Scientia Horticulturae 318 (2023) 112128

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Dissecting a vegetable landrace: Components of variation in Spanish 'Moruno' tomatoes as a case studio

J. Villena [a,1], C. Moreno [a,1], S. Roselló [b], J. Cebolla-Cornejo [c], M.M. Moreno [a,*]

[a] Higher Technical School of Agricultural Engineering in Ciudad Real, University of Castilla-La Mancha, Ronda de Calatrava 7, Ciudad Real 13071, Spain
[b] Joint Research Unit UJI-UPV - Improvement of Agri-Food Quality, Biology, Biochemistry and Natural Sciences Department, Universitat Jaume I, Avda. Sos Baynat s/n, Castelló de la Plana 12071, Spain
[c] Joint Research Unit UJI-UPV - Improvement of Agri-Food Quality, COMAV, Universitat Politècnica de València, Cno. de Vera s/n, València 46022, Spain

ARTICLE INFO

Keywords:
Solanum lycopersicum L.
Traditional variety
Genetic resources
Variability
On-farm conservation

ABSTRACT

The variability of landraces is dissected and related to farmers' practices through surveys. Spanish 'Moruno' tomatoes, recognised for their excellent flavour, were selected as a case study. For this purpose, 30 populations were characterised in a four-year program. Higher intra-population than inter-population variability was found. Variability is generally reduced in the traits used by farmers as selection criteria. Farmers rarely used flavour as a selection criterion, but it is one of the main characteristics used by them to define the landrace. Seed exchange and growing different landraces simultaneously are commonplace, and outcrossing might occur, thus justifying in part the seed degeneration problems experienced by some farmers. At the same time, farmers select seeds for the next year on a per-fruit basis rather than a per-plant basis, justifying the maintenance of a high level of variability. In such cases, high pressure would be applied to key morphological traits but not to flavour. Accordingly, the sugars, acids and volatiles profiles related to flavour in the landraces' populations are highly variable, though the environment also exercises a high effect. It is necessary to make population selections to offer farmers materials combining the best organoleptic perception and a high stable yield. This would reduce the gap between the price premium received by farmers and the expected fair price. It would also be necessary to develop consumer information campaigns to exploit their willingness to pay for the extra value offered by landraces. Only then long-term on-farm conservation would be economically feasible. Although achieving a trade-off between yield and flavour is difficult, it is possible to identify populations that reach a compromise between them. In germplasm banks, it is impossible to evaluate all the materials in the same year. The use of hybrid controls, with no genetic variation, is helpful in considering the environmental effects. Still, genotype x environment interactions are evident, and using selected control populations of landraces is necessary to evaluate possible performances closer to the type of materials being evaluated.

1. Introduction

Crop landraces represent not only a cultural heritage of incalculable value but also the vast majority of the diversity present in the cultivated species. They should therefore be conserved as a different part of our cultural heritage and as a necessary repository of sources of variation for the development of plant breeding programs. However, a considerable amount of this diversity has been lost due to genetic erosion processes. Despite these losses, a significant portion of this diversity was saved in seedbanks during the last part of the 20th century. Nonetheless, *in situ* conservation is necessary to complement *ex situ* efforts, allowing the interaction of landraces with farmers and the environment and, thus, enabling their evolution in a climate-changing scenario.

But what is a landrace? Zeven (1998) defined them as varieties with a high capacity to tolerate biotic and abiotic stress, resulting in high yield stability and intermediate yield level under a low input agricultural system. Camacho-Villa et al. (2005) redefined them as dynamic populations of a cultivated plant that have a historical origin, distinct identity and lack formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems. Alternative definitions have also been proposed but usually express similar ideas. For example, as reviewed by Conversa

* Corresponding author.
E-mail address: martamaria.moreno@uclm.es (M.M. Moreno).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.scienta.2023.112128
Received 12 March 2023; Received in revised form 13 April 2023; Accepted 3 May 2023
Available online 9 May 2023

et al. (2020), landraces have also been defined as crops that have developed their unique characteristics through *in situ* selection operated by growers, have never been subjected to formal plant breeding and represent a constituent part of rural cultures and landscapes.

As these definitions express, one of the main characteristics of landraces is their genetic diversity. This diversity is structured at different levels (between and within populations of the landrace), and it continues to evolve when they are grown *in situ* (Negri et al., 2009). Indeed, each farmer applies a different selection method and criteria in a somewhat different environment. Consequently, the populations of a landrace grown by different farmers are diverse. On the other hand, mass selection, usually unconsciously performed by farmers, leads to the configuration of landraces as population varieties. That is a mix of different genotypes with shared features.

This diversity represents the cornerstone of the traditional agricultural homoeostatic systems, which are able to offer certain yield stability, as expressed by Zeven in his definition. Indeed, genetic diversity enables a minimum production to be maintained when an external factor strikes an area. This traditional agricultural scenario opposes the current intensive agricultural systems in which production relies on a narrow genetic diversity represented in a few commercial varieties, thus resulting in a high level of genetic vulnerability.

In order to further study the phenotypic diversity present in landraces of vegetable crops, tomato was selected as a model species in the present study, as it is highly valued in on-farm conservation. In fact, in recent surveys, tomato is, by difference, the most represented species in recent field surveys of landraces, as reported, for example, in the Puglia region of Italy (Conversa et al., 2020).

Within tomato, the 'Moruno' landrace was selected as a representative example of Spanish landrace, which has not been thoroughly studied in the past, and it is highly appreciated by its organoleptic profile (Moreno et al., 2019). Spain and Italy can be considered as secondary centers of diversity of tomatoes due to their early introduction in the area in the sixteenth century by Spaniards. Both countries introduced it rapidly in their diet. Matthioli, 2023, an Italian physician, described the consumption of tomato in 1544 fried with salt and pepper as the eggplants, and tomato appeared on the shopping list of a hospital in Seville in 1608 (Hamilton, 1976). In a short time, descriptions of tomato fruits with different colours, shapes and sizes arise in Europe in the sixteenth century (van Andel et al., 2022), confirming the existence of a great diversity.

Few studies have been focussed in the analysis of phenotypic variation in its different scales: between tomato landraces, between populations and within populations. An initial evaluation performed with Eastern Spanish tomato landraces confirmed that the variability is so high that the spectra of variation of different landraces even overlap (Cebolla-Cornejo et al., 2013). It was then already suggested that farmers may have applied strong selection pressure on morphological traits, including size, shape, ribbing or colour, after accidental crossings to depurate segregating generations. Even in some cases, the differentiation of a landrace relies on a single trait. It would be the case of long-life 'Penjar' tomatoes, in which the *alc* allele of the *nor* gene was introgressed and selected in different genetic backgrounds (Casals et al., 2012).

This is not always the case, as differences even in fruit shape have been identified within landraces in different European tomato varieties remarking differences in fruit shape within the same landrace. That would be the case of 'Santorini' in Greece, with different morphologies depending on the use (Terzopoulos and Bebeli, 2010) or 'A pera Abruzzese' in Italy with marked differences in fruit shape (Mazzucato et al., 2010).

The present study is therefore targeted to further contribute to the dissection of the variability present in landraces. For that purpose, several populations of the 'Moruno' tomato landrace, selected as a model, were characterized, and the variability within and between populations was studied. Following a common practice in germplasm banks, different populations were grown in different years to evaluate which would be the best control in germplasm evaluations. This work also tries to cover a gap in previous studies, aiming to establish a relationship between the diversity in landraces and farmers' practices.

2. Materials and methods

2.1. Surveys conducted with 'Moruno' farmers

Farmers (17) growing the 'Moruno' landrace in Ciudad Real (central Spain) were approached by the research team and asked to complete a survey regarding different aspects of the landrace. A 15-item survey was designed (Supp. Table 1) to analyse i.a. how farmers define the landrace, which are the most important traits, size of the smallholding and targets of production, coexistence with other materials, seed exchange and seed degeneration, seed selection procedures, the incidence of pests and diseases, yield and prices (received and expected). These questions would enable the identification of links with the different levels of diversity between and within populations of the landrace and aspects related to the genetic erosion process.

Some of the farmers did not answer specific questions or provided vague answers. It was the case of questions related to the number of plants grown (15 answers), price aspects (11–13 answers), losses by diseases (11 answers), and the age of farmers (15 answers).

2.2. Research site, plant materials and field trials

A collection of 30 populations (accessions) belonging to the to the Spanish ''Moruno'' tomato landrace (Table 1) was analysed in a 4-year open-air study regarding morphological and agronomical traits. The accessions were collected at different times, and they were evaluated as they were accessed. All of them were obtained from local farmers except for SL-41, kindly provided by the germplasm bank of Centro de Investigación y Tecnología Agroalimentaria (BGHZ-CITA, Spain). They are currently maintained at the seedbank located at the Higher Technical School of Agricultural Engineering (University of Castilla-La Mancha) in Ciudad Real (Spain).

These populations have an indeterminate growth habit and are characterised by medium to large sized fruits, a dark red or brown colour, strong to medium-ribbing intensity, dark shoulders and a predominantly flattened shape. The study was developed using ten different populations per year during the Years 1 to 3; in the Year 4, seven of the populations studied in the previous seasons were selected considering the results of previous years as well as their sensory evaluation (Villena et al., 2023). For all the field trials, the commercial 'Royesta' F1 hybrid was used as control.

The spring-summer field trials (May to Sept) were carried out in adjacent plots at the experimental farm of the Research Centre "El Chaparrillo", Regional Institute for Agro-Food and Forestry Research and Development (39°0′N, 3°56′W, altitude 640 m), in Ciudad Real (Central Spain). The climate of this region is continental Mediterranean, with a mean, maximum and minimum air temperatures during the four cropping seasons at a range of 20.4 to 22.3 °C, 28.3 to 31.0 °C and 11.4 to 13.0 °C, respectively. The soil was a Xeralfs, Petrocalcic Palexeralfs (USDA, 2010).

The field trials were performed using a randomised complete block design with four replicates. Each experimental plot consisted of eight plants (32 plants per population) separated 2.0 m between rows and 1.0 m between plants. For the different evaluations, the central six plants of each plot were considered.

