



Breeding Ox-heart Tomato Populations through Participatory Plant Breeding in Italy

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Abstract

Participatory Plant Breeding (PPB) and Evolutionary Plant Breeding (EPB) offer a practical alternative to centralized conventional breeding, with the special aim to improve farmer's participation and to develop genetic materials adapted to specific pedo-climatic conditions, especially those linked to organic and/or low input agriculture. In that frame, an ox-heart tomato PPB program was conducted in Italy by Rete Semi Rurali (RSR) between 2018 and 2020. An F_4 population, derived from a half-diallel cross of 4 outstanding local varieties, was grown and selected in 4 different organic farms in Italy during two consecutive years (2018-2019). In 2018 and 2019, for each location, a group of farmers and other participants evaluated plots of 400 plants, and the 20 plants with the highest score were selected to constitute a Farmer's Selection (FS) population. Simultaneously, in every location, the seeds from a random fruit per plant were taken to constitute Natural Selection (NS) populations. In 2020, a Multi-Location Trial was performed in the 4 locations, using all of the FS and NS from each of the 4 locations, as well as 4 local controls and 2 widely grown varieties, for a total of 14 genotypes. After performing Spatial Analysis, Tukey HSD tests and GGE Biplots, the results show evidence of efficiency in participatory selection, especially for the yield at first harvest. This trait is highly important for the farmers, as it guarantees better market prices earlier in the season. A noteworthy effect of local adaptation was also demonstrated in one of the locations (Rotonda, Basilicata). Altogether, these results confirm the viability of using PPB and EPB in tomato, and set a milestone as one of the firsts documented examples of EPB in vegetable crops.

Key words: Participatory Plant Breeding, Farmer's Selection, Local adaptation, GGE Biplot, Spatial Analysis

Resumen

El Mejoramiento Genético Participativo y Evolutivo ofrece una alternativa práctica al mejoramiento convencional centralizado y tiene como principales objetivos aumentar la participación de los agricultores y desarrollar materiales genéticos adaptados a condiciones pedo-climáticas específicas, sobre todo para aquellas ligadas a la agricultura ecológica y/o de bajos insumos. En este marco, se llevó a cabo un programa de Mejora Participativa de tomate corazón de buey en Italia liderado por la Rete Semi Rurali (RSR). Se cultivó una población F_4 , proveniente del cruce compuesto entre 4 materiales locales sobresalientes, en 4 fincas orgánicas durante dos años consecutivos (2018-2020). En 2018 y 2019, para cada ubicación, un grupo de agricultores y otros actores evaluaron parcelas de 400 plantas y se seleccionaron las 20 plantas mejor evaluadas, de este modo se constituyeron las poblaciones de selección participativa. Simultáneamente, se tomaron las semillas de un fruto por planta para constituir poblaciones seleccionadas naturalmente. En 2020, se realizó un ensayo multi-localidad en las 4 fincas, utilizando las poblaciones derivadas de la selección participativa y natural para cada ubicación, además de 4 controles locales y 2 controles comunes de toda Italia, para un total de 14 genotipos. Se realizó un análisis espacial para tomar en cuenta la heterogeneidad del campo en el desempeño de los materiales, la prueba HSD de Tukey para diferenciar entre genotipos y se usaron *GGE Biplots* para analizar visualmente la interacción genotipo x ambiente. Los resultados muestran evidencia importante de eficacia en la selección participativa, especialmente para el rendimiento en la primera cosecha. Este rasgo es muy importante para los agricultores, ya que garantiza mejores precios de mercado al comienzo de la temporada. Además, solo en una localidad, (Rotonda, Basilicata), Se demostró también, para una sola una localidad, un efecto notable de adaptación local de los materiales. En conjunto, estos resultados confirman la viabilidad del uso de mejora participativa y de la mejora evolutiva en tomate, y marcan un hito como uno de los primeros ejemplos documentados de mejora evolutiva en el cultivo de hortalizas.

Palabras clave: Fitomejoramiento participativo, Selección de Agricultores, Adaptación local, GGE Biplot, Análisis espacial

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1. Introduction

1.1. *Times for change in agriculture*

Humanity is now facing unprecedented challenges regarding the fate of planet Earth. The interconnected processes of climate change, biodiversity loss and the cycle of N have already been altered in irreversible ways, and others such as ocean acidification, chemical pollution and the P cycle threat to do so (Rockström et al., 2009). Thus, a need for radical rethinking of our economy (as in the management - *nemein-* of our house –*oikos-*) is compulsory (Raworth, 2017) and agriculture plays a central role in this scenario.

In particular, industrialized and high input agriculture is at the limelight for, despite largely contributing to the world's food supply, producing a large amount of negative externalities such as soil erosion, eutrophication, biodiversity loss and chemical pollution (Campbell et al., 2017). In that context, various calls have been made for the Sustainable Intensification (SI) of agriculture, so that food is produced with satisfactory quantity and quality, but that environmental damages are reduced or even reverted (Volger, 2013). One of the main approaches for the SI process is embracing organic agriculture, which has proven sustainability benefits and is now rapidly growing across the world (Eyhorn et al., 2019). Though many challenges prevail, transition towards organic agriculture is beneficial in the sense that it addresses many of the above mentioned negative externalities of conventional agriculture (Reganold and Wachter, 2016)

However, there is an ongoing (and heated) debate around the difference in yields between conventional and organic agriculture, and in consequence, around the feasibility for the latter to contribute more substantially to the world's food production in the context of a growing population (Badgley et al., 2007; Connor, 2018). In this discussion, setting aside the fact that food production already exceeds world's demands by large, meta-analytic evidences show that yield differences lie around 20% in most cases, and can be reduced to less than 10% with adequate rotations (Ponisio et al., 2015).

Moreover, the utterly needed changes are not only on technical aspects, but also on the socioeconomic ones. This implies recognizing the farmers as the managers of complex agro-ecological systems, and not only as mere food suppliers (Volger, 2013). Thus, the need for developing cooperative agricultural knowledge-based economies has also been asserted, as it will likely diminish the "free-rider" kind of activity that so strongly contributes to environmental degradation (Pretty et al., 2018).

It is not only necessary to mitigate the effects of human activity on the environment, but it is also imperative to develop adaptation strategies to face these effects, especially for those linked to climate change. In addition to the long term trend in global warming that will affect agricultural production, the higher variability of precipitation and extreme temperature events will profoundly influence agriculture (Siepielski et al., 2017). So, holistic strategies for building agricultural resiliencies are of paramount importance, and the use of plant breeding will be a cornerstone in this process, as it can contribute by providing genetic materials that are up to the challenge.

1.2. Participatory, Decentralized and Evolutionary Plant Breeding

Participatory Plant Breeding (PPB) is an emerging strategy that aims to increase efficiency in plant breeding and improve farmer's involvement in research (Ceccarelli, 2015). This approach was initially born as a way to combine the classical principles of plant breeding, with the emerging current of participatory research in agriculture, which proposed to "put farmer's first" or "at the start and the end" of research programs (Chambers and Ghildyal, 1985; Dawson et al., 2008; Rhoades and Booth, 1982). Rather than a static concept, PPB describes a wide spectrum of breeding practices with different goals and degrees of participation.

Among the many goals and principles of PPB, many are shared with those of conventional plant breeding programs, such as increasing production gains and (sometimes) quality, all while maximizing cost efficiency. However, PPB sets specific emphasis in properly targeting the user needs, particularly the needs of farmers (Sperling et al., 2001). In a pristine manner, PPB can be described as plant breeding with a very high client orientation (Witcombe et al., 2005). First described thoroughly in 1995 (Eyzaguirre and Iwanaga, 1995), PPB is a way of formalizing a long history of farmer's participatory research and breeding (Weltzien et al., 2003; Witcombe et al., 2005). In that sense, the formalization of institutionally-led PPB programs owes much to the pioneering work of Salvatore Ceccarelli in the Middle East and that of John Witcombe in the Indian Subcontinent (Dawson et al., 2008).

Any classical plant breeding program can be separated in the following 5 main stages: i) Definition of the breeding targets, ii) Generation of variability, iii) Selection among segregating populations, iv) Genotype testing and evaluation and v) Variety release and adoption (Schnell, 1982). While many conventional programs only include farmers in the last step, a PPB program would ideally include them during most, if not all, the stages of the process (Sperling et al., 2001). This frame allows for an initial distinction between Participatory Plant Breeding (PPB) and Participatory Varietal Selection (PVS). In the latter, farmer's participation is restricted mostly to participation at late stages of the process when a small number of materials have been already selected (Weltzien et al., 2003).

Even if some argue that farmer's participation should not be considered as a goal itself and that it might bring unnecessary costs (Witcombe et al., 2005), it is however true that enhancing their participation can have additional positive impacts like: i) a better understanding of the farmer's preferences, ii) the empowerment of the participants involved in the process and iii) an enhanced confidence from them in the executing institutions (Weltzien et al., 2003). Furthermore, if participation is designed and executed with a gender perspective, improvement of gender equality can also be pursued by these means (Galiè et al., 2017; Tufan et al., 2018)

Moreover, as the origins of formal PPB trace back to selection for marginal environments, it has been also strongly associated with breeding decentralization; this means that breeding is practiced at the target environments, *ie.* the farmer's fields (Ceccarelli and Grando, 2007). This approach takes advantage of the genotype-by-environment ($G \times E$) interactions and can thus make selection significantly more efficient (Ceccarelli, 2015; Dawson et al., 2008). However, although first conceived and applied for solving immediate needs of farmers in marginal contexts, PPB has extended and proven useful for a wide variety of contexts. As of 2020, PPB has been documented in at least 47 different crops around 69 countries, comprising a great diversity of economic scales, crop types and agricultural environments (Ceccarelli and Grando, 2020a).

When decentralized, PPB is particularly useful for the case of low input and/or organic agriculture, where a lack of adapted genotypes with the specific ideotypes required is among the biggest constraints in production (Lammerts van Bueren et al., 2011). For example, plant breeding in environments with high inputs of synthetic fertilizer has weakened the capacity of modern genotypes to beneficially interact with soil microbes (Kiers et al., 2007). Both agriculture in marginal environments and under organic and/or low input conditions share a large deal of similarities, and they are often overlooked by conventional breeding programs, mainly due to profitability concerns.

Under conventional breeding conditions, selection is still done through most stages at Research Stations, and submitted to multi environmental trials late in the process. However, when plant selection only takes place at research stations and optimal environments, materials that could better adapt to marginal conditions might be quickly discarded (Ceccarelli, 1989). Selection exclusively in optimal environments could be justified only if: i) heritability is significantly higher in these environments and ii) there is a high genetic correlation between these and the target environments (Ceccarelli, 2015; Dawson et al., 2008). The second condition would mean that GxE crossover interactions are nil or few. Nonetheless, this interaction is sometimes so strong that the genetic correlation is significantly reduced and the best genotypes under optimal conditions will rank well below the top in sub-optimal environments (Reid et al., 2009; Simmonds, 1991).

As for the adoption of new varieties, PPB, and specially decentralized PPB, is also expected to increase the use of new genetic material by the farmers, as they are closer to the process and they get to see each variety growing under their own field conditions (Casals et al., 2019). This is yet another way in which PPB might contribute to efficiency in breeding, if the number of adopted varieties per breeding cycle is taken into the equation (Ceccarelli, 2015).

What's more, PPB can also make use of the natural selection pressures in each environment and be combined with Evolutionary Breeding (EB). In brief, EB consists in a mixture of the plant breeding principles with those of natural selection. Thus, an EB program comprises mainly four main stages: i) assorting a stock of diverse genetic material, ii) recombination of these stocks by hybridization, iii) bulking of the F1 population and iv) submitting these materials to the selection environment for prolonged natural selection (Suneson, 1956).

The first formal experiments in this sense were done by Harlan and Martini, when a mixture of 11 elite barley materials from all over the world was created and tested along 10 different environments for at least 15 years, and they noted how the least adapted varieties for each environment disappeared quickly and the genotypes with greater fitness prevailed (Harlan and Martini, 1938, 1929). Later on, Suneson (1956), who actually coined the term "Evolutionary Breeding", created different Cross Composite Populations (CCP) of barley that were set to evolve for 15 years in different environments. When compared to an elite variety, CCPs had similar yields, but higher stability. The author argued that this efficient and cost effective approach was being underused, and also noted the economic and political implications of this method as it could result in an important variation from the "pure line economy".

The biological foundations of this approach lie in the proper exploitation of high levels of biodiversity and the specific fitness for each genotype at the given environment, as well as the positive correlation between fitness and agronomic value (Allard and Hansche, 1964). The use of CCPs and EB have been more recently proposed as a viable alternative to breed germplasm for low input environments, as the obtained populations are expected to be highly resilient and able to "respond dynamically to complex natural and artificial selection from abiotic and biotic stresses" (Phillips and Wolfe, 2005). For the same reasons, it has also been considered as powerful tool to respond to the ever growing complexity of climate change (Ceccarelli and Grando, 2020b). Recently, cases of success in EB have been documented in wheat (Bocci et al., 2020; Döring et al., 2015) and barley (Raggi et al., 2017).

Genetic erosion concerns are also addressed by decentralized PPB and EPB (Ceccarelli and Grando, 2007; Joshi et al., 1997), as each breeding cycle ends with diverse genotypes, genotype combinations or populations in every location, instead of the recommendation of one or two varieties to be grown across different environments. Additionally, as farmer's selection tend to prioritize yield stability over

yield potential, which is often linked with high allelic diversity within the populations, this is yet another way in which PPB favors biodiversity (Ceccarelli et al., 2001)

However, important policy bottlenecks arrive in this sense, especially in the more industrialized countries. In the 1970s, the European Union (EU), in compliance with the UPOV treaties, established that every commercialized variety must be registered in a national catalogue, and comply with Distinction, Uniformity and Stability (DUS) standards. But, more often than not, the DUS standards are not met, nor desired, by certain landraces, or by genotypes obtained through PPB/EPB. This in fact, has led to marginalization of the informal seed systems and of the often heterogeneous landraces. Most likely, this legal framework has also largely contributed to the notable genetic erosion present in most commercial crops, despite the ongoing narratives held by scientists and policy makers about the importance of actively preserving agrobiodiversity (Louwaars, 2018)

In 1998, the EU first introduced the concept of conservation variety and in 2008 gave the first directive to formally allow landraces and heirloom varieties to be sold and grown under specific cases and conditions. Among these conditions, the varieties that are to be grown must have a specific historical bond to a particular territory. So, the directive did not bring any solutions to varieties/populations that were created through PPB/EPB programs and were heterogeneous *by design* (Bocci, 2009). Then, in order to give a proper legal status to those materials (which were in part developed by EU research project), the European Commission's executive decision 2014/150/EU established a "temporary experiment" allowing the commercialization of heterogeneous populations of oats, wheat, barley and corn (Petitti, 2018). Starting in January 2022, the new European organic regulation (EU) 2018/848 is extending the possibility to market heterogeneous populations' seed to all species (Organic Heterogeneous Material) through a simplified notification process (Costanzo et al, 2019).

1.3. *Breeding Tomato for Organic Cultivation in Italy and Europe.*

Tomato (*Solanum lycopersicum*) is the most popular vegetable in the world and the seventh most produced crop species, after maize, rice, wheat, potatoes, soybeans and cassava (Bergougnoux, 2014). Its world production accounts for over 180 million tons a year, more than 5 million ha and a share of 14% of world's vegetable production (FAO, 2021). In Europe, Italy is the largest producer, with over 5 million tons per year in an area of approximately 90 000 ha (FAO, 2021). Its ability to grow in diverse climatic conditions, its unique taste and the versatility to be consumed fresh, or as paste, juice, soup and other preparations, are among the reasons of its great importance.

The species is native from the Andean region in South America, and its domestication process is still somewhat obscure to date, but is presumed to have

occurred between South and Central America around 7000 years ago (Razifard et al., 2020). In the early 16th century, during the conquest of America, small yellow tomatoes from Tenochtitlan (current Mexico City) arrived to Spain. From there, they moved through Europe, and came to Italy through Naples, which was under Spanish rule at the time. However, because of the physical resemblance to other toxic species in the Solanaceae family (like *Atropa belladonna* and *Mandragora officinarum*) its use in Europe remained as ornamental until the 17th century (Gentilcore, 2010; McCue, 1952).

