estimate objectively and not amenable

for exhaustive assays for being time-consuming and expensive. Therefore, the horticultural industry has been using indexes highly correlated with flavour and consumer acceptability for selecting and breeding (Beckles, 2012). In tomato, Baldwin et al. (1998) reported that total sweetness index or its ratio to titratable acidity were closer than soluble solids content or its ratio to titratable acidity to their acceptability based on sweetness we perceive ($r \geq 0.80$). In the present study, while total sweetness index significantly decreased with LN treatment, soluble solids content was not affected. Despite the high correlation between sugars and soluble solids content and that the latter is easier and faster to measure, soluble solids content includes other compounds that do not contribute to sweetness. Thus, it could be interesting to quantify individual sugars (Fernández-Ruiz et al., 2004). On the other hand, as acidity level influences the perception of sweetness, parameters related to sweetness-acidity balance are more likely to correlate with taste preferences than sugar and acid contents alone (Baldwin et al., 1998; Beckles, 2012). In this experiment, a significant decrease in average sugars associated with low N inputs was reflected as a slight reduction of their average ratios with organic acids and acidity related traits. Remarkably, this reduction was not significant; thus, low N inputs may not change a great deal the taste of 'de penjar' tomatoes. On the other hand, taking into consideration the traditional conservation and consumption of the 'de penjar' tomato, up to a minimum of 2 months after harvest, having lower sugar content under low N treatment could be detrimental to its taste during post-harvest life, since a decrease, first sharply and then more gradual, of sugar content in this type of tomato, has been reported at 2 to 4 months post-harvest, while organic acids decreased to a lesser degree in the same period (Casals et al., 2015). However, further studies are needed to draw an accurate conclusion, using experienced sensory panels and investigating the postharvest performance of the accessions evaluated, as diversity for these traits has been observed among 'de penjar' accessions (Casals et al., 2015).

One possible mitigation of the reduction of sugars under low N conditions would be the use of foliar sprays as a nutritional complement. The main advantage of fertilizing by foliar treatments is its efficiency with a minimal contribution to environment pollution. Recently, applications of 1 mg L⁻¹ sodium selenate or 500 mg L⁻¹ abscisic acid have reported to improve fructose, glucose and vitamin C, in tomato fruits (Barickman et al., 2017; Zhu et al., 2018). On the other hand, the best approach might be an optimized fertilization and irrigation management. As reported by Fullana-Pericàs et al. (2019), 'de penjar' tomato local varieties withstand low irrigation conditions with minimum yield losses, and even with enhanced sugar content in fruits. In any case, these are possible paths for further studies about the cultivation of 'de penjar' tomato using less N fertilization.

Aspartic and glutamic acids are major amino acids in tomato fruits. Tomato accumulate several-fold higher content of glutamic acid in their fruits than other vegetables such as

pepper, onion or carrot (Haytowitz et al., 2011). On the one hand, aspartic and glutamic acids function as other amino acids precursors in plants, e.g. glutamic acid constitutes the first element of the GABA (γ aminobutyric acid) synthesis, a bioactive molecule of recent interest for its health-promoting potential (Gramazio et al., 2020). On the other hand, those are the only amino acids that are related to taste, especially glutamic acid. In their ionized forms and in presence of sodium salt, they give the fifth basic taste, umami, known as savoury and taste-enhancing (Lioe et al., 2010). Due to its molecular N basis, the amount of amino acid in the plant will depend on the N content of the plant. In the case of aspartic and glutamic, no significant differences were found in our ‘de penjar’ collection by reducing nitrogen fertilization to one third of the usual supply.

Regarding bioactive compounds, tomatoes owe their antioxidant power mainly to their vitamin C and carotenoid content (Adalid et al., 2010). Although the effects of lowering N fertilization on these compounds are still not clear (Hernández et al., 2020; Truffault et al., 2019), in our experiment they were not significantly affected by N dosage. Interestingly, vitamin C showed a significant positive environmental correlation to sweetness (soluble solids content, fructose, total sweetness index). A positive correlation between fruit content in sugars and vitamin C has been already reported in other works (Causse et al., 2003), and could be explained by the role of sugars as precursors for vitamin C biosynthesis. On the other hand, sugars also function as signaling molecules in source-sink regulation and as regulators of gene expression, what could also be involved in this correlation (Eveland and Jackson, 2012). These positive correlations could help breeders to identify trends in several compounds measuring just a few easier ones.

4.4. Selection based on ideotype and comparison of local vs. commercial materials

Considering all the traits analysed in the present work, there are prospects for selecting the best ‘de penjar’ varieties based on an ideotype under low N inputs with the aim to include them in breeding programs or directly cultivate them under those conditions. The desirable attributes pursued in our study would be high yield in the first place; high nitrogen content, due to its direct relationship with protein content; great sweetness, given by high content in sugars (mainly fructose), but moderate content in organic acids resulting in high sweetness:acidity ratio; high glutamic and aspartic acid contents, which would potentiate taste; and great content in antioxidant compounds (vitamin C, carotenoids) due to their reported bioactive role in the human body. In this regard, among the best ten yielding varieties under low N treatment, both MT1 and MO1 seemed to present the best ideotype. In addition, they showed opposite average fruit weight, which could be an advantage in breeding for different markets and maintaining the morphological variability within ‘de penjar’ type. On the other hand, MO2 showed very good resilience in yield ranking third, besides being the fourth best yielding variety under low N treatment. Among the 19 out of 44 accessions that showed significant differences between the two N treatments for some of the traits evaluated (lightness of

colour, fructose:glucose ratio, contents in lycopene, β -carotene, total carotenoids and carbon), the already mentioned MT1 was very interesting for showing increased average contents of lycopene, β -carotene and total carotenoids by 43-52% under low N treatment. In addition, TO1 stood out for showing increased mean values under the low N treatment compared to the high N in all the mentioned traits, except for lightness of colour. Although TO1 did not rank in the top 10 positions for yield in any of the N treatments, nor for yield resilience, the cultivation and conservation of this local variety could be interesting for future breeding programs aimed at improving fruit quality under low N inputs.

Commercial tomato varieties are the result of the last fifty years of breeding towards high yield, pest resistance and fruit uniform appearance (Casañas et al., 2017). However, in the last decade, with consumers demanding more tasteful and healthful fruits and agriculture facing the challenge of producing more with fewer resources and with less impact on the environment, local tomato varieties are being “rediscovered” as an important source of variability. Several studies have been carried out in order to properly characterize the ‘de penjar’ tomato (Casals et al., 2015, 2012; Cebolla-Cornejo et al., 2013; Figàs et al., 2015). However, there is a lack of comparison of local vs. commercial varieties for this varietal type. Herein we have found significant differences for three (soluble solids content:titratable acidity, total sweetness index:titratable acidity and vitamin C) out of the 29 traits analysed. For all three traits and both N treatments, local varieties showed higher average value, which reinforces their appreciation for their organoleptic and nutritional quality.

