Characteristics and Selection of the 'Almagro' Heirloom Eggplant and Potential for Further Development

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Abstract

'Almagro' eggplant (*Solanum melongena* L.) is an heirloom native to the Spanish region of Castilla-La Mancha used for making pickles. Pickled 'Almagro' eggplants from the "Campo de Calatrava" municipalities have a Protected Geographical Indication status, which has boosted its cultivation and importance in the last years. In order to make high quality pickles, fruits are harvested when the berry has not completed growth. An important characteristic of the 'Almagro' eggplant is that fruits are covered by an acrescent calyx which at the time of harvest covers all or most of the fruit. Given the interest of this heirloom for the local development, we have been studying its characteristics and we have performed a selection and breeding programme. Our studies show that this variety presents specific morphological and composition features when compared to other similar eggplant varietes. The molecular characterization with AFLP and SSR markers shows that 'Almagro' eggplant is different from other similar pickling varieties and that genetic variation exists within this heirloom, which allows the selection of the best materials. At the nutritional level, 'Almagro' eggplant presents a high content in antioxidant phenolics and represents a source of variation for breeding programmes aimed at improving the bioactive constituents of eggplant. In this respect, we have made a selection ('H15') which has low calyx prickliness and a high yield. Furthermore, we have initiated a backcross programme in order to develop new 'Almagro' eggplant materials with lower prickliness. In this respect, marker assisted selection will be helpful in this programme in order to recover the genetic background of the 'Almagro' eggplant.

INTRODUCTION

Spain presents a plethora of eggplant (*Solanum melongena* L.) heirlooms, and because of this it is considered a secondary center of diversity (Prohens et al., 2005). Two

of the most internationally renowned Spanish local varieties of eggplant are the 'Listada de Gandía' (Muñoz-Falcón et al., 2008) and the 'Almagro' eggplants (Muñoz-Falcón et al., 2009). The former is characterized for having semi-long fruits with purple stripes on a white background and has excellent cooking properties. The latter has small fruits covered by an acrescent calyx and is the only eggplant variety recognised in Europe with an official Protected Geographical Indication (PGI) (Castro, 2005). The 'Almagro' name refers to the city of Almagro, which is the capital of the "Campo de Calatrava" county (situated in the province of Ciudad Real, in the center of Spain), and makes reference to the place of origin of this heirloom.

Contrarily to most of the Spanish eggplant heirlooms, the 'Almagro' eggplant is mainly used for the elaboration of pickled fruits of eggplant (Seseña et al., 2004). At present, the production of 'Almagro' eggplant under the Protected Geographical Indication (PGI) "Berenjena de Almagro" oscillates between around 1500-2000 t (Prohens et al., 2009) and provides extra income to farmers in the region, which normally cultivate vegetable crops, such as onion, tomato, pepper, or melon for the fresh produce market, as well as field crops such as cereals.

The 'Almagro' eggplant has not been subjected to formal breeding programmes, and the materials grown by farmers correspond to the result of an empirical selection made for several generations of farmers under the agroecological conditions of the "Campo de Calatrava" county (Castro, 2005). This has resulted in the generation of some variants of the 'Almagro' eggplant, which may be amenable to selection. Also, during the last years, introduction of materials used for pickling from other regions of Spain, like Andalusia, have threatened the genetic integrity of the traditional 'Almagro' heirloom, which may result in a decrease of the uniformity and quality of the produce (Muñoz-Falcón et al., 2009).

The added value of the 'Almagro' eggplant can be increased by having a detailed knowledge of the characteristics that distinguish it from other varieties and also by studying the traits for which this variety is outstanding over other varieties. Also, given the diversity of the 'Almagro' eggplant, selection can be done in order to get improved materials of this heirloom. In addition, breeding programmes based in crosses can be useful to develop new materials that, while having the typical quality characteristics of 'Almagro' eggplant have some improved traits, like a reduced prickliness (Prohens et al., 2009).

During the last years we have initiated a programme for the study of the characteristics and diversity of the 'Almagro' eggplant, as well as for the selection and improvement of this local heirloom. Here we review the work done up to now, the main results obtained, and the prospects for further development.

CHARACTERISTICS

Agronomic and morphological

The 'Almagro' eggplant is an heirloom adapted to open field cultivation during the warm summer season of the center of Spain (Castro, 2005). It has multiple inflorescences, allowing the production of many fruits, which are harvested when they have a size of 4 to 9 cm long (Prohens et al., 2009). This heirloom is similar in gross morphology to other heirlooms used for making pickles, like the 'Andalusian' pickling eggplant, from the neighbour region of Andalusia, but it can be distinguished by several traits or by a combination of them (Table 1). For example, in our characterization trials we have found that the 'Almagro' eggplant has smaller leaves, shorter fruit pedicel length, a smaller

proportion of the berry covered by the calyx, and more prickles in the calyx than the 'Andalusian' eggplant (Muñoz-Falcón et al., 2009). This latter trait is an inconvenient for the production of 'Almagro' eggplant as it difficults the harvesting, can cause accidents to agricultural workers, can damage the fruits when processed, and requires their removal by manual means before processing.

