Enhancing conservation and use of local vegetable landraces: the *Almagro* eggplant (*Solanum melongena* L.) case study

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Abstract

We have used the *Almagro* pickling eggplant landrace as a model for the enhancement of a local vegetable landrace. The programme has included characterization, selection, and breeding activities. Considerable intra-landrace diversity has been found for morphological traits and molecular markers. Characteristic morphological traits have allowed its registration as conservation variety. Also, universal and specific simple sequence repeat (SSR) markers have been found for the *Almagro* landrace. The chemical characterization revealed that *Almagro* eggplant has a high content in bioactive phenolics. Organoleptic tests revealed that pickles produced with *Almagro* eggplant are superior to those of the related *Andalusian* landrace. The selection programme has led to the development of a pure line (H15) with increased yield and reduced fruit calyx prickliness. A participatory breeding programme, in which selection is made by farmers in their own field, has been initiated to introduce the no-prickles trait from three other varieties in the genetic background of the *Almagro* eggplant. The results of the programmes show that plants with the *Almagro* eggplant ideotype and with reduced prickliness can be selected in the backcross generations. As a result of the enhancement programme the acreage and total production of *Almagro* eggplant has tripled in the last decade.

Keywords Backcross breeding · Characterization · Diversity · Landrace · Markers · Phenolics · Selection · *Solanum melongena*

Introduction

The use and enhancement of local landraces may have an important role for the development of horticulture. During the last years there has been an increasing interest for the recovery of local landraces by consumers and markets (Spataro and Negri 2013). Landraces of vegetables are associated to better flavour ("flavour of the past"), local tradition, and environmentally friendly production (Trichopolou et al. 2007). Because of this, consumers accept paying higher prices for the local landraces. In addition, cultivation of local landraces contributes to on-farm conservation of genetic resources and to preserving the ethnobotanical knowledge associated to them (Hammer et al. 2003, Polegri and Negri 2010).

Contrarily to commercially important cultivars, local landraces of vegetable crops often have been neglected and poorly studied, and little scientific information exists on their characteristics and unique features (Piergiovanni and Laghetti 1999, Polegri and Negri 2010, Cianconelli et al. 2013, Torricelli et al. 2013). We hypothesize that added value of local landraces can be increased by limited investments in their scientific study, characterization, selection, and breeding, leading to an improvement in its production, quality, or both, and contribute to the enhancement and demand of local landraces (Trichopolou et al. 2007).

We present the work done and new results of an enhancement programme directed to the *Almagro* pickling eggplant (*Solanum melongena* L., 2n = 2x = 24) landrace. We show how, with a limited effort it is possible to make an effective contribution to improving the commercial production, demand, and on-farm conservation of local landraces of vegetable crops.

The model landrace used: *Almagro* eggplant

The *Almagro* eggplant landrace is used for pickles and is native to the county of Campo de Calatrava, situated in the center of Spain (Muñoz-Falcón et al. 2009). The *Almagro* name of this landrace refers to the historic city of Almagro, which is the capital of the Campo de Calatrava county. This eggplant landrace has a small plant and fruit size (Fig. 1) and is very prolific, as it presents multiple inflorescences which often set several fruits. Like the rest of eggplants (Pessarakli and Dris 2004), *Almagro* eggplant is mostly autogamous. The fruits are obovoid-shaped and are harvested when they are 4 to 9 cm in length (Prohens et al. 2009). The berry is covered in a large proportion by an acrescent, usually prickly, calyx (Fig. 1). The berry skin presents a uniform green background colour and anthocyanins in the part exposed to the sun. The fruits of the *Almagro* eggplant are used by several local industries of the Campo de Calatrava county for elaborating *Almagro* eggplant pickles. The pickling process, which has been studied in detail (Seseña and Palop 2007), involves grading of fruits, blanching, lactic fermentation, stuffing or cutting into pieces (where appropriate) and canning in a brine solution (Fig. 1). Pickles made with *Almagro* eggplant have a long tradition in their region of origin, as well as in other parts of the Center and South of Spain, and in 1994 were recognized with a Protected Geographical Indication status (Muñoz-Falcón et al. 2009). In the last decades, other pickling eggplants from the neighbouring region of Andalucía (referred to as *Andalusian* landrace) have been introduced into the

Campo de Calatrava county (Muñoz-Falcón et al. 2009). The introduced *Andalusian* eggplants present productive advantages over the *Almagro* landrace, as they have higher yields and lower prickliness. However, the pickles obtained from the *Andalusian* eggplant landrace have lower organoleptic quality (Prohens et al. 2007a).