The 19th century saw a big increase in the amount of different varieties and ecotypes of tomato, mainly obtained through the efforts and observation skills of farmers and enthusiasts, by means of natural mutation, natural outcrossing or recombination of pre-existing genetic variation. As tomato is mostly self-pollinating, selection was usually done for individual plants, whose progenies were fairly similar to the mother plant. This process led to a plethora of varieties and landraces that are still grown, highly valued and strongly linked to culture and gastronomy in certain regions (Bauchet and Causse, 2012). Although somewhat wide and subject to discussion, the term landrace is usually related to “cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems” (Camacho Villa et al., 2005).

With the start of 20th, the interest for formal breeding in tomato using the mendelian principles arose, especially in the United States (where it was re-introduced from Europe). One of the prominent figures in these context was Charlie Rick from University of California at Davis, who noted the bottleneck trough which tomato was submitted and was the first to promote the concept of using wild accessions as a source of variation in breeding programs (Rick, 1974). Moreover, with the increasing popularity of hybrid crops, the first hybrid F1 tomato was set into the market in 1946 with the name “Single Cross” (Bai and Lindhout, 2007). Eventually, F1 hybrids have become the single most dominant type of seed in the market.

At the European level, half a billion euros worth of tomato seed are sold annually, mostly hybrid seed bearing fruits for fresh consumption (Bai and Lindhout, 2007). It is a very competitive industry, with an expected turnover rate of 5 years, in which it is normal for breeding companies to make use of the competence’s genetic material for their own programs, as a means to accelerate breeding and which is perfectly allowed according to Plant Breeder’s Rights. Nonetheless, the market is highly concentrated; in 2013, 95% of the total market share and 43% of the registered varieties belonged to only 5 companies. The top 2 of those 5 companies (Bayer and Syngenta), are also world leaders in the production and sale of chemical inputs, especially for pest control; this in turn makes the modern genotype’s dependence on these inputs fairly expectable (Mammana, 2013). In addition, the domestication and intensive breeding process in tomato during the last centuries has

led to a steep decline in allelic diversity in tomato, and it is estimated that cultivated tomato only accounts for < 5% of variation found in its wild relatives (Bai and Lindhout, 2007).

In terms of nutrition, tomato has a wide variety of important compounds with antioxidant, anti-inflammatory and anticancer activities, such as carotenoids, flavonoids and vitamin C (Salehi et al., 2019). However, there is a growing body of evidence showing that modern varieties, in comparison with landraces or older varieties, contain substantially less total soluble solids, titratable acidity, ascorbic acid and volatile aromatic compounds, which are also linked to the nutritional value and the sensorial attributes of tomato (Rodríguez-Burruezo et al., 2005; Tieman et al., 2017). In fact, for these and other reasons, the heirloom tomato varieties have significantly increased popularity during the last decades in the industrialized world and are now being re-conceptualized as cultural objects linked to taste, territory and tradition (Jordan, 2007).

Not only have they better nutritional and sensorial qualities, but important agronomic traits have also been linked to heirloom varieties and landraces. For example, European Mediterranean landraces have been shown more stable than modern genotypes when facing water deficit (Fullana-Pericàs et al., 2019), saline stress (Massaretto et al., 2018), or specific diseases (Boziné-Pullai et al., 2021). As a downside, many landraces are now still scarcely cultivated due to reasons like: lower potential yields, susceptibility to low temperatures, blossom tip rot, abnormal fruit shapes at the blossom end, blotchy ripening at high temperatures and, in some cases, susceptibility to specific diseases (Campanelli et al., 2015; Casañas et al., 2017).

One of the most prominent examples of tomato landraces is the ox-heart or beef-heart type of tomato (*Coer de Beuf* in French; *Cuor di Bue* in Italian). This typology encompasses a wide array of diverse local varieties which share specific morphological traits: they bear sweet large fruits (usually > 150 g), with very prominent ribs and usually wider at the stem end than at the blossom end (that which gives the characteristic heart shape). Ox-heart tomatoes also have often a pinkish color in their skin, as the result of *colorless epidermis* (*y*) mutation (Mazzucato et al., 2010). Moreover, there is no clear absolute characterization for these typology and there is discussion if some varieties belong or not into this category.

Even though Italian heirloom tomatoes languished as a result of the increasing importance of round and smooth tomatoes in the mid-decades of the last century, they resurged in the 1980s with the appearance of denominations of protected origin like “San Marzano”, which is a rather long and soft fruit (Gentilcore, 2010). Moreover, despite being a secondary center of diversity with large amount of local diverse heirloom varieties (Mazzucato et al., 2008), many of the varieties cultivated nowadays are bred and selected outside of Italy (Campanelli et al., 2015), and, as in many other crops species, genetic erosion has been documented (Casals et al.,

2011). Also, despite the historical and cultural importance of tomato in Italy, as of 2010, only one landrace (“San Marzano”) had received a Protected Designation of Origin (Mazzucato et al., 2010), later joined by the Sicilian “Pomodoro di Pachino” in the form of a Protected Geographical Indication (Italian Ministry of Agriculture, 2021). Five tomato conservation varieties are currently listed in the Italian National variety catalogue indicating a growing interest by commercial operators for local varieties (Canestrino di Lucca, Fiaschello battipagliese, Pisanello, Pizzutello dell'Agro Ericino, Riccio di Parma).

In this context, organic agriculture has had a significant role in the preservation and ongoing evolution of landraces in many different crops, and tomato is not the exception; the robustness and resiliency of these varieties may largely explain this link (Boziné-Pullai et al., 2021; Dwivedi et al., 2019). Also, organic tomato cultivation has been significantly growing in the world, especially in the US (Hoagland et al., 2015) and Europe (Fruit Logistica, 2020).

But, still, one of the biggest constraints in organic tomato yield and expansion might be genetic, as it is estimated that around 95% of the plant material used was bred under conventional farming conditions (Lammerts van Bueren et al., 2011). Thus, a case has been made to stress the importance of breeding specially for organically managed environments (Murphy et al., 2007). Key traits such as weed competitiveness, increased nutrient use efficiency, tolerance or resistance to pests and pathogens and an enhanced capacity to beneficially interact with the soil microbes, should be central in breeding for organic agriculture. Though these characteristics are also important in conventional breeding, their lack can be often compensated with high amounts of chemical inputs.

While some argue that breeding for organic farming should be done exclusively (or at least mostly) at organic fields (Reid et al., 2009), others state that it could be framed into breeding for conventional agriculture, mainly by including trials in organically managed environments with the advanced segregation populations (Crespo-Herrera and Ortiz, 2015). For the reasons exposed before, namely the high importance of exploiting GxE interactions at early segregation stages, the first option would seem more reasonable.

In the specific case of tomato, previous experiences point towards this direction. For example, after screening more than 3500 tomato genotypes at different locations in Germany under organic management, most of the best performing genotypes came from an previous organic screening or breeding background (Horneburg and Becker, 2008). For this case, great importance was given to the tolerance/resistance to tomato late blight (*Phytophthora infestans*), as it represents a major constraint in tomato production and genetic defense is likely the best tool for organic farmers against this disease (Hoagland et al., 2015).

Previous PPB programs for tomato organic cultivation have been already undertaken in Italy (Campanelli et al., 2019, 2015), as well as in Catalonia (Casals

et al., 2019) and the United States (Hoagland et al., 2015). The results offer promising prospects, as high yielding, locally adapted and flavorful genotypes have been delivered. Nonetheless, there is still plenty of room for breeding tomatoes, as a diversity of ideotypes in terms of fruit characteristics and local adaptation are required. Even traditional landraces and heirloom varieties, which are often thought as static and inalterable, can be subjected to breeding programs in order to attain goals of adaptation or improvement of specific characteristics (Casañas et al., 2017).

Finally, regardless of the breeding condition of materials, according to the European Organic Regulation (834/2007), crops growing under organic certification must come from organically certified seed. This is true unless there is a proven non-availability, in which case a derogation of the norm is allowed. Many European countries, including Italy, list available organic certified seed on a public database to facilitate transactions between farmers and seed suppliers and increase transparency of the organic seed market. However, despite this, out of the approximately 360 million transplants of organic tomato done yearly in Southern Europe, 70% are done with conventional seed through derogation of the norm, whereas 20% came from certified seed and 10% came from farm saved seed (Solfanelli et al., 2019). This situation shows that there are still great important gaps to close in order to fulfill the demand of tomato organic seed.

1.4. The FP7 SOLIBAM and the H2020 LIVESEED European Projects

As a response to the growing need of genetic material suitable for the above mentioned needs, an European Project was initiated in 2010 and named “Strategies for Organic and Low Input Integrated Breeding and Management” (SOLIBAM, www.solibam.eu). The project involved 12 countries, 23 organizations and over 50 field experiments with crops such as wheat, barley, maize, tomato and broccoli (Petitti, 2018). For tomato, different projects regarding cover crops, drip irrigation and sensorial attributes were executed. Most importantly, tomato was used as a model to conform the first documented Composite Cross Population on a vegetable species, emulating what was done before in cereals (SOLIBAM, 2015). This was done in collaboration with seed companies in France (Gautier Semences) and Italy (Arcoiris).

The project phased out in 2015 and the breeding process on the tomato CCP was halted. The work on this population was continued with another European initiative, the LIVESEED project (www.liveseed.eu), which started in 2018 with the objective of enhancing the use of organic seed and adapted cultivars. The Università Politècnica de Valencia in Spain conducted participatory evaluations of Spanish and Italian local varieties of tomato, whilst the SOLIBAM tomato population was used for experimentation by the Rete Seme Rurali (RSR) in Italy. The latter is an NGO that works as an umbrella organization integrating several farmer’s associations across

the country. The population was grown in five different locations the first year, reduced to 4 locations in the second year. In each location, a part of each population was submitted to Farmer's Selection (FS) or Natural Selection (NS). The third and final year, all the populations selected at each environment (4 FS and 4 NS) were tested in a Multi-Location Trial in the 4 selection farms. For the purpose of this study, we consider only the 4 farms/locations that completed the two cycles of participatory selection and hosted the final comparative trial. The results of this process will be the object of analysis of the present thesis.

2. Research Objectives

The main objective of the present thesis is to determine the effectiveness of decentralized farmer's selection for breeding superior genetic material of ox-heart type tomato for Italian organic farms.

The specific objectives are:

1. To compare the agronomic characteristics of the Farmer's Selection, Natural Selection and Control Populations grown under 4 different Italian organic farms.
2. To analyze the main changes between the Farmer's Selection and Natural Selection populations bred at the same locations.
3. To quantify the effects of local adaptation for the Farmer's Selection and Natural Selection populations to their respective breeding environments.
4. To identify the main correlations between the participant's evaluations and the measured traits in the ox-heart tomato plots.

3. Materials and Methods

3.1. Plant material

The tomato population used for this study originated from the European Research Project SOLIBAM. In 2011, 35 different landraces were evaluated in four locations in France and Italy. Following a participatory evaluation, four genotypes were selected and used to generate a composite-cross in 2012, thus constituting the foundation of the population (Table 1). The selection comprised two ox-heart types from France and Italy (*Cuor di Bue* and *Cœur de bœuf*, respectively), and two Spanish landraces (the Marmande type *Muchamiel* and the pear shaped *Allungato de Alicante*). Half-diallel crosses were performed between the 4 genotypes, resulting in 6 different F₁ progenies. In the same year, these were grown in isolation and self-pollinated, and the seed harvested from each sub-population was mixed to constitute one sole F₂ population.

400 plants of this population were grown in 2013 at Il Lombrico Felice Farms (Perugia, Italy) and were subjected to selection by Antonio Lo Fiego (responsible for selection at Arcoiris, an organic seed Italian company) and Bruno Campion (Researcher at the Italian Council for Agricultural Research and Agricultural Economy Analysis) using plant health, productivity and fruit shape as selection criteria. The main goal of this selection was to discard off types with deformities or extreme susceptibility to disease. The resulting F₃ population was planted in 2014 at Orti Corti di Sant'Arcangelo di Romagna (Emilia-Romagna, Italy) and submitted to selection using the same criteria. The harvest of this second cycle of selection constitutes the F₄ population that was used as starting material for this project.

Table 1. Landraces used to constitute the tomato participatory breeding population. Source: Petitti (2020).

| Name | Allungato de Alicante | Muchamiel | Cuor di Bue | Cœur de bœuf |
|------------------|-----------------------|------------|--------------|--------------|
| Origin | Spain | Spain | Italy | France |
| Type | Long shape | Marmande | Ox-Heart | Ox-Heart |
| Maturity | Late | Medium | Early | Medium |
| Growth Habit | Indetermined | Determined | Indetermined | Indetermined |
| Fruit weight (g) | 150 – 190 | 190 - 250 | 190 - 250 | 190-250 |

3.2. Trial Locations

In the frame of the H2020 LIVESEED European project, seeds from the F₄ populations were sent to different organic farms in Italy to conduct a three-year Participatory Plant Breeding Project. Four different locations, representing diverse pedoclimatic conditions, were selected. In three of the four locations, the project was implemented in the same farm throughout the three years. These farms were: Alle Fontanine Farm (Sestola, Emilia-Romagna), Silvano Di Leo's Farm (Castronuovo di Sant'Andrea, Basilicata) and Pollino Experimental Farm (Rotonda, Basilicata). In the case of Molise, the fourth location, three different farms were involved, these were: Vincenzo Battezzato's Farm (Campobasso, 2018 and 2019), Agribio Petacciato (San Giuliano di Puglia, 2019) and Primo Sole (Campobasso, 2020). A fifth location was included in 2018 (Diversamente Bio Farm, Padova), but not in the following years, and thus the related information is not included.. The geographic distribution of the 4 locations can be seen in Figure 1. It is also important to note that the farms at Sestola, Castronuovo and Molise were all privately owned by small-medium farmers, whilst the farm of Rotonda is the experimental farm of a public funded regional research station (ALSIA Basilicata).



Figure 1. Trial Locations in Italy for the Ox-heart tomato Participatory Breeding Project.

The summary for the climatic conditions is presented in Fig. 2. Although patterns throughout locations and years are fairly similar, important differences should be pointed out. In 2020, Castronuovo had an important rainfall event including a hailstorm, which severely damaged the crop. Days with extremely high rainfall were also observed in other locations that same year, and the most extreme event was in Sestola in July, in which the accumulated daily rainfall was as high as 85 mm. As for the temperature, the warmest of locations was Castronuovo, where the highest temperature recorded was 35°C. Rotonda, despite being very close to Castronuovo, had lower temperatures as it is located at a higher altitude. Sestola, the northernmost location, was also the one in which the lowest temperatures through the growing cycles were recorded.