5. Conclusions

The present work provided the first comprehensive characterization of the variability of ‘de penjar’ tomato varieties under contrasting levels of N fertilization. A wide diversity in our collection for agronomical, morphological and fruit organoleptic and nutritional quality traits was revealed. Our data support the evidence of a current over-fertilization in ‘de penjar’ tomato cultivation. Under the experimental conditions tested, reducing to one third the usual nitrogen supply did not show any significant effect on yield and most of the traits evaluated related to fruit nutritional and organoleptic quality, except for a decrease in soluble sugars. Several varieties showed excellent results under low N supply conditions, being within the best ten yielding varieties with good fruit quality parameters. In addition, the present work highlights the value of local varieties for selection and breeding of ‘de penjar’ tomatoes and enhances their potential as a very useful gene pool for future tomato breeding programs for resilience under restrictive environmental conditions. Further studies on association of genetic and phenotypic data and on postharvest performance under low N fertilization conditions, as well as developing segregating generations for the *alc* mutation will provide relevant information for the enhancement of the ‘de penjar’ tomato for selection and breeding for resilience.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions: J.P., S.S. and M.P. planned the study. J.P., S.S. and M.P. supervised the research. E.R.-M., L.A. and R.B. performed the morphological and chemical composition characterization. A.A. and E.R.-M. performed the chemical analyses by HPLC. L.P.-D., C.C., E.S., M.F., R.B., L.A. and E.R.-M. performed the agronomic characterization. M.G.-M. performed the soil analysis. L.P.-D., C.C., E.S., M.F. and S.S. supervised the crops. E.R.-M. and R.B. curated the data. L.P.-D. and E.R.-M. performed the statistical analyses. E.R.-M., J.P., S.S. and M.P. drafted the manuscript.

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Data Availability Statement: The raw data supporting the conclusions of this manuscript will be made available by the authors without undue reservation, to any qualified researcher

Supplementary Material: Supplementary data associated with this article are the following:

Table S1. Accession name, code used in the present work and geographical origin within Spain or breeding company or institution of the ‘de penjar’ tomato local and commercial varieties used for their evaluation under low and high nitrogen fertilization treatments.

Table S2. Mean values and standard error of the soil chemical composition for each soil of both low and high nitrogen fertilization field plots ($n = 5 \times 2 = 10$).

Table S3. Mean values of the three replicates per accession and treatment ($n = 3 \times 2 = 6$) for all the traits evaluated. (Note: acronyms LN and HN refer to low nitrogen and high nitrogen treatment, respectively).

Figure S1. Daily maximum (purple line), average (red line) and minimum (green line) temperatures (A), and radiation (orange area) and pluviometry (blue columns) (B) since the transplant, on May 8th 2019, until the end of cultivation, on September 30th 2019.

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GENERAL DISCUSSION

Agriculture is facing new challenges. The advent of climate change effects (Ruiz de Galarreta et al., 2018), the loss of soil fertility (Zeng et al., 2016), the lack of arable lands (Panagos et al., 2019), the exponential growth of the world population (Godfray et al., 2010), and the consumer increasing demand for healthier and tastier fruits and vegetables (Asensio et al., 2019), have been the focus for the establishment of new agricultural policies and for the development of new varieties. In this way, some authors have reported the need for a new Green Revolution, increasing yields, genetic diversity and nutritional content of fruits and vegetables, without causing further harm to the environment (Raza et al., 2019; Zhang et al., 2015).

It was against this background that the present doctoral thesis was conducted. We carried out a comprehensive characterization of fruit quality parameters, along with important agronomic traits, of a set of genetic resources scarcely explored up to date, within the tomato, eggplant and pepper germplasm. Our studies have addressed potential genetic resources for the incorporation of new variation into the genetic background of these three crops, encompassing traditional varieties and a set of eggplant introgression lines carrying genome fragments of its wild relative *Solanum incanum* L. We hope the results obtained will make a valuable contribution to the scientific knowledge of the *Solanaceae* vegetables and will boost conservation and utilization of these resources in future breeding programs for nutritional and organoleptic quality, as well as for adaptation to a more sustainable agriculture.

1. Diversity among cultivated tomato, eggplant and pepper germplasm for fruit quality

Since the Green Revolution (1960s), a limited number of modern varieties have progressively displaced the cultivation of a large number of traditional varieties conserved locally by small farmers and adapted to specific agroclimatic conditions (Fu and Dong, 2015; Lin et al., 2014; Van De Wouw et al., 2009). These modern varieties are characterised by high uniformity, high yields, resistance to pathogens and longer shelf-life. The introduction of modern varieties was a milestone in the history of agriculture enabling a dramatic yield increase to meet the needs of a growing world population. However, the disregard for nutritional and organoleptic aspects in breeding programs during that time led to a loss of quality attributes in fruits and vegetables. Consumers are now increasingly complaining about that loss, demanding more health-promoting products and looking for the “taste of the past” (Causse et al., 2010, 2003; Klee and Tieman, 2018; Tieman et al., 2017). As a result, in the last decade, plant researchers and breeders are focusing more efforts into developing tools and building knowledge for the improvement of quality traits.

On that regard, tomato, eggplant and pepper are highly valued as a source of fibre, vitamins, minerals and antioxidant compounds to the human diet (Bursać Kovačević et al., 2020; Yahia et al., 2019). The fact that they are amongst the top ten vegetables most

produced worldwide (FAOSTAT, 2019) and are present in the cuisine of almost every culture, makes them an attractive target for the improvement of fruit quality. In this sense, many works on the characterization of tomato, eggplant and pepper quality have been published in the last decades, including commercial and traditional varieties and wild relatives (e.g., Ayaz et al., 2015; Chassy et al., 2006; Docimo et al., 2016; Fratianni et al., 2020; García-Salas et al., 2014; Giuffrida et al., 2013; Lemos et al., 2019; Martí et al., 2018; Martínez-Ispizua et al., 2021a, 2021b; Massaretto et al., 2018; Mennella et al., 2018, 2012; Meza et al., 2020; Plazas et al., 2014; Prohens et al., 2013; Raigón et al., 2008; Siddiqui et al., 2015; Slimestad et al., 2008; Stommel and Whitaker, 2003; Wahyuni et al., 2014). A large inter- and intra-varietal diversity has been found between and within these studies, which reflects the main challenge in breeding for quality: the high complexity of its genetic basis (Carli et al., 2011; Tieman et al., 2017), and the strong influence of the environment on the final phenotype (Figàs et al., 2015; Panthee et al., 2012; Stommel et al., 2015; Tripodi et al., 2018). Furthermore, the influence of the consumer culture and subjectivity on the organoleptic component are difficult to evaluate or predict. The existing diversity dependent on genotype and environment, as well as the resulting selection efficiency for all the traits studied were analysed and discussed throughout the different works in this doctoral thesis, by using different parameters, such as the range of data, standard error, multivariate analysis of principal components, genetic and phenotypic coefficients of variation, genotype \times environment interaction or the estimation of heritability in a broad sense. This information will be of great relevance for breeders to plan efficient breeding programs.