The *Almagro* eggplant germplasm has traditionally been conserved by individual farmers. This strategy favours the maintenance of diversity of the landrace (Gómez et al. 2005). Until we began our enhancement programme, no scientific breeding and selection had been applied for the improvement of *Almagro* eggplant.

Enhancement through characterization

A detailed characterization is essential to assess the diversity and relationships of a landrace, to identify characteristics that make it distinct and unique, as well as to provide information on composition traits and properties that may be of interest to consumers (Hammer and Diederichsen 2009, Ciancaleoni et al. 2013, Torricelli et al. 2013). In the case of *Almagro* eggplant, we undertook the characterization from different points of view: morphological, molecular, chemical, and organoleptic.

Morphological characterization

Morphological characterization allows the description of a landrace as well as identifying the traits or combinations of traits which allow distinguishing it from other landraces or cultivars (Spataro and Negri 2013). The *Almagro* eggplant may be easily distinguished from eggplant varieties for regular use (cooking, frying) by a combination of multiple traits (e.g., small plant size, multiple inflorescences, fruit size, acrescent calyx, fruit colour). However, distinguishing it from other pickling landraces, like the *Andalusian* eggplant, may be more challenging as the gross morphology of the plant and the fruit is very similar (Prohens et al. 2007a; Muñoz-Falcón et al. 2009).

In order to obtain a morphological profile of the *Almagro* eggplant landrace, we characterized *Almagro* and *Andalusian* pickling eggplant accessions for morphological descriptors (Muñoz-Falcón et al. 2009). We found that the *Almagro* eggplant has smaller leaves, shorter fruit pedicels, smaller fruit area covered by the acrescent calyx, and higher prickliness than the *Andalusian* eggplant (Muñoz-Falcón et al.

2009). Therefore, measurement of a few morphological fruit traits can be of great utility for a rapid discrimination of *Almagro* eggplant fruits from the closely related *Andalusian* pickling eggplant. These morphological differences were essential for the registration of the *Almagro* eggplant as conservation variety in 2008, which may contribute efficiently to its conservation and enhancement (Spataro and Negri 2013).

However, environmental effects may difficult the distinction of authentic *Almagro* landrace materials from similar materials, especially if they have been cultivated under different environmental conditions. In this case, molecular characterization may be very useful for unambiguous identification.

Molecular characterization

The diversity and relationships of the *Almagro* eggplant with other regular use and pickling eggplant materials has been studied with AFLP and SSR markers (Muñoz-Falcón et al. 2009; Hurtado et al. 2012; Vilanova et al. 2012). A study of *Almagro* eggplant accessions with AFLP and SSR markers revealed that the *Almagro* eggplant materials presented considerable genetic intra-landrace diversity and were highly homozygous (Muñoz-Falcón et al. 2009), probably a consequence of autogamy (Pessarakli and Dris 2004). Genetic diversity is a typical characteristic of landraces (Hammer and Diederichsen 2009; Polegri and Negri 2010) and indicates that selection within the *Almagro* landrace may be effective.

Regarding the relationship of the *Almagro* landrace with other eggplant varieties, AFLP and SSR markers showed that *Almagro* eggplant clusters with other Occidental (i.e., from Europe, Middle East, Africa, America) eggplants (Hurtado et al. 2012, Vilanova et al. 2012). SSRs also clearly distinguished *Almagro* and *Andalusian* materials (Muñoz-Falcón et al., 2009), and two SSR alleles universal and specific to the *Almagro* eggplant were found. These markers can be used as a diagnostic tool to distinguish between *Almagro* and *Andalusian* pickling eggplant landraces and detect fake *Almagro* produce elaborated with the *Andalusian* landrace.

Chemical characterization

The chemical composition, in particular of bioactive compounds, of a vegetable landrace can give added value to the produce, as consumers are increasingly valuing this type of information (Botonaki

et al. 2006). Recently, San José et al. (2013) have studied the composition of one accession (H11) of *Almagro* eggplant and found it has higher values for protein content, vitamin C, glucose and total phenolics content than regular use varieties. Also, the high values of dry matter content, are probably the consequence of selection for good quality for pickling, as fruit with high moisture may result in pickles that do not present good firmness. Regarding bioactive constituents, Prohens et al. (2007b) found that *Almagro* eggplant ranked first out of 69 eggplant varieties for total phenolics content, a value that was up to four-fold higher than other eggplant varieties. The comparison of the content of chlorogenic acid content between *Almagro* eggplant, and the wild relative *S. incanum* L., which presents high content in chlorogenic acid (Prohens et al. 2013), has also revealed that *Almagro* eggplant presents chlorogenic acid content values higher than those of *S. incanum*. This may be of high relevance for the promotion of eggplant, as the healthy properties attributed to eggplant phenolics (Plazas et al. 2013), may contribute to increase demand substantially (Botonaki et al. 2006). In this respect, the reference to the high levels of phenolics of *Almagro* eggplant has been used in promotional advertisements of pickles elaborated with this landrace.

