Breeding strategies for improving the performance and fruit quality of pepino (*Solanum muricatum*): a model case for the enhancement of neglected exotic fruits

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Abstract

Pepino (*Solanum muricatum* Aiton) is a neglected Andean crop that has recalled an increasing interest from exotic fruit markets. Pepino is a highly diverse crop and by using adequate breeding strategies based on the exploitation of diversity it has been possible to develop successful breeding programs for this crop. Here we review the strategies used and advancements made in the genetic improvement of pepino for several traits, including agronomic performance and fruit quality traits. Different strategies, like the use of a wide diversity of genetic resources, exploitation of genotype×environment interaction, use of clonal hybrids, and introgression of genes from wild species have allowed important developments in increasing the commercial exploitation of pepino and in developing new cultivars adapted to new agroclimatic conditions. Agronomic performance of pepino has been improved by the use of genetic parthenocarpy, resistance to *Tomato mosaic virus*, and by developing heterotic hybrids. Improvements of yield resulting from these strategies did not affect negatively fruit quality. Breeding for quality has focused mostly in improvement of sweetness. Despìte the limited available intraspecific diversity for sugar content it has been possible to develop materials with improved soluble solids content (SSC) and aroma profile. Further increases in SSC have been obtained by using wild relatives in interspecific breeding programmes. As a result of the breeding efforts performed, several cultivars with improved agronomic performance and fruit quality have been obtained. The use of genomic tools represents an opportunity to use the extensive genomic information in related species, like tomato or potato, for the future improvement and enhancement of pepino. In summary, the results obtained in pepino with limited breeding efforts show that ample opportunities exist for improving the commercial exploitation of neglected exotic fruits by means of breeding programs.

Keywords: breeding, chemical composition, exotic fruits, organoleptic quality, pepino, *Solanum muricatum*

1. Introduction

The pepino (*Solanum muricatum* Aiton) is an herbaceous crop native to the Andean region grown for its juicy fruits, which are mostly consumed as a refreshing dessert fruit like melon (National Research Council, 1989; Prohens et al., 1996). Pepino fruits can also be used in salads in the same way than cucumber (Prohens et al., 2002), or in juices or milk drinks as other exotic fruits (Cruz et al., 2009; Schwartz et al., 1988). The most prominent features of this exotic fruit are its attractive appearance and the properties derived from its chemical composition. Most common varieties have a golden yellow background covered by purple stripes in the area exposed to the sun, which makes pepino a visually attractive fruit differentiated from other exotic fruits already present in the market. At the composition level, pepino has a high content of water (>92%), is low in calories (0.25 kcal/kg), and contains significant amounts of vitamin C (200-800 mg/kg) and K (>1000 mg/kg) (Pluda et al., 1993a; Redgwell & Turner, 1986; Sánchez et al., 2000). In addition, pepino has been attributed some properties of medicinal interest, like hypotensive, diuretic, and antitumoral properties (Redgwell & Turner, 1986; Ren & Tang, 1999; Sánchez-Vega, 1992), which may increase its demand.

Pepino was very important during pre-Columbian times in its region of origin. Thus, there is an abundance of pottery representations and depictions of pepino from the Mochica, Nazca, and other Andean cultures, as well as many references to this fruit by the first Spanish chroniclers (Prohens et al., 1996). However, in post-Columbian times, pepino did not reach the prominence of other American *Solanaceae* like peppers (*Capsicum* spp.), potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), or tomato (*Solanum lycopersicon*), and it remained as a locally important neglected crop. For that reason, the production of pepino was mostly devoted to local markets in Andean countries like Colombia, Ecuador, Perú, Bolivia, and Chile (National Research Council, 1989; Prohens et al., 1996).

After centuries of neglection, there was a rediscovery of pepino for commercial exploitation in the 1970s-80s. This was stimulated by the attempts of introduction of exotic fruits undertaken in New Zealand after the success of kiwifruit (Dawes & Pringle, 1983; Morley-Bunker, 1983; National Research Council, 1989), and several cultivars were released at that time in New Zealand (Dawes & Pringle, 1983). This initiative was subsequently followed by other countries like Spain, France, Italy, The Netherlands, USA, Israel, Korea, or Australia (Prohens et al., 1996). In the last years, other adaptation programs are being developed in other countries, like Turkey and Iran (Cavusoglu et al., 2009; Nemati et al., 2009).

An important shortcoming that has hampered the attempts to introduce pepino under new agroclimatic conditions is that the organoleptic quality of the fruit frequently does not reach the expectations of the potential consumers, especially when the intended use is as a dessert fruit. Pepino fruits should exhibit a mixture of melon/pear-like aroma with exotic fruit notes and should have a soluble solids (mostly sugars) content (SSC) of at least 8% to have an acceptable degree of sweetness (El-Zeftawi et al., 1988; National Research Council, 1989). Furthermore, the increasing interest of consumers for health-promoting foods favors the acceptance of new exotic fruits containing high levels of nutraceutical compounds. In consequence, the improvement of fruit quality is a major breeding objective in pepino. However, in order to be commercially successful, new pepino cultivars must combine this improved quality with good yields, and therefore yield must also be taken into account in real breeding programs aimed at improving fruit quality.

An important fact to be taken into account in breeding programs, is the reproductive biology of the crop. Although most pepino cultivars are sexually fertile and produce viable seeds, in the agricultural practice pepino is vegetatively propagated by stem cuttings, and pepino cultivars do not breed true due to its high level of heterozygosis (National Research Council, 1989; Prohens & Nuez, 1999). Therefore, pepino cultivars are highly heterozygous clones, and sexual propagation is only used for breeding programs.