The tomato seeds were sown in a commercial nursery during the first half of April, and transplanted in the open air on beds mulched with black plastic 60 μm thick between the 6th and the 23rd of May, depending on the year. Fertilisation consisted of organic vermicompost (1 kg $l.m.^{-1}$, 2.2% N, 1.5% P and 2.3% K in organic forms), and no chemical fertilisers or pesticides were applied, adopting organic farming

Table 1
Populations evaluated of the 'Moruno' tomato landrace and year of cultivation.

Year	Accession	Local name	Origin		
			Town	Province	Coordinates
1	SL-2	"Plano de El Avellanar"	San Pablo de los Montes	Toledo	39°32′N 4°19′W
1	SL-6	"Moruno de San Pablo"	San Pablo de los Montes	Toledo	39°32′N 4°19′W
1	SL-11	"Moruno de El Avellanar"	San Pablo de los Montes	Toledo	39°32′N 4°19′W
1,4	SL-25	"Moruno"	La Malaguilla	Guadalajara	40°49′N 3°15′W
1	SL-27	"Morado"	Anchuras	Ciudad Real	39°28′N 4°50′W
1,4	SL-33	"Negrillo"	Almoguera	Guadalajara	40°18′N 2°59′W
1	SL-41*	"Negro rosa"	Elche de la Sierra	Albacete	38°27′N 2°3′W
1,4	SL-62	"Moruno"	Socuéllamos	Ciudad Real	39°17′N 2°47′W
1,4	SL-72	"Bonito"	Ciudad Real	Ciudad Real	38°59′N 3°55′W
1	SL-74	"Moruno"	Ciudad Real	Ciudad Real	39°0′N, 3°56′W
2,4	SL-112	"Moruno de Aguas Nuevas"	Aguas Nuevas	Albacete	38°55′N 1°55′W
2	SL-113	"Moruno"	Aguas Nuevas	Albacete	38°55′N 1°55′W
2	SL-114	"Moruno"	Aguas Nuevas	Albacete	38°55′N 1°55′W
2	SL-116	"Moruno"	Aguas Nuevas	Albacete	38°55′N 1°55′W
2	SL-122	"Morao"	Aguas Nuevas	Albacete	38°55′N 1°55′W
2	SL-136	"Morao"	La Poblachuela	Ciudad Real	38°59′N 3°55′W
2	SL-154	"Moruno"	Elche de la Sierra	Albacete	38°27′N 2°3′W
2	SL-160	"Moruno"	Albacete	Albacete	38°59′N 1°51′W
2	SL-163	"Morao"	Arroba de los Montes	Ciudad Real	39°09′N 4°32′W
2	SL-165	"Morado"	Navas de Estena	Ciudad Real	39°29′N 4°31′W
3	SL-20	"Gordo"	Priego	Cuenca	40°27′N 2°19′W
3	SL-140	"Morao"	Arenales de San Gregorio	Ciudad Real	39°18′N 3°01′W
3	SL-143	"Moruno"	Socuéllamos	Ciudad Real	39°17′N 2°47′W
3	SL-149	"Negro"	Riópar	Albacete	38°30′N 2°25′W
3	SL-150	"Negro"	Riópar	Albacete	38°30′N 2°25′W
3,4	SL-204	"Morao dulce"	Priego	Cuenca	40°27′N 2°19′W
3	SL-207	"Negro plano"	Brihuega	Guadalajara	40°45′N 2°52′W
3	SL-208	"Morao"	Priego	Cuenca	40°27′N 2°19′W
3	SL-209	"Moruno"	Elche de la Sierra	Albacete	38°27′N 2°3′W
3,4	SL-252	"Moruno"	El Alcornocal	Ciudad Real	40°44′N 3°52′W

[*] Provided by BGHZ-CITA, Spain.

practices (EC n.834/2007). Irrigation amounts, estimated from the reference evapotranspiration and the phenological stage of the crops, were applied daily by a trickle irrigation system following the methodology proposed by Allen et al. (1998). Removal of lateral shoots and basal leaf pruning operations were carried out on plants. The crop cycles ranged from 130 to 140 days.

2.3. Morpho-agronomical characterisation

Fruits were harvested at the red-ripe stage. They were hand-picked through a total of 7 to 9 harvests depending on the year (August to September). At each harvest, all the fruits were classified as commercial or non-commercial, and then counted and weighed separately. The total yield and fruit number were calculated as the sum of commercial and non-commercial fractions. Mean fruit weight was determined from yield and fruit number. Then, the variables considered for the quantitative agronomical study were: Commercial fruit number (cFN), Commercial yield (cY), Mean commercial fruit weight (cFW), Non-commercial fruit number (ncFN), Non-commercial yield (ncY), Mean non-commercial fruit weight (ncFW), Total fruit number per plant (tFN), Total yield per plant (tY), Mean fruit weight (tFW).

In the middle harvest (first half of September), one representative fruit was obtained from each plant for the morphological study. For that, different qualitative and quantitative descriptors were used based on the International Plant Genetic Resources Institute's guidelines (IPGRI, 1996), complemented with added descriptors based on previous works (Cebolla-Cornejo et al., 2013; Cortés-Olmos et al., 2015).

The quantitative morphological descriptors used in the evaluation and the corresponding units and abbreviatures, were: Fruit length (mm, FL), Fruit highest width (mm, FHW), Fruit lowest width (mm, FLW), Fruit mean width (mm, FWD), Fruit length to width ratio (LWR), Fruit estimated volume (cm^3, estimated by considering tomato fruit as a sphere, FV), Fruit weight (g, FW), Fruit estimated density (g cm^{-3}, FD), Depth of fruit corky vasculature (mm, DV), Width of fruit corky vasculature (mm, WV), Pericarp thickness (mm, PT), Skin thickness (mm, ST), Locule number (LN), Fruit firmness (measured with a Bertoluzzi FT327 penetrometer with a 8 mm probe, kg cm-2, FIR), Fruit conservation (fruits kept at 8 ± 0.5 °C and judge by appearance, days, FC), Dry matter (expressed as grams per 100 g fresh weight,%, DM), Consistence (measured as the distance that the homogenised fruits flowed in 30 s under its own weight along a level surface with a standard Bostwick consistometer, CSC Scientific, 1–800–458–2558, USA, cm Bostwick, CON). Additionally, fruit shoulder colour was also measured using CIE Lab coordinates (L*, a*, b*, a*/b* ratio) with a colourimeter (Minolta Chroma meter CR400/410). Measurements were also taken separately in the equatorial (eL*, eA*, eb*, ea*b*) part of the fruits (two measurements for each part).

The qualitative morphological descriptors used were: Fruit shape (FS), Fruit cross-sectional shape (FCS), Shape of pistil scar (SLS), Width of pistil scar (WLS), Width of pedicel scar (mm, WPS), Fruit shoulder shape (FSS), Fruit blossom end shape (FBS), Fruit ribbing (FR), Fruit cracking (FCR), Fruit hollowness (FH), Intensity of greenback (IG).

2.4. Statistical analysis

For each descriptor, different coefficients of variation were calculated: within population (using fruit measurements), between populations (using mean values for each population) and between years (calculated as the coefficient of variation of the mean coefficient of variation of both years being considered). In the case of qualitative variables, IPGRI descriptors provide a number for each descriptor, usually related with intensity of the descriptor (*i.e.* fruit shape varies from 1 for flattened to 3 rounded, other shapes were not observed). The coefficients of variation were calculated using these values.

A comprehensive study of the effect of population x environment combination on morphoagronomical traits was conducted using a graphical MANOVA biplot representation. Bonferroni circles were used to represent the confidence intervals ($P = 0.05$). Their projection on each variable enables the identification of significant differences between groups. For the variables in which the MANOVA biplot did not detect significant effects of the cultivar-environment of cultivation, the vectors were marked in dashed lines. Prior to MANOVA biplot analysis, variables were normalised using Autoscaling (mean centre and scaling each variable to unit standard deviation) and Haar transformation (wavelet transform). Normality of transformed variables were assessed with Shapiro-Wilk test in SPSS 22.0 software (NYSE: IBM, Armonk, USA). Multibiplot, a freeware licensed software, was used to perform the MANOVA biplot analysis (Vicente-Villardón, 2015).

In order to complement the information on morphoagronomical traits with metabolomic traits and flavour perception, the data regarding sensory evaluation, sugar, acid and volatile accumulation (10.5281/zenodo.6963114) obtained in the same field study and published in Villena et al. (2023) were reanalysed statistically. The methods can be consulted in the original publication and the results presented here are different and complementary to those published in the first instance. For that purpose, principal component analysis (PCA) of the accumulation of soluble solids including sugars and acids (Supp. Fig. 3) and volatile organic compounds, VOCs (Supp. Fig. 4) were performed using S-Plus v.8.01 (Insightful Corp., Seattle, WA, United States). In both cases, the size of the score label of each population in the biplots of PCAs was proportional to the global acceptability of the population in sensory evaluations.