For the first chapter of the thesis, we tried to minimize the effect of the environmental component by growing a collection of ten representative varieties of tomato (*Solanum lycopersicum* L.), ten of eggplant (*S. melongena* L.) and ten of pepper (*Capsicum annuum* L.) under the same cultivation conditions, in order to compare the three species and to spotlight the variability in terms of their internal fruit composition. This work represents a very comprehensive evaluation encompassing 36 parameters associated to taste, major nutrients, and to the most relevant bioactive compounds in the *Solanaceae*. Our work extends the information that can be found in nutritional databases, insofar as we provided ranges of varietal variation in each species for each compound evaluated, which we believe will be very useful for the selection of the best genotypes and for an integral improvement of fruit quality. To our knowledge, no other evaluation and comparison of such an extensive fruit composition traits panel has been performed for a diverse set of these crops grown under the same conditions.

For the studies carried out within the doctoral thesis, we combined the evaluation of individual metabolites using high-throughput techniques, such as High-Performance Liquid Chromatography (HPLC) coupled to a UV detector and a refractive index detector, atomic absorption spectrophotometry and stoichiometric flame photometry, with widely used aggregated parameters using traditional less-sensitive techniques such

as titration, UV-VIS spectrophotometry or refractometry. On that regard, the development and increasing use of sensitive high-throughput metabolomic analysis techniques in the last decades, such as HPLC, HPLC-mass spectrometry (MS), nuclear magnetic resonance (NMR) or Fourier transform mid-infrared (FT-MIR) spectroscopy, has allowed the identification and quantification of individual metabolites in large sample sizes in relatively short times, using small sample amounts or even non-destructive techniques (Zampieri et al., 2017). The analysis of individual metabolites over the use of aggregated parameters is very useful for a better understanding of metabolic pathways and their regulation. Besides, combining targeted metabolomics with genomic analysis has proved to be an advantage in determining the genetic basis of flavour and nutritional quality of fruits, facilitating the detection of quantitative trait loci (QTLs) and enabling precise gene and gene function dissection (Pott et al., 2021; Sulli et al., 2021; Tieman et al., 2017). Nevertheless, high-throughput metabolomic techniques are still very expensive, and the use of aggregate parameters such as soluble solids content (ssc), titratable acidity (ta) and their ratio, or total sweetness index (tsi) are still widely used in screenings for variability and quality control of fruits and vegetables, as they are easily measurable traits, which highly correlate with consumer appreciation. We believe that the combination of the analysis of simple aggregated variables such as ssc or ta with the identification of the metabolites responsible for them can be advantageous for the improvement of fruit internal quality.

The evaluation of fruit composition of tomato, eggplant and pepper varieties showed a considerably different and complementary quality profile among species. The results confirmed the interest of these *Solanaceae* to the human health associated to their consumption, due to their content in bioactive compounds (Yahia et al., 2019). Increasing the content in bioactive compounds of fruits and vegetables is gaining relevance in breeding programs, since a growing number of studies support their health-promoting potential, as they have shown antioxidant, antimicrobial, antitumoral, antidiabetic, neuro- and cardio-protective activities (D'Amelia et al., 2018; Hounsome et al., 2008; Kyriacou and Rouphael, 2018; Pott et al., 2019; Vincente et al., 2014; Wahyuni et al., 2013; Weng and Yen, 2012; Yahia et al., 2019). Within this context, our work showed that, under the same growing and environmental conditions, pepper stood out for its high content in vitamin C, so that a 100 g serving would far exceed the recommended daily allowance (RDA) of this nutrient. Tomato also represented a good dietary source for vitamin C, covering 30-36% of the RDA, and was also rich in the flavonoid rutin. Eggplant, on the other side, represented the best source of phenolic acids, mainly chlorogenic acid, which contributes to its high antioxidant activity. These are not surprising results at all, being in agreement with several other works for pepper (Fратиanni et al., 2020; García-Closas et al., 2004; Howard et al., 2000; Sánchez et al., 2018; Wahyuni et al., 2013), tomato (Figàs et al., 2015; García-Closas et al., 2004; Slimestad et al., 2008; Slimestad and Verheul, 2009) and eggplant (Luthria et al., 2010;

Plazas et al., 2013; Prohens et al., 2013; Singh et al., 2009; Stommel and Whitaker, 2003).

There are still few studies aimed at characterizing the mineral content of tomato, eggplant and pepper. However, mineral intake is essential for the proper functioning of the human body, as they play important roles in oxygen transport, osmosis balance, as enzyme cofactors, components of bones and teeth or DNA (Quintaes and Diez-Garcia, 2015). Our study included the evaluation of the most abundant minerals in plants (K, P, Ca, Mg, Na, Fe, Cu, Zn). Among the three species, eggplant stood out for being the best source of mineral compounds, although the proportion of each mineral over the total amount was similar among the three species, with K being the most abundant. These results were in agreement with other sources (Ayaz et al., 2015; Fernández-Ruiz et al., 2011; Guilherme et al., 2020; Raigón et al., 2010; U.S. Department of Agriculture, 2021).

The large variability observed within each species for all traits studied demonstrates that fruit internal quality is highly dependent on genotype, which is in agreement with several other studies (Figàs et al., 2015; Plazas et al., 2013; Sánchez et al., 2018). In this way, the highest coefficients of variation in our work were found for sucrose and bioactive compounds (>90%), i.e., chlorogenic acid or myricetin in pepper, naringenin chalcone in tomato and β -carotene in eggplant. These results offer substantial room for improvement of the functional potential of tomato, eggplant and pepper fruits. The large variability found within species will allow the selection of the best genotypes for their conservation and use in breeding programs. Crossing the best varieties for different compounds and subsequent rounds of selfing and selection might be a good strategy leading to new varieties with improved internal fruit content.