In this work we review the breeding strategies used to develop high quality cultivars of pepino adapted to the Mediterranean climates. The exploitation of genetic diversity, genotype-by-environment interaction, the use of marker assisted selection, and the utilization of wild relatives, among others, have been of great relevance to achieve this objective. The approach followed by us may serve as a model for other breeders interested in adapting exotic fruits to new agroclimatic conditions and improving their quality.

2. The genetic resources for pepino breeding

Despite the interest of the introduction of pepino in several regions of the World, many attempts have been unsuccessful. In most cases, a low genotypic diversity, even a single cultivar, was used in the trials, which restricted the possibilities for adaptation to local conditions (Heiser, 1985; National Research Council, 1989; Prohens et al., 1996).

The exploitation of the genetic resources is essential in order to develop new materials adapted to new conditions and with improved characteristics. In this respect, pepino is a highly diverse species for many morphological and horticultural traits (Prohens et al., 2004a) (Figure 1), which is matched by a high molecular diversity (Anderson et al., 1996; Blanca et al. 2007) (Table 1). Furthermore, the heterozygous nature of pepino clones can be exploited by breeders to obtain segregating populations by selfing a particular cultivar or by crossing different cultivars. In both ways, breeders can obtain new genotypic combinations, which are susceptible of being selected (clonal selection) and vegetatively propagated for subsequent cycles of evaluation (Prohens & Nuez, 1999).

Nevertheless, the available intraspecific variation within the pepino might be insufficient to improve the content in sugars and other fruit quality traits of pepino above certain limits, and wild relatives may serve as sources of variation for such traits. Pepino is a member of section *Basarthrum* of genus *Solanum* and it is phylogenetically related to the species included in this section (Anderson, 1979; Anderson & Jansen, 1998; Anderson et al., 1996, 2006). Four wild species of this section, namely *S. caripense*, *S. tabanoense*, *S. cochoae*, and *S. basendopogon*, have been successfully crossed with pepino (Anderson, 1979; Anderson & Bernardello, 1991; Anderson & Jansen, 1998), giving fertile offsprings, which facilitates its use for pepino breeding. Among them, *S. caripense* and *S. tabanoense* (Figure 2) are considered the closest relatives of pepino and the ones with the highest probabilities of being its ancestors (Anderson et al., 1996; Anderson & Jansen, 1998; Blanca et al., 2007; Heiser, 1964). Furthermore, both species are edible and sweeter than pepino, which means that they represent sources of variation of great interest for improving pepino fruit quality.

3. Breeding for yield and adaptation

The successful introduction of an exotic crop requires developing techniques and/or materials that allow obtaining good yields. For that reason, our first efforts in pepino breeding were devoted to the improvement of yield.

In the first screenings performed, several cultivars from the Andean region as well as segregating populations raised from seeds of diverse origins were grown under greenhouse. A great diversity for yield was found, with many cultivars and individual genotypes having very low yield, although individuals with good yield were also identified. These screenings also revealed that fruit set and, consequently, yield were affected by high temperatures, which caused low pollen fertility (Ruiz et al., 1996). However, some materials, despite having low pollen fertility, were able to give a high load of fruits due to their facultative parthenocarpic ability. Parthenocarpy has been reported in pepino cultivars since the 19th century (Bailey, 1891), and parthenocarpic clones are more productive than non-parthenocarpic ones due to their ability to circumvent the lack of fertilization and to set fruits in a wider range of conditions (Prohens et al., 1998a). Thus, parthenocarpy provides pepino breeders with an alternative to increase yield stability. We determined that parthenocarpy in pepino was under the control of one dominant gene, which we called *P* (Prohens et al., 1998a), and which is a useful tool for improving yield and stability of pepino production.

Greenhouse experiments performed with a comprehensive collection of pepino clones in Mediterranean conditions, showed that cultivation in the autumn-winter growing season gave, in most cases, a higher yield (45% on average) than in the spring-summer growing season (Rodríguez-Burruezo et al., 2000) (Figure 3). This was probably due to the fact that temperature conditions in autumn-winter season are within the optimum range of temperatures for the development of the pepino plant. Yield showed, in both growing seasons, high values for heritability (>0.70), which indicates that response to clonal selection would be efficient for this trait (Rodríguez-Burruezo et al., 2000).

Another factor that limited the adaptation of pepino to Mediterranean conditions was the susceptibility to *Tomato mosaic virus* (ToMV), which is the causal agent of the most limiting disease of pepino in our conditions, as it causes severe yield losses (Prohens et al., 1998b). Consequently, studies focused on the search and utilization of sources of resistance were undertaken. The first screenings allowed identifying several sources of hypersensitivity-mediated resistance within both the cultivated pepino and wild relatives. The studies of inheritance confirmed that most alleles for resistance to ToMV were dominant (Pérez-Benlloch et al., 2001), which facilitates the use of these sources of resistance to develop new cultivars.

Finally, further experiments showed that irrigation with moderately saline water, a condition very frequent in Mediterranean areas, reduced yield, but this effect was lower in heterotic hybrids. In fact, hybrids under saline conditions had higher yields than their corresponding parents under non-saline conditions, which was probably due to heterosis, hybrid homeostasis, or both (Prohens et al., 1999). Such findings reinforced the utility of developing heterotic hybrid clones for cultivation under Mediterranean conditions.