3. Results

3.1. Farmers' survey

The survey was answered by 17 local farmers, which is rather representative considering that landraces are experiencing a deep genetic erosion process in Spain. When farmers were asked to define the 'Moruno' landrace and identify the peculiar traits of the landrace, interestingly, a vast majority (70.5%) included flavour as a defining trait (Fig. 1A). Mostly, it was described as very sweet, flavourful, and with specific aroma tinges. But apart from it, 88% of them recognised the

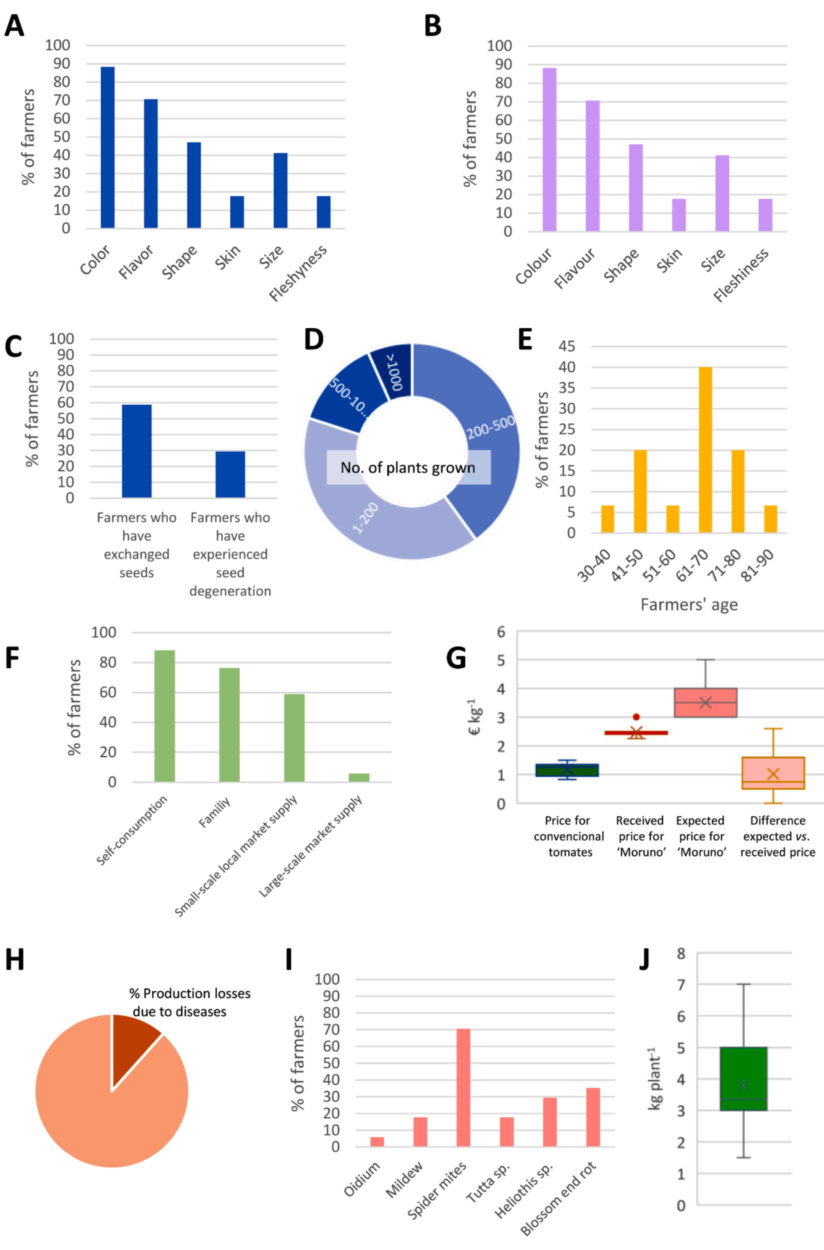

Fig. 1. Results of the surveys conducted with 'Moruno' farmers. **(A)** Percentage of farmers that referred to each trait as important in their definition of the 'Moruno' landrace. **(B)** Percentage of farmers that used each trait as a criterion of the selection of seed for the next year. **(C)** Incidence of seed exchange and seed degeneration. **(D)** Distribution of the number of plants grown by each farmer. **(E)** Distribution of farmers' age. **(F)** Destination of production. **(G)** Prices received and expected by farmers. **(H)** Production losses due to diseases. **(I)** Relative importance of pests, diseases and physiological disorders in the production. **(J)** Mean yield per plant.

variety by its typical purplish colour. Farmers also included shape and size as important traits (47% and 41.1%). In general, they identified medium-sized slightly-flattened fruits with different degrees of ribbing. Nonetheless, discrepancies were identified between some farmers in terms of fruit size (medium to big) and fruit shape (flattened to round). 17.6% of the farmers also highlighted the thinness of the skin and its fleshiness. An ideal representation of these traits is depicted in Supp. Fig. 1.

They selected the seed for the next year, usually using external appearance as selection criteria, as only 12.5% used flavour as a selection criterion (Fig. 1B). Interestingly, none of them highlighted yield or fleshiness as a selection criterion, and all of them confirmed that they made the selection on a fruit basis and not on a plant basis. Most farmers (58.8%) had previously exchanged seeds with other farmers (Fig. 1C), while only a few had experience seed degeneration (29.4%). All of them used to grow other tomato varieties at the same time.

In general, the size of the smallholdings was reduced, and 40% of them planted less than 200 plants, 40% between 200 and 500, 13.3% between 500 and 1000, and 6.6% more than 1000 plants (Fig. 1D). The size of the smallholdings is highly related to the destination of the production. The majority of the farmers (88.8%) dedicate part of their production to self-consumption and 76,5% share production with family and friends. Only 58% of the farmers targeted part of the production to retail in small local markets and 5.9% to large-scale markets (Fig. 1F). In general, farmers selling in retail markets reserved between 10 and 20% of the production for self-consumption and distribution between family and friends. Most of the farmers were older than 60 years (Fig. 1E). Nonetheless, a certain degree of generational renewal was detected with more than 25% of them being younger than 50 years old.

In the retail market, the price received by farmers was relatively high, with a mean value of 2.48 € kg^{-1}, and relatively stable amongst the farmers (Fig. 1F). This price is considerably higher (x2.1) than the one received for standard commercial varieties. Still, this price was not considered fair, and farmers confirmed higher expectations in the selling price, with a mean fair price estimation of 3.5 € kg^{-1}, but with a high level of variability. That means that they received 70% of the expected fair price. In part, the high price expectations might be related to yield issues rather than with the incidence of pests and diseases. It seems that pests and diseases, being important, would not be crucial, causing, on average, an 11.6% loss of production (Fig. 1G). In this case, most farmers (70.6%) had problems with spider mites (Fig. 1H). The incidence of other pests was lower, with 17.6% referring *Tuta absoluta* as a problem and 29.4% *Heliothis* sp. As regards diseases and physiological disorders, oidium (5.9%) and mildew (17.6%) seemed to have a low incidence, being more important the problems with blossom end rot. It is clear, then, that the limiting step is the low yield of their populations, as most of them, when asked, reported yields with a wide range between 1.53 and 7 kg $plant^{-1}$and a mean of 3.8 kg $plant^{-1}$ (Fig. 1I).

3.2. Decomposition of within population, between population and between years variation

Coefficients of variation were calculated for each descriptor and population both for quantitative and qualitative traits (Supp. Table 2). In the case of the commercial hybrid control 'Royesta', three unusually high values were identified in year 4. These values were identified as outliers (Grubb's test, $P = 0.05$) and discarded during the calculation of mean values.

The mean level of variation within populations of 'Moruno' landrace, considering all the descriptors evaluated, was roughly stable during the 4 years (approximately 16%), even though different populations were assayed each year. Slightly higher values were obtained, though, in year 2. These values were similar to the mean level of variation of the landraces assayed during two years and almost doubled the variation found in the commercial hybrid 'Royesta' (Fig. 2).

The analysis of the levels of variation in the populations assayed in Years 1 and 4 enabled a comparison of the variability within populations, between populations and between years for each descriptor. The highest levels of variation within population in the traditional materials evaluated were found for ecuatorial colour parameters (a* and a*/b* ratio), followed by commercial and non-commercial yield and fruit number, fruit cracking, the width of the corky area of fruit vasculature, and fruit firmness and hollowness (Fig. 3), all of them with coefficients of variation within population higher than 19%. In the case of colour, it should be considered that colour distribution in the fruit, both in the green shoulder and equatorial area, is not uniform and represents *per se* a typical characteristic of the fruit (Supp. Fig. 1).

The profile of variation in the commercial hybrid was very similar, usually with levels lower than in the 'Moruno' landrace (Fig. 3). Only in fruit conservation, fruit colour perceptual lightness in the equatorial area (L*), the shape of the pistil scar, the width of the pistil scar, fruit shoulder shape and ribbing and the intensity of greenback, the mean variation found in 'Royesta' was considerably higher than that of the landrace. On the contrary, the lowest levels of variation in 'Moruno'

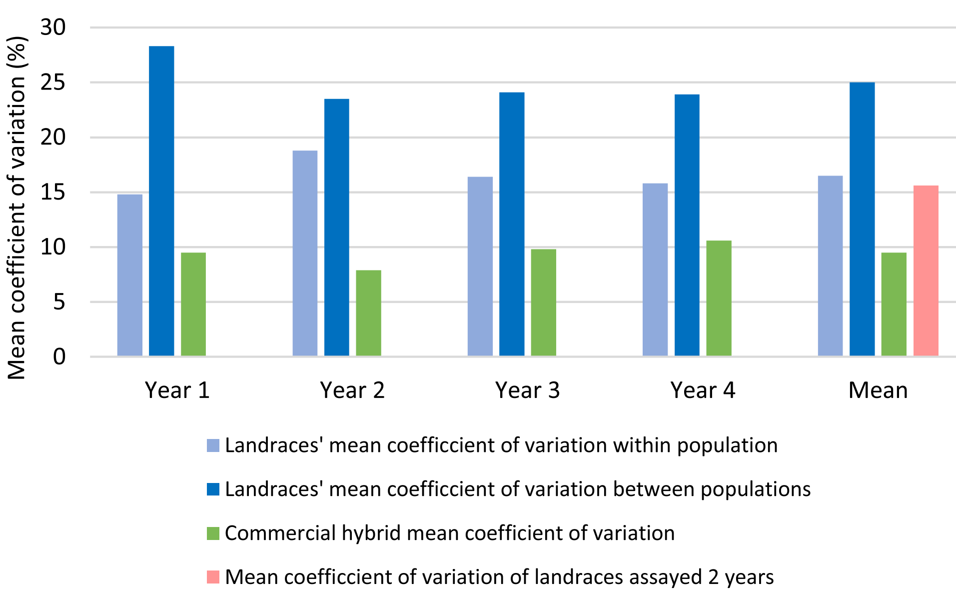

Fig. 2. Mean coefficient of variation (%) of the descriptors evaluated in landraces and the commercial hybrid control 'Royesta'.