On the one hand, this study has evidenced the diversity of the *Solanaceae* family, which is in agreement with genetic studies (Arnoux et al., 2021). In addition, it suggests the existence of certain differences in the expression of metabolic pathways for the synthesis of metabolites, mainly secondary metabolites, among these species. On the other hand, the complementarity of the metabolic profiles ensures that a daily diet rich in these three vegetables would largely provide the recommended intake of macro and micronutrients, as well as a large amount of antioxidant compounds of different nature. Finally, this study establishes the basis for defining the main objectives for improving fruit quality in each crop, as well as provides sufficient variability to be used in new breeding programs.

The phenotyping of the natural variation is the first essential step towards the development of new improved varieties. At present, large-scale characterization projects of the *Solanaceae* vegetables have been carried out in recent years, supported by public funding; i.e., the international projects TRADITOM (www.traditom.eu), BRESOV (www.bresov.eu), G2PSOL (www.g2p-sol.eu) or HARNESSTOM

(www.harnesstom.eu). In this way, the characterization of tomato, eggplant and pepper presented in the doctoral thesis is embedded within the international G2PSOL project, aimed at restoring the complexity and the richness of the original germplasm of the four *Solanaceae* food crops through phenotyping and genomic studies of tens of thousands of accessions stored at genebanks worldwide. The work of genebanks should be highlighted in this regard, which are devoted to collect, document, regenerate and publicly distribute a great number of accessions including landraces, modern varieties, pre-breeding materials, semi and wild relatives, etc., thus preserving the diversity of several species (Alonso et al., 2018; Díez et al., 2018). The continuous feedback between genebanks and plant researchers constitute the wheels of the vehicle that moves breeding towards new goals.

Finally, the plants included in the first chapter were grown under organic farming conditions. This will allow breeders to select the best genotypes for its cultivation and breeding for adaptation to a sustainable agriculture model with low inputs.

2. Genetic resources for breeding for quality for a more sustainable agriculture with low inputs

Paradoxically, intensive agriculture practices have been one of the main contributors to environmental pollution and climate change, and now agriculture has to face the consequences. Climate change conditions will involve higher atmospheric CO₂ and O₃ concentration, the increase in global average temperatures, huge variations in rainfall patterns leading to increased flooding and periods of drought, and therefore the increase of extreme adverse conditions worldwide. In addition, the extension of arable lands over wild natural habitats and the overexploitation of soil for cultivation has led to a loss of biodiversity and soil fertility. Furthermore, the excessive, unlimited use of inorganic fertilizers has led to pollution of water bodies, eutrophication and loss of biodiversity, as well as soil acidification and greenhouse gases release into the atmosphere. In this sense, since nitrogen (N) is an essential nutrient for plant growth and development, N-enriched fertilizers have been the most used and therefore the most polluting (Ruiz de Galarreta et al., 2018; Stevens, 2019; Zhang et al., 2015).

On that regard, new agricultural policies aim to shift towards a more sustainable agriculture. In this scenario, the European Union has recently adopted a new common agricultural policy (CAP) for the 2023-27 period, setting nine key objectives (European Commission, 2018). These include “the contribution to climate change mitigation and adaptation” and the “efficient management of natural resources such as water, soil and air”. For addressing these issues and from a plant researcher and breeder point of view, a change in cultivation practices should be implemented hand in hand with the development of improved varieties adapted to adverse conditions and low inputs, with a more efficient use of nutrients, especially N (European Commission, 2018).

First, the domestication process and then, the development of modern varieties indirectly led to a loss of genes and allelic variability of utmost interest for the adaptation to climate change conditions and low inputs, as well as for the improvement of functional potential and quality of fruits (Bebeli and Mazzucato, 2008; Van De Wouw et al., 2009). In this sense, the development of modern varieties has taken place in the context of an unlimited use of inorganic fertilizers, making them overly dependent on inputs. To overcome the new agricultural challenges, breeders have to “restore” that lost variability. In this way, researchers are pointing at new interesting sources of variation such as crop wild relatives (CWRs) (Gramazio et al., 2021; Zhang et al., 2017), which usually grow under harsh environments and maintain a large genetic diversity, and local varieties (Hurtado et al., 2014; Massaretto et al., 2018; Parisi et al., 2017), which are adapted to specific agroclimatic conditions, are produced and maintained traditionally by small-scale farmers under low input conditions, and are appreciated for their richness in flavour and nutritional content.

CWRs have been historically used for introgressing resistance genes against pest and diseases into cultivated varieties. For instance, in tomato, more than 40 resistance genes found in cultivated varieties have been derived from wild relatives, mostly *Solanum pimpinellifolium* L., *Solanum pennellii* Correll, *Solanum peruvianum* L. and *Solanum cheesmanii* (L. Riley) Fosberg (Rick and Chetelat, 1995). Also, resistance to potato late blight was obtained from *Solanum demissum* Lindl. and *Solanum stoloniferum* Schltld. and Bché (Hajjar and Hodgkin, 2007). Local varieties, meanwhile, have shown promising resilient characteristics against abiotic stresses such as drought and salinity (Fullana-Pericàs et al., 2019; Iglesias et al., 2015; Massaretto et al., 2018; Meza et al., 2020; Sumalan et al., 2020), as well as higher content in flavour-related and nutritional compounds (Figàs et al., 2015; Massaretto et al., 2018; Parisi et al., 2017; Raigón et al., 2008; San José et al., 2013; Sánchez et al., 2018; Tieman et al., 2017) than commercial cultivars.

In this context, for the present doctoral thesis we proposed to evaluate, on the one hand, a population of eggplant lines with genome introgressions of its CWR *Solanum incanum* L., and on the other hand, a collection of local varieties of ‘de penjar’ tomato, in order to characterize the existing variability for fruit quality and agronomic traits, as well as to analyse the response of these traits to low N fertilization conditions. With all this information, we wanted to determine the potential of these two poorly exploited genetic resources for the improvement of eggplant and tomato quality and adaptation to low N inputs.

2.1. Eggplant interspecific introgression lines

Introgression lines (ILs) have proved to be a powerful resource for the introduction of traits of interest from CWRs into the domesticated genetic pool by avoiding the linkage drag of undesirable traits usually present in these species, such as low yield, the presence

of prickles or the content of anti-nutritional compounds in the fruit. In addition, ILs show a great advantage over other mapping populations, such as F₂ or RILs, for the elucidation of the genetic components of complex traits. This potential is due to the fact that each line carries a single fragment of the donor parent genome, which is incorporated into the genetic background of the cultivated parent (Lippman et al., 2007; Pratap et al., 2021; Prohens et al., 2017).