When grown in environments different to those of their native region, some differences in the development of the plants may be observed. In this respect, the comparison between 'Almagro' plants grown in its region of origin in the Campo de Calatrava county with plants grown in coastal Valencia, we have found that plants in Valencia are shorter and have smaller leaves, and the fruits have shorter fruit pedicel length and are more round in shape than those grown in its region of origin (Table 1). This is probably due to the important differences in environment, which in eggplant can lead to important differences in phenotype (Prohens et al., 2004) and confirms that cultivation in its region of origin plays a key role in achieving a high quality. It also is an indication that phenotyping and selection in breeding programmes should be made in the "Campo de Calatrava" county.

The performance of 'Almagro' eggplant when grown under greenhouse is very poor and the plant develops long internodes, very large leaves, high prickliness, and has a very low fruit set due to deficient fertilization produced by low temperatures and insufficient light. Similar results have been observed in other Spanish heirlooms adapted to open field cultivation when are grown in greenhouse during the winter season (Prohens et al., 2004). Also, some non-fertilized pseudofruits may develop under these conditions, and although the ovary arrests development after they have reached 2-3 cm in diameter and frequently rots, the calyx may continue its growth producing an abnormal development of this organ.

Molecular

As occurs with many heirlooms of vegetables (e.g., Rao et al., 2006), the 'Almagro' eggplant is genetically heterogeneous. We have demonstrated this fact with both AFLP and SSR markers. However, the levels of diversity detected were rather limited, with total diversity (H_T) values obtained with AFLPs of H_T=0.0107, compared to a diversity of H_T=0.0152 for 'Andalusian' pickling eggplant. Also, when using SSR markers the number of polymorphic markers in 'Almagro' eggplant was lower, with 13.9% of SSR markers being polymorphic compared to 22.2% for 'Andalusian' eggplant (Muñoz-Falcón et al., 2009). Therefore, despite being less diverse than 'Andalusian' eggplant, diversity exists within the 'Almagro' eggplant indicating that this heirloom may be amenable to selection.

Characterization with these markers has also allowed studying the relationships of 'Almagro' eggplant with related materials. In this respect, when using AFLPs, we found that 'Almagro' and 'Andalusian' pickling eggplants are clearly separated from the rest of eggplants for fresh consumption, but that both heirlooms share a common genetic background, probably linked to a common origin in the distant past. In this respect, when studying the relationships between these heirlooms using multivariate analyses the 'Almagro' and 'Andalusian' materials are intermingled and can not be distinguished with AFLPs (Muñoz-Falcón et al., 2009). Also, no AFLP band specific and universal to all 'Almagro' materials could be found. However, when studying the relationships of 'Almagro' and 'Andalusian' heirlooms with SSR markers, the picture is completely different, as SSRs separate clearly these heirlooms (Figure 1). This is a clear indication

that SSRs are much better than AFLPs at resolving the relationships of closely related plant materials (Tam et al., 2005). Furthermore we have found two SSR alleles, which correspond to alleles 206 of marker EM127 and allele 360 of marker EM145 (Nunome et al., 2003a, 2003b) that are unique and universal to all 'Almagro' accessions (Muñoz-Falcón et al., 2009). This shows that the 'Almagro' eggplant is genetically unique and different from similar materials. This differentiation is probably the result of the action of microevolutive forces, including natural and artificial selection, and genetic drift. The fact that some markers that are fixed in 'Almagro' eggplant and present but not fixed in 'Andalusian' materials may suggest that genetic drift may have had an important role in the genetic differentiation of 'Almagro' eggplant.

Composition

At the composition level, the 'Almagro' eggplant presents levels of soluble solids content of 5.5% to 6.3% and a pH of 5.4 to 5.8, which are values similar to those of other pickling and fresh consumption eggplants (Prohens et al., 2009). However, the Almagro eggplant presents very high values of antioxidant phenolic compounds, with values between 0.7 and 0.9 g/kg, and in an experiment including 69 *S. melongena* varieties, it was the common eggplant variety with the highest total phenolics content (Prohens et al., 2007a). Given that the most important phenolic compounds in the eggplant fruit are hydroxicynnamic derivatives, in particulary chlorogenic acid (Whitaker and Stommel, 2003) and this compound has a high antioxidant potential and is very stable even after being subjected to cooking and processing (Lo Scalzo et al., 2010), the 'Almagro' eggplant has a very high nutraceutical quality. In fact, it could be a source of variation for breeding programmes aimed at increasing the nutraceutical quality of eggplant.