4. Does breeding for yield and adaptation affect pepino quality?

Improvement of yield may result in a lower quality of fleshy fruits, due to the so-called “nutrient dilution” effect caused by the counter-balance between yield and concentration of sugars and other nutrients (Davis, 2009). When studying the effects of improvement of yield in pepino fruit quality, a preliminary experiment did not show any negative genotypic correlation between yield and fruit quality traits (Prohens & Nuez, 1999), suggesting that the selection of pepino clones for high yields might not be at the expense of fruit quality. A subsequent experiment encompassing a representative collection of pepino clones confirmed this fact, showing that, with the only exception of SSC in the spring-summer season, no negative genotypic correlations were found between yield and several quality traits like fruit weight and size, SSC, titratable acidity (TA), or ascorbic acid content (AAC) (Rodríguez-Burruezo et al., 2002). In addition, other experiments conducted with F_2 and BC_2 populations segregating for gene *P* (Prohens & Nuez, 2001) showed that the use of genetic parthenocarpy on pepino to improve yield does not have undesirable effects on fruit weight or SSC (Table 2).

Regarding the effects of improvement of yield in ToMV resistant materials under conditions of infection, it was found that, given that ToMV infection affects negatively the quality of pepino fruits, up to the point that in the most susceptible cultivars infected plants may have no marketable fruits (Pérez-Benlloch et al., 2001), resistance to ToMV resulted in an improvement of fruit quality under conditions of infection (Table 2). Therefore, the use of ToMV resistant cultivars would be useful not only to avoid yield losses, but also to keep the commercial quality of fruits.

Finally, we also found that irrigation with moderately saline water, although it resulted in a reduction in yield, it was compensated by an increase in the SSC and TA (Table 2), and also improved fruit flavour and earliness (Prohens et al., 1999). These results were in agreement with the findings reported by other authors for the effect of salinity on pepino SSC (Pluda et al., 1993a).

5. Breeding for quality

High fruit quality is of paramount importance to make the pepino fruit attractive to consumers (Welles, 1992). When taking into account composition traits related to fruit quality in pepino, total sugars, SSC, maturity index (SSC/TA), and AAC increase during the ripening process of pepino, while TA, decreases (Sánchez et al., 2000). Sucrose is the predominant sugar in fully ripe fruits and its concentration, as well as the total sugars content, is highly correlated with SSC, indicating that SSC readings might be utilized by breeders as a rapid and accurate alternative to other time-consuming methodologies, like HPLC, to evaluate the soluble sugars content of pepino. This high correlation between SSC and soluble sugars was also confirmed in subsequent studies (Prohens et al., 2005a). Therefore, indirect selection for sweetness can be made by selecting for high SSC.

The study of available diversity is of great relevance to improve the fruit quality of pepino. Although some reports on pepino fruit composition existed at the beginning of our breeding program (Esquivel & Hammer, 1991; Heyes et al., 1994; Redgwell & Turner, 1986; Shiota et al., 1988), these studies did not provide much information on the variation available in the crop, and therefore were very limited to assess the opportunities for improving the organoleptic and nutritional quality of this fruit. Therefore, we studied the intraspecific and interspecific diversity of fruit quality for pepino breeding and used it to improve the fruit quality of this crop.

5.1. Intraspecific variation

The evaluation of a collection of pepino clones grown in different seasons (autumn-winter and spring-summer), showed that SSC and AAC were higher and TA slightly lower under the autumn-winter conditions (Rodríguez-Burruezo et al., 2002) (Figure 4). This study also revealed the existence of an important genotype×environment (G×E) interaction, suggesting that separate breeding programs should be made for each growing season. It also showed that heritability values for SSC (0.17-0.39), TA (0.18-0.43), and AAC (0.54-0.57) were moderate. This experiment also revealed that the coefficient of genotypic variation was low for SSC and TA, while it was moderate for AAC, indicating that genetic advances for SSC and TA would be low or moderate if only intraspecific variation were used.

Considering the results obtained, we concluded that future efforts for adaptation and quality improvement of pepino should be focused on the autumn-winter growing season, as it allows higher yields and better fruit quality. Nevertheless, a higher genotypic variation would be necessary for obtaining relevant improvements in the SSC, which might be achieved by obtaining new genotypic combinations by crossing different clones or by exploiting the variation present in the wild related species.

Aroma is also a highly relevant trait for the organoleptic quality of pepino. Thus, the aroma volatile constituents of pepino and its inheritance in pepino clones with complementary aroma profiles (from intense fruity/exotic to intense vegetable/cucumber-like) and hybrid clones from crossings among these parents were studied (Rodríguez-Burruezo et al., 2004a). We found that aroma differences between pepino clones could be explained on the basis of 17 odor contributing volatiles (OCVs). Fruits with fruity/exotic aromas were characterized by a predominance of esters, complemented by the exotic notes of several minor compounds like β-damascenone, lactones or mesifurane. By contrast, the intense vegetable/cucumber-like aromas of other cultivars was due to very high levels of aldehydes like hexanal, (E)-hex-2-enal, and several nonenals which have been described as relevant for the aroma of melons and cucumbers. We also found that total content on OCVs of hybrids was highly correlated (0.91) with the mean value of the corresponding parent clones. Furthermore, we observed that by means of complementary crossings it is possible to obtain hybrid clones showing intermediate, or even transgressive, levels of most OCVs (Figure 5), which had either a more intense fruity or a more herbaceous aroma. Therefore, this strategy could lead to the selection and development of hybrid clones with improved aroma.