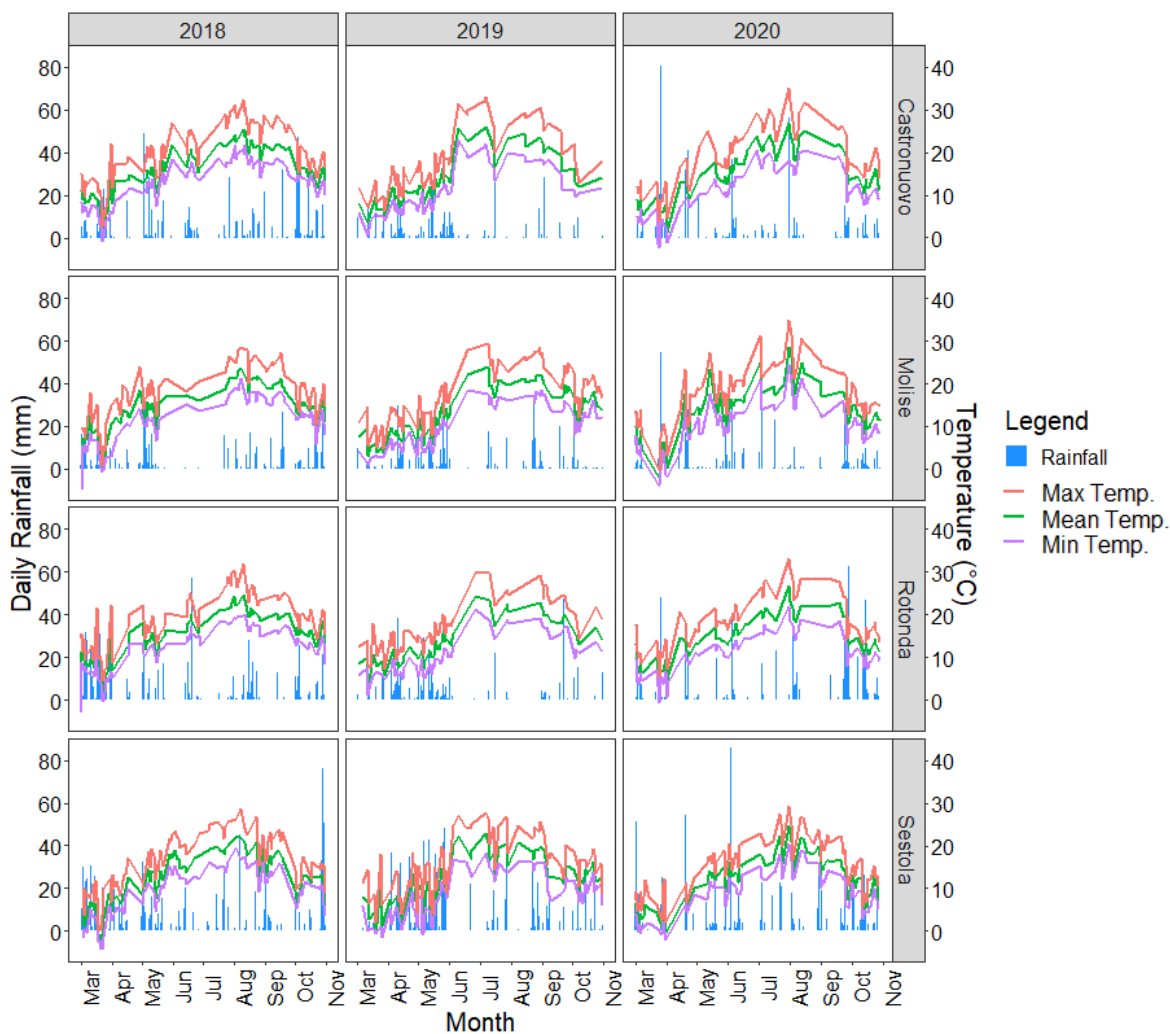


Figure 2. Daily rainfall and temperature (mean, minimum and maximum) for the tomato growing season in 2018, 2019 and 2020 in the four trial locations for the tomato PPB project. Data for Molise, Castronuovo and Sestola come from the regional Civil Protection Service, whilst the source for Sestola is ARPAE Emilia-Romagna.

3.3. Participatory Selection

In 2018, for each of the four locations, plots of 400 plants from the aforementioned F₄ population were planted, following agronomical management in line with each farm's practices. Right before the first harvest, a group of participants (mainly farmers, but also researchers, technicians, students, activists, chefs, etc.) were invited to the field and asked to evaluate each plant. Each of them was given a set of sheets with a row for each of the 400 plants, and asked to independently give a mark between 1 and 4 to each individual plant (1= "Not satisfying at all", 2= "Unsatisfying", 3= "Satisfying", 4= "Very satisfying"). The evaluations from all participants were averaged and 20 seeds from each of the 20 plants which had received the best evaluation were taken to constitute the *Farmer's Selection Population* for next year. Even though selection was not only done by farmers, the name *Farmer's Selection* (FS) will be adopted for simplicity. At the same time, one random fruit per plant in the whole 400 plant population was taken, and their seeds were mixed to constitute the *Natural Selection Population* (NS) for next year.

In 2019, for each location, the 400 plants of the FS populations were planted and evaluated in the same way as the previous year. In all farms, spatial arrangement consisted of 10 rows of 40 plants each, in order to allow for an even distribution of the 20 plants deriving from each selected plant the previous year. Participatory evaluation was done equally, and a new 400 plant FS population per location was constituted with 20 seeds from the 20 plants which had received the best evaluation. Simultaneously, 400 plants from the NS originated the previous year were planted and, again, the seeds from one random fruit per plant were collected to advance one generation in each NS population. Thus, after two growing cycles and two generations, four FS populations and four NS populations, one from each location, were obtained. In the case of Molise, in which three farms were part of the project, the 2018 FS and NS were obtained from Vincenzo Battezzato's Farm. In 2019, the FS was planted and selected at the same farm, but the NS was done at Agribio Petacciato's. The summary for the breeding scheme can be seen in Fig. 3.

3.4. Multi Location Trial

In 2020, a Multi-Location Trial (MLT) was performed in the four locations. In the case of Molise, the trial was done at Primo Sole Farm, whereas in the other three locations, the trial was done in the same farms in which the breeding scheme was implemented. All the four Farmer's Selection (FS) and the four Natural Selection (NS) populations were sent to all 4 locations. A local variety (LV) or local control from each location was also chosen according to the will of the local participants. The "Costoluto di Parma" was the variety chosen for Sestola, while "Rosa di Castronuovo" (donated by Silvano di Leo) and "Miscuglio di Rotonda" (donated by

Domenico Cerbino, from Pollino Experimental Farm) where the local varieties for their respective locations. For Molise, the “Kéro F1” was used as the local control. Also, an Open Pollinated Variety (Belmonte, Sementi Biologici, Italy) and a popular F1 Hybrid (Déko F1, ISI Sementi, Italy) were included, for a total of 14 genotypes evaluated.

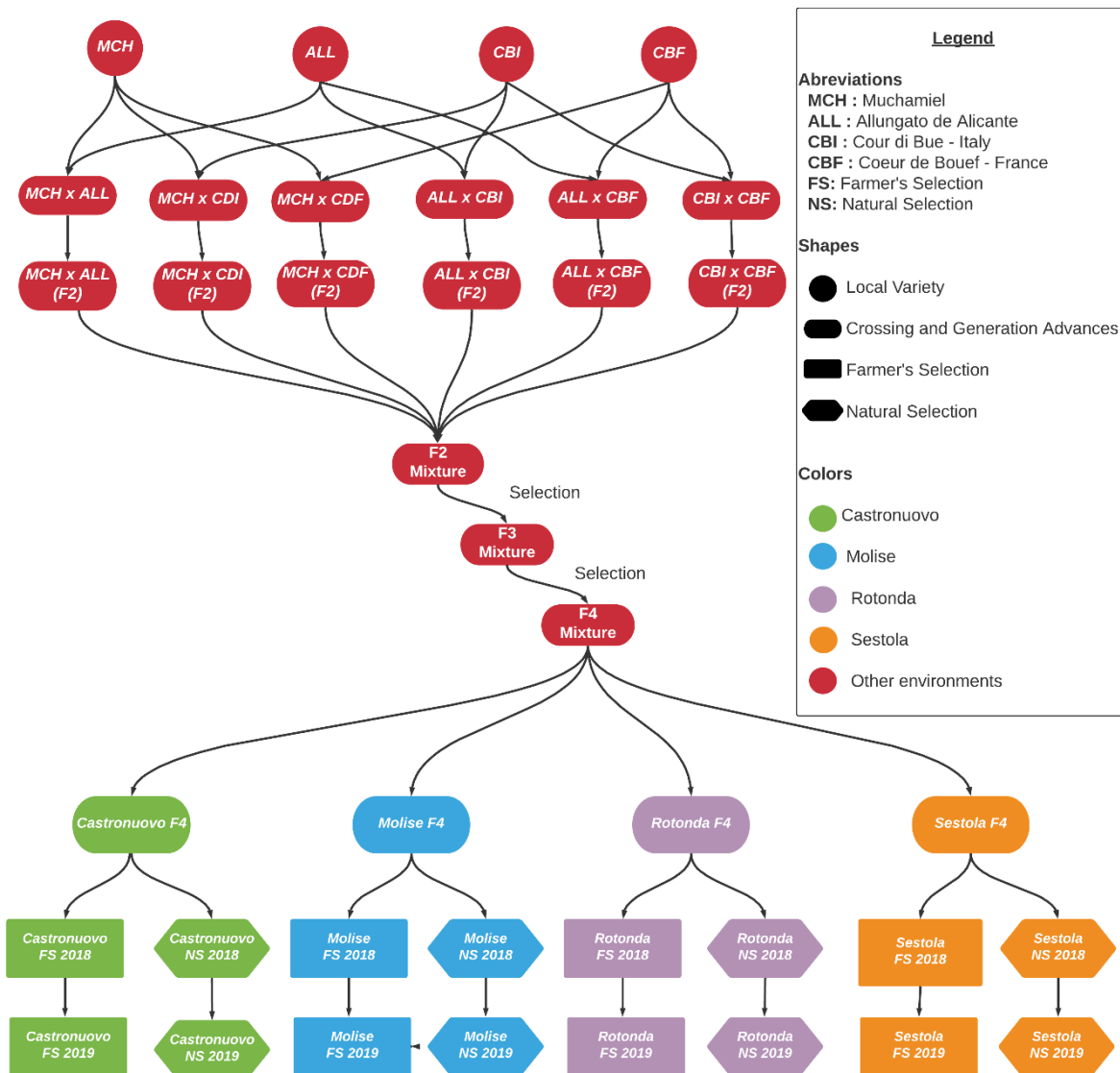


Figure 3. Breeding Scheme for the Participatory Plant Breeding Program for ox-heart tomato.

Trials were set in a display of 28 plots per field, so that each entry had two repetitions. Each plot consisted of 20 plants, subdivided into two adjacent rows of 10 plants each. The total number of rows per field was 14, each row had 40 plants and every pair of rows contained 4 plots. The distance between rows was of 1.5 m and distance between plants varied from 20 cm to 40 cm depending on the farm. A picture for each of the fields showing the basic aspects of plot layout per farm is seen at Fig. 4. Randomizations for the field position of each plot were done with the software DiGGer, which produces non-factorial experimental designs for plots arranged in rows and columns (Coombes, 2009)

Yield data was collected in all four locations by the host farmers. The plots were harvested in four different occasions, and in each of those occasions the number of plants harvested, the number of fruits and the total fruit weight were recorded. The yield was also divided into commercial and non-commercial harvest, according to the criteria from the corresponding farmers.

Finally, for each of the four locations, a group of participants (mostly farmers, but with the participation of other kinds of actors) were also invited to evaluate each plot. This time, evaluations were also given in a 1 to 4 scale, but five different parameters were considered: i) disease resistance, ii) homogeneity, iii) plant vigor, iv) perceived productivity and v) overall evaluation. Each participant was given a sheet with the plot numbers and was asked to do the evaluation independently. The identity of each plot was revealed only after all evaluations were given.

3.5. Statistical Analysis

Although plots started with 20 plants, hail storm and extreme rain events reduced plot stand, especially at Sestola where the plants per plot varied between 13 and 20 plants (mean of 15.9 ± 1.96). Therefore, the yield components were divided by the actual plot stand, to obtain weight and number of fruits per plant. Although we considered ANCOVA as one option, by using the actual number of plants per plot as a covariate, it was discarded because: a) the basic assumptions were not met (specifically, linearity between covariate and response for all groups), and b) the plants were widely spaced, thus diminishing the competition effect that might have biased the per plant mean in the case of missing plants.

All the data analysis and plots were made through R and the RStudio platform (RStudio Core Team, 2021). First, ANOVA tests were used to determine the significance of factors contributing to the observed variance in the measured variables. The ANOVAs were performed with location, genotype and genotype x location interaction as factors. Additionally, the repetition factor was added as a nested effect within the location effect.

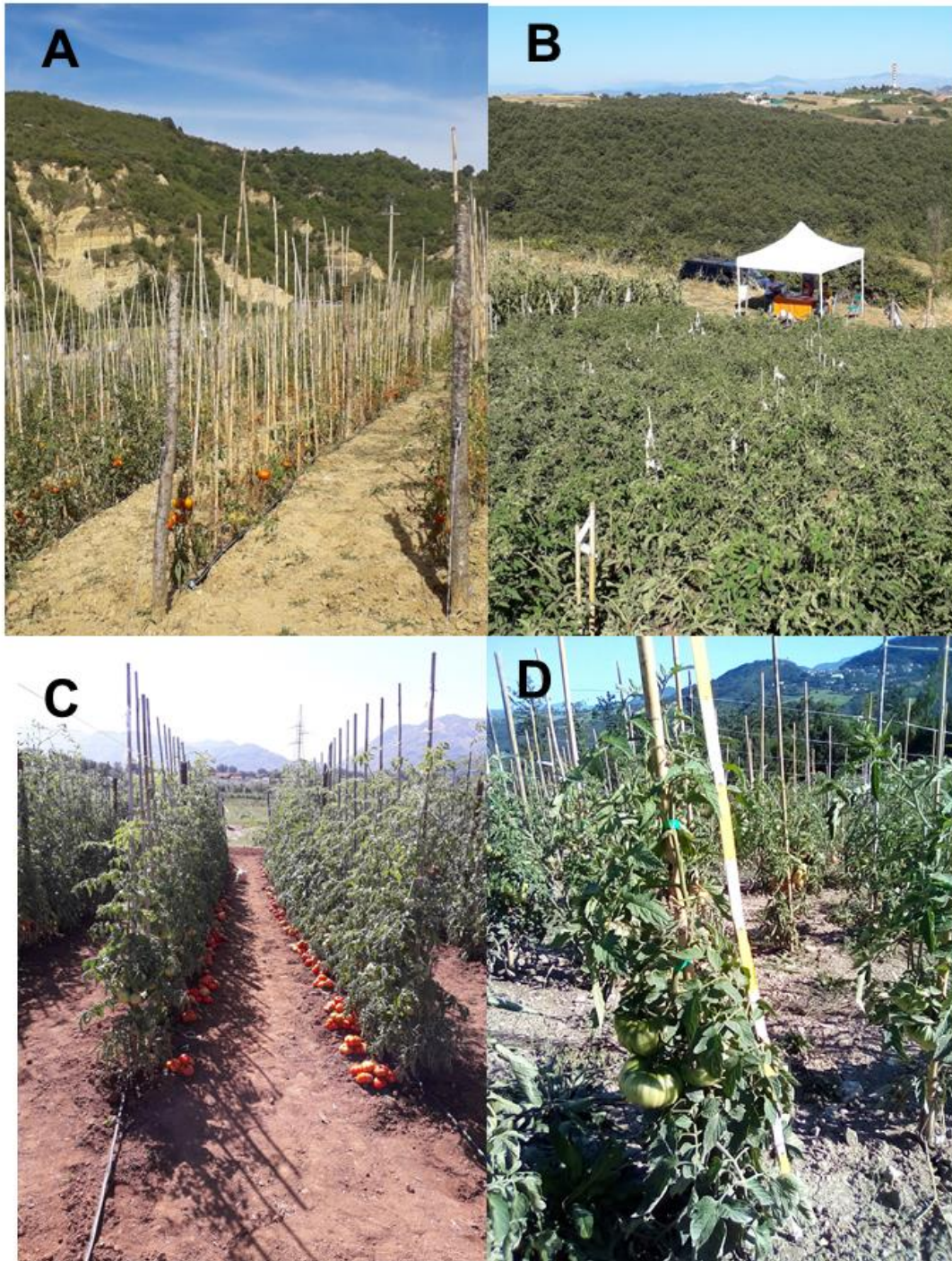


Figure 4. Picture of the Multi-Location Trial plots for tomato established in 2020 at four different organic farms. A= Castronuovo, B= Molise, C= Rotonda, and D= Sestola. Credits: Salvatore Ceccarelli (A, B and C) and Matteo Petitti (D)

Further on, Spatial analysis was performed to correct for the field heterogeneity and to calculate the Best Linear Unbiased Estimators (BLUEs) for each genotype in each location, as well as spatially fitted values for each plot. This was done with the R package “SpATS”, which uses two-dimensional P-splines with anisotropic smoothing within the mixed model framework (Rodríguez-Álvarez et al., 2018). The model used is the following:

$$y = f(u, v) + Z_c c_c + Z_r c_r + X_g \beta_g + \varepsilon$$

Where y is the response variable and $f(u, v)$ is the “smooth-by-smooth interaction trend jointly defined over the row and column directions”. $Z_c c_c$ and $Z_r c_r$ correspond to the random effects of column and row, and their associated design matrixes. $X_g \beta_g$ stands for the genotypic effect considered as fixed. Finally, ε represents the error or residuals of the model (Rodríguez-Álvarez et al., 2018). As this was done for each location separately, this factor is not included in the model. The adjusted means (the sum of the BLUEs and the intercept) and its associated standard error were used to plot the values for each genotype at each location. These values were also used to perform Tukey HSD mean comparison tests in each environment, in order to statistically separate the genotype means ($\alpha = 0.05$).