Another component for which the 'Almagro' eggplant is outstanding is the vitamin C content, with values above 20 mg/100 g of fresh weight. Although the vitamin C is mostly lost when cooking, it prevents browning of tissues during processing (Macheix et al., 1990) and this is an interesting property of this heirloom.

Organoleptic quality

The outstanding organoleptic quality of the 'Almagro' eggplant is the result of the interaction between the raw material used and the processing for producing the pickles (Seseña et al., 2004; Prohens et al., 2007b). We have evaluated the quality of several accessions of 'Almagro' and 'Andalusian' eggplant and have found that the 'Almagro' eggplant obtains better scores than the 'Andalusian' eggplant for fruit color, firmness, texture, and global appreciation (Prohens et al., 2009), which confirms that the use of the 'Almagro' eggplant heirloom is a prerequisite for obtaining high standards of quality in the final pickled produce.

SELECTION AND BREEDING

The 'Almagro' eggplant presents genetic diversity and therefore is amenable to selection. The SSR analyses we have performed confirm that the materials of 'Almagro' eggplant are highly homozygous, probably as a consequence of its mostly autogamous reproduction (Pessarakli and Dris, 2004), and therefore the selection of outstanding individuals can result in the production of improved pure lines. These pure lines not only may have better characteristics, but can also result in higher homogeneity. As a result of a participatory selection programme for yield and low prickliness established several years ago, we have selected an improved pure line (H15) (Prohens et al., 2009). This selected

line presents the typical morphological characteristics and the intrinsic quality of the 'Almagro' eggplant, and a higher yield and lower prickliness than the non-selected heirloom (Table 2). Therefore, an appropriate strategy for improving the 'Almagro' eggplant without losing the typical characteristics of the variety is by selecting the best individuals, which should then be selfed and the progeny evaluated for uniformity and performance. Another alternative is the mass selection, in which the selfed seed of the best individuals is bulked to produce a new population (Acquaah, 2007). This alternative, although may not produce dramatic improvements allows maintaining an important part of the diversity of the local heirloom. In this respect, 'Almagro' farmers are involved in a participatory breeding programme in which they have been instructed how to select the best individuals for seed production.

Exploitation of heterosis resulting from crossing genetically divergent individuals (Sidhu et al., 2004; Rodríguez-Burruezo et al., 2008) might also be exploited for improving the 'Almagro' eggplant. We have tested the potential utility of hybrids between different pure lines derived from the 'Almagro' heirloom, as well as between 'Almagro' and 'Andalusian' accessions (Muñoz-Falcón et al., 2009). However, the results show that the 'Almagro' x 'Almagro' hybrids do not represent any productive advantage over the pure lines, probably because of the genetic similarity among the different 'Almagro' pure lines. The 'Almagro' x 'Andalusian' hybrids are intermediate in morphological characteristics but they do not present heterosis for yield and give low quality pickles (Prohens et al., 2007b). Furthermore, the production of hybrid seed represents an additional cost. Therefore, we have abandoned the strategy of developing hybrids for improving the production of 'Almagro' eggplant.

Another alternative for the improvement of the 'Almagro' eggplant is the establishment of a backcross programme in which the objective is to obtain 'Almagro' materials which introgress the lack of prickles in the calyx trait from other eggplant materials. We have initiated a backcross programme in which the recurrent parent is the H15 selection and the donor parents are 'Andalusian' pickling eggplants with low prickliness on one hand, and varieties for fresh consumption without prickles on the other. The reason for using 'Andalusian' eggplants is that they are genetically very similar to the 'Almagro' heirloom (Muñoz-Falcón et al., 2009) and, therefore the number of backcross generations required in the backcross breeding programme is lower (Acquaah, 2007). However, the 'Andalusian' pickling varieties are not completely devoid of prickles and, in consequence, we also used prickles-free modern eggplant varieties as donors of this trait. Molecular markers distributed throughout the genome (Vilanova et al., 2010) will be used to help in selecting, among the individuals with the desired trait, those with a higher proportion of 'Almagro' eggplant genome. In this way, we pretend to obtain improved 'Almagro' accessions with reduced prickliness. Also, Doganlar et al. (2002) have found that a major QTL accounts for an important part of the variation in the presence of prickles in generations derived from a *S. linneanum* x *S. melongena* cross. It still remains to be investigated if markers linked to this QTL could be useful for selection in the 'Almagro' eggplant breeding programme.