5.2. Interspecific variation

The limited genotypic variation found within *S. muricatum* for quality traits, particularly SSC, led us to evaluate the potential of *S. caripense* and *S. tabanoense* as sources of variation for this trait (Prohens et al., 2003). Both species showed the highest fruit weight among wild pepino relatives, particularly *S. tabanoense*, and the yield of selected interspecific hybrids was comparatively high (30-40 t/ha), while their fruit weight, though intermediate (40-60 g), was considerably higher than those of the corresponding wild parents. Finally, most accessions of both wild species showed a high SSC (10-14%) and very high TA (70-150 mmol/kg citric acid), with values of TA of up to 15-fold higher than those of the cultivated pepino (9-15 mmol/kg) (Figure 6). Although these high TA values are undesirable, increases in SSC must be accompanied by comparatively equivalent increases in TA in order to maintain the balance between sugars and acids. Furthermore, some wild accessions showed AAC values two-fold higher than those of pepino clones.

On the basis of those findings we developed interspecific families from each of the crosses *S. muricatum*×*S. caripense* and *S. muricatum*×*S. tabanoense*, to study the inheritance of SSC and TA in these materials (Rodríguez-Burruezo et al., 2003a). An important genetic effect was found for differences of SSC and TA between wild parents and cultivated pepino, confirming the suitability of both species in the improvement of these traits. Contrarily to what occurred for SSC and TA when using only interspecific variation, heritability estimates for these traits were intermediate (0.4-0.5) and would allow high genetic advances with moderately low selection pressures of 5-10%. Generation mean analyses revealed that for both families, the additive effect was the only significant effect controlling the inheritance in SSC. By contrast, genetic effects associated with additivity and dominance were detected in both families for TA. For this latter trait, alleles from the wild species are recessive to those of the cultivated *S. muricatum*. This explained that, despite having wild parents with very high TA values, TA in the F1 generation was closer to those of the cultivated species than to those of the wild species (Figure 6). In addition, fruit weight in the first backcross to the cultivated parent was high and in some individuals it was close to the values of *S. muricatum*, particularly in *S. muricatum*×*S. tabanoense* family. All these results suggested that a successful breeding program should include several backcross generations towards the cultivated pepino for a fast recovering of fruit weight, including selection of those individuals with the highest fruit weight, SSC, and TA. After this, the selected backcrossing individuals should be selfed to obtain individuals in which the favorable alleles for SSC from the wild species are fixed in homozygosis. As a result of applying this approach we have been able to improve the fruit quality of pepino.

6. Development of new cultivars and improved materials

A relatively simple program of clonal selection among segregating generations of materials from Chile resulted in the first pepino cultivars adapted to Mediterranean climates, ‘Sweet Long’ and ‘Sweet Round’, which were released in 1997 (Ruiz et al., 1997). ‘Sweet Long’ and ‘Sweet Round’, have moderate yield and levels of SSC, but represented the first materials specifically selected for Mediterranean climates (Ruiz et al., 1997). Subsequently, parthenocarpy was exploited for the development of cultivar ‘Puzol’ (Prohens et al., 2002). ‘Puzol’ is a facultatively parthenocarpic hybrid obtained in 1994 as a result of clonal selection in a cross between 9-2, a homozygous clone for gene *P* (Prohens et al., 1998a), and 6-21, an experimental clone which is a full sib of ‘Sweet Round’. Thanks to its parthenocarpic *Pp* genotype, ‘Puzol’ has a remarkably higher yield than either ‘Sweet Long’ or ‘Sweet Round’ (Table 3). However, in this cultivar, SSC values are low and AAC values are moderate-low for this species. All these properties make it suitable for use in salads but not for dessert fruit.

The lack of a good combination of high yield and quality encouraged us to plan an intraspecific breeding program which tried to combine high yields and fruit quality, and adapted to the autumn-winter season under greenhouse cultivation. In order to achieve this objective, we performed a hybridization program taking into account: i) exploiting hybrid vigour for yield resulting from crossing nonrelated parents; ii) using parents complementary for traits of interest, particularly those with monogenic dominant nature (parthenocarpy, ToMV tolerance), but also polygenic traits (e.g. SSC, TA, AAC); and iii) selecting among the segregating hybrid populations within each crossing, which would allow clonal selection of those individuals with the best combinations of yields and fruit quality. After complementary crossings were made, a high degree of segregation was found within each hybrid population, and transgressive individuals were identified in many traits. Thus, mean values of 25% of the best individuals within the hybrid populations were 35-66 t/ha for yield, 250-430 g for fruit weight, and 9.4-10.7% of SSC (Rodríguez-Burruezo et al., 2003b). Such level of diversity offered the opportunity for selecting many individuals with satisfactory combinations of yield and fruit quality. In addition, we found that heterosis was highly correlated with genetic distance (estimated with AFLP molecular markers) between parents. This indicates that marker assisted selection can be used to improve yield by obtaining hybrids from parents situated at high genetic distance.

From these hybrid segregating populations, individuals were selected for their combination of yield and fruit quality, and clonally replicated for subsequent evaluations. After several trials in different locations, two new improved hybrid clones, ‘Valencia’ and ‘Turia’ were developed (Table 3). ‘Valencia’ is currently the best pepino cultivar for dessert fruit adapted to Mediterranean conditions (Rodríguez-Burruezo et al., 2004b). It was developed from the crossing between ‘Sweet Long’ (moderate yield, medium-high SSC) and clone Sm-26 (ToMV resistant and high SSC). It represents a considerable improvement over previous dessert cultivars like ‘Sweet Long’ and ‘Sweet Round’, with higher yield, SSC, and AAC (Table 3). Furthermore, it has a balanced TA, and exotic fruity aroma, resembling in this trait to its ‘Sweet Long’ parent (mostly due to esters like butyl, 3-methyl-2-buten-yl, and 3-methyl-3-buten-yl acetates, as well as β-damascenone, mesifurane, and lactones) (Rodríguez-Burruezo et al., 2004b).