Organoleptic characterization

Good organoleptic properties are main drivers in the decision to purchase local varieties by consumers (Botonaki et al. 2006). The organoleptic properties of different accessions of *Almagro* and *Andalusian* pickling eggplants, as well as of hybrids between them, grown under the same conditions and subjected to the same pickling processing, was evaluated by a taste panel (Prohens et al. 2007a). The results from the panel showed that accessions of the *Almagro* eggplant presented higher scores for fruit colour, firmness, texture and global appreciation than the *Andalusian* landrace and inter-landrace hybrids. This is a clear indication that in order to obtain a final pickled produce with high quality it is necessary to use the local *Almagro* landrace instead of the introduced *Andalusian* landrace or hybrid materials. In consequence, the certification that the elaborated prickles are produced with the local *Almagro* landrace provides an added value to the produce as it ensures a high standard of quality.

Enhancement through selection

Selection within a genetically heterogeneous landrace may result in an increase of favourable genetic combinations for the traits of interest. A participatory selection scheme was designed in order to select individuals of the *Almagro* eggplant landrace with high yield and low prickliness (Prohens et al. 2007a, 2009). Selected individuals could be used for bulk propagation, in order to maintain genetic diversity within the *Almagro* eggplant landrace or, alternatively, to obtain uniform *Almagro* eggplant pure lines. Fields of *Almagro* eggplant from farmers associated to the PGI were systematically screened by farmers, who looked in their own exploitation for plants that presented high yield and low prickliness. Selected plants were marked by farmers and a final selection of 27 plants from different farmers' fields was made by the team of breeders and farmers. The finally selected plants were pruned, uprooted, transplanted to pots into a greenhouse in Valencia and selfed during the winter season.

The increased seed of the selected plants was assessed for uniformity and tested in the fields of different farmers and evaluated for *Almagro* eggplant ideotype, including uniformity, yield, prickliness, and conformation with the typical characteristics of the *Almagro* eggplant. As a result of this process a pure line (H15), with higher yield and lower prickliness than the original *Almagro* landrace, was selected and registered as commercial variety (Prohens et al., 2009). This shows how individual selection within an autogamous landrace of a vegetable crop is an efficient and fast method to select pure improved pure lines with a limited effort.

Enhancement through breeding

Breeding programmes aimed at improving certain specific deficiencies of landraces while maintaining the genetic integrity of the landrace can result in the development of improved materials that maintain the characteristic traits of the landrace. In these cases, backcross breeding is an appropriate breeding method.

In the case of *Almagro* eggplant the presence of prickles in the calyx is the most detrimental characteristic. Therefore, we initiated a participatory backcross breeding programme in which we used the *Almagro* eggplant H15 selection as recurrent parent and three non-prickly eggplants as donors of the no-prickles trait. The three non-prickly donor parents were two black eggplants for regular use, of which one was a commercial F1 hybrid and the other a Spanish landrace, and a non-prickly *Andalusian* pickling eggplant accession. The latter accession, despite presenting some prickles under stress conditions was

used as donor parent, as the genetic background is very similar to that of the *Almagro* eggplant (Muñoz-Falcón et al. 2009) and therefore, less backcross generations may be needed to recover the characteristics and genetic background of the *Almagro* eggplant.

Selection has been performed using a participatory approach in which segregating generations (500-1000 plants per selection cycle) are cultivated by farmers in the Campo de Calatrava county using the typical cultivation conditions (open field during summer season) for *Almagro* eggplant (Fig. 2). This allows an optimal exploitation of the genotype x environment interaction, as the selection is made under the specific conditions in which the finally selected materials will be grown. A pre-selection of individual plants most interesting on the basis of reduced prickliness and *Almagro* ideotype characteristics is made by farmers. A final selection of plants to be used for further backcrossing is performed by the breeders together with the farmers and the selected plants are uprooted and transplanted to greenhouses for making the backcrosses during the winter season so that seed from the next generation of backcrosses is available for the next summer season (Fig. 2).