Several interspecific ILs have been developed in tomato and other important crops in the last decades. In this way, as we investigated in our review entitled “Genetics and breeding of phenolics content in tomato, eggplant and pepper fruits” (draft, not published), the most exploited tomato IL collection is developed from *S. lycopersicum* × *S. pennelli* (Eshed and Zamir, 1995). Thanks to its availability, hundreds of QTLs related to the nutritional and organoleptic quality of the fruit have been identified (Alseekh et al., 2015; Ballester et al., 2016; Gürbüz Çolak et al., 2020; Hanson et al., 2014; Ökmen et al., 2011; Tripodi et al., 2020). Furthermore, the crossing of two ILs from this population, which harboured positive alleles for five QTLs associated to total phenolics, ascorbic acid and soluble solids content, enabled the pyramiding of these QTLs in four genotypes of the F₃ population (Sacco et al., 2013).

On the other hand, despite being one of the most produced vegetables worldwide, the development of mapping populations and genomic tools in eggplant has lagged behind other important crops, including tomato, pepper or potato. In this regard, the first interspecific population of ILs has been recently developed by our group using the CWR *S. incanum* as donor parent (Gramazio et al., 2017). *S. incanum* is part of the eggplant secondary gene pool (Syfert et al., 2016), and naturally grows in dry regions from northern Kenya to Pakistan (Knapp et al., 2013). In various characterization studies, *S. incanum* has shown tolerance to drought and several diseases (Knapp et al., 2013; Mishra et al., 2021; Yamakawa and Mochizuki, 1979), as well as higher levels of antioxidant activity, total phenolics and chlorogenic acid content compared with *S. melongena* (Kaur et al., 2014; Prohens et al., 2013). Therefore, the ILs from *S. melongena* × *S. incanum* could be of great interest for eggplant breeding. In fact, the IL collection has already been characterized for morphological and agronomic traits (Mangino et al., 2021, 2020), revealing their interest for genetic improvement of eggplant for several of these important traits. However, the incorporation of these ILs by breeders in their breeding pipelines requires ensuring that the anti-nutritional compounds present in *S. incanum* are not dragged along and that they produce fruits safe for consumption. In this context, eggplant wild relatives have been reported to present high concentrations of glycoalkaloids (Mennella et al., 2012; Sánchez-Mata et al., 2010), often above the internationally accepted safety limit (200 mg kg⁻¹ of fresh weight) established for new potato cultivars (OECD, 2020).

With this scenario, in the present doctoral thesis, a set of 16 ILs from *S. melongena* × *S. incanum* (Gramazio et al., 2017) was evaluated for the first time for their fruit

composition through the analysis of 25 traits, including proximate composition, sugars, acids, phenolics, minerals and glycoalkaloids. In addition, the cultivated and wild parents, and the F₁ hybrid were included in the evaluation, which allowed the assessment of heterosis. The evaluation was carried out under two environments (open field and greenhouse), which allowed the evaluation of the environmental effect on the quality traits evaluated and the occurrence of genotype × environment interactions.

The parents of the ILs showed large significant differences in fruit composition, and the mid parent heterosis was only significantly positive for malic acid content and negative for fructose and chlorogenic acid content. Despite these differences, the ILs generally exhibited similar nutritional and functional quality to the recipient *S. melongena* parent. This statement includes the glycoalkaloid content, which remained below the safety limit in the ILs, although *S. incanum* had contents well above this limit, with high plant-to-plant variation. The avoidance of high glycoalkaloid drag-in from CWRs with the development of ILs was also reported in eggplant advanced backcrosses with *Solanum sodomaeum* L. and *Solanum integrifolium* L. (Mennella et al., 2010). Our results are fundamental for the future use of the ILs collection in breeding programs, since it demonstrates the safety of their fruits for human consumption, in addition to having good internal quality characteristics.

The genotyping of the IL collection was previously performed by HRM (high-resolution melting) platform using 62 SNPs selected from the set detected by genotyping-by-sequencing (GBS) in the first round of selfing (Gramazio et al., 2017). The availability of genotype data of the ILs was very useful in the present work for the detection of QTLs associated with fruit composition and nutritional quality. Since the ILs were fixed with only one fragment per chromosome, significant differences between one IL and the recipient parent were indicative of the existence of a QTL associated with the trait of interest in the introgressed genome fragment of the donor parent (Dunnett, 1955; Prohens et al., 2017). In total, six stable putative QTLs were detected in the two environments evaluated, which were scattered over chromosomes 3 for crude protein, 5 for malic and total acids, and 7 for chlorogenic acid and solamargine. These QTLs constitute a very interesting tool for future breeding programs using the ILs, as it might enable marker-assisted breeding for both positive and negative selection of genotypes at early stages of development. In this way, marker-assisted selection and pyramiding of genes and QTLs has been proven to be an effective strategy for improving several traits simultaneously or a single quantitative trait with polygenic control (Rigano et al., 2014; Sacco et al., 2013; Salgotra and Neal Stewart, 2020).

The advantage of having tomato, a *Solanaceae*, as a model organism for research on fruit development and metabolism has greatly facilitated the identification of QTLs and genes of interest in eggplant. This has been possible thanks to the availability of the reference genomes of the three *Solanaceae* together with the respective gene annotations in a single publicly accessible platform (www.solgenomics.net) with a user-friendly

interface, as well as synteny studies between tomato, eggplant and pepper. With the use of these tools, potential candidate genes were discussed for most of the QTLs identified, which provide new relevant information for eggplant breeding.

In view of the resilient characteristics of *S. incanum* (Knapp et al., 2013), we believed that the eggplant IL collection with *S. incanum* as donor parent could also be a source of variation for the adaptation to low N inputs. Therefore, once confirmed the safety for consumption and good quality of their fruits, we evaluated a set of ten ILs along with their *S. melongena* recipient parent under two N fertigation conditions. We aimed at evaluating the response of the ILs under low N inputs, in order to select potential materials, as well as generate valuable genetic information, for their use in breeding for eggplant adaptation to a more sustainable agriculture.