The BLUEs values were then used to analyze the information through GGE Biplots (Yan and Kang, 2002), executed through the R package “metan” (Olivoto and Lúcio, 2020). GGE Biplots are a useful tool for the analysis of Genetic and Genotype x Environment effects, where the PC1 (x-axis) is proportional to the first and the PC2 (y-axis) to the latter. For this case, values were standardized and plots centered around the environmental values. Two specific features of the GGE BiPlots were used. The “Which-Won-Where” type draws a polygon along the genotypes that are the farthest away from the origin, this means that they are the genotypes with longest vectors and are thus the most responsive. As a rule of thumb, genotypes which are on the polygon vertex had the best performances in the environments found in the same sector (Yan et al., 2007). The “Mean vs. Stability” feature draws a first line, the mean environmental axis, passing through the origin with an arrow representing the “ideal” environment, and then a second line perpendicular to the first, which represents the GxE interaction. Genotypes with projections close to the arrow in the first axis would be high performers in this ideal environment, while large projections on the second axis indicate low stability or high GxE interaction. For the particular case of this work, GxE interaction is interpreted only as Genotype x Location interaction, as the trials were only done in one year.

In order to address the second and third specific objectives, two main questions drove the analysis for the BLUEs data from the MLT. First, if farmer’s selection was effective for the evaluated traits. And second, if there were any evident effects of local adaptation of the populations to the selection environments. Each of these questions had their specific comparison of interest (Table 2).

Table 2. Research questions and comparisons of interest that drove the statistical analysis of the data coming from the tomato PPB project.

| Research Question | Comparison of Interest |
|---|--|
| Was farmer's selection effective for the evaluated traits? | Farmer's Selections vs. Natural Selection from the same environment |
| Are there evident effects of local adaptation in the populations to the selection environments? | For each location, Local Natural Selection Populations vs. Non-local Natural Selection Populations |

Further on, to better understand the changes between the farmer's selection and the natural selection population, a comparison of the yield components was made. Thus, the yield of each population at each environment was dissected by time (the four different harvests), mean fruit weight and fruit number. A two-sample t-test was made at every possible point of comparison and the respective p-values were calculated to determine the probability of significance differences in the comparisons.

Moreover, to identify correlations among the participant's evaluations and the measured traits, the Pearson's correlation coefficient (r) was estimated for each pair of traits at the overall level, and then at every location. The measured traits included were: yield at first harvest, total yield, mean fruit weight, number of fruits per plant and percentage of marketable yield. Also, the five different evaluation scores were included, that is, overall evaluation, perceived productivity, vigor, disease resistance and homogeneity. The Overall Evaluation was also divided between male and female participants, to explore gender trends in the evaluation. Additionally, a special mean for the overall evaluation is included, estimated only with the evaluations of consistent actors, that is, those who participated in 2020 and also in at least one year before (2018 or 2019). Trends according to age were not evaluated, as participant's were not asked to declare their age before evaluations. Finally, a correlation plot was built with all the r coefficients and their corresponding significance level, using the R package "metan" (Olivoto and Lúcio, 2020).

After comparing genotypes with individual variables, a multivariable analysis was done through a Principal Component PCA. The PCA was performed at an environment level, using the mean value of each genotype for each of variables described in the correlation analysis. These were done using the R package "factoextra" with the default parameters (Kassambara, 2017).

4. Results

4.1. *Participation in Breeding and Evaluation of Genotypes*

A wide variety of diverse actors took part in this project across the three years and the four locations. A grand total of 174 different participants (out of which the 44% were farmers) evaluated the plots at least once during the three years. The location with the highest participation was Rotonda, and 2020 was the year with most participants across all locations. In most cases, farmers comprised the largest percentage of evaluators and technicians were the second most numerous group (Figure 5, upper panel). Researchers, teachers and students, were also professions with high frequency among the evaluators. Men were more represented than women as 130 of these actors were male and only 44 were female. During the 2020 Multi Location Trial (MLT), 60 men and 22 women participated.

Regarding consistency in participation across the years, a relatively small number of participants were present in more than one year (Figure 5, lower panel). In 2019, the percentage of evaluators who were also present in 2018 varied from 18% in Rotonda to 67% in Molise (but with only 3 evaluators in total). For the 2020 MLT, the number of evaluators who participated in both previous years was between 6% and 8% for Rotonda, Castronuovo and Molise, while in the case of Sestola none of the evaluators had that level of previous experience. Finally, regarding participants in 2020 with only one year of previous experience (being that 2018 or 2019), the percentage varied between 18% and 38% for Sestola, Rotonda and Molise, and was of 0% in Castronuovo.

Overall, Rotonda was the only location where farmer's comprised the majority of evaluators in all three years, and also the only one to have a majority of famers in 2020. Also, this location is the one with the highest percentage of consistent participants in 2020. On the other hand, Molise and Sestola had years with very scarce participation, with minimums ranging from 3 (Molise in 2019) to 7 participants (Sestola in 2020).

4.2. *ANOVAs for agronomic characteristics*

The ANOVAs reveal that for all traits, excepting mean fruit weight, the location effect was the most influencing (Table 4). Moreover, a highly significant genetic effect ($p < 0.001$) was found for mean fruit weight, fruit number and percentage of marketable yield, and a significant ($p < 0.05$) effect was also found for the mean overall evaluation, yield at first harvest and total yield. The genotype x location effect

was also significant for all traits and its effect was stronger and more evident for total yield and percentage of marketable yield.

Regarding location effects, the data reveals deep dissimilarities on the crop conditions at the different farms. For most of the traits, the overall best location was Rotonda, which had the highest total yield (g/plant), number of fruits per plant and percentage of marketable yield (Table 5). Nonetheless, the highest yield in the first harvest was found at Sestola. Overall participant's evaluation also varied across locations, as the plots received a higher score in Rotonda and Molise, followed by Sestola and lastly Castronuovo. In this last location there was no marketable yield at all (according to the corresponding farmer who did the evaluation), as a hailstorm severely damaged the crop.

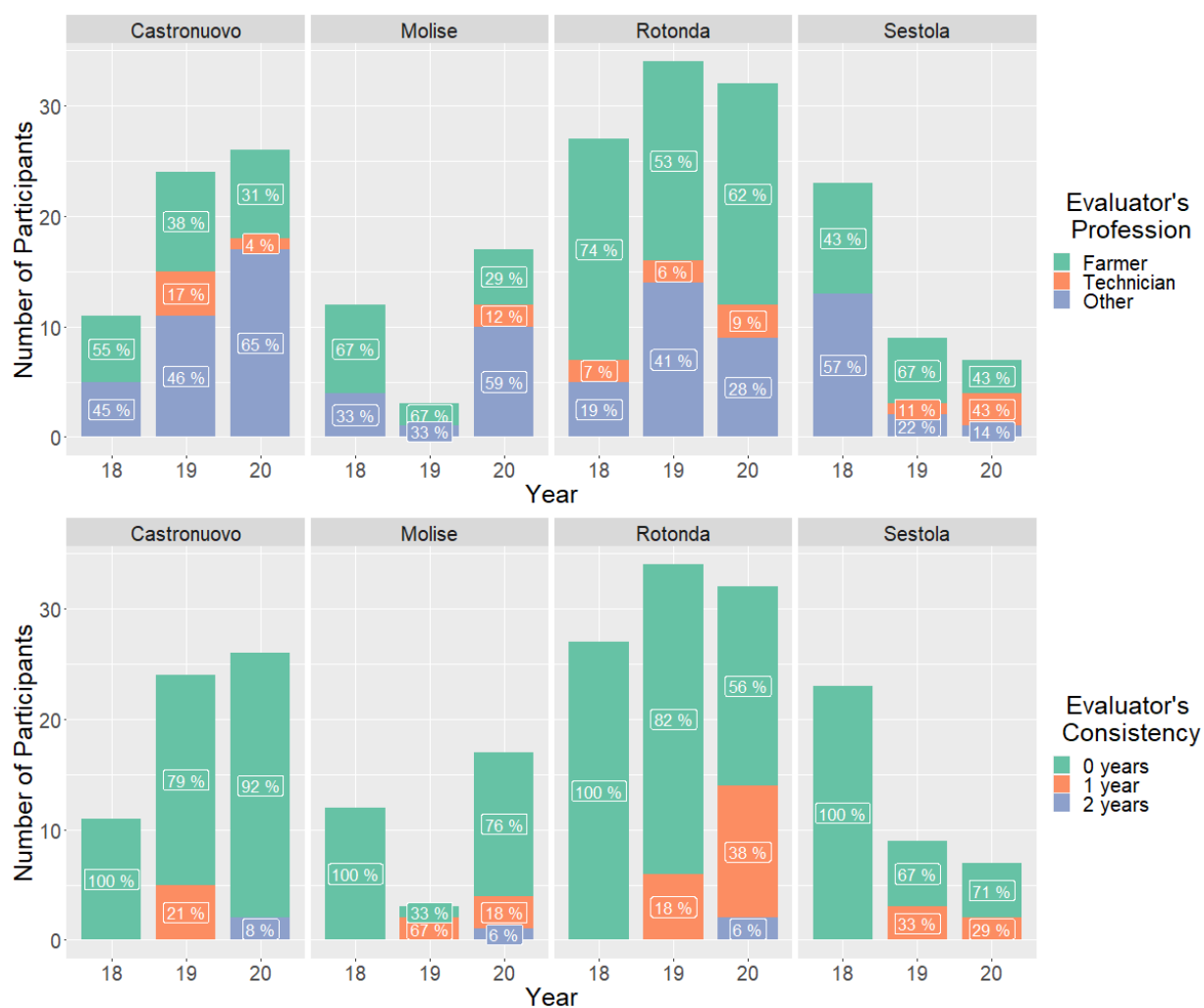


Figure 5. Evaluator's profession (upper) and consistency (lower) across years and locations in the participatory plant breeding program in tomato.

Table 4. Percentage of the total sums of squares and significance level for the sources of variation considered in the ANOVA model for the agronomic traits evaluated in the Multi Location Trial for tomato. *p < 0.05; **p < 0.01; ***p < 0.001.

| | Genotype | Location | Genotype * Location | Repetition/ Location | Residuals |
|-------------------------------|-----------|-----------|------------------------|-------------------------|-----------|
| Degrees of Freedom | 13 | 3 | 39 | 4 | 52 |
| Overall Evaluation | 12.75 ** | 35.07 *** | 32.56 ** | 0.53 | 19.08 |
| Yield at First Harvest | 15.59 ** | 45.75 *** | 23.19 ** | 0.79 | 14.69 |
| Total Yield | 1.46 * | 88.40 *** | 7.47 *** | 0.13 | 2.54 |
| Mean Fruit Weight | 66.30 *** | 0.91 | 18.37 * | 0.74 | 13.66 |
| Total Fruit Number | 10.21 *** | 80.52 *** | 5.19 * | 0.10 | 3.96 |
| % of Marketable Yield | 2.54 *** | 87.06 *** | 9.12 *** | 0.09 | 1.18 |

Table 5. Mean and standard error at each location for the main traits evaluated in the Multi Location Trial for tomato. Different letters indicate different groups according to the Tukey HSD test ($\alpha = 0.05$).

| | Castronuovo | Molise | Rotonda | Sestola |
|--|----------------|----------------|------------------|----------------|
| Overall Evaluation | 2.3 ± 0.1 c | 3 ± 0.1 a | 3 ± 0.1 a | 2.6 ± 0.1 b |
| Yield at First Harvest (g/ plant) | 148.5 ± 16.6 b | 19.4 ± 3.8 c | 123.5 ± 17.4 b | 216.6 ± 17 a |
| Total Yield (g/ plant) | 441.4 ± 39.8 c | 872.6 ± 30.9 b | 3010.1 ± 117.1 a | 993.2 ± 53.6 b |
| Number of Fruits per plant | 2.8 ± 0.4 c | 5.5 ± 0.5 b | 17.1 ± 0.7 a | 5.7 ± 0.4 b |
| Mean Fruit Weight (g) | 174.3 ± 11.7 a | 175.6 ± 8.6 a | 183.7 ± 9 a | 185.7 ± 10 a |
| % of Marketable Yield | 0 ± 0 d | 64.3 ± 1.3 b | 80.1 ± 1.6 a | 39.1 ± 4 c |

4.3. BLUEs estimation and genotype comparison

To better estimate the performance of each breeding material, the BLUEs values for each genotype and each variable were estimated at the environment level through the spatial analysis methodology previously described. So, the results for the variable of overall evaluation indicate the preference for some genotypes across locations, but no single genotype ranked in the top for all the environments (Fig. 6). At Castronuovo, the genotype with the highest score was the F₁, followed by the Molise Local Variety (Mol LV). In Molise, the best ranked was the Castronuovo NS and in Sestola it was the Molise FS. Finally, in Rotonda, the best two ranked genotypes are the Rotonda FS and the Sestola NS.

In a general comparison between the FS and the NS counterparts (*ie.* those bred at the same location), no consistent pattern is found. In some cases, the FS outperforms the NS, like in the case of the Molise Populations at Sestola or the Castronuovo populations at Castronuovo and Molise. In other cases, the opposite happens, as for example with the Molise, Rotonda and Sestola populations at Castronuovo. If only NS populations are compared to identify specific effects of adaptation in this trait, the only locations with significant differences between NS populations are Castronuovo and Molise. However, in none of those cases did the local NS population outperform the NS populations from the other locations.

For this trait, the GGE Biplots show an interesting pattern regarding Rotonda (Fig. 6 B), where both the local FS and the NS were notably better evaluated here than when tested elsewhere, indicating strong evidence for local adaptation and/or differences in local participant's preferences. Additionally, the GGE Biplots indicate that Rotonda (aligned to the left) is very different than the other locations (aligned to the right). In fact, the Mean vs. Stability Biplot (Fig. 6 C) draws a line with an arrow in the hypothetical mean location, but it completely draws apart the best performing genotypes at Rotonda and those better performing at Sestola and Castronuovo. For the latter, the entries with the highest means are the F₁, Mol FS, Ses LV and Mol LV, although their scores are very unstable, as they have high projections in the secondary axis. Lastly, it is noteworthy that, on the GGE Biplots, Mol FS is an outstanding genotype in Sestola and, in a reciprocal manner, Ses FS and Cas NS are outstanding genotypes in Molise.

Regarding total yield at first harvest, notable differences were found in all locations (Fig. 7 A). At Sestola, the location with the highest yields at first harvest, a remarkable situation occurs, as all FS have higher BLUEs values than their NS counterparts. Interestingly enough, the FS from Sestola had also higher values than the Sestola NS at Rotonda and Molise.