We have found that plants of the F1 between the non-prickly parents and the H15 *Almagro* selection present a degree of prickliness intermediate between both parents. In the backcross generations, segregation has been observed being compatible with a model in which one major partially dominant gene and several minor genes control the prickliness trait (Doganlar et al. 2002). Therefore, it has been possible to select plants with lower prickliness in the backcross generations. Although segregation for fruit characteristics, like size, shape, and colour are observed in the early backcross generations (BC1, BC2), rapid recovery of the characteristics typical of the *Almagro* eggplant has been observed in the three backcross families. Once the *Almagro* eggplant genetic background has been recovered to a desired level (e.g., BC3: 93.75%; BC4: 96.88%; BC5: 98.44%), individual plants presenting the *Almagro* ideotype will be selfed or subjected to anther culture (Salas et al. 2012) in order to obtain pure lines in which the introgressed gene/s conferring low prickliness are in homozygous state. Preliminary results performed in the selfed generations from selected BC1 and BC2 plants show that there is segregation for prickliness and it is possible to select segregants with low or no prickliness in the selfed generations.

Development of F1 hybrids, which usually are heterotic for yield traits (Rodríguez-Burruezo et al. 2008), was also attempted to improve the *Almagro* eggplant. However, the *Almagro* hybrids were not more productive than the pure lines, probably because the genetic differences between accessions are too low to obtain heterosis for yield. Also, complementary crosses between *Almagro* and *Andalusian*

eggplants were made. However, again, no heterosis was observed for yield and hybrids were intermediate for the morphological characteristics and prickliness (Prohens et al. 2007a; Muñoz-Falcón et al. 2009). In addition, the pickles elaborated with them did not reach the standards of quality of the *Almagro* eggplant landrace (Prohens et al. 2007a). Therefore, given that no productive advantage was evident for the F1 hybrids and that the production of F1 seed represents an additional cost, this breeding strategy was discarded.

Conclusions

Here we have shown that participatory enhancement programmes can make an effective contribution to recovering and providing added value to the *Almagro* eggplant landrace through different characterization, selection and breeding activities (Table 1). The use of a participatory approach, in which farmers have been involved in the characterization, selection, and breeding processes has facilitated the enhancement of the *Almagro* eggplant, including the registration as conservation variety and the selection of an improved line. In fact, since the programme was initiated, the cultivation of this landrace has increased steadily in the last 10 years from between 15-20 ha to 50-60 ha, while the total production has risen from 500-700 t to 1500-2000 t. Given the success with the *Almagro* eggplant landrace, we consider that the approach we have used can also be used as a model for the enhancement of other vegetable crop landraces (Table 1).

Landraces and their elaborated products may be recognized by the European Union with a protected denomination, indication, or status (Trichopolou et al. 2007), which guarantees the quality of the produce and provides protection against imitation. In this respect, the morphological, molecular, chemical, and organoleptic characterization of landraces of vegetable crops, can make a relevant contribution to achieving and maintaining this recognition as they may support the claims that the landrace and/or its elaborated products are traditional, unique and have a high quality (Polegri and Negri 2010, Spataro and Negri 2013, Torricelli et al. 2013). Also, the information on the properties and characteristics of *Almagro* eggplant, in particular its high content in phenolics, could be relevant for increasing its demand and even for opening new markets (Botonaki et al. 2006).

The acreage devoted to landraces of vegetable crops is often limited, as the market is frequently local (Hammer and Diederichsen 2009; Spataro and Negri 2013). Therefore, seed companies usually do

not establish selection and breeding programmes aimed at this type of materials. However, we have shown that a limited investment could result in a considerable enhancement of a vegetable landrace, which can result in an economically interesting alternative for farmers and that at the same time favours on-farm conservation of agricultural diversity (Piergiovanni and Laghetti 1999, Hammer et al. 2003, Polegri and Negri 2010).