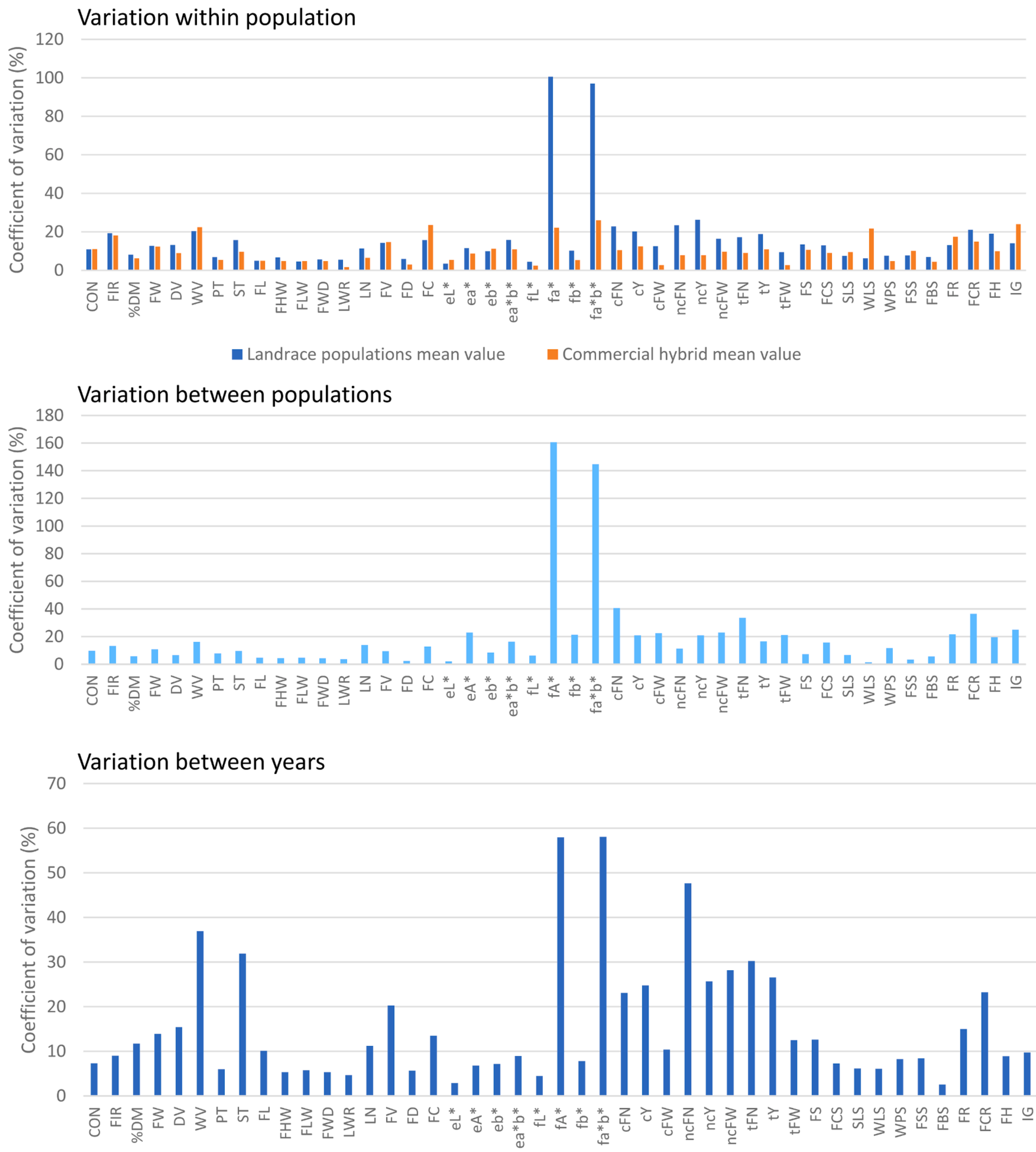

Fig. 3. Mean coefficient of variation of descriptors **(TOP)** within populations grown both in Years 1 and 4 of the landrace 'Moruno' and the comercial hybrid 'Royesta', **(MIDDLE)** between populations of 'Moruno' grown both in Years 1 and 4 **(BOTTOM)** between year means considering landraces grown both in Years 1 and 4. CON: Consistence, FIR: Fruit firmness,%DM:%Dry matter, FW: Fruit weight, DV: Depth of fruit corky vasculature, WV: Width of fruit corky vasculature, PT: Pericarp thickness, ST: Skin thickness, FL: Fruit length, FHW: Fruit highest width, FLW: Fruit lowest width, FWD: Fruit mean width, LWR: Fruit length to width ratio, LN: Locule number, FV: Fruit estimated volume, FD: Fruit estimated density, FC: Fruit conservation, eL*: Fruit ecuatorial colour L*, eA*: Fruit ecuatorial colour a*, eb*: Fruit ecuatorial colour b*, ea*b*: Ecuatorial colour a*/b* ratio, fL*: Fruit shoulder colour L*, fA*: Fruit shoulder colour a*, fb*: Fruit shoulder colour b*, fa*b*v: shoulder colour a*/b* ratio, cFN: Commercial fruit number, cY: Commercial yield, cFW: Mean commercial fruit weight, ncFN: Non-commercial fruit number, ncY: Non-commercial yield, ncFW: Mean non-commercial fruit weight, tFN: Total fruit number per plant, tY: Total yield per plant, tFW: Mean fruit weight, FS: Fruit shape, FCS: Fruit cross-sectional shape, SLS: Shape of pistil scar, WLS: Width of pistil scar, WPS: Width of pedicel scar, FSS: Fruit shoulder shape, FBS: Fruit blossom end shape, FR: Fruit ribbing, FCR: Fruit cracking, FH: Fruit hollowness, IG: Intensity of greenback.

(<7%) were found in fruit colour perceptual lightness in both areas (L*), fruit width, fruit length, fruit length-to-width ratio, fruit estimated density, width of pistil scar, pericarp thickness and blossom end shape.

The inter-populations effect was even higher than the intra-population effect (Fig. 2). Effectively, higher variation was observed between populations of 'Moruno' grown in the same year (mean value of 25%) than within populations (16.5%). Between populations, the lowest variation (<8%) was found for fruit colour perceptual lightness in both areas (L*), fruit width and length, fruit length to width ratio, fruit shape, estimated density, shape and with of pistil scar, pericarp thickness, blossom end shape (Fig. 3). Again, the highest variation (>30%) was found for colour parameters, fruit number and fruit cracking.

The year effect was also notable (Fig. 2), and the mean coefficient of variation of averages of descriptors in the populations assayed two years (17.3%) was similar to the mean variation found within population (16.2%). In the landrace, the lowest variation between years (<7%) was found for blossom end shape, fruit equatorial colour perceptual lightness in both areas(L*), fruit width and length to width ratio, fruit estimated density, pericarp thickness width and shape of pistil scar and fruit colour a* in the equatorial area (Fig. 3). The highest variation was found on some of the a*, b* colour parameters, fruit number, non-commercial and total yield, width of the corky area of the vasculature, skin thickness, fruit cracking and volume, with values higher than 20%.

A MANOVA biplot analysis was performed with the phenotypic values of the populations grown over two years to gain a deeper insight in the different levels of variation present in the landrace. The biplot clearly separated the commercial hybrid from the landrace population in its first component (Fig. 4). This component was negatively related mainly to locule number and fruit conservation and positively with consistence, ea* and fruit estimated volume. Indeed, the populations of 'Moruno' have a higher size and number of locules.

The four-year replicates of the commercial hybrid plotted over a wide area, confirming a high level of variability between years, that was considerably higher than that found. This inter-year variation was only slightly lower than the inter-population variability found between 'Moruno' populations. In this case, some of the populations, including SL-112, SL-25 and SL-62, were considerably more stable through the years than others, such as SL-252, SL-72 and especially SL-204, whose populations were plotted at a considerable distance (Fig. 4). Those populations with the highest stability also represented the core area of distribution of the landrace variation. Only the repetitions of SL-204 and SL-252 in the third year and SL-72 in the first year were plotted outside this core area of distribution.

The MANOVA biplot was reanalysed using only those accessions grown in years 1 and 4 to provide a better idea of the effect of the environment. In general, the populations of 'Moruno' were affected in a similar way by the change of environment (Supp. Fig. 2). All of them reduced the values on the first principal component (PC) and especially on the second PC. Only SL-62 was affected in a higher degree. Interestingly SL25, SL-33 and SL-72 were grouped together in both years. In year 1 SL-62 did not group with the rest of populations of the landrace, but in thear 4, I came closer to the core of variation. The effect on the first PC was lower for 'Royesta' and with a different direction, though a high decrease in the second PC was also observed. Thus, a genotype x environment interaction was detected, with a differential behaviour between landrace populations and the commercial hybrid control (see evolution vectors in Supp. Fig. 2, that have different directions for landrace populations and the commercial hybrid).

3.3. *Variation in agronomically essential traits*

The level of variability was further studied in two essential agronomical traits: commercial weight and yield. Mean commercial fruit weight varied through the years (Fig. 5). This variation was related to not only environmental effects, but also genetic effects, as different populations were assayed each year. Nonetheless, the impact of the environment is evident when the results from the commercial hybrid 'Royesta' are analysed, as no genetic variation is expected in this material. 'Royesta' presented a commercial fruit weight close to the populations of 'Moruno' with a lower weight. Mean weight, in this case, was higher in Year 1 and lower in Year 3, with intermediate values in Years 2 and 4. Nonetheless, within each year, the variability for commercial