N deficiency has been associated with a limitation of plant growth, photosynthetic rate, yield (Duran et al., 2016; Fernandez et al., 2020; Fontes et al., 2010; Hawkesford et al., 2012; Pérez-Jiménez et al., 2019; Sylvestre et al., 2019; Tosti et al., 2016; Wang and Li, 2004; Zhu et al., 2016). Although the present thesis aimed at the improvement of nutritional and organoleptic quality, the success of new released varieties is highly dependent on the yield, since it is a trait of utmost interest for the economic benefit of the farmer. Therefore, we decided to evaluate a total of twenty morpho-agronomic and composition traits, including traits related to yield, plant growth, N and C distribution in leaves and fruits, fruit size, morphology and content in phenolic compounds, and to analyse how reducing the N input could affect them.

We used two N fertilization doses applied as a nutritive solution with the irrigation system. The difference was the addition of N as ammonium nitrate for the high N treatment in a similar concentration to that optimal for eggplant cultivation (Baixauli and Aguilar, 2002), while no external N fertilizer was supplied for the low N treatment.

Our study showed that, for the ten ILs evaluated plus the recipient parent, lowering N inputs significantly decreased the average values of SPAD, plant height and biomass, early yield, total number of fruits, fruit dry matter, N and C content in leaf and N content in fruit, while it did not significantly affect final yield, fruit morphology, size, and content in phenolic compounds. These results are promising insofar as, although plant development was affected by the low N treatment resulting in lower early yields, there seemed to be a progressive adaptation of the plants so that they managed to maintain final yields similar to those obtained under high N conditions, as well as to produce fruits with similar content in phenolic compounds, thus maintaining their functional potential. The absence of genotype \times treatment interaction for most of the traits indicated that we can expect a similar trend in the phenotype response to lowering N inputs for all the lines. However, we found three ILs, which carried the introgressed fragment on chromosomes 5, 9 and 12, performing similarly under both N treatments, which could be interesting for breeding for eggplant adaptation to low N inputs.

Linking the phenotyping of the ILs with the genotyping led to the identification of 36 QTLs associated with most of the traits analysed scattered across 9 out of the 10 chromosomes explored. In this way, we considered the putative QTLs in each N treatment separately as they all can be useful for improvement in a particular environment (Toppino et al., 2016; Tripodi et al., 2020). Among the QTLs detected, 12 were specific to the high N treatment, 17 were specific to the low N treatment and 7 were stable across the two N treatments. Chromosomes 2 and 12 harboured the highest number of QTLs detected under the low N and the high N treatment, respectively. The cluster of QTLs detected in the introgressed fragment of chromosome 2 were associated to plant growth, yield and fruit morphology and weight. The high inter-trait correlation among these could indicate the existence of genetic linkage or a pleiotropic locus (Causse et al., 2002; Fulton et al., 1997; Portis et al., 2014). These results are really interesting since are in agreement with major QTLs reported on chromosome 2 in both eggplant and tomato experimental populations (Doganlar et al., 2002; Frary et al., 2014, 2000; Nesbitt and Tanksley, 2001; Portis et al., 2014; Swamy and Sarla, 2008). Furthermore, four QTLs identified in a previous two-environment evaluation of a set of the ILs with introgressions from *S. incanum* (Mangino et al., 2020) were validated herein. The search for candidate genes was challenging due to the large size of the introgressions, in some lines covering almost 100% of the chromosome, although some possibilities were discussed.

Unfortunately, the evaluation of the ILs did not result in the identification of QTLs for Nitrogen Use Efficiency (NUE), and the wild alleles had a negative effect on the trait compared to the recipient parent for most of the QTLs identified. However, besides the large size of the introgressed fragments, NUE is a very complex trait in which several plant physiological processes are involved. In this way, we discussed some of them, which we believed could be related to our results. The possible strategies for addressing these issues could be the obtention of sub-ILs, with shorter and overlapping introgression fragments for fine mapping, a better control of the growing conditions and the dissection of the complex NUE trait into several simpler traits.

Fertilization also affects soil properties. In this way, the analysis of cultivated soils after long-time fertilization periods has shown soil acidification, reduction of organic matter and microbial communities, and thus, soil fertility (Zeng et al., 2016). Likewise, soil properties and composition interact with the plant nutrient absorption capacity (Jones Jr., 2012). Our work in the present doctoral thesis emphasizes the need of soil composition analysis as an essential task when planning the most appropriate fertilization for a sustainable agriculture model, which has not been a customary practice in the past (Soto et al., 2015). For the evaluation of eggplant ILs under two N fertilization levels, we performed soil and intake water analyses in order to prepare the adequate nutritive solutions for each of the two N treatments. We also performed a soil analysis of each of the N treatment fields at the end of the cultivation period. No significant

differences were observed between the soils of the high N and low N treatments, except for N and Fe contents after the cultivation period, which were slightly lower in the high N treatment, probably as a consequence of higher nutrient removal from soil by plants and even a decreased microbial activity associated to an excess of N, although studies of soil biological and enzymatic activity would be required to confirm this assumption. Our results suggested that a soil analysis before and after cultivation would be helpful to determine if the crop is being over-fertilized, in agreement with other studies (Salinas-Garcia et al., 1997; J. Sun et al., 2020).

2.2. Tomato local varieties

Local varieties are commonly associated by consumers to the “taste of the past”. They have important cultural roots and are traditionally anchored to the local market. Although they usually present lower yields and are more susceptible to pest and diseases than commercial varieties, they are adapted to specific agroclimatic conditions, which makes them more suitable for organic farming and sustainable agriculture (Casañas et al., 2017; Conesa et al., 2020; Gonzalez-Cebrino et al., 2011; Ribes-Moya et al., 2018). These characteristics have increased their demand in a current market prone to the ‘Zero’ concept (‘Zero’ km, ‘Zero’ waste, ‘Zero’ pesticides, etc.). Their strong appeal to consumers nowadays is boosting local economy and small-scale farmers income, which are also key objectives in new government policies for a more sustainable and fair agriculture (European Commission, 2018). Within this context, a growing interest for local varieties has emerged for broadening and improving the genetic base of modern varieties gene pool. In the last years, an increasing number of characterizations of the diversity of local varieties of *Solanaceae* vegetable crops has been performed (Casals et al., 2011; Cortés-Olmos et al., 2014; Figàs et al., 2015; Martínez-Ispizua et al., 2021b, 2021a; Massaretto et al., 2018; Parisi et al., 2017; Ribes-Moya et al., 2018; Sánchez et al., 2018), including large-scale international projects, such as TRADITOM (<http://traditom.eu/>), which aimed at characterize the large diversity hidden in local varieties of tomato throughout Europe.