In any case, for a successful selection programme, the materials finally selected have to conform to the traditional 'Almagro' eggplant variety as recognised by the PGI, must have an adequate agronomic performance, and give a high quality final produce. In this respect, the collaboration in the breeding programme of farmers, manufacturers, and consumers is of high relevance (Witcombe et al., 2005).

POTENTIAL FOR FURTHER DEVELOPMENT

The study of the characteristics and relationships of the 'Almagro' eggplant gives an added value to this unique eggplant heirloom. In particular, the availability of a genetic fingerprint that allows certifying that the materials used for elaborating the PGI pickled 'Almagro' eggplant correspond to the local heirloom is an important tool for protecting against fake materials being marketed as 'Almagro' eggplant (Muñoz-Falcón et al., 2009). In this respect, new markers distributed throughout the genome can be found so that they allow a better certification process.

The discovery that 'Almagro' eggplant has a high content of antioxidant phenolics also contributes to increasing the added value of the produce elaborated with this heirloom and increases its market value. The study of the particular phenolic compounds of 'Almagro' eggplant may also provide relevant information on the beneficial effects of the consumption of the 'Almagro' eggplant. Finally, eggplant genomics studies (Wu et al., 2009), which are not as advanced as in other related crops, like tomato or pepper may provide tools of great utility for the improvement of 'Almagro' eggplant.

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Tables

Table 1. Mean values for several traits for ‘Almagro’ and ‘Andalusian’ eggplant grown in the open field in its region of origin in the Campo de Calatrava county (center of Spain) and in the coastal region of Valencia (adapted from Muñoz-Falcón et al., (2009) data).

	Campo de Calatrava county		Valencia	
Trait[a]	‘Almagro’	‘Andalusian’	‘Almagro’	‘Andalusian’
Plant height (cm)	82.2 a	91.2 a	68.7 b	77.2 ab
Leaf blade length (cm)	20.1 b	23.5 a	13.8 c	15.3 c
Leaf blade width (cm)	16.6 b	20.0 a	8.8 c	10.4 c
Fruit pedicel length (cm)	8.84 b	11.22 a	6.06 c	7.71 b
Fruit calyx length[b]	6.48 c	8.51a	5.80 c	7.00 b
Fruit calyx prickles[c]	2.22 a	0.69 b	2.35 a	0.77 b
Fruit length/width ratio	2.16 a	2.18 a	1.60 b	1.54 b

[a]Means separated by different letters within a row are significantly different according to the Student-Newman-Keuls multiple range test ($P<0.05$).

[b]Fruit calyx length relative to the fruit length measured on a 1 to 9 scale (1=<50%; 3=50-70%; 5=70-90%; 7=90-99%; 9=100%).

[c]Measured on a 0 to 9 scale (0=none; 1=1-2; 3=3-7; 5=8-15; 7=16-25; 9=>25).

Table 2. Values of yield and prickliness of H15, relative to the non-selected ‘Almagro’ heirloom (reference value of the ‘Almagro’ heirloom is 100) during several years (adapted from Prohens et al. (2009) data).

	2006		2007		2008	
	Yield	Prickliness	Yield	Prickliness	Yield	Prickliness
H15 value	156.7	60.0	108.6	73.7	135.7	72.2

Figures

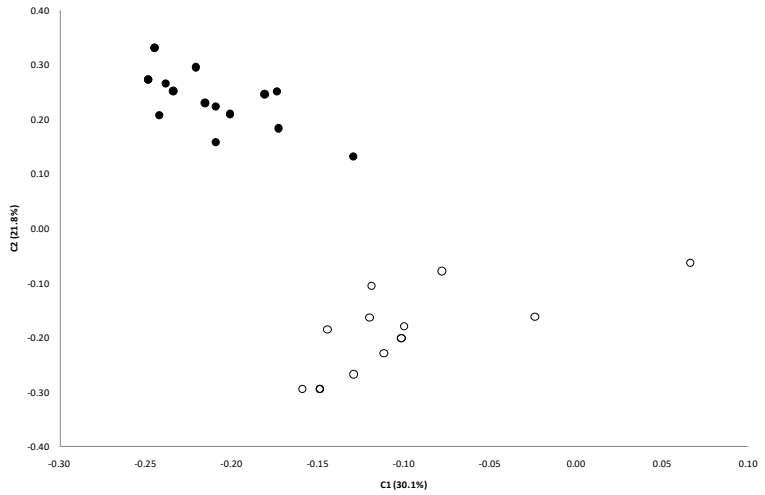

Fig. 1. SSR-based relationships between ‘Almagro’ (●) and ‘Andalusian’ (○) pickling eggplants materials based on principal coordinates analysis. The first (C1) and second (C2) components account for 31.1% and 21.8% of the total variation, respectively (adapted from Muñoz-Falcón et al. (2009) data).