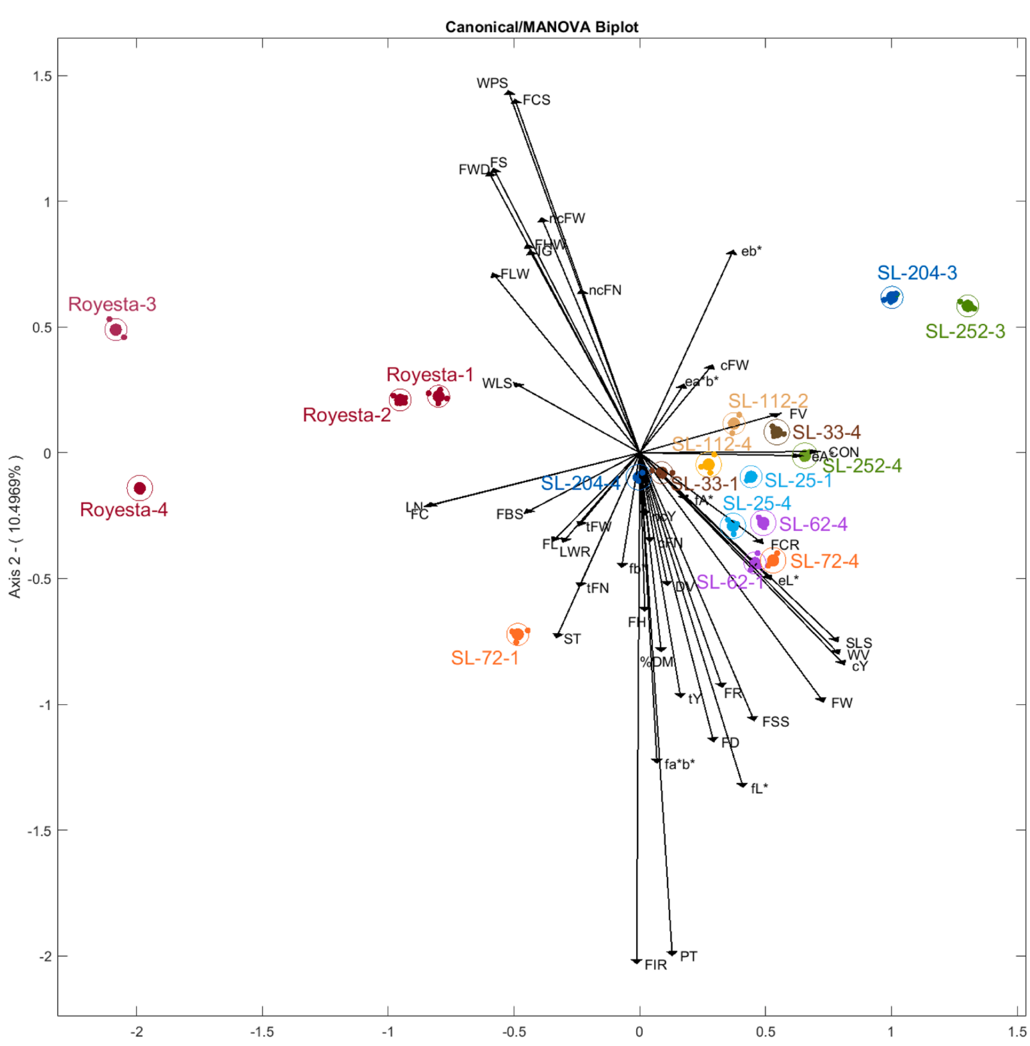

Fig. 4. MANOVA biplot analysis of the phenotypic values of the populations of 'Moruno' grown two years and the commercial hybrid control 'Royesta'. Circles represent Bonferroni confidence intervals. The effect of genotype was significant for all variables. CON: Consistence, FIR: Fruit firmness,%DM:% Dry matter, FW: Fruit weight, DV: Depth of fruit corky vasculature, WV: Width of fruit corky vasculature, PT: Pericarp thickness, ST: Skin thickness, FL: Fruit length, FHW: Fruit highest width, FLW: Fruit lowest width, FWD: Fruit mean width, LWR: Fruit length to width ratio, LN: Locule number, FV: Fruit estimated volume, FD: Fruit estimated density, FC: Fruit conservation, eL*: Fruit ecuatorial colour L*, eA*: Fruit ecuatorial colour a*, eb*: Fruit ecuatorial colour b*, ea*b*: Ecuatorial colour a*/b* ratio, fL*: Fruit shoulder colour L*, fA*: Fruit shoulder colour a*, fb*: Fruit shoulder colour b*, fa*b*v: shoulder colour a*/b* ratio, cFN: Commercial fruit number, cY: Commercial yield, cFW: Mean commercial fruit weight, ncFN: Non-commercial fruit number, ncY: Non-commercial yield, ncFW: Mean non-commercial fruit weight, tFN: Total fruit number per plant, tY: Total yield per plant, tFW: Mean fruit weight, FS: Fruit shape, FCS: Fruit cross-sectional shape, SLS: Shape of pistil scar, WLS: Width of pistil scar, WPS: Width of pedicel scar, FSS: Fruit shoulder shape, FBS: Fruit blossom end shape, FR: Fruit ribbing, FCR: Fruit cracking, FH: Fruit hollowness, IG: Intensity of greenback.

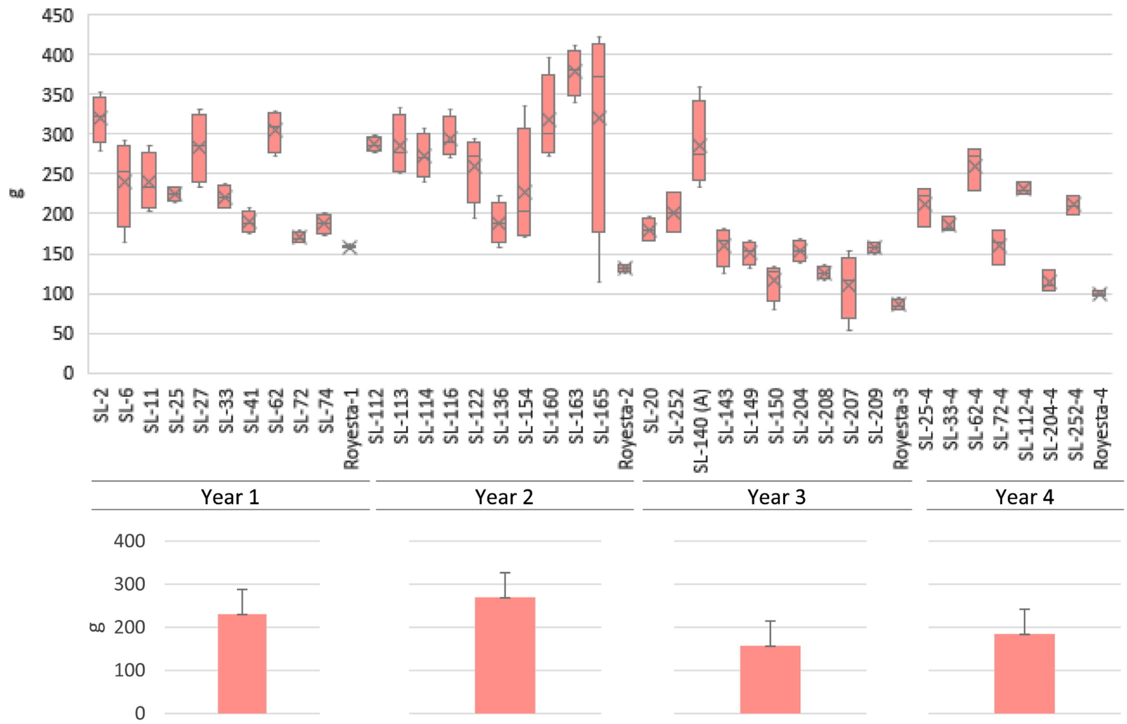

Fig. 5. Boxplots of commercial fruit weight (g) of 'Moruno' populations and the commercial hybrid 'Royesta' and mean commercial fruit weight per year (bottom). Year of cultivation is indicated following population name for populations grown more than one year.

fruit weight in 'Royesta' was minimum, with values oscillating between 1.4% in Year 1 and 7.4% in Year 3 (Supp. Table 2).

Variability was also found between populations of 'Moruno' in commercial weight (Fig. 5). Although all the populations were characterised by medium to big fruit sizes, an environmental effect over the means was also evident in the populations grown over two years. For example, fruit weight in Year 2 was higher than in Year 1, and this one was higher than Years 2 and 3.

Not only the means varied amongst the accessions but also the level of variation within population. Indeed, the variability in commercial

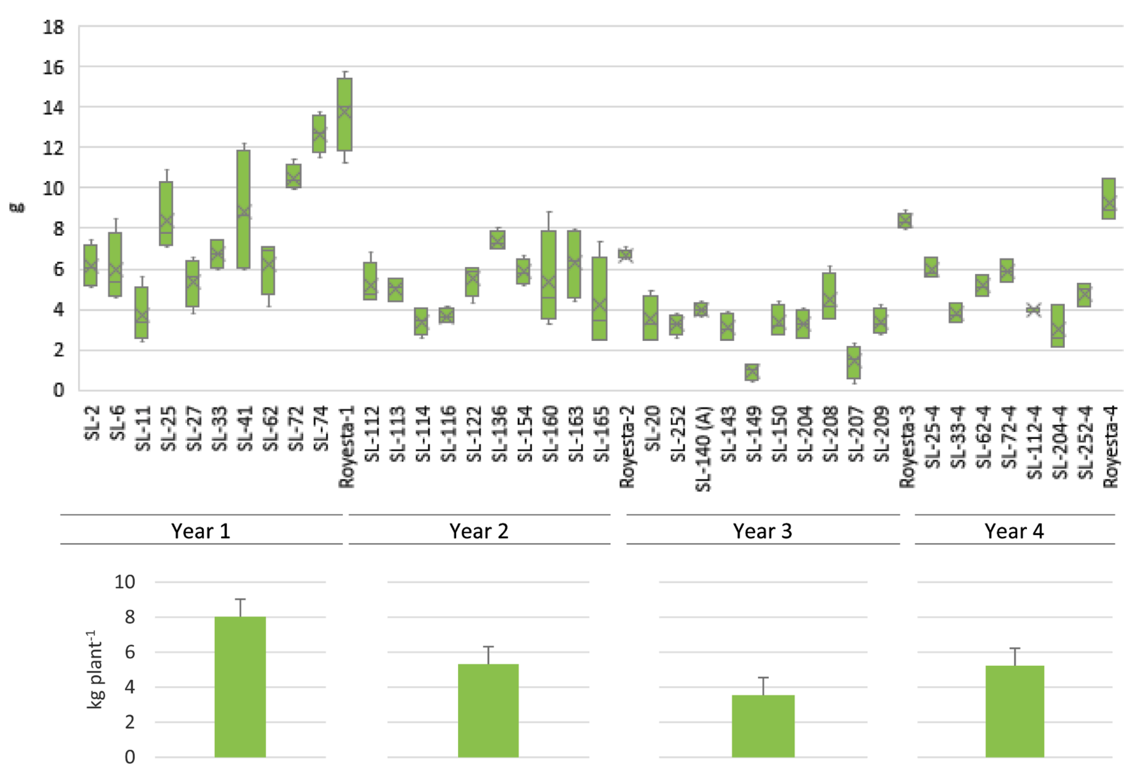

Fig. 6. Boxplots of commercial yields (kg plant^{-1}) of 'Moruno' populations and the commercial hybrid 'Royesta' and mean yields per year **(bottom)**. Year of cultivation is indicated following population name for populations grown more than one year.

fruit weight considerably varied amongst the accessions of 'Moruno'. For example, in the first year, the lower coefficient of variation for commercial fruit weight ranged from 4.01% for SL-25 to 22.69% for SL 6, with values 2.9 to 22.4 times higher than the level of variation obtained in the commercial control (Supp. Table 2). Several populations, such as SL-25, SL-33, SL-62 and SL-72, presented reasonable levels of variation. This constrained variability was also maintained during Year 4 when they were grown again. Nonetheless, the values obtained this year were slightly higher (Fig. 5, Supp. Table 2). Even higher variability for this parameter was found during Year 2, but again, the accession SL-112 (grown in Years 2 and 4) showed a contained level of variation. This trend was also found in the accessions SL-204 and SL-252 grown in Years 3 and 4.