Apart from ‘Valencia’, we also released cultivar ‘Turia’, which is specifically adapted to be used for salad use (Rodríguez-Burruezo et al., 2004c). ‘Turia’ is a clonal selection from a crossing between ‘Puzol’ (salad use, high yield, parthenocarpic) and the clone Sm-4 (androsterile, medium-high SSC, ToMV tolerance). ‘Turia’ represents an improvement over ‘Puzol’, with higher yield, SSC, and AAC (Table 3). It also shows balanced TA values (7 mmol/kg citric acid) and fruits with firm flesh and intense vegetable/cucumber-like aroma (mostly due to a high content of aldehydes hexanal and nonenals and a low contribution of esters and other “fruity” volatiles) (Rodríguez-Burruezo et al., 2004c). All these characteristics make ‘Turia’ the best pepino cultivar currently available for salad use and specifically adapted to Mediterranean climates.

In parallel, and in order to further improve the organoleptic quality of pepino, particularly by increasing SSC, an interspecific program was started from the *S. muricatum*×*S. caripense* and *S. muricatum*×*S. tabanoense* materials. Thus, several individuals of the first backcross towards *S. muricatum* (BC_1) were selected for high SSC (>9%; selected individuals: 9.2-11.7%), yield (>20 t/ha; selected individuals: 23-121 t/ha), and fruit weight (>50 g; selected individuals: 65-262 g), as well as balanced TA. These BC_1 selected individuals were selfed in order to accumulate favorable alleles from the wild species for SSC in homozygosis in the segregating offspring ($BC_{1\varnothing}$). A preliminary clonal selection was then performed in these $BC_{1\varnothing}$ populations and clones were propagated and subsequently evaluated. These evaluations allowed us to select the best BC1ø clones, which were then utilized to perform a second backcross towards *S. muricatum* ($P_1 \times BC_{1\varnothing}$). In order to avoid inbreeding depression, pepino clones not related to the original P_1 parent were used as recurrent materials to develop these BC_2s (Figure 7) Again, clonal selection was performed for yield, fruit weight, and SSC. Clonal propagation of the selected BC_2 clones and subsequent evaluations allowed us selecting six clones characterized by high yields (38-82 t/ha), commercial fruit weights (200-300 g), and high SSC (8.4-11.2%) (Prohens et al., 2005a). Most of them were developed from the *S. muricatum*×*S. tabanoense* crossings, because, as expected, the proportion of individuals at this BC level with commercial fruit size was higher in this family than in the *S. muricatum*×*S. caripense* family. These selected clones from the interspecific program have been evaluated at a higher scale and some materials have entered into the phase of extensive evaluation to be released as new cultivars

7. Further prospects in pepino breeding

Despite being in the era of genomics, few molecular works have been performed in pepino, and it is obvious that pepino breeding may benefit from these new available tools. In this respect, the only applications of molecular markers used in pepino up to now have been for establishing the phylogenetic relations of pepino (Anderson et al., 1996; Anderson & Jansen, 1998; Blanca et al., 2007) and for marker assisted selection by selecting parents situated at high genetic distances to obtain highly heterotic hybrids (Rodríguez-Burruezo et al., 2003b). Although a very preliminary genetic map has been made in the wild relative *S. caripense* (Nakitandwe et al., 2006), a genetic map is not yet available for pepino.

The pepino is phylogenetically close to other economically important Solanaceae, like tomato or potato (Lester, 1991; Spooner et al., 1993), for which a wide array of molecular tools are available for breeding programs. This opens the door to to perform synteny studies and localization of genes in pepino from information of the tomato and potato genomes. In this respect, the use of conserved orthologous sequence (COS and COSII) markers may help to produce a pepino genetic map which can be anchored to the tomato or potato genomes and help in the identification of genes and genomic regions of interest in the genetic improvement of pepino. Also, the development of co-dominant markers, like SSRs and SNPs would also be helpful to study some characteristics of relevance for pepino breeding, like the degree of heterozygosis of the materials, and would provide new types of markers for marker assisted selection. For example, it is known that pepino is variable for the type of respiratory activity, with some materials behaving as climacteric and others as non-climacteric (Martínez-Romero et al., 2003). Evaluation of materials and identification of genes involved in the regulation of the respiratory performance could lead to the selection of pepino clones with extended postharvest life and improved quality.

Pepino is amenable to genetic transformation with Agrobacterium, and efficient protocols for genetic transformation exist (Atkinson & Gardner, 1991). This opens the way to the improvement of many fruit quality traits. However, given the difficulties in introducing genetically modified organisms into the market, it seems that no commercial transgenic pepinos will be found in the market in the short term.

Finally, given the increasingly reduced costs of DNA sequencing we can foresee that in the coming years the full sequence of the pepino genome will be available, which will further contribute to the identification of genes and will provide tools that will increase the efficiency of breeding programs aimed at improving the fruit quality and other attributes of the pepino crop, as well as to face the challenges posed by the climatic change on crop physiology and fruit quality (DaMatta et al., 2010).

7. Conclusions

Pepino is an exotic fruit with ample potential for introduction in different agroclimatic conditions. Here we have shown that with a limited breeding effort based on the utilization of the genetic resources and in establishing appropriate breeding strategies it is possible to obtain new cultivars adapted to Mediterranean climates. Improvement of quality, mostly sweetness, which is a main limitation for the acceptance of pepino has been achieved by developing intraspecific and interespecific breeding programs, which have resulted in new cultivars and improved materials. Further efforts in pepino breeding probably will result in new cultivars with better characteristics that will contribute to adding the pepino to the list of important exotic fruits already available in the markets. The approach used here for pepino may be successfully applied to improve other neglected vegetatively progagated exotic fruit crops.