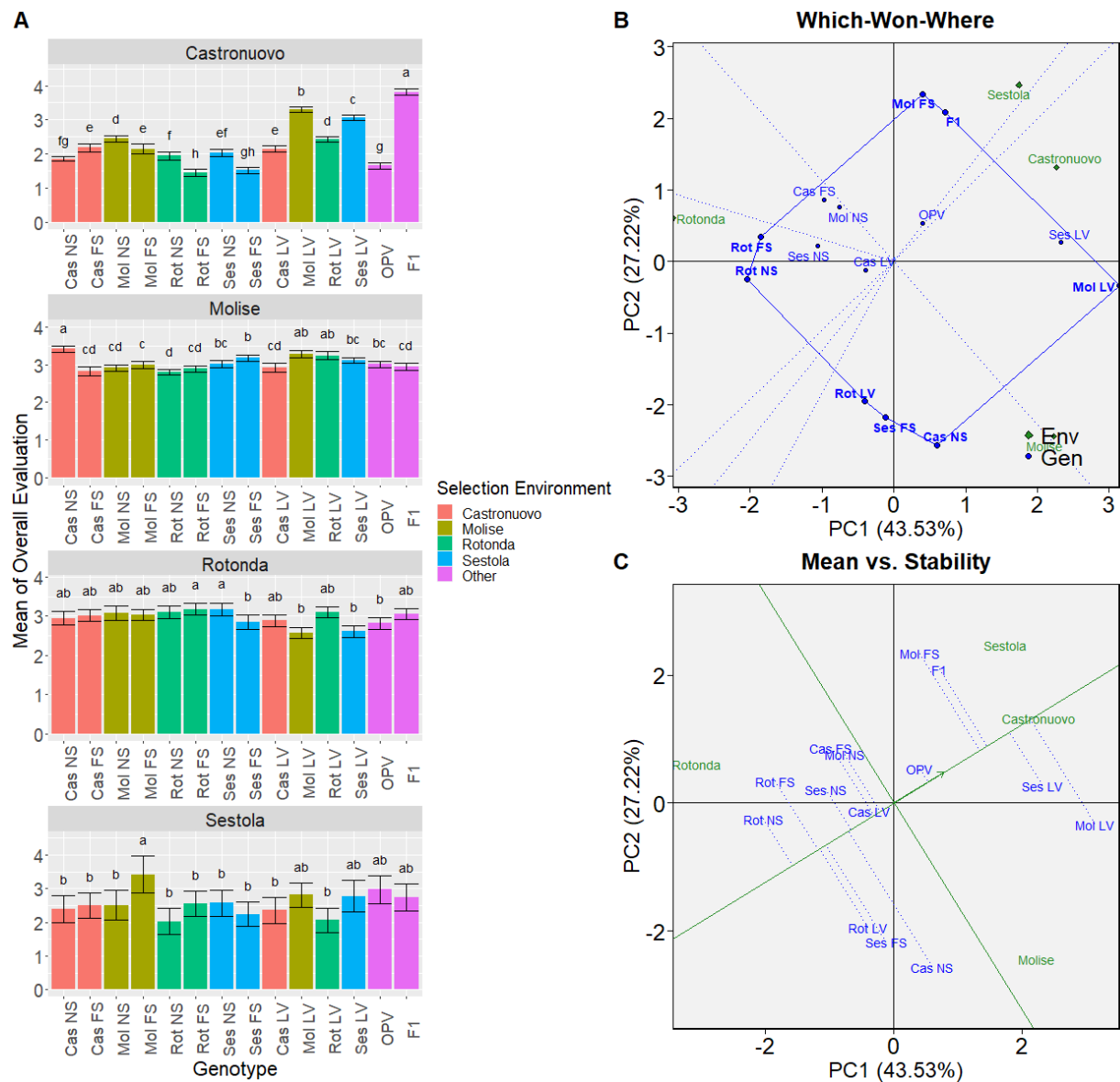


Figure 6. Overall evaluation (A) and corresponding Which-Won-Where (B) and Mean vs. Stability (C) features of the GGE Biplots. The bars represent the BLUES adjusted means and their associated standard error. Different letters indicate different groups according to the Tukey HSD test performed at each location ($\alpha = 0.05$). NS, FS and LV mean respectively Natural Selection, Farmer's Selection and Local Variety.

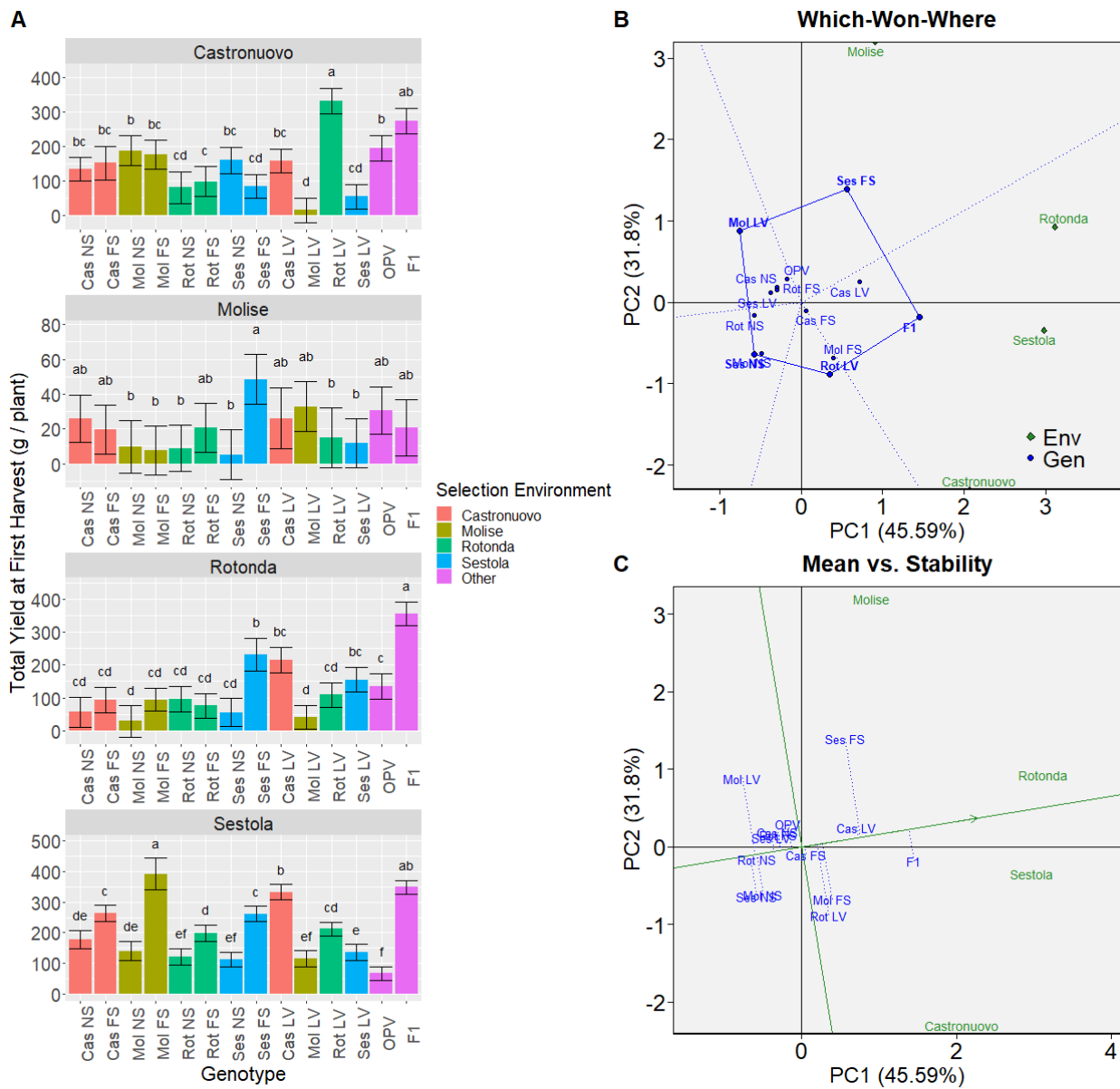


Figure 7. Yield (g/plant) at First Harvest (A) and corresponding Which-Won-Where (B) and Mean vs. Stability (C) features of the GGE Biplots. The bars represent the BLUEs adjusted means and their associated standard error. Different letters indicate different groups according to the Tukey HSD test performed at each location. ($\alpha = 0.05$). NS, FS and LV mean respectively Natural Selection, Farmer’s Selection and Local Variety.

For this trait, no single genotype was the best performer in all environments, which confirms and explains the high contribution of the genotype-by-location interaction detected in the ANOVA for most traits. However, the Mean vs. Stability Biplot (Fig. 7 C), shows that the modern F1 hybrid control had the highest mean and a very high stability for this trait. Also, as seen in the plots for Overall Evaluation, the Sestola FS was the winner at Molise; this genotype had a very high mean as well, but with much less stability. Finally, the effect seen in the bar plots as a higher BLUEs for the FS when comparing to their respective NS is also visible in the GGE Biplots, where all the FS align more to the right (and thus closer to the location points) than their respective NS.

For the total yield per plant, all environments except Sestola could discriminate significantly between genotypes (Fig. 8 A). Moreover, the comparisons between FS and NS pairs only indicate significant differences in Rotonda, and the results indicate that two NS (Molise and Sestola) have actually higher yields than their comparable FS populations. The opposite cases, where FS populations actually out yielded the NS in the comparison, are only significant at α levels smaller than 0.05, for example for the Castronuovo populations at Castronuovo ($p = 0.058$) or the Molise populations at Molise ($p = 0.052$). It is noteworthy too that the F1 was the highest yielding only at Castronuovo, whereas at Molise and Rotonda the higher yielding genotypes were Local Varieties, NS or FS populations.

Regarding the local adaptation of the NS, there is small evidence for one case and that is the one of the Rotonda NS at Rotonda. Although this is not evident in the bar plot and the Tukey test, the GGE Biplots (Fig. 8 B) show it as the "winner" genotype for the environment of Rotonda, along with the Molise NS, which shows however less stability than the Rotonda NS (Fig. 8 C). In overall, the GGE Biplots show, again, a large difference between Rotonda, aligned to the left, and the other three locations, aligned to the right

As for the two main yield components, number of fruits per plant and mean fruit weight, the results are shown in Fig. 9. As the Local Variety, the F1 and the OPV are varieties with different genetic backgrounds and fruit characteristics, important differences were expected for these traits. For example, the Molise local control (Kéro) has notably more fruits but with smaller size. Also, the OPV has bigger fruits than the F1, but it has a lower number of fruits.

When analyzing the effects of farmer's selection and natural selection on these traits, no evident overall pattern was found, but two interesting cases arise. The Castronuovo's FS had larger fruits than its NS counterpart at Castronuovo, and had also a higher number of fruits at Rotonda. In the other hand, only at Rotonda, the Sestola FS had higher fruit number but with less weight than the Sestola NS. Moreover, no important adaptation effects of the local NS were found and thus the GGE Biplots were omitted, as most of the genotypes clustered tightly in the center.

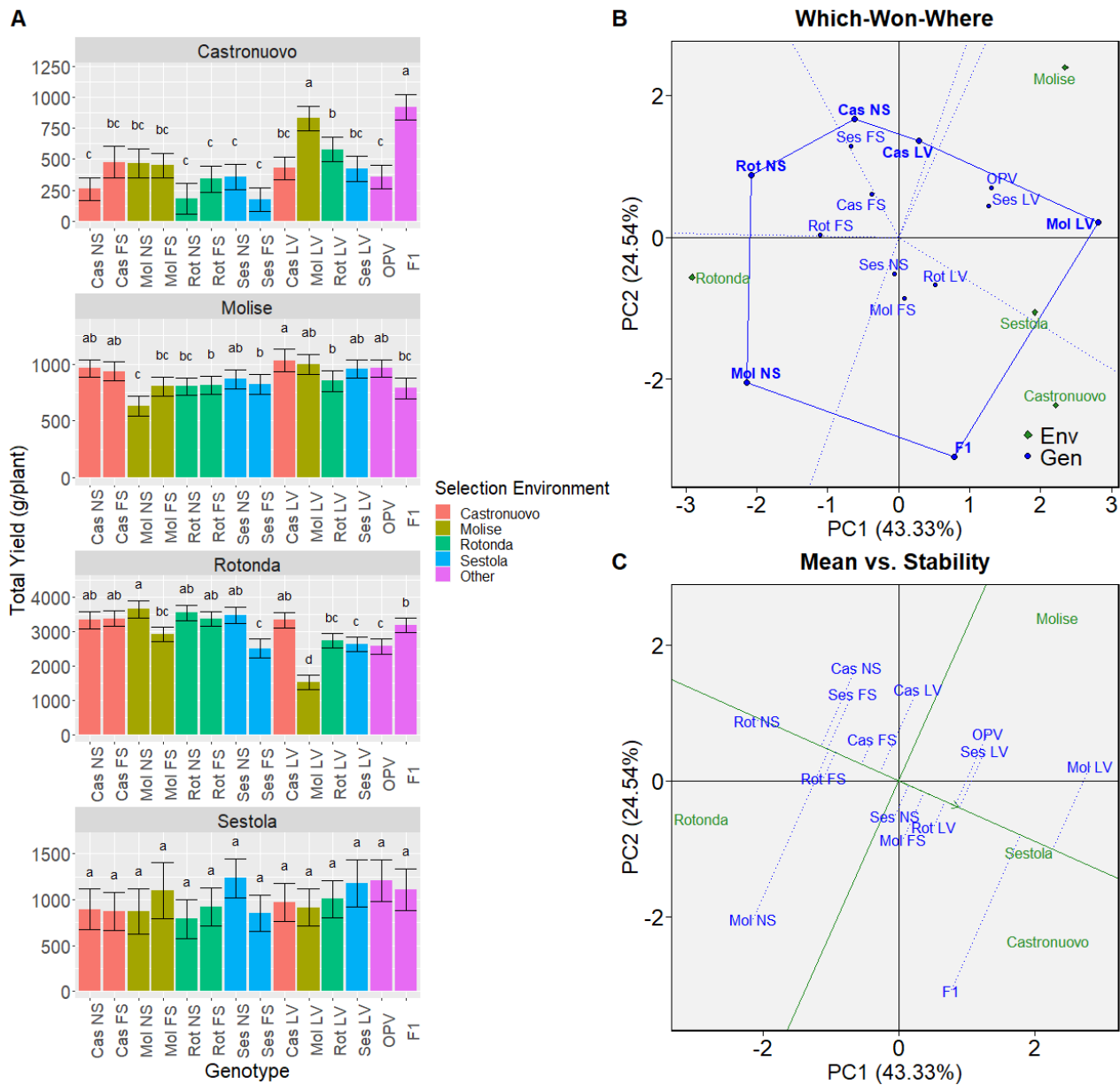


Figure 8. Total Yield (g/plant, A) and corresponding Which-Won-Where (B) and Mean vs. Stability (C) features of the GGE biplots. The bars represent the BLUES adjusted means and their associated standard error. Different letters indicate different groups according to the Tukey HSD test performed at each location ($\alpha = 0.05$). NS, FS and LV mean respectively Natural Selection, Farmer's Selection and Local Variety.

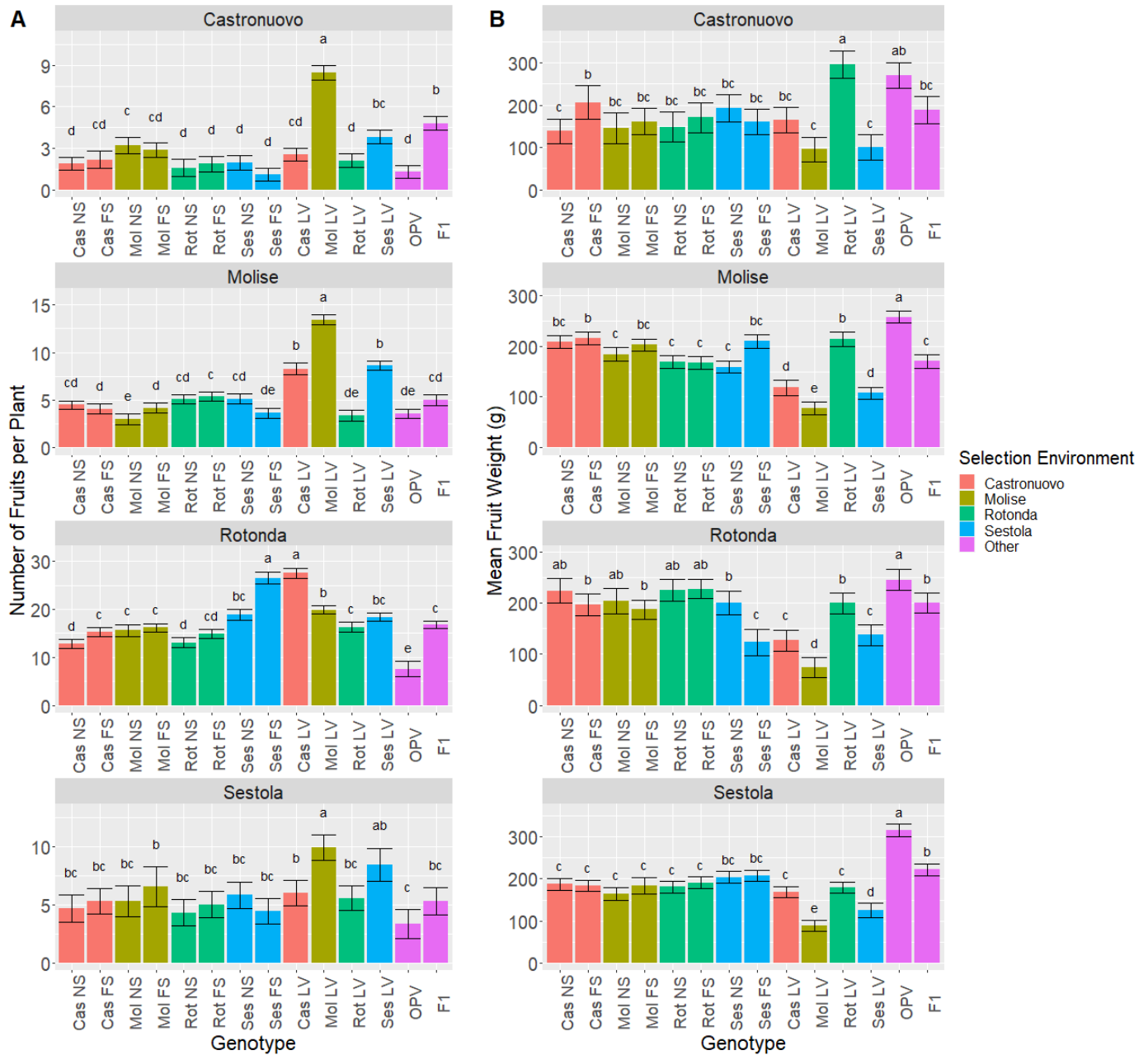


Figure 9. Number of Fruits (A) and Mean Fruit Weight (B) by genotype in the Multi Location Trials for ox-heart tomato. The bars represent the BLUEs adjusted means and its associated standard error. Different letters indicate different levels according to the Tukey HSD test performed at each location ($\alpha = 0.05$).