The better environmental conditions of Year 1 were also evident for commercial yield, which was higher in 'Royesta' and the repeated 'Moruno' populations in this year. During the first year, the mean yield of 'Moruno' populations averaged 7.6 kg plant^{-1}, a bit more than one-half (55%) of the yield of 'Royesta', 13.8 kg plant^{-1} (Fig. 6). Nonetheless, it was possible to identify populations of the landrace (SL-72 and SL7–4) that exceeded 10 kg plant^{-1}. In Year 2 the environmental conditions were not as favourable for the commercial hybrid, and the mean yield of the 'Moruno' populations was 77% of that of 'Royesta', as its mean yield dropped to 6.7 kg plant^{-1}. Again, in Year 2 it was possible to identify a population that SL-136 that with 7.4 kg plant^{-1} surpassed the control. This accession, though, was relatively variable in fruit weight.

Year 3 was slightly better for 'Royesta', with a yield reaching 8.3 kg plant^{-1}, but the performance of the populations assayed this year was not so good, with a mean yield of 3.1 kg plant^{-1}, a 37% of the yield of the control (Fig. 6, Supp. Table 2). The performance in Year 4 was similar to Year 3, and 'Royesta' yielded 9.3 kg plant^{-1}, doubling the mean yield of the 'Moruno' populations (4.6 kg plant^{-1}). The good performance observed in year 1 in SL-72 and SL-25 was not repeated in Year 4, but their yields were higher than the rest of the 'Moruno' populations grown two years. Other populations, such as SL-62, were not as affected by the environment, but their best performance only approached the worst performance of SL-72.

In order to evaluate the impact of environment on yield, the environmental conditions were reviewed (Supp. Table 3). Probably the lower yields registered in the commercial control 'Royesta' during Year 2 were related to considerably lower mean and minimum values of relative humidity during the whole cycle that may have resulted in a higher degree of flower abortion and lower fruit set, especially in the initial stages. Additionally, mean and maximum temperatures were considerably higher during the last three years.

As a whole, the 'Moruno' populations only reached 55% of the yield of the commercial control (Fig. 6). Nonetheless, SL-72, one of the best populations through the years, averaged 70% of the corresponding yield of 'Royesta'.

The variability in commercial yield followed a similar trend to that found for commercial fruit weight (Fig. 6, Supp. Table 2). In fact, the correlation between the coefficients of variation of both variables was 0.52. Nonetheless, the variability found in commercial yield in the hybrid control was considerably higher than that found for commercial weight, with coefficients of variation between 3.7% and 13.7%, with Years 1 and 4 being more variable. Amongst the populations of 'Moruno', high differences were found in the level of variability of commercial yield. Populations such as SL-41 in Year 1 or SL-160, SL-163 and SL-165 in Year 2 were highly variable. On the other extreme, population SL-72, with a good performance in terms of commercial yield, had coefficients of variation in Years 2 and 4 lower than the commercial hybrid control (5,8% *vs.* 13,7% and 9.4% *vs.* 11.2%, respectively).

3.4. Variation in the accumulation of sugars and acids and volatile organic compounds

Principal component analysis of the accumulation of sugars and acids (Supp. Fig. 3) and VOCs (Supp. Fig. 4) confirmed a high degree of variation. Nonetheless, populations grown on the same year tended to group together, especially in the case of VOCs. This fact suggests that the impact of the environment on metabolomic traits related to flavour is higher than the effect of genotype. Even the commercial F1 hybrid 'Royesta' was highly affected by the environment.

Amongst the populations grown in year 1, SL-72, SL-27, SL-62 and SL-33 presented high overall flavour acceptability in the sensory evaluation by a consumer panel. In Years 2 and 3, outstood SL-112, SL-208, SL-204, SL-20 and SL-252. In the last year, in which the best populations were grown again, the highest values of overall flavour acceptability were obtained by SL-112, SL-62, SL-33, SL-252 and SL-204. SL-72 and SL-25 presented slightly lower values.

4. Discussion

Several factors threaten the survival of landraces grown *in situ*. Amongst them, it has often been reported that their cultivation is related to old farmers who cultivate a small number of plants for self-consumption or targeted at local markets. This was the case on the East of Spain in 2007, as reported by Cebolla-Cornejo et al. (2007), Missio et al. (2018). In the former case, one third of the visited farms had less of 40 plants of landraces, maintained by farmers older than 60. Another third, with up to 100 plants, targeted production to local markets and only one third sold production to wholesalers, with an average plant number of 640. In general, 43% of the farms had a commercial profile. In the case of the 'Moruno' landrace, sixteen years later and in central Spain, these figures seem to have evolved, and 64% of the smallholdings had a commercial profile, and 60% planted more than 200 plants of the landrace.

Interestingly, the appreciation of the landraces was commonplace, and even with commercial profiles, farmers still saved part of the production for self-consumption and distribution between family and friends. Cebolla-Cornejo et al. (2007) pointed out that younger farmers tended to maintain commercial cultivation of landraces. Accordingly, it seems that a generational renewal progressively increases this profile amongst 'Moruno' growers. Indeed, more than one-quarter of the surveyed farmers were younger than 50 years old. This situation that seems common in Europe, is different in other areas. For example, in the case of Mexico, Estrada-Castellanos et al. (2011) found that the majority of farmers growing landraces were under 55 years old.

Professionalisation in quality markets linked to landraces might be an alternative for young farmers in order to help them to overcome the barriers that commonly affect young European farmers, and which hamper the establishment and consolidation of their farming enterprises (Eistrup et al., 2019).

Pests and diseases were not the main factors determining landrace cultivation viability. Less than 12% of the production is jeopardised by this factor, mainly related to the incidence of pests, such as mites and *Tuta,* and diseases such as mildew. A low incidence might be related to adaptation to local conditions through evolution. In general, it is admitted that landraces count with some level of resistance or tolerance to those diseases to which they have been continuously exposed. An adaptation favoured by farmer intervention. For example, *Ve* alleles of resistance to *Verticillium dahliae* Kleb. have been found in Italian tomato landraces and reflect farmer selection since the wilt fungus is endemic in the area (Acciarri et al., 2010).

Nonetheless, the low impact of pests and diseases on landrace cultivation is not generalisable, especially in cases without coexistence of landraces and pathogens. In this sense, farmers have pointed out that they tomato landraces are highly susceptible to viral diseases of recent apparition (Cebolla-Cornejo et al., 2007). It seems clear that the importance of this factor depends on the incidence of virus in the cultivation area. This was not the case for the 'Moruno' landrace in central Spain, as farmers did not emphasise the incidence of viral diseases. On the other hand, on the East coast of Spain, Cebolla-Cornejo et al. (2007) reported a high incidence of viral infections that compromised the production. In fact, virus resistant selections of tomato landraces have been developed in areas in which the production is not viable otherwise (*e.g.* 'Muchamiel' and 'de la pera' selections developed by Carbonell et al. (2018), in the South East of Spain).

This last approach has some limitations, though. Some genes, such as *ty-1* conferring tolerance to TYLCD, imply genetic drag with deleterious effects that negatively affect yield (García-Martínez et al., 2016), and it is necessary to recover the flavour profile of the original materials valued by consumers. Other criticisms affect the concept of landrace itself. Several definitions imply that landraces should not have been subjected to formal plant breeding programs. Nonetheless, some researchers emphasise the concept of continuous evolution of landraces, including human intervention and claim that conventional or modern breeding methodologies should be contemplated for these materials (Casañas et al., 2017).

More important than the incidence of diseases, the main limitation of landrace cultivation is the lower yields obtained from these materials. We have seen in our study that depending on the year and accessions considered, mean yield of landraces is slightly higher (55%) than one-half of the yield of a commercial F1 hybrid. However, this lack of competitivity varies between years (36.1% in year 3 up to 79.1% in year 2). It also varies in the specific populations being considered. It can be as low as 11.2% for SL-149 in Year 3 or even surpass the commercial hybrid in years with bad conditions for the commercial hybrids, as in the case of SL-136 in Year 3 (110%). Although different populations were grown each year, it seems evident that landraces, despite having low productivity, show a homoeostatic performance in years not so suitable for tomato cultivation. This point is noticeable in Year 2 when the commercial hybrid 'Royesta' was highly affected by environmental conditions and dropped its yield, while 'Moruno' populations maintained some yield stability, thus, resulting in an increased relative yield, up to 79%, compared to 'Royesta'.

The high variability in yield in tomato landraces has already been described in other works (Cebolla-Cornejo et al., 2013; Donoso and Salazar, 2023). Cebolla-Cornejo et al. (2013), recorded a mean coefficient of variation for yield in Spanish landraces of 0.54, 3.4 times higher than the one found in the commercial F1 hybrid control. It was already suggested the necessity to tackle this variability in order to offer the farmer a sustainable alternative. It is necessary to offer high, uniform and stable yields. But, is it possible to identify and offer a selection of populations that provide the external phenotype claimed by farmers and a high and stable yield?