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Table 1

Estimates of genetic diversity in pepino, tomato and eggplant obtained with AFLP markers using three combinations of primers (adapted from Nuez et al. (2004), Prohens et al. (2005b) and Blanca et al. (2007)).

Crop	Number of accessions	Number of loci screened	Number of polymorphic loci (%)	Total gene diversity (H_T)[a]
Pepino	27	298	204 (68.5)	0.190
Tomato & close relatives[b]	38	242	99 (0.41)	0.091
Eggplant	32	339	65 (0.19)	0.046

[a]Total gene diversity according to Nei (1973).

[b]Includes the cultivated tomato (*L. esculentum*) and the closely related *L. pimpinellifolium* and *L. cheesmanii*.

Table 2

Changes (in percentage) over controls induced by parthenocarpy (in a BC2 and F2 segregating generations), ToMV infection (in two susceptible clones), and salinity (increase of 5 dS/m in the electrical conductivity of the irrigation water in four parents and two hybrids among them) for yield, fruit weight, soluble solids content (SSC), titratable acidity (TA), and ascorbic acid content (AAC) of pepino fruits (adapted from Prohens et al. (1999), Pérez-Benlloch et al. (2001), and Prohens and Nuez (2001).

	Effect					
Trait	Parthenocarpy		ToMV infection		Salinity	
	BC2	F2	Clone 1	Clone 2	Parents	Hybrids
Yield	$+47^{**}$	$+67^{**}$	-95^{***}	-100^{***}	-40^{**}	-24^{*}
Fruit weight	$+19^{ns}$	-17^{ns}	-26^{***}	$+5^{ns}$	-28^{**}	-17^{*}
SSC	-3^{ns}	-6^{ns}	$+9^{ns}$	-16^{**}	$+23^{***}$	$+22^{***}$
TA	--	--	--	--	$+12^{ns}$	$+9^{ns}$
AAC	--	--	--	--	-26^{**}	-27^{**}

^{ns}, *, **, *** indicate non significant, or significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

Table 3

Comparative results of yield, SSC, and AAC for the pepino cultivars released in the breeding program grouped for their year of release (adapted from Rodríguez-Burruezo et al., 2004b, 2004c).

Cultivar (main use)	Yield (t/ha)	SSC (%)	AAC (mg/kg)
1997			
Sweet Long (dessert)	19-30	6.7-8.3	283-349
Sweet Round (dessert)	22-33	7.1-8.2	260-410
2001			
Puzol (salad)	40-60	5.1-7.0	235-328
2004			
Valencia (dessert)	27-47	8.8-10.0	405-574
Turia (salad)	49-72	7.2-8.0	260-340


Fi. 1. Diversity for fruit size, shape and colour in cultivated pepino.

Fig. 2. Fruits of *S. caripense* (left) and *S. tabanoense* (right), the two wild species most closely related to pepino, and which represent a source of variation for improving fruit composition.