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Table 1 Contribution of different characterization, selection, and breeding activities used for the enhancement of the *Almagro* eggplant landrace with have potential application to other landraces of vegetable crops

Activity	Contribution to landrace enhancement
Morphological characterization	Identification of traits that allow the fast and easy distinction from other similar landraces Information of relevance for the registration as conservation variety and for registration of improved selections as commercial varieties
Molecular characterization	Demonstration of the uniqueness of the landrace Specific and unique genetic fingerprint that ensures that the produce is elaborated with the landrace and protects from fakes
Chemical characterization	Identification of relevant nutritional and bioactive traits for which the landrace presents outstanding values
Organoleptic characterization	Demonstration of the superior value of the landrace over fakes and other similar materials
Selection	Development of materials by means of mass or individual selection, with improved performance while maintaining the characteristics of the landrace.
Breeding	Backcross breeding for the introgression of specific traits that improve the agronomic performance of the landrace while maintaining the characteristics of the landrace

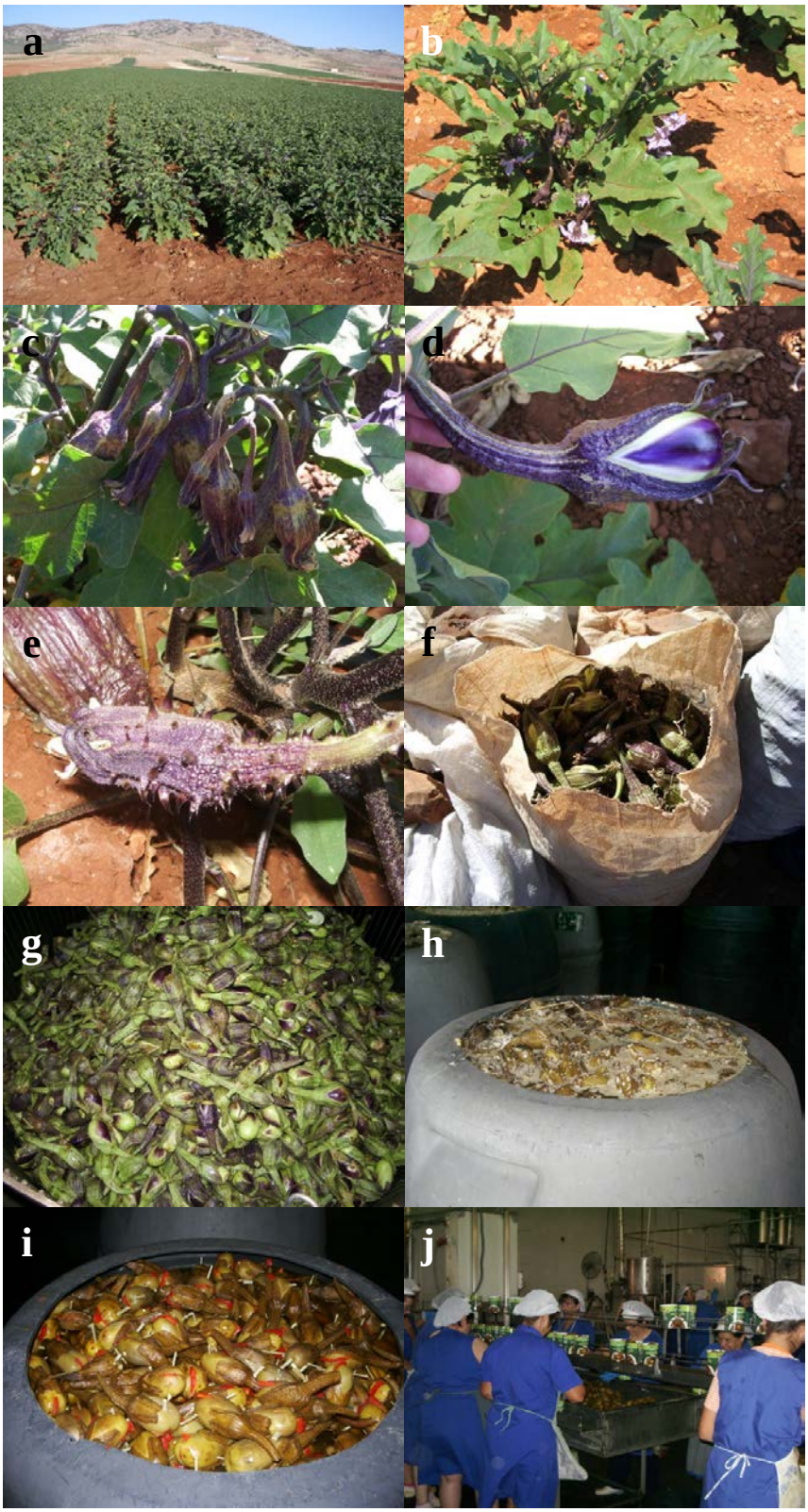

Fig. 1 The *Almagro* eggplant landrace: commercial plantation in the Campo de Calatrava county (a), young plant (b), multiple infrutescences (c), typical fruit ready for harvest (d), highly prickly calyx (e), bag of harvested fruit ready to be transported (f), fruits ready for blanching (g), lactic fermentation (h), stuffing with pepper (in the case of stuffed eggplants) (i), and canning (j).