It seems evident that not all the landrace populations do offer the productivity level necessary to reach economic sustainability. Nonetheless, populations such as SL-25 and SL-72 offer relatively high yields (within the limitations of the landrace) that are somewhat stable within and between years. It seems evident that 'Moruno', like other landraces, presents populations with extremely low yields (Cebolla-Cornejo et al., 2013; Bota et al., 2014). Therefore, it requires some level of depuration to offer farmers economically viable materials that minimise the gap between the yields of landraces and commercial hybrids to promote sustainable on-farm conservation. With this aim, depuration programs have been recommended for other tomato landraces such as 'Valenciano', 'Montserrat', 'Pera Girona' or 'Muchamiel', i.a., selecting between populations and within populations (Casals et al., 2011a; Cebolla-Cornejo et al., 2013; Cortés-Olmos et al., 2014; Donoso and Salazar, 2023).

Obviously, any depuration program would not completely avoid the lack of competitivity of landraces with commercial hybrids in terms of yield. But, this gap can be overcome thanks to the price premium that consumers pay for the high organoleptic quality of landraces. This price premium can sometimes reach differences of up to 4.7 times the price paid for conventional varieties (Cebolla-Cornejo et al., 2007). In our study, we have analysed this price premium effect. Farmers are paid a relatively high price for 'Moruno' tomatoes, reaching more than 2.5 € kg^{-1}, which more than doubles the price received for standard varieties. A price that seems generally stable in the area, as the coefficient of variation for perceived prices is extremely low. But it also seems evident that this price does not reflect farmer expectations that place the expectation of a fair price at least 1 € kg^{-1} higher.

In the case of 'Pomodoro di Mercatello' in Italy, the actual price for the landrace was higher than the one paid for conventional tomatoes, as in our case, but the authors identified amongst consumers a willingness to pay primes 1.4 to 2.4 € kg^{-1} higher (Rocchi et al., 2016). This willingness to pay, though, is not always so high. For example, Posadinu et al. (2022) reduced the willingness to pay a price premium to 0.9 € kg^{-1}. Anyway, despite the amplitude of the price premium, it seems clear that it would still be possible to reach farmers' expectations through valorisation programs, as it has been reported for other crops (Krishna et al., 2010). That would be possible promoting the organoleptic and functional quality of the materials or even exploiting the functional value already described in tomato landraces (Cortes-Olmos et al., 2014).

Regarding organoleptic quality, the preference of consumers for landraces has been assumed to be commonplace. The development of breeding programs has obviously played a key role in the flavour degeneration of commercial materials. In this sense Tieman et al. (2017) evidenced the loss of key alleles related to volatile production during breeding programs as one of the main reasons behind the differences between commercial varieties and landraces. Furthermore, Baldina et al. (2016) confirmed that the metabolic profile of tomato landraces with certain shapes and colours are different to those of commercial similar materials.

Nonetheless, it has been reported that the link between tomato landraces and the best flavour is not always valid. In fact, within a certain landrace, there is a considerable variation in the accumulation of sugars and acids influencing taste perception (Cebolla-Cornejo et al., 2013; Carillo et al., 2019) or even volatiles influencing aroma perception (Casals et al., 2011b). In the case of 'Moruno', a recent study confirmed a relatively high variability in both traits (Villena et al., 2023). The reanalysis of these data for this work adds more information in this sense. It has been shown that despite a high level of variation between populations in the accumulations of sugars, acids and volatiles, the truth is that the effect of the environment is even higher (Supp. Fig. 3 and 4). Indeed, the 'Moruno' populations had a quality profile highly dependant on the environment, especially in the case of volatiles. Accordingly, the populations could be separated considering the year of cultivation, even assuming that different populations were grown each year.

In this context, we should bear in mind that depuration programs and selection for high yield can affect negatively flavour. Indeed, in a previous work with this landrace (Villena et al., 2023) it was concluded that yield is negatively related with flavour perception. Nonetheless, despite the difficulty of identifying populations of tomato landraces that combine high yields and a high accumulation of sugars, acids and volatiles, resulting in better flavour acceptability, it was still possible to achieve a trade-off. One of the populations of 'Moruno' that should be selected for on-farm conservation programs, SL-62, combined a morphology representative of the 'Moruno' ideal with intermediate yields with a high overall flavour acceptability (Supp. Figs. 3 and 4) and a high level of uniformity and stability. Another interesting population, SL-72, offers a higher yield, but its overall flavour acceptability was influenced by the year of cultivation. In other cases, it was not possible to reach a trade-off. It would be the case of SL-33, which was appreciated for its flavour, but had low productivity, while the opposite applied to population SL-25.

This problem has been raised in previous works. For example, in the selection of tomato landraces adapted to high temperature, Scarano et al. (2020) found an association between secondary metabolites and traits influencing organoleptic quality. Nonetheless, amongst the three populations with the best metabolomic profile, only one showed acceptable yields (Scarano et al., 2020). On the other hand, all the better populations in terms of yield with one exception exhibited lower yields.

Interestingly, most farmers included flavour as a critical trait of the 'Moruno' landrace. However, only a tiny part of them used flavour as a selection criterion for choosing the seeds to be grown the following season. This would partially explain the variation in the accumulation of solutes and volatiles related to flavour. It has been described that seed mixing and spontaneous cross-pollination can be frequent in tomato landraces (Cortés-Olmos et al., 2015). After such events, the farmers would apply strong selection pressure to few external traits. Our results confirm this evolution. Farmers confirmed that they usually exchange seeds with other farmers and grow several landraces at the same time, enabling the occurrence of spontaneous crossings. In fact, some of them experienced seed degeneration. Additionally, they confirmed that they selected the seeds considering external traits that define the landrace: shape, size and colour, and placed a very low emphasis on internal traits, including flavour and fleshiness.

The importance of shape in differentiating different landraces is well known. In fact, Sacco et al. (2015) found that a 45% of the variation present in a wide collection of Italian landraces relies in fruit shape. Although farmers used colour (purple) as a main selection criterion, the colour measured with a colourimeter was highly variable within populations both in the equatorial and shoulder parts of the fruit. In this case, the typical green shoulder of the 'Moruno' populations probably justifies this variability, as it is challenging to provide a stable measurement even in the same fruit. On the other hand, fruit shape traits were highly conserved within populations, as well as those reflecting the fleshiness of the fruit: estimated density and pericarp width. The higher variation in shapes between populations reflects each farmer's different parameters for selection. In fact, there were some discrepancies in the definition of the landrace in terms of size (mid to big sized) and shape (more or less flattened fruits). Accordingly, a high variation was found for fruit size within and between populations.

In some cases, the variability found within population was even 22 times higher than that found in the commercial F1 hybrid, which is genetically uniform. This trend has also been found in other tomato landraces. It would be the case, for example, of the Spanish landrace 'Valenciano', whose fruits varied from 113 g to 303 g while they maintained their typical heart-shaped morphology (Cebolla-Cornejo et al., 2013). In the Spanish 'Muchamiel', this effect has also been described with weight ranges between 199 g and 356 g but maintaining a flat and ribbed shape.

One of the limitations present at germplasm banks performing an *ex situ* conservation of genetic resources is the impossibility to evaluate all the materials in the same environmental conditions. This work faces these problems, as different materials were evaluated in different years, while only a selected part of them were re-evaluated during a second year. Our results confirm the importance of the environment on agronomical and morphological traits in a tomato landrace. This effect can be even more important than the genotypic effect in the evaluation of populations of the same landrace. The effect of environment on the metabolic profile had already been suggested, reinforcing the ideal of double evaluation, as these traits are highly affected by the environment (Galiana et al., 2018). In fact, Casals et al. (2021) reported that the accumulation of soluble solids was one of the main characteristics affected not only by environment but also by genotype x environment interactions in the 'Penjar' tomato landrace. In the same line, Lázaro (2018) reported the existence of considerable genotype x environment interactions in consumer acceptability of tomato landraces, and evidenced a strong environmental effect on the perception of tomato fruit quality.

Additionally, the effect on morphoagronomical traits is also considerable. It reinforces the idea to include controls in order to enable some level of comparison. In our case we selected a commercial hybrid to provide a genetically uniform material, thus having a better estimation of the effect of the environment. Nonetheless, our own results identify a genotype x environment interaction. Interestingly, the response amongst populations of the landrace is more similar than the response of the hybrid to the change in the environment. It is necessary to incorporate not only hybrid materials as controls, but also a representative population of the landrace to provide a representation of the effect of the environment on these homoeostatic materials.

5. Conclusions

Landraces maintain a high degree of intra- and inter-populations variability. This variability is related to the selection criteria used by farmers when saving seed for the next generation. Accordingly, those traits tend to show a lower level of variation. This intra-population variability is maintained by the way farmers perform the selection. They select specific fruits for their shape rather than specific plants for their global performance. Inter-population variability is maintained by the way each farmer uses different selection criteria. Probably, growing different landraces at the same time and the natural outcrossing values of tomato favours accidental cross-pollination resulting in segregation or what the farmers understand as seed degeneration. The evolution of these populations would be then redirected applying strong selection on external traits, and not on internal traits such as flavour. This would led to the existence of important variation in the accumulation of compounds related to flavour (sugars, acids and volatiles). The high diversity existent affects dramatically key agronomical traits such as fruit size and yield. It is therefore necessary to offer farmers selected populations with the highest yield and flavour as possible. Nonetheless, it is difficult to combine yield and flavour at the same time. Notwithstanding, it is possible to achieve a compromise between both traits in selected populations.

CRediT authorship contribution statement

J. Villena: Investigation, Writing – review & editing. **C. Moreno:** Conceptualization, Investigation, Formal analysis, Writing – review & editing, Supervision, Project administration. **S. Roselló:** Formal analysis, Visualization, Writing – review & editing. **J. Cebolla-Cornejo:** Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **M.M. Moreno:** Conceptualization, Investigation, Formal analysis, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marta M. Moreno Valencia reports financial support was provided by Ministry of Science and Innovation (Spain).

Data availability

Morphoagronomical data: 10.5281/zenodo.7715955. Metabolomic data: 10.5281/zenodo.6963114.