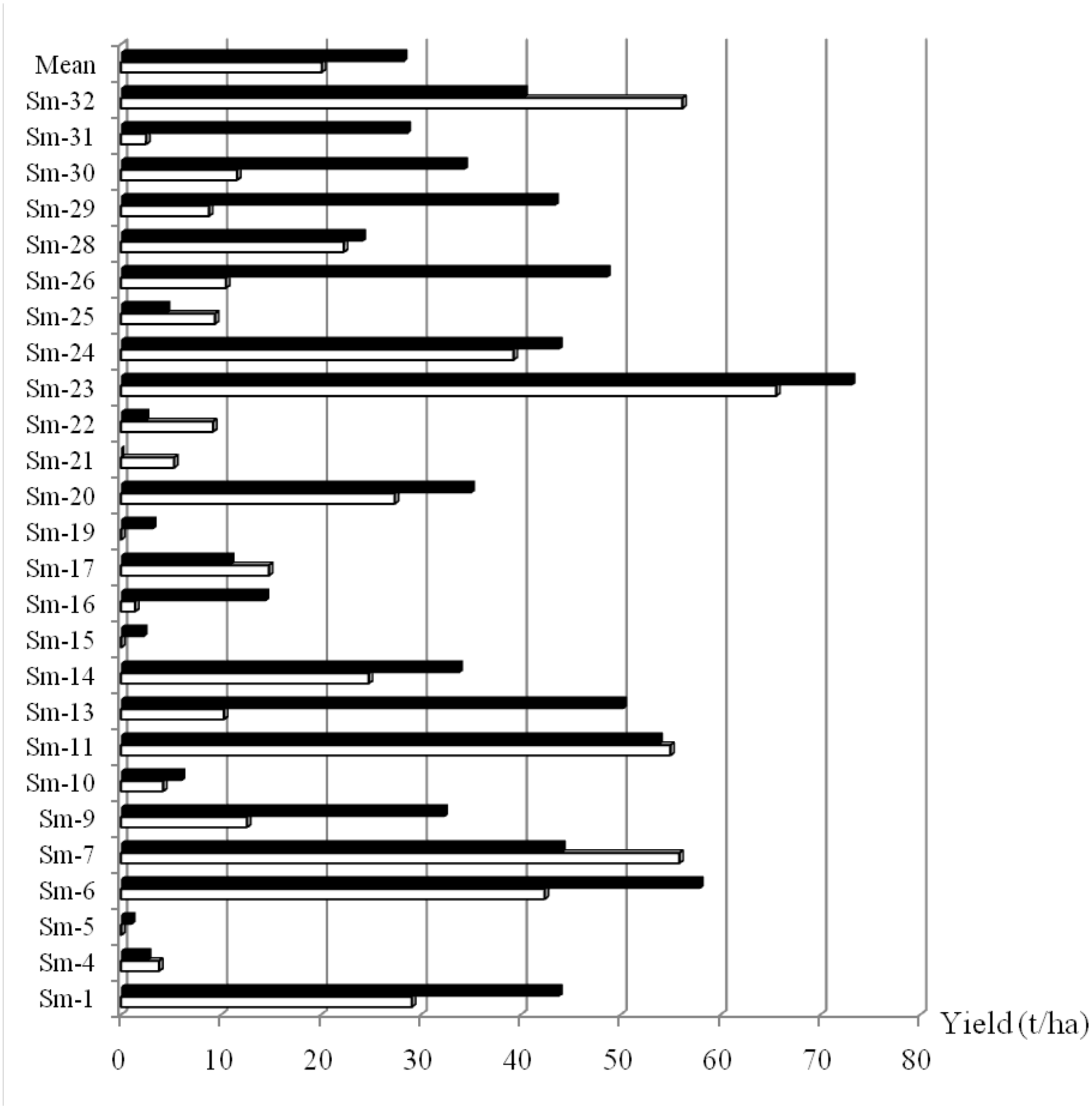

Fig. 3. Effect of cultivation under autumn-winter (black bars) and spring-summer (white bars) growing cycles on yield (t/ha) in a collection of 26 pepino clones (adapted from Rodríguez-Burruezo et al., 2000).

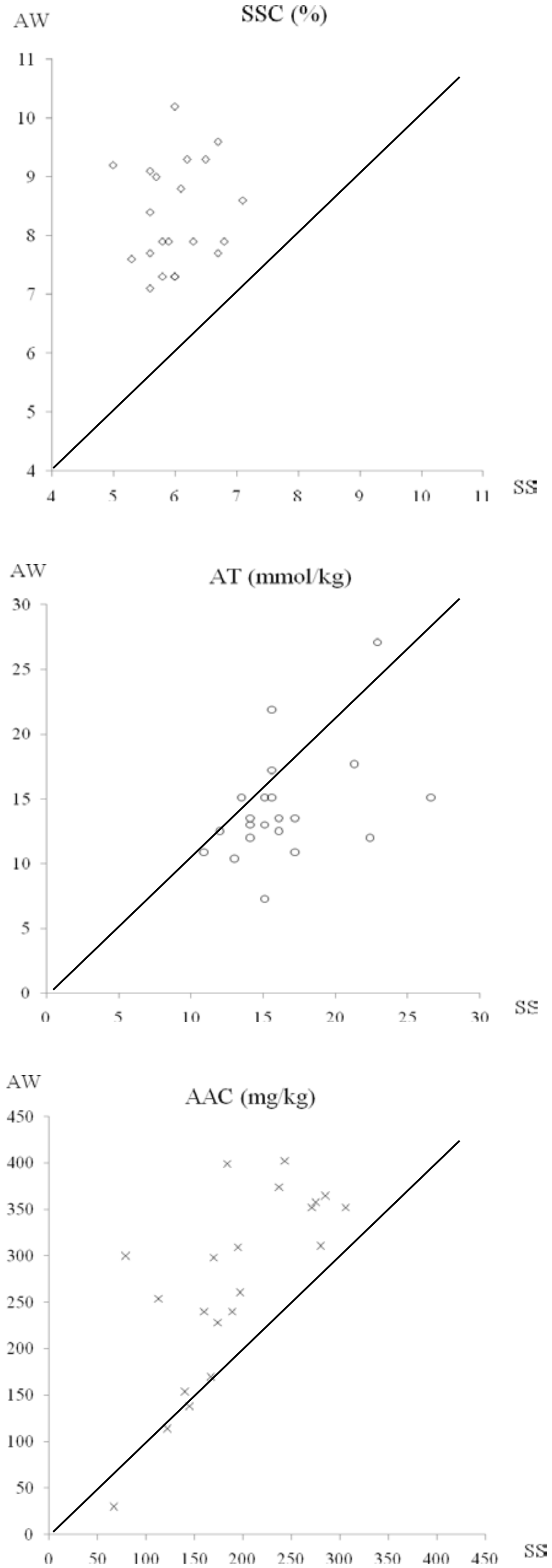

Fig. 4. Comparison of soluble solids content (SSC), titratable acidity (TA), and ascorbic acid content (AAC) between autumn-winter (AW) and spring-summer (SS) growing seasons in a collection of 21 pepino clones (adapted from Rodríguez-Burruezo et al., 2002). The solid line indicates a β=1 slope.