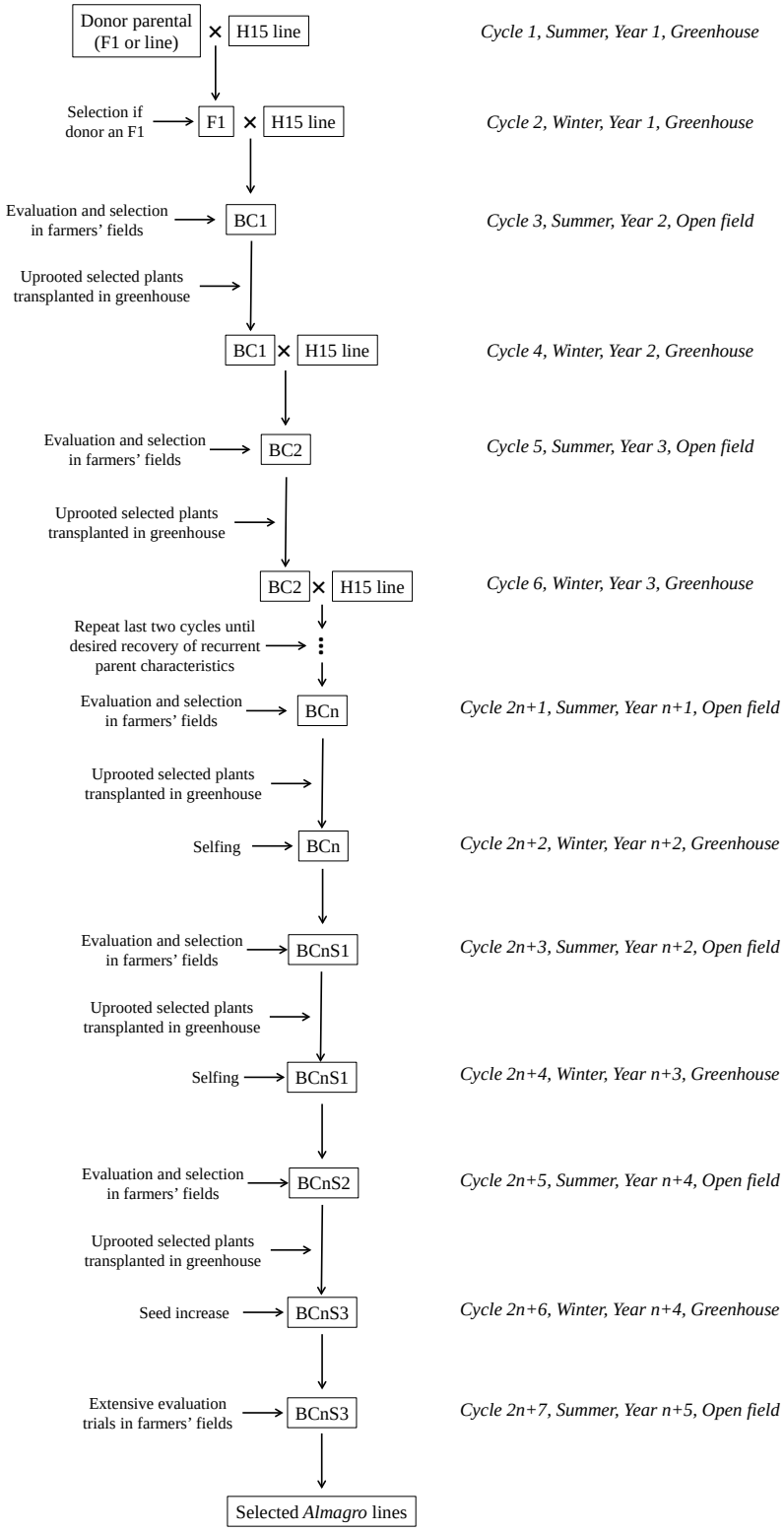

Fig. 2 Scheme of the participatory breeding programme for introgressing the no-prickles trait from three eggplant materials (a commercial F1 hybrid and a Spanish landrace for regular use, and a non-prickly *Andalusian* pickling eggplant accession). A single selection cycle is performed every year by farmers in their own fields in the Campo de Calatrava county during the summer season, and crosses are made in greenhouse in Valencia during the winter season