Among local varieties of tomato, long shelf-life (LSL) varieties, distributed throughout the Mediterranean basin, from Spain to Italy (also called ‘de penjar’ in the Valencian Region and Catalonia, ‘de ramellet’ in the Balearic Islands or ‘da serbo’ in Italy), have been recently targeted by breeders due to their interesting characteristics. Besides the long shelf-life of their fruits, up to 6 to 12 months after harvest (Casals et al., 2012), they have shown higher acidity and soluble solid content than standard tomato (Cebolla-Cornejo et al., 2013; Figàs et al., 2015), and their traditional cultivation under rain-fed semiarid summer conditions have resulted in the selection of resilient genotypes. However, they also maintain a great variability for other important traits such as fruit morphology and quality according to the area of cultivation and to their uses (Bota et al., 2014; Casals et al., 2015; Cebolla-Cornejo et al., 2013; Figàs et al., 2015). The genetic determinants of the long shelf-life have been widely studied in the past for

varieties specific to an area of cultivation (Bota et al., 2014; Casals et al., 2012). More recently, a large-scale genomic study using double digest restriction-site associated sequencing (ddRAD-seq) and 152 LSL varieties from different areas of the Mediterranean basin has given interesting insight into the genetic footprint of this group of varieties (Esposito et al., 2020). In this way, the authors found high frequencies of several mutations in genes related to stress tolerance and fruit maturation within this germplasm collection, reaffirming their interest for breeding for the adaptation to a more sustainable agriculture and climate change conditions. In addition, several SNP markers have been developed associated to these mutations, which will be incredibly helpful for the assisted selection of the best genotypes (Esposito et al., 2020).

Tomato is one of the horticultural crops that has abused N fertilization the most. Furthermore, in the last decades, growers have been supplying N fertilizers well above the official reported requirements of the crop (Thompson et al., 2007). In this way, a drastic reduction of N inputs has become imperative. LSL tomato varieties are a genetic resource scarcely explored, which attracted our attention as a source of variability for low N inputs due to its already demonstrated resilience to drought, as well as the N needs of this type of tomato being far below the N fertilization levels required for intensive standard tomato cultivation (Seda and Muñoz, 2011). Herein we evaluated the largest collection of Valencian ‘de penjar’ tomato up to date, under two N fertilization levels, for agronomic and fruit quality traits. The two N doses were applied in the form of manure, according to the organic farming practices, and a soil physicochemical analysis before cultivation showed no differences between the two neighbouring field plots where plants were grown. The high N treatment corresponded to a similar concentration to that optimal for ‘de penjar’ tomato cultivation (Seda and Muñoz, 2011), while for the low N treatment a 1/3 lower N concentration than that of the high N treatment was used as fertilizer. Our work provided the first comprehensive characterization of the variability of ‘de penjar’ tomato varieties under contrasting levels of N fertilization. We evaluated an extensive set of compounds and parameters related to fruit nutritional and organoleptic quality including colour, fruit N and C content, sugars, acids, vitamin C and carotenoids. We also evaluated the taste-enhancing amino acids glutamic and aspartic acids, which are of growing interest among breeders, as they are responsible for the umami taste, participate in flavour intensity, and glutamic acid is the precursor of GABA (γ aminobutyric acid) synthesis, a bioactive compound of recent interest for its health-promoting potential (Gramazio et al., 2020b). Furthermore, we evaluated yield and fruit weight due to the relevancy of agronomic traits to the farmer. This extensive characterization provided information for an integral understanding of ‘de penjar’ tomato fruit quality and its response to lowering N inputs.

The analysis of the results showed that reducing to one third the usual N input to ‘de penjar’ tomato did not significantly affect yield parameters and most of the traits evaluated related to fruit nutritional and organoleptic quality, except for a slight change

in colour and a decrease in soluble sugars and total sweetness index. Interestingly, higher contents in soluble sugars have been reported for Italian LSL tomato varieties compared to the Spanish varieties, which are more acidic (Conesa et al., 2020). Gene transfer between these two populations could increase sweetness in our collection. We also proposed the use of foliar sprays as a nutritional complement, which have shown high efficiency with a minimal impact on the environment (Barickman et al., 2017; Z. Zhu et al., 2018). With all this, further studies for plant breeding for the adaptation to sustainable agriculture and climate change conditions will be directed towards the evaluation of the best genotypes under different combined stresses. In this way, the best approach for improving ‘the penjar’ tomato quality using less N fertilization might be an optimized fertilization and irrigation management (Fullana-Pericàs et al., 2019).

We also confirmed the high diversity for fruit morphology and quality among the ‘de penjar’ tomato varieties, in agreement with other phenotypic and genetic studies (Esposito et al., 2020; Figàs et al., 2018). The large intra- and inter- varietal variation and the generally low heritability of fruit composition and quality parameters was also highlighted, which is in agreement with several studies for different species, including the ones of the present doctoral thesis. This translates into a lower efficiency in genotypic selection and, therefore, the need for a greater effort in the characterization of the genetic basis of complex quality traits. Variation was usually higher in secondary metabolites accumulation, confirming the results of the comparative study of quality in *Solanaceae* vegetables presented in the first chapter, which might be the result of domestication (Pott et al., 2019), as well as a stronger influence of the environment compared to primary metabolism, since their synthesis is usually triggered by a stressful condition (Yang et al., 2018).

In addition, the assessment of the differences among genotypes highlighted several local varieties showing excellent results under low N conditions, being within the best ten yielding varieties with good quality. The inclusion of five ‘de penjar’ commercial varieties allowed the identification of local varieties that performed better under both N conditions, which highlights the value of the latter for selection and breeding of ‘de penjar’ tomatoes. Ultimately, our work enhanced the potential of ‘de penjar’ tomatoes as they constitute valuable genetic resources with good quality characteristics for future tomato breeding programs for resilience under restrictive conditions.

3. Concluding remarks and future perspectives

Uncovering the variability of crops is a fundamental task for the conservation and improvement of genetic resources. This, together with the goal of improving varieties for a more sustainable, environmentally-friendly agriculture model, are the basis on which the studies compiled in the present doctoral thesis were developed. In this sense, we have characterized the diversity for fruit quality that we could find within and among the three major *Solanaceae* vegetable crops, tomato, eggplant and pepper, while grown

under organic farming. We explored the variability for fruit quality and important agronomic traits of a set of eggplant interspecific introgression lines under different environments, including the characterization of their response to minimizing N fertilization inputs. Likewise, we characterized the diversity for fruit quality and yield within a large collection of ‘de penjar’ long shelf-life tomato varieties, as well as their response to lowering N inputs.

These works provide new useful phenomic and genomic information for plant researchers and breeders. With our results, we enhanced the gene pool available to contribute to broadening and improving the genetic base of current modern varieties. Specifically, we demonstrate the utility of CWRs and local varieties.