Acknowledgments

This research has been funded by the National Institute for Agricultural and Food Research and Technology (INIA), Ministry of Science and Innovation (grant code: RTA 00083-C02-01).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112128.

References

Acciarri, N., Sabatini, E., Ciriaci, T., Rotino, L.G., Valentine, D., Tamietti, G., 2010. The presence of genes for resistance against Verticillium dahliae in Italian tomato landraces. Eur. J. Hortic. Sci. 75 (1), 8–14.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration. Guidelines for computing crop water requirements. Food and Agriculture Organization of the United Nations (FAO), Rome. Irrigation and Drainage.

Baldina, S., Picarella, M.E., Troise, A.D., Pucci, A., Ruggieri, V., Ferracane, R., Mazzucato, A., 2016. Metabolite profiling of Italian tomato landraces with different fruit types. Front. Plant Sci. 7, 664.

Bota, J., Conesa, M.À., Ochogavia, J.M., Medrano, H., Francis, D.M., Cifre, J., 2014. Characterization of a landrace collection for Tomàtiga de Ramellet (*Solanum lycopersicum* L.) from the Balearic Islands. Genet. Resour. Crop Evol. 61, 1131–1146.

Carbonell, P., Alonso, A., Grau, A., Salinas, J.F., García-Martínez, S., Ruiz, J.J., 2018. Twenty years of tomato breeding at EPSO-UMH: transfer resistance from wild types to local landraces-from the first molecular markers to genotyping by sequencing (GBS). Diversity 10 (1), 12.

Carillo, P., Kyriacou, M.C., El-Nakhel, C., Pannico, A., dell'Aversana, E., D'Amelia, L., Rouphael, Y., 2019. Sensory and functional quality characterization of protected designation of origin 'Piennolo del Vesuvio'cherry tomato landraces from Campania-Italy. Food Chem. 292, 166–175.

Casañas, F., Simó, J., Casals, J., Prohens, J., 2017. Toward an evolved concept of lan drace. Front. Plant Sci. 8, 145.

Casals, J., Pascual, L., Cañizares, J., Cebolla-Cornejo, J., Casañas, F., Nuez, F., 2011a. The risks of success in quality vegetable markets: possible genetic erosion in Marmande tomatoes (*Solanum lycopersicum* L.) and consumer dissatisfaction. Sci. Hortic. 130 (1), 78–84.

Casals, J., Cebolla-Cornejo, J., Roselló, S., Beltrán, J., Casañas, F., Nuez, F., 2011b. Long-term postharvest aroma evolution of tomatoes with the alcobaça (alc) mutation. Eur. Food Res. Technol. 233, 331–342.

Casals, J., Pascual, L., Cañizares, J., Cebolla-Cornejo, J., Casañas, F., Nuez, F., 2012. Genetic basis of long shelf life and variability into Penjar tomato. Genet. Resour. Crop Evol. 59, 219–229.

Casals, J., Martí, M., Rull, A., Pons, C., 2021. Sustainable transfer of tomato landraces to modern cropping systems: the effects of environmental conditions and management practices on Long-Shelf-Life tomatoes. Agronomy 11 (3), 533.

Cebolla-Cornejo, J., Soler, S., Nuez, F., 2007. Genetic erosion of traditional varieties of vegetable crops in Europe: tomato cultivation in Valencia (Spain) as a case study. Int. J. Plant Prod. 1, 113–128. https://doi.org/10.22069/IJPP.2012.531.

Cebolla-Cornejo, J., Roselló, S., Nuez, F., 2013. Phenotypic and genetic diversity of Spanish tomato landraces. Sci. Hortic. 162, 150–164.

Conversa, G., Lazzizera, C., Bonasia, A., Cifarelli, S., Losavio, F., Sonnante, G., Elia, A., 2020. Exploring on-farm agro-biodiversity: a study case of vegetable landraces from Puglia region (Italy). Biodivers. Conserv. 29, 747–770.

Cortés-Olmos, C., Leiva-Brondo, M., Roselló, J., Raigón, M.D., Cebolla-Cornejo, J., 2014. The role of traditional varieties of tomato as sources of functional compounds. J. Sci. Food Agric. 94 (14), 2888–2904.

Cortés-Olmos, C., Valcárcel, J.V., Roselló, J., Díez, M.J., Cebolla-Cornejo, J., 2015. Traditional Eastern Spanish varieties of tomato. Sci. Agric. 72, 420–431.

Donoso, A., Salazar, E., 2023. Yield Components and Development in Indeterminate Tomato Landraces: an Agromorphological Approach to Promoting Their Utilization. Agronomy 13 (2), 434.

Eistrup, M., Sanches, A.R., Muñoz-Rojas, J., Pinto Correia, T., 2019. A "young farmer problem"? Opportunities and constraints for generational renewal in farm management: an example from Southern Europe. Land 8 (4), 70 (Basel).

Estrada-Castellanos, J.B., Carrillo-Rodriguez, J.C., Jerez-Salas, M., Chavez-Servia, J.L., Perales-Segovia, C., 2011. Small farmer practices for production improvement of the kidney-type tomato landrace: a case study in Oaxaca. Afr. J. Agric. Res. 6 (13), 3176–3182.

Galiana-Balaguer, L., Ibanez, G., Cebolla-Cornejo, J., Rosello, S., 2018. Evaluation of germplasm in Solanum section Lycopersicon for tomato taste improvement. Turk. J. Agric. For. 42 (5), 309–321.

García-Martínez, S., Grau, A., Alonso, A., Rubio, F., Carbonell, P., Ruiz, J.J., 2016. New breeding lines resistant to Tomato Mosaic Virus and Tomato Spotted Wilt Virus within the 'De la Pera'tomato type: UMH 1353 and UMH 1354. HortScience 51 (4), 456–458.

Hamilton, E.E., 1976. What the new world economy gave the old. In: Chiappelli (ed.). In: First Images of America: The Impact of the New World On The Old, 2. University of California Press, Los Angeles, pp. 853–884.

Krishna, V.V., Pascual, U., Zilberman, D., 2010. Assessing the potential of labelling schemes for *in situ* landrace conservation: an example from India. Environ. Dev. Econ. 15 (2), 127–151.

Lázaro, A., 2018. Tomato landraces: an analysis of diversity and preferences. Plant Genet. Resour. 16 (4), 315–324.

Mattioli, P.A., 1544–1554. I discorsi di M. Pietro Andrea Matthioli... ne i sei libri di Pedacio Dioscoride Anazarbeo Della Materia Medicinale...Vincenzo Valgrisi, Venezia. http://alfama.sim.ucm.es/dioscorides/consulta_libro.asp?ref=X532874063.

Mazzucato, A., Ficcadenti, N., Caioni, M., Mosconi, P., Piccinini, E., Sanampudi, V.R.R., Ferrari, V., 2010. Genetic diversity and distinctiveness in tomato (*Solanum lycopersicum* L.) landraces: the Italian case study of 'A pera Abruzzese'. Sci. Hortic. 125 (1), 55–62.

Missio, J.C., Rivera, A., Figàs, M.R., Casanova, C., Camí, B., Soler, S., Simó, J., 2018. A comparison of landraces *vs.* modern varieties of lettuce in organic farming during the winter in the Mediterranean area: an approach considering the viewpoints of breeders, consumers, and farmers. Front. Plant Sci. 9, 1491.

Moreno, M.M., Villena, J., González-Mora, S., Moreno, C., 2019. Response of healthy local tomato (*Solanum lycopersicum* L.) populations to grafting in organic farming. Sci. Rep. 9 (1), 1–10.

Negri, V., Maxted, N., Veteläinen, M., 2009. In: Veteläinen, M., Negri, V., Maxted, N. (Eds.). In: European Landraces: On-Farm Conservation, Management and Use, Bioversity Technical Bulletin, 15. Bioversity International, Roma, pp. 1–22.

Posadinu, C.M., Rodriguez, M., Madau, F., Attene, G., 2022. The value of agrobiodiversity: an analysis of consumers preference for tomatoes. Renew. Agric. Food Syst. 37 (3), 237–247.

Rocchi, L., Paolotti, L., Cortina, C., Boggia, A., 2016. Conservation of landrace: the key role of the value for agrobiodiversity conservation. An application on ancient tomatoes varieties. Agric. Agric. Sci. Procedia 8, 307–316.

Sacco, A., Ruggieri, V., Parisi, M., Festa, G., Rigano, M.M., Picarella, M.E., Barone, A., 2015. Exploring a tomato landraces collection for fruit-related traits by the aid of a high-throughput genomic platform. PLoS ONE 10 (9), e0137139.

Scarano, A., Olivieri, F., Gerardi, C., Liso, M., Chiesa, M., Chieppa, M., Rigano, M.M., 2020. Selection of tomato landraces with high fruit yield and nutritional quality under elevated temperatures. J. Sci. Food Agric. 100 (6), 2791–2799.

Terzopoulos, P.J., Bebeli, P.J., 2010. Phenotypic diversity in Greek tomato (*Solanum lycopersicum* L.) landraces. Sci. Hortic. 126 (2), 138–144.

Tieman, D., Zhu, G., Resende Jr, M.F., Lin, T., Nguyen, C., Bies, D., Klee, H, 2017. A chemical genetic roadmap to improved tomato flavor. Science 355 (6323), 391–394.

Van Andel, T., Vos, R.A., Michels, E., Stefanaki, A., 2022. Sixteenth-century tomatoes in Europe: who saw them, what they looked like, and where they came from. PeerJ 10, e12790.

Vicente Villardón, J.L., 2015. MULTBIPLOT: A package for Multivariate Analysis Using Biplots. Departamento de Estadística. Universidad de Salamanca.. http://biplot.usal.es/ClassicalBiplot/index.html

Camacho Villa, T.C., Maxted, N., Scholten, M., Ford-Lloyd, B., 2005. Defining and identifying crop landraces. Plant Genet. Resour. 3 (3), 373–384.

Villena, J., Moreno, C., Roselló, S., Beltrán, J., Cebolla-Cornejo, J., Moreno, M.M., 2023. Breeding tomato flavor: modeling consumer preferences of tomato landraces. Sci. Hortic. 308, 12, 111597.

Zeven, A.C., 1998. Landraces: a review of definitions and classifications. Euphytica 104, 127–139.