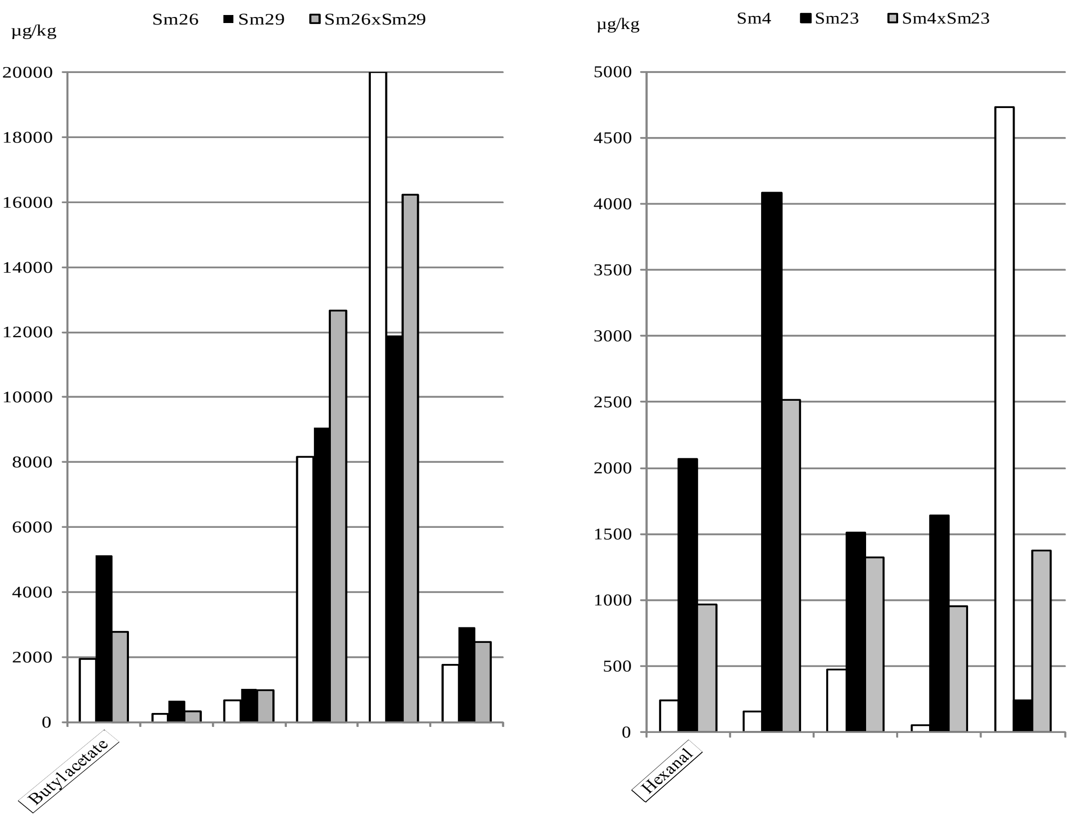

Fig. 5. Patterns of odor contributing volatiles in parent clones and their hybrids. Left: crossing involving parents (Sm26 and Sm29) with predominant fruity aroma (pattern based on esters and alcohols) and its hybrid (Sm26×Sm29). Right: crossing involving parents (Sm4 and Sm23) with predominant green/vegetable aroma (pattern based on aldehydes) and its hybrid (Sm4×Sm23) (adapted from Rodríguez-Burruezo et al., 2004a).

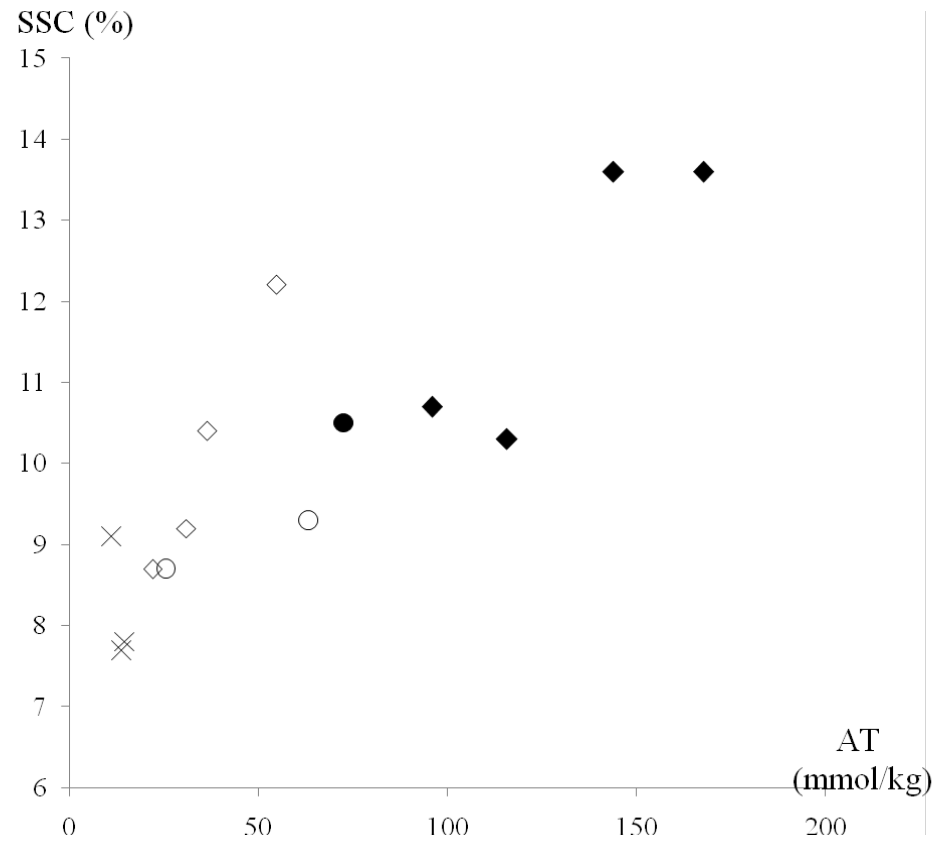

Fig. 6. Comparison of soluble solids content (SSC) and titratable acidity (TA) between clones of cultivated pepino (*S. muricatum*, ×), wild relatives (*S. caripense*, ♦; *S. tabanoense*, ●), and interspecific hybrids (*S. muricatum*×*S. caripense*, ◊; *S. muricatum*×*S. tabanoense*, ○) (adapted from Prohens et al., 2003).

Fig. 7. Example of BC2 segregating generations (before clonal selection) derived from *S. muricatum*×*S. caripense* and *S. muricatum*×*S. tabanoense* crossings (left) and comparison of fruit size and appearance to cv. Turia (rigth).