Firstly, the large variability found within the genetic resources of tomato, eggplant and pepper will pave the way for the selection of the best genotypes, for future studies of other traits of interest and for the integral improvement of nutritional and organoleptic quality of fruits of these *Solanaceae*, as well as for the adaptation to low input conditions. The evaluation of genotype \times environment interactions will allow breeders to select elite varieties with the best response under specific conditions, such as low N inputs.

Secondly, we demonstrated that the eggplant introgression lines carrying introgressions of *S. incanum* produce fruits with similar quality to those of the cultivated *S. melongena* parent and, most importantly, fruits that are safe for human consumption. This opens the door to the use of these introgression lines directly as pre-breeding materials. In this sense, the evaluation of the lines under different abiotic and biotic stresses, even combined stresses, is foreseen. Furthermore, linking the phenotyping studies with the available genotyping of the lines led to the identification of novel QTLs, as well as the validation of some QTLs previously described in the population. That information will broaden the eggplant genetic knowledge, taking a step further towards narrowing the existing gap with other important *Solanaceae*, such as tomato.

Finally, our work confirmed the possibility of reducing the usual N inputs for both eggplant and tomato cultivation, without jeopardising yields and most quality traits. In this way, we provided useful information for the potential utilization of the eggplant ILs and the ‘de penjar’ tomato varieties on that regard. Further studies are needed to find the optimum N fertilization level so that a synchronization between N inputs and N uptake by the plant occur, maximising yields without reducing fruit quality.

Future research strategies include the development of new eggplant interspecific introgression lines, using wild relatives of the eggplant primary (*S. insanum* L.), secondary (*S. dasyphyllum* Schumach. & Thonn.) and even tertiary (*S. elaeagnifolium* Cav.) germplasm. Advanced backcrosses of these developing lines are currently being evaluated under high and low N fertilization conditions (Villanueva et al., 2021). Interesting results have already been obtained regarding resilience to low inputs, as well

as increased phenolic content in fruit. In fact, related to this line of research, the group found a particularly interesting phenolic profile on *S. elaeagnifolium*, compared to the usual profile of cultivated eggplant in which chlorogenic acid represents 70-80% of total phenolics. The identification by HPLC-ESI-MS of the phenolic profile of both the wild parent and several advanced backcrosses, showed the presence of high contents of compounds derived from chlorogenic and caffeic acids with high interest for their bioactive potential and benefit to human health. This new information, together with the genotyping of the advanced backcrosses, may lead to the detection of useful QTLs and to new potential breeding targets which ultimately could translate into eggplants with new profiles of bioactive compounds.

As for the resilient 'de penjar' tomato, further association studies and postharvest performance evaluations under low N fertilization conditions will provide relevant information regarding the selection of elite materials and breeding for resilience.

Overall, this thesis provides relevant information on the utilization of genetic resources of the three major *Solanaceae* vegetable species for future breeding programs aimed at improving fruit quality and resilience to low N inputs. We enhanced the gene pool of crop wild relatives and local varieties to broadening the cultivated genetic background of eggplant and tomato, respectively, and we encouraged their conservation and exploitation. We believe the phenomic and genomic information laid out in this thesis will be of great relevance for the development of new varieties more prepared for the advent of a more sustainable agriculture, which has to be less harmful to the environment, as well as for meeting the consumer increasing demand of more flavourful and healthful fruits.

GENERAL CONCLUSIONS

1. The comprehensive characterization of tomato, eggplant and pepper varieties for fruit composition profile provided insight into the different regulation patterns of metabolite accumulation among the species, and enabled the detection of remarkable ranges of variation for fruit quality compounds and parameters.
2. Pepper, tomato and eggplant had a clearly distinct composition profile. Pepper showed the highest average content in total sugars and organic acids. Fructose and glucose were the major sugar compounds in the three species, although in pepper and tomato sucrose was present only in trace amounts. Citric acid was the major organic acid in pepper and tomato, while in eggplant it was malic acid. Pepper and eggplant had the highest total antioxidant activity. Vitamin C content was much higher in pepper than in tomato and eggplant, while eggplant accumulated high concentrations of chlorogenic acid. Furthermore, eggplant was the species with higher content in most minerals, particularly for K, Mg and Cu, while pepper was the richest in Fe. These findings may be of interest for nutrition programs guidance and will be very useful for the selection of varieties with better nutritional and organoleptic properties as well as for potential utilization in breeding programs.
3. The set of 16 eggplant introgression lines with genome fragments of *S. incanum* generally exhibits a nutritional and functional quality similar to that of the *S. melongena* recipient parent. This demonstrates the potential of the ILs as pre-breeding material and their safety for human consumption, since linkage drag of undesirable quality traits such as glycoalkaloids is avoided.
4. Removing the external N supply to a set of ten eggplant lines with *S. incanum* introgressions resulted in a limitation of plant growth, N and C distribution, number of fruits and fruit dry matter. Reducing to one third the usual N supply for ‘de penjar’ tomato cultivation negatively affected only soluble sugars accumulation in fruits. Nevertheless, the results confirmed the possibility of reducing the usual N inputs for both eggplant and tomato cultivation, without jeopardising final yields and most quality traits. These results enhance the eggplant ILs and ‘de penjar’ tomato as potential genetic resources for breeding for the crop adaptation to low N inputs. An optimal fertilization between the two N fertilization levels used for both eggplant and tomato cultivation should be investigated in order to maximise plant yields and fruit quality without causing damage to the environment.
5. The soil composition analyses performed in our studies were essential for planning the most suitable fertilization for eggplant and tomato cultivation. Our

results also indicated that a soil analysis before and after cultivation would be helpful to determine if the crop is being over-fertilized.

6. A set of novel and validated QTLs were identified in the eggplant IL population for fruit composition and morpho-agronomic traits. This set includes stable QTLs across environments, as well as QTLs specific of the N fertilization treatment, which provides a useful tool for marker assisted breeding in eggplant. In addition, that information will broaden the eggplant genetic knowledge, taking a step further towards narrowing the existing gap with other important *Solanaceae*, such as tomato.
7. The present work highlighted the value of local varieties over the commercial materials for selection and breeding of 'de penjar' tomatoes and enhanced their potential as a very useful gene pool for future tomato breeding programs for resilience under restrictive environmental conditions.
8. The data analyses confirmed that fruit composition and quality traits have low values of heritability and are influenced by a strong environmental effect. The genotype \times environment interactions found within our studies may be useful in identifying optimal combinations for future field trials by breeders aiming to improve fruit quality of tomato, eggplant and pepper.

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