Finally, for the percentage of marketable yield, only the results for three locations are shown, as no marketable yield was obtained in Castronuovo (Fig. 10). Regarding the effects of selection on this trait over FS/NS pairs, in three cases was the FS significantly superior to its NS pair (Molise and Sestola populations at Sestola) while in two cases it was the opposite (Molise populations at Rotonda and Castronuovo populations at Sestola). So, no general effect of selection over this trait can be pointed out.

A notable superiority of the Local Varieties for this trait stands out. This is clear in the bar plots for Molise and Sestola, and the GGE Biplots point out the respective local varieties as winners in these environments, while most of the other genotypes are plotted in the center. Moreover, no evident effect was found for local adaptation of the locally evolved NS populations.

4.4. Selection efficiency in terms of yield components

In order to better understand the direction of the farmer's and natural selection, a decomposition of yield components was done, dividing total yield into time (corresponding to three or four different harvests), number of fruits and mean fruit weight. Fig. 11 shows the decomposition of yield through the four different harvest dates, and it also shows the p-value of the t-test done to compare of the FS/NS pairs (only shown when $p < 0.3$). The trend seen before, where the FS from Sestola outperformed the NS all environments is seen again. But, also, it can be seen that this effect reverses at the end of the cycle, where actually the Sestola NS has higher yields than the Sestola FS.

Fig. 12 is yet an elaboration from Fig. 11, where yield is further decomposed into number of fruits and mean fruit weight. In this case, the p-values of the t-test are shown in black for fruit number and in red for mean fruit weight (also, only shown when $p < 0.3$). This plot allows to dissect the differences found in the populations from Sestola, where the FS had higher yields at the beginning, but the yields for the NS populations were higher towards the end of the season. Also, it can be seen that the most stable effect (the one that is more repeated through environments) is that of the number of fruits. In the other hand, the mean fruit weight appears to be less reliable, as there are even some extreme cases, like the Molise populations at Molise, where differences change direction from the third harvest (where the FS fruit weight $>$ NS fruit weight) to the fourth (where the opposite happens).

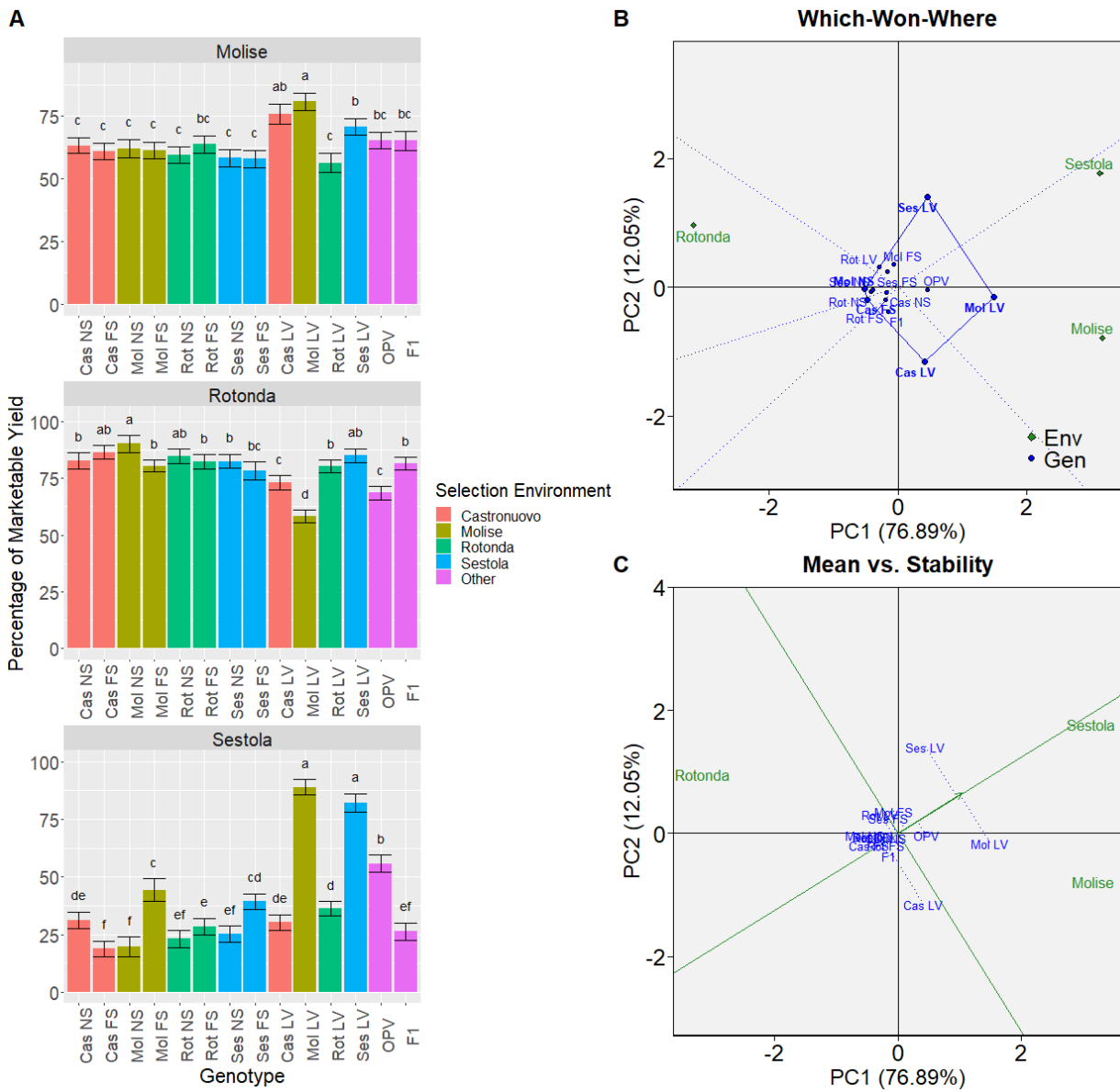


Figure 10. Percentage of Marketable yield (A) and corresponding Which-Won-Where (B) and Mean vs. Stability (C) features of the GGE biplots. The bars represent the BLUEs adjusted means and their associated standard error and different letters indicate different groups according to the Tukey HSD test performed at each location ($\alpha = 0.05$). NS, FS and LV mean respectively Natural Selection, Farmer’s Selection and Local Variety.

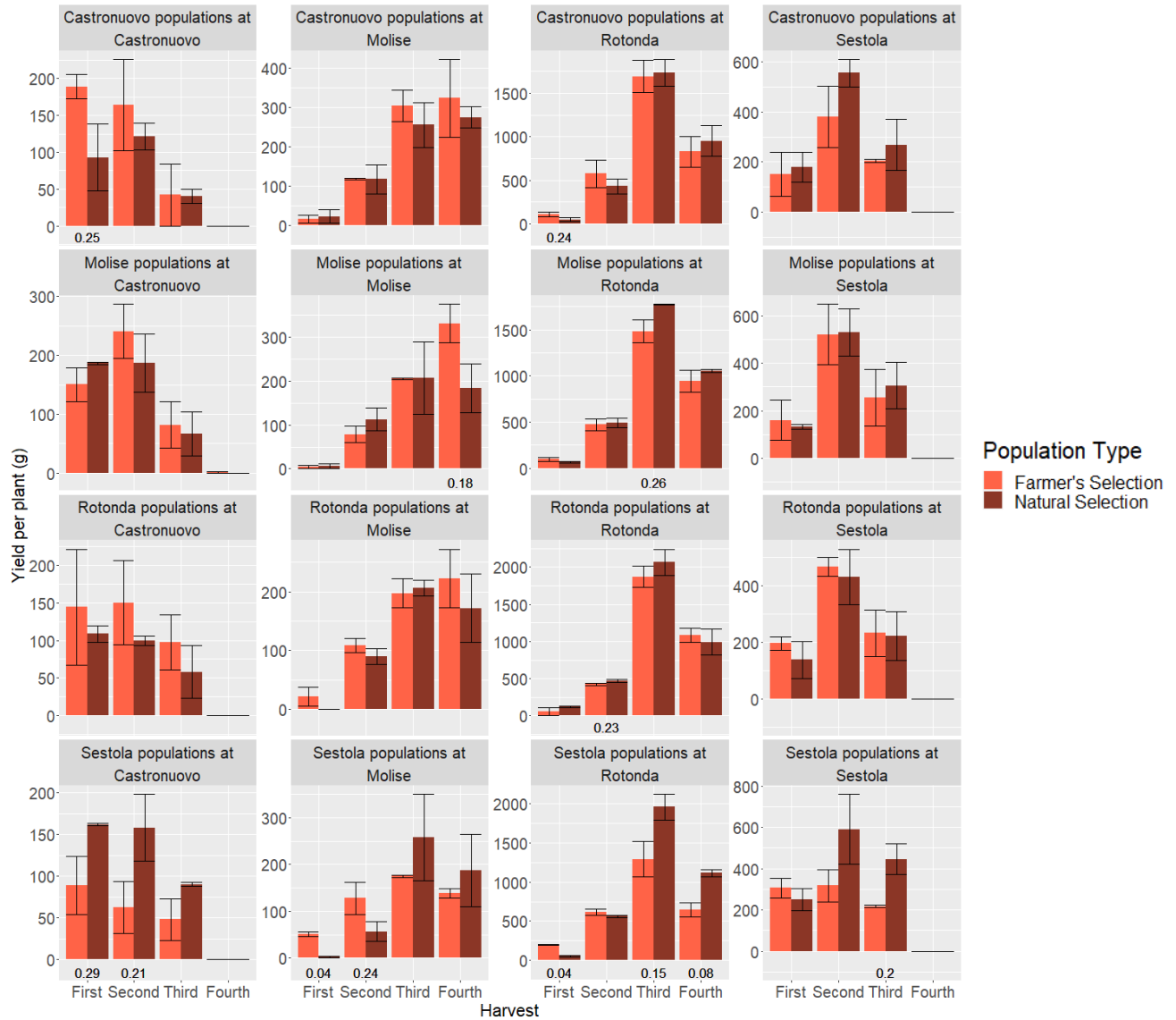


Figure 11. Yield per plant (g/plant) along the four harvest dates in the Multi – Location Trial for tomato. The numbers below the bars indicate the p-value of the t-test for each Farmer’s Selection/Natural Selection comparison (only shown when $p < 0.3$).

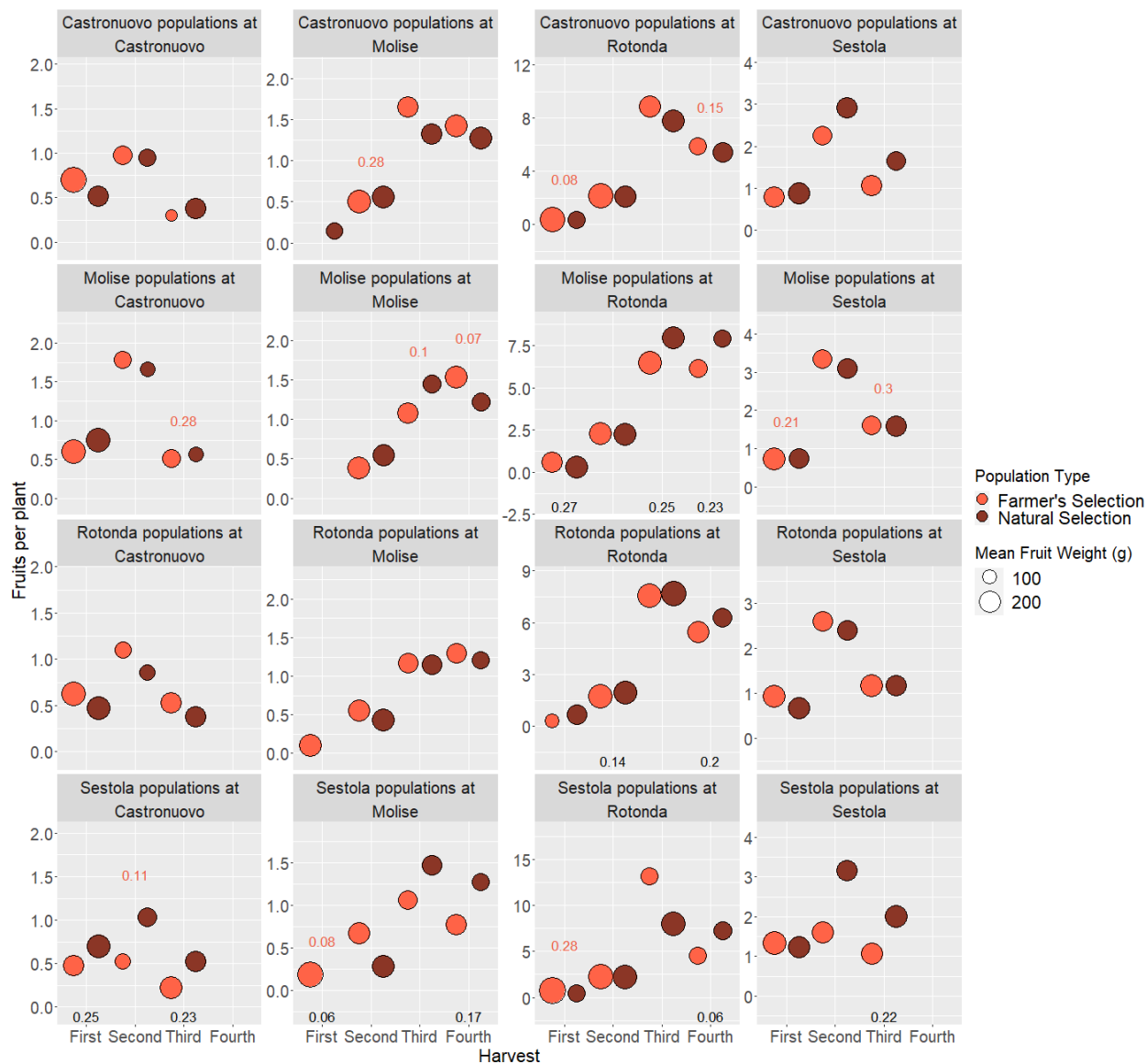


Figure 12. Number of fruits per plant and mean fruit weight (g) along the four harvest dates in the Multi – Location Trial for tomato. The numbers in the plot indicate the p-value of the t-test for each Farmer's Selection/Natural Selection comparison (only shown when $p < 0.3$). Black numbers correspond to comparisons in fruits per plant, and red numbers to comparison in mean fruit weight.

4.5. Correlation analysis

To better appreciate the relationship between the measured traits and the participant's evaluations, a correlation plot was generated (Fig.13). The main variables already analyzed are included, but also the supplementary participant's evaluations for resistance, vigor, uniformity and productivity. Two variables containing only the overall evaluations from male or female evaluators were included, in order to analyze if there were any differences in evaluations according to gender. Also, a special variant of the overall evaluation is added, which is estimated only with the evaluations from the consistent actors, that is, those who participated also in the earlier years of the project (2018 or 2019). Because this were the only actors who actually participated in the selection process (and not only on the 2020 MLT), this could allow for intuitions about the variables that were important for participants during the previous years, when the Farmers Selection Populations were constituted.

The results show that, in general, the measured variable with the higher correlation with the overall evaluation from participants was the percentage of marketable yield, followed by the total yield and the number of fruits. The number of plants per plot and the mean fruit weight were not all correlated with the overall evaluations. Surprisingly, the yield at first harvest was negatively, but weakly, correlated with the participant's overall evaluation. This is true also when considering only evaluations from consistent or female participants but not when only considering males (which show no significant correlation).

This in fact is the most important observation to point out regarding gender differences in the evaluations and their respective correlations. For all the rest of the variables, the results show no significant difference, except for slight differences in the r coefficient, that could also be due to the fact that there were fewer female evaluators than men. A similar situation occurs when comparing the evaluations only from consistent participants and that of the whole group of participants.

When observing only the measured variables, it is noteworthy that total yield had no correlation with the yield at first harvest or with mean fruit weight, and a very high correlation with number of fruits (as expected). In the other hand, percentage of marketable yield is very highly correlated with total yield, but negatively with the yield at first harvest. Finally, the only variable positively correlated with yield at first harvest is the mean fruit weight.

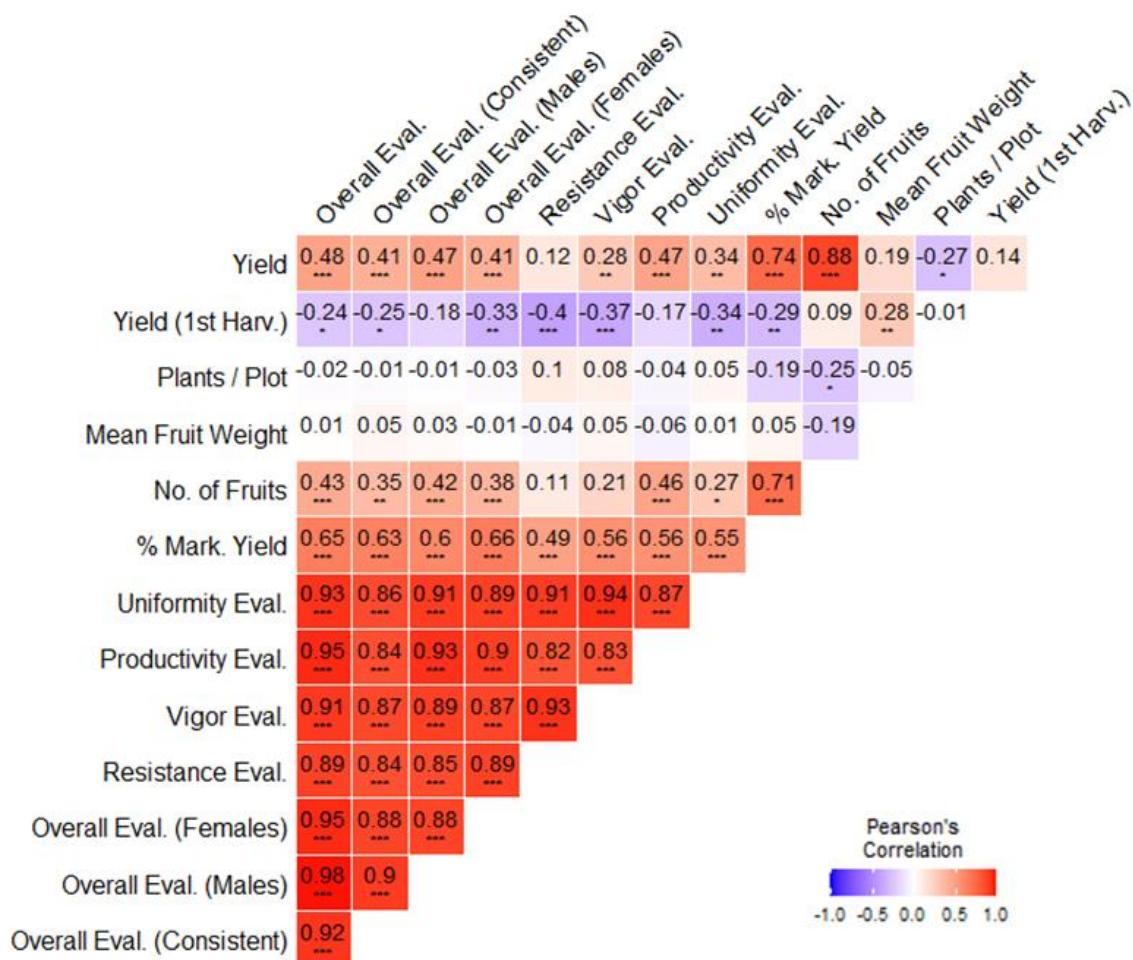


Figure 13. Correlation between measured variables and participant’s evaluations in the Multi-Location Trial for ox-heart tomato. The numbers inside the box indicate the Pearson’s Correlation Coefficient, and the number of stars indicate its degree of significance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

To further elaborate about these correlations across locations, a plot for each farm was generated (Fig.14). Again, the variable most consistently correlated with the overall evaluation was the total yield, and the location where the correlation was strongest was Castronuovo ($r = 0.84$). The weakest correlation was found in Molise ($r = 0.51$). Among the evaluation of specific traits (disease resistance, vigor, uniformity and productivity), the variables that had strongest correlations with the overall evaluation varied across locations. For example, productivity was better correlated with the overall evaluation in Sestola and Molise, whereas it was resistance at Castronuovo and vigor at Rotonda. Moreover, for the latter two locations, correlations among evaluations for the different traits were generally very high and very significant, and can be graphically seen as a stronger red color in the lower left corner of these correlation plots.

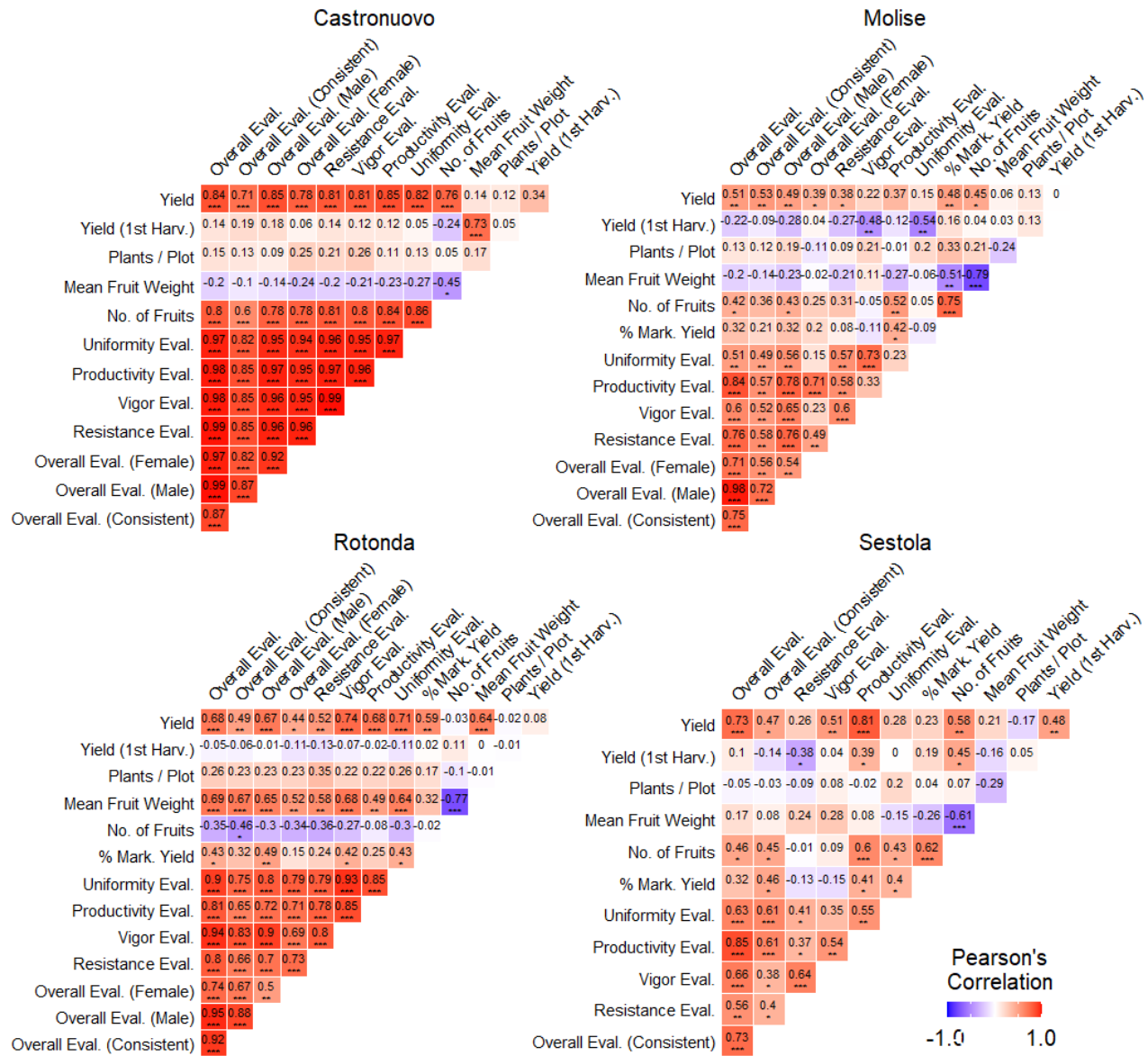


Figure 14. Correlation between measured variables and participant's evaluations in different locations of the Multi-Location Trial for ox-heart tomato. The numbers inside the box indicate the Pearson's Correlation Coefficient, and the number of stars indicate its degree of significance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The mean fruit weight was positively correlated with participant's evaluations at Rotonda, but was negatively correlated or not correlated at the other locations. Conversely, the number of fruits was very highly correlated with the overall evaluation at Castronuovo, weakly correlated at Sestola and Molise, and negatively correlated at Rotonda. And again, the yield at first harvest had none or weak

correlations with the overall evaluations in most locations, although results indicate that selection was very strong for this trait.

Regarding the evaluation of the farmers and actors who participated in the selection years (*ie.* consistent participants), yield was in general the better correlated variable, although the correlation was lower than when taking the whole group of evaluators. At Rotonda, where, as seen in Fig. 6, the local FS was the among the best evaluated genotypes, an even stronger correlation was found between the consistent farmer's evaluation and mean fruit weight. However, as seen on Fig. 9, no important effect of selection is seen on this trait for this population. For the rest of the locations, the correlation between measured traits and consistent farmer's evaluation were hardly different than the correlations described on the previous paragraph.

The separation by gender was only done at three locations, because in Sestola no women participated to the evaluation in 2020. In Castronuovo, no important differences in correlations were found when dividing evaluations by gender. Whereas, at Molise and Rotonda, notable differences were indeed found. At the first location, the number of fruits, the uniformity and the vigor evaluation were highly correlated with the overall evaluation of men, but not with that of the females. In Rotonda, percentage of marketable yield was highly correlated with the overall evaluation from male, but not from women. Additionally, in this location, the correlation between yield and the overall evaluation is significantly higher in men than in women.

Lastly, the correlation between the measured traits shows surprising differences across locations. For example, mean fruit weight was strongly correlated with yield only at Rotonda, and in the other locations this correlation was nil or very low, while yield was strongly correlated with the number of fruits. This indicates important variation regarding the importance of the yield components across locations. Moreover, mean fruit weight was strongly correlated with yield at first harvest only at Castronuovo, presumably because of the fast decay of hailstone damaged fruits. Another important correlation noted was that of total yield with the percentage of marketable yield, which was strong and positive in Molise and Rotonda.

4.6. Principal Component Analysis

After the analysis of genotypes and variable correlations at the univariable level were done, a Principal Component Analysis was performed to identify the main trends of variation in the whole data at multivariate level. Because of the notable differences described across location level, this was also done independently for the

results at each farm and results are in Fig. 15. The results from each location produce unique plots that should be analyzed carefully.

The data from Castronuovo shows that the first dimension is mostly composed from all the evaluation variables (Overall, resistance, vigor, uniformity, productivity), and that the second dimension is mainly correlated with the yield first harvest and mean fruit weight. In that context, a very high projection from the F1 hybrid is seen at both axes, and very modest or even negative projections are noted in the FS and NS populations, most clustering at the center of the plot. For Molise (upper right), variables are more disperse through the plot and axes are harder to interpret. However, the first dimension is still mostly correlated with the overall evaluation ($r = 0.95$) and evaluations for resistance and productivity, while yield is also partly represented ($r = 0.60$). In the other hand the second dimension is related with other two evaluations, vigor ($r = 0.89$) and uniformity ($r = 0.89$), and negatively with the yield at first harvest ($r = -0.77$). Thus, it must be noted that the FS materials (as circles) are all located lower in the y-axis than their NS counterparts (as triangles). This indicates both a higher yield at first harvest and a lower vigor and uniformity for our FS when comparing the to the respective NS population.

At Rotonda (lower left panel), variables are also clustered together. Again, the overall evaluation is the main driver for the first dimension, along with other evaluations like vigor, uniformity, productivity and resistance. Whereas the second dimension is mostly correlated with the yield at first harvest ($r = 0.52$) and, surprisingly, plants per plot ($r = -0.77$), even though variation of plants per plot was very low in this environment (19.6 ± 0.57). The FS from Sestola, Rotonda and Castronuovo are well above their respective NS in the y-axis, and the first two are also more to the left in the x-axis. The first condition could imply a higher yield at first harvest, while the second, a lower overall yield, vigor and uniformity. In the other hand, the Molise FS and NS stand closely together. Additionally, the Molise Local Variety was not plotted because it's coordinates ($x = -6.32$ and $y = -3.02$) were too far away from the center and reduced readability.

Finally, for Sestola, the first dimension is mostly correlated with the productivity evaluation, although highly correlated to the overall evaluation and total yield, while the second dimension is very highly correlated with mean fruit weight ($r = 0.77$), and negatively with yield at first harvest, number of fruits and percentage of marketable yield. As for the FS/NS comparisons, only the FS Sestola clearly differentiates from its NS counterpart, as it has a more negative coordinate on the x and y axis. The FS from Castronuovo does the same with a smaller magnitude, while the FS from Rotonda behaves the opposite way with regards to its NS, that is, it has more positive coordinates on both axes. As seen in Rotonda and Castronuovo, the NS and FS populations from Molise stand closely together.

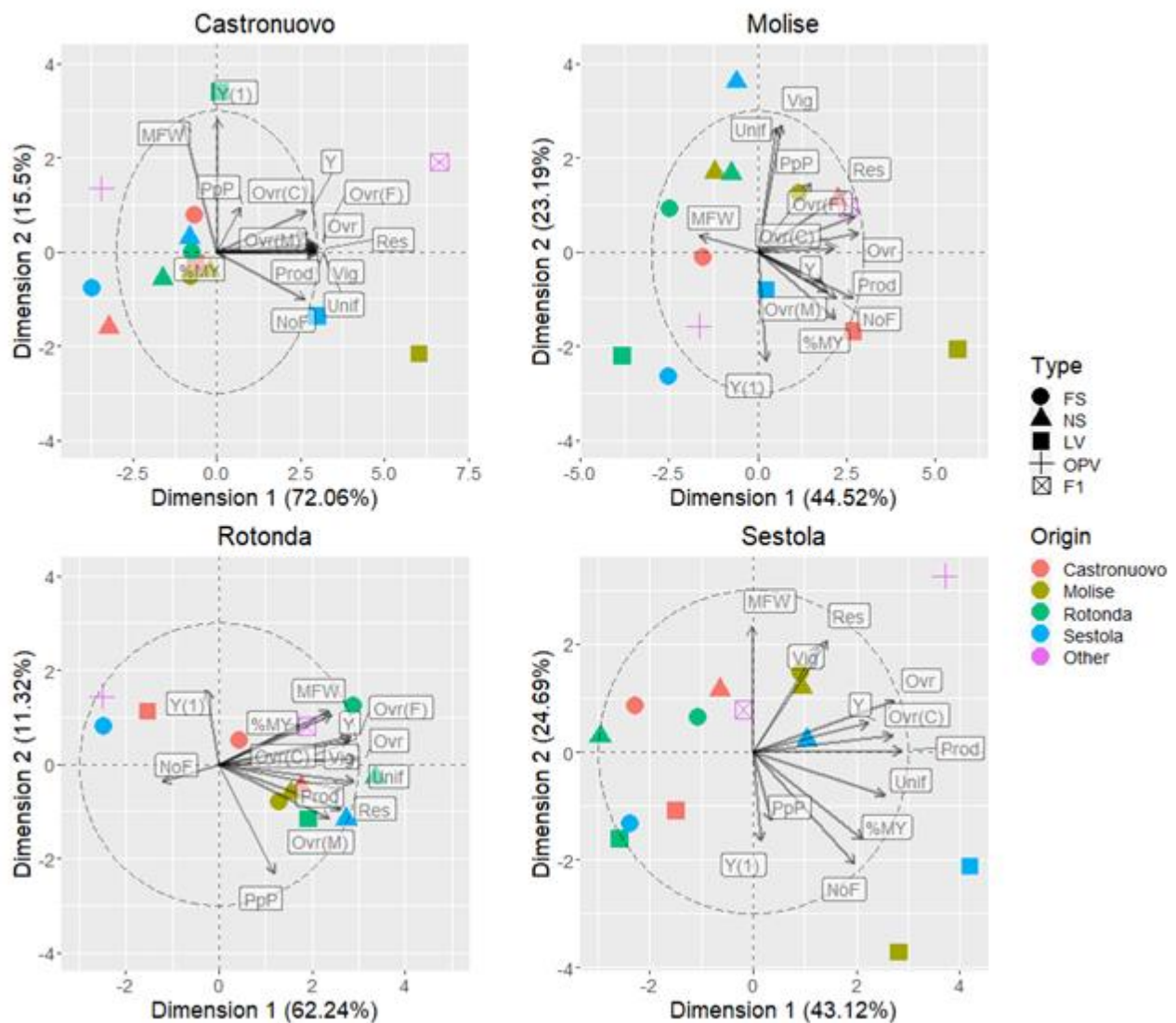


Figure 15. Principal Component Analysis Biplot of the genotypes and the main variables divided by environments. The circle represents a correlation of 1 for the shown variables. Variables abbreviations are the following: Overall Evaluation ("Ovr"), Overall Evaluation from Consistent Actors ("Ovr(C)"), Overall Evaluation from Male Actors ("Ovr(M)"), Overall Evaluation from Female Actors ("Ovr(F)"); Resistance Evaluation ("Res"), Vigor Evaluation ("Vig"), Productivity Evaluation ("Prod"), Uniformity Evaluation ("Unif"), Percentage of Marketable Yield ("%MY"), Number of Fruits ("NoF"), Mean Fruit Weight ("MFW"), Plants / Plot ("PpP"), Yield at 1st Harvest ("Y(1)"), Yield ("Y").

5. Discussion

The objective of the present work was to determine the effectiveness of decentralized participative selection for breeding ox-heart tomato heterogeneous populations adapted to Italian organic farms. For that purpose, the same F₄ population was sent to 4 different locations and submitted to Farmer's or Natural Selection during two years; then, the resulting breeding populations were evaluated in a Multi-Location Trial (MLT).

The overall results show a significant effect for the genetic, environmental and interaction effects for most of the evaluated variables. However, the most important factor for all traits, excepting mean fruit weight, was consistently the environment, as it is expected and as has been seen in previous works (Casals et al., 2019; Figàs et al., 2018; Panthee et al., 2013). Environmental effects tend to be even stronger in organic conditions, as they are highly influenced by natural soil properties and long-term farm management, that cannot be easily evened out with the use of external inputs (Maeder et al., 2002). In this context, high variability in soil conditions within farm and within fields is also of great importance and could also be a large source of variance (Dawson et al., 2008). However, this was addressed by having at least two repetitions in each field (the nested repetition effect was not significant in the ANOVA) and by correcting the evaluations and measured variables according to the row – column spatial effect through SpATS.

Noteworthy differences were seen regarding the mean yield among locations. At first harvest, for example, the yield in Sestola was 10 times higher than at Molise. However, in this last trait, the precise moment where the farmer decided to harvest might have had some influence on the result. As for the total yield, the results at Rotonda were 3 to 6 times higher than at the other environments and relatively low yields were found across 3 out of 4 environments (less than 1 kg per plant). Besides from the evident pedo-climatic differences across farms, crop management might have had a big influence on these results. For example, as can be seen in Fig. 4 B, plants in Molise were not tutored, and that can have an important negative effect on yield. Moreover, it has been seen that when evaluation is done in low yielding environments, the genetic component of variance tends to decrease and the error tends to increase, thus making selection more difficult (Dawson et al., 2008).

The overall genotype comparison shows that, with the exception of the results from Castronuovo, none of control materials (including local varieties and commercial hybrids) consistently outperformed the FS and the NS populations. Similar results were obtained by Casals *et al* (2019), who found that, when evaluated by farmers in organic conditions, breeding lines obtained from local landraces had equal or higher yields when comparing to the modern cultivar that was currently occupying the landrace's niche. If agronomic performance and fruit quality are similar, using the presently bred populations could be more advantageous for the

farmer's that using hybrid seed for two main reasons: i) seeds could be saved and reused by the farmers without legal constraints or yield penalties, and ii) because of its diverse background, they could continue evolving and thus adapting to the given environment of the farm, whilst providing some buffering against biotic and abiotic stresses in light of their higher intra-cultivar diversity. Casals et al. (2019) also concluded that it was not only yield or quality driving the replacement of landraces with hybrids, but other reasons including the relative facility with which they could get or buy the seed. And this has led to a process of replacement of historic landraces or diverse populations with completely uniform hybrids, which is the single major cause of genetic erosion in cultivated plants (van de Wouw et al., 2010). This is particularly true in Italy, where farmers rely on tomato transplants offered by horticultural nurseries, which represent a major bottleneck when it comes to cultivar choice and access to material adapted to organic or marginal environments.

An important limitation in this project was that the MLT was only performed for one year, and thus, the local variations due to changing weather conditions in different years could not be assessed. The most evident case for this situation is at Castronuovo, where a hailstorm event severely hampered plant development and made virtually all harvest non-marketable. Thus, more robust results for this and all locations could be obtained if evaluations were repeated more years. Also, the presence of some diseases is naturally stronger in some years, so that doing multi-year trials could better allow to identify selection's efficiency in tolerance, as confirmed by Horneburg and Becker (2008) regarding tomato late blight in their organic tomato PPB program. As a matter of fact, in most field crops it has been demonstrated that climate variation can explain up to a third of yield variation (Ray et al., 2015).

5.1. Efficacy of Farmer's Selection

The single variable in which the efficacy of farmer's selection was most evident was the yield at first harvest, the population where it was best seen was the Sestola Farmer's Selection and the location where the FS populations performed better than the NS populations was Sestola.

Selection might have been directed so strongly towards yield at first harvest for several reasons. One is the fact that having early yield is very important for farmers because it allows for better market prices, as the offer of local tomatoes is rather low in early moments of the season. For these reasons, the evaluating farmers weighted heavily this aspect when evaluating. In a similar case, Bocci *et al* (2020) showed that farmer's selection on wheat evolutionary populations notably sped up the process of adapting the genotypes towards farmer's needs. However, counterintuitively, this variable was not correlated in 2020 to the participant's evaluations, even when only considering consistent participants. The other important

reason factor might have been the moment when evaluation was made during 2018 and 2019. That is, because participants were only invited to see the field once per cycle, and mostly in the early part of the cycle, they could only see a photograph of what was happening exactly on that moment; so, they made their evaluation based on that information.

The fact that this efficacy was visible with all the populations at Sestola, and with the Sestola FS in the majority of locations can be clearly explained by temperature. In tomato, the fruit set of the first three clusters can be affected by low temperatures (lower than 10–12 °C) during the early season (Campanelli et al., 2015), and Sestola was the location which recorded the lowest temperatures. Thus, a double effect can be pointed out. First, that the selection pressure was very strong for that trait at Sestola, and in consequence, that the selection response was very high for the Sestola FS. Second, that because of these specific environmental conditions at Sestola, smaller genetic changes in the other FS populations were more phenotypically evident when they were evaluated in this environment in 2020.

Additionally, the variable we name as yield at first harvest is also affected by the precise moment of harvest. Because these yields were higher at Sestola than in the rest of the locations, it is also possible that the first harvest was done later in this location than in the others and thus, because of the phenological moment of evaluation, the differences were more evident for all the FS populations in that location.

Furthermore, we saw a very weak correlation between yield at first harvest and total yield, and most importantly, we saw that many NS populations out yielded their FS pairs, even when they were initially lower yielding in the first harvest. A very likely explanation for this situation is the competition for assimilates and the sink-source ratio of the plants. As there is a rivalry for resources between fruit development and vegetative development, the growth of big trusses early in the season is done at the expense of vegetative growth (Bertin, 1995; Heuvelink, 1997; Heuvelink and Buiskool, 1995). And, if vegetative growth is constrained at the beginning of the cycle, less axillary buds and functional leaves will be available later for the development of new trusses. This hypothesis is supported by the consistent negative correlations and divergent projections on the PCA between yield at first harvest and vigor. Thus, a clear effect of trade-off between high early harvest and high total harvest can be inferred.

Also, regarding the efficacy of selection, we saw very small evidence in selection response for the trait of overall participant's evaluation, and to explain this, many reasons could be attributed. Most notably, only two selection cycles and one evaluation year might have been insufficient to demonstrate genetic gain for this complex trait. Also, the trait as it was measured might have low heritability because of two main reasons: firstly, the fact that evaluators changed through the years and, as it has been clearly seen, different evaluators might have different opinions and

preferences; secondly, it is possible that the ideotype to which farmers are used in each environment was different to the one from the bred populations, so that they were consistently ranked poorly when compared to local varieties and other controls.

5.2. Evidences for Local Adaptation

Evidence for local adaptation of the NS and FS populations was present but very modest and the only environment where this manifested evidently was Rotonda. Here, the locally bred FS population was the best evaluated by participants. Additionally, the local NS performed notably better at this environment than at the rest, as seen by the GGE Biplot for Overall Evaluation and Total Yield.

It is likely that the increased use of external inputs in this location, as it is a research station and not a private farm, is partially responsible for these results, as this created a more stable environment in which the direction of selection and adaptation was more consistent through the years. It has been described that, in natural populations, selection tends to be very low, but that it can grow exponentially in certain specific contexts (Kingsolver et al., 2001). Campanelli et al (2015), also did a PPB program for tomato in organic farms and research stations, and saw a higher efficacy when selecting in the research station. However, although the general climate and agronomical characteristics of the selection environments are known, it is hard to dissect the precise causes that drove natural selections towards a given direction, unless an ad-hoc experimental design is done for that purpose (Wade and Kalisz, 1990).

Another interesting effect is the apparent adaptation of the Molise FS population to Sestola and of Sestola FS to Molise. This was seen both in the overall evaluation and in the total yield at first harvest. The direction of selection is known to change along locations and years, as the relationship between a given trait and conferred fitness may change. It is for those reasons, that Horneburg and Becker (2008) insist in recommending “multilocational” breeding approaches with frequent exchange of breeding material and data. Moreover, this is also the rationale that drove the “shuttle breeding” strategy designed by Norman Borlaug on wheat, where breeding material was constantly brought back and forth from contrasting locations in Mexico (Ortiz, 2008). It is possible, thus, that the particular environmental conditions in the previous years at Molise, implied a selection pressure which led to increased fitness for that FS at Sestola in 2020 (and vice versa).

Furthermore, the effect local adaptations were only seen in terms of yield and not in disease related variables, like percentage of marketable yield or the evaluation for resistance (not shown). This could be explained by the fact that, in the context of natural selection, it has been observed that selection via survival tends to be weaker than selection via fecundity or mating success (Kingsolver et al., 2001).

5.3. Participant's preferences

A total of 174 different persons stated their evaluation about individual plants or plant plots during the three-year process. The sole fact of involving farmer's and civil society in the process of breeding already carries its own benefits, as it can trigger synergic empowerment processes that lead to more cooperation and inclusion (Weltzien et al., 2003). Moreover, the process had special complications, as movement restrictions and social distancing measurements were being imposed by national authorities worldwide, due to the COVID-19 pandemic. A critical point in this sense was that selection at Molise in 2019 had only three participants, and this could potentially hamper the robustness of the participatory evaluation and selection. In fact, selection efficacy was not confirmed in this location and the PCA plots cluster the Molise FS and NS together. Moreover, the differences in participation across locations are hard to explain, as they depend on many factors, such as the nature of the social web already weaved in the location, as well as the efficacy of the diffusion methods used to invite participants, which were mainly done by word of mouth within the farmer's networks.

The correlation analysis showed different results when the whole Multi-Location Trial was analyzed as a whole than when each location had its own analysis. This is most likely due to the fact that every environment had really different characteristics and that the data was not previously normalized. When analyzing each environment separately, total yield was consistently the trait most highly correlated to the participant's preferences. A very high correlation between yield and farmer's evaluation has been previously found in tomato PPB programs (Casals et al., 2019), as well as in other crops (Annicchiarico et al., 2019; Casals et al., 2018; Sperling et al., 1993). This confirms the capability of farmer's and other participants to visually estimate quantitative traits with precision, and thus allows to rethink the role of technicians and plant breeders, as more of their attention could be focused on other activities (such as data analysis and communication) rather than in the evaluation of agronomic traits.

The overall evaluation was also divided according to different criteria: only participants who were involved in the program before 2020, only male participants and only female participants. Nonetheless, only small differences were found in the correlation analysis when taking the evaluations from these groups separately. Thus, no relevant conclusions can be made about the differences in male or female perceptions on our populations, neither about the differences between consistent and first-time evaluators. Regarding the absence of clear gender differences in preferences, this corresponds to the results of a similar analysis done before in wheat in Italy (Bocci et al., 2020), but does not imply that a gender perspective should be ignored when designing and executing a breeding program, as notable differences in preferences might arise in other contexts (Tufan et al., 2018). In the other hand, very marked differences across locations were found regarding the

preferences of the participants, which accentuates the need for decentralizing breeding and conducting site-specific programs (Ceccarelli and Grando, 2007).

Finally, taste and nutraceutical qualities have an increasing importance in tomato breeding, and even more in the context of breeding for landraces and for cultivars adapted to organic agriculture, which are usually sold at higher prices with the associated high quality expectations (Casals et al., 2011). However, this was not part of the components of these trials, but evaluating these characteristics in our populations could be of great use and interest for the future.

6. Conclusions

In the present work, the results of a participatory and decentralized breeding program for ox-heart type tomato were deeply analyzed, in order to determine its effectiveness for delivering superior organic heterogeneous material adapted to Italian organic farms. Overall, the analysis showed a deep influence of the environment, genotype and genotype x location interaction in most traits. For the latter, important crossover interactions were found, where genotypes ranked differently across environments. However, in most environments and for most traits, the FS and NS populations were equally or better performing than most of the controls used, indicating clearly the viability of breeding elite materials through this means.

The effectiveness and the response to selection was observed through the comparison between FS and NS populations bred at the same location. We confirmed specially selection response for yield at first harvest, where the Sestola FS was superior to the Sestola NS in all environments, and the other FS were superior to their respective NS in at least one location. However, this effect was reverted in the total yield, possibly due to a trade-off effect and a fierce competition for resources early in the plant life cycle. Considerations upon how important is a yield at first harvest against total yield should be taken, and also about the precise moment in which participant's evaluation is done.

In the traits analyzed, the effects of local adaptation on Natural Selection Populations were very scarce. The only clear example of this kind of adaptation was at Rotonda, with both the FS and the NS populations with evident adaptation in farmer's perception and yield at first harvest. The special condition of this location as a research station might largely explain these results

Finally, regarding the main correlations between evaluations and the measured traits, very important differences were seen across environments, remarking the importance of decentralizing selection. However, it was noteworthy that correlations between yield and farmer's evaluation were high in most environments (between 0.5 and 0.8) and this confirms the viability of using participatory evaluation in quantitative traits.

Now, these populations are accessible to all farmers across Italy, and may constitute an important asset in the further development of new genetic material adapted and adaptable to a wide array of environments and management practices.

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