Scientia Horticulturae 133 (2012) 23–30

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

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

Anomalous embryo sac development and fruit abortion caused by inbreeding depression in almond (*Prunus dulcis*)

Pedro J. Martínez-García, Federico Dicenta, Encarnación Ortega*

Plant Breeding Department, CEBAS-CSIC, P.O. Box 164, E-30100, Campus Universitario de Espinardo, Murcia, Spain

ARTICLE INFO

Article history:
Received 29 July 2011
Received in revised form
29 September 2011
Accepted 1 October 2011

Keywords:
Prunus dulcis
Flower morphology
Self-fertilization
Embryo sac development
Fruit set
Inbreeding depression

ABSTRACT

The decrease in fitness of the inbred offspring in relation to the outbred offspring is termed inbreeding depression, and is attributed to the expression of deleterious recessive alleles. This may represent an important problem for future almond growing, since the use of a very limited germplasm base as source of self-compatibility in breeding programmes has presumably led to a considerable increase of the inbreeding degree in subsequent progenies, from which new cultivars will be released. In the present study, the effects of inbreeding depression on flower morphology, pollen viability, pollen tube growth and fruit set were evaluated after self- and cross-pollination of two self-compatible almond cultivars and 12 descendants obtained from successive self-fertilizations. Moreover, in those descendants with very low or null fruit set values and in the cultivar 'Tuono' (first generation), the embryo sac development was studied. The self-compatible cultivar 'Antoñeta' was included as external control in the experimental assays. In general, there were no differences between both types of pollination, and only those individuals with higher inbreeding levels were affected by inbreeding depression, which was manifested by lower pollen germination, decelerated growth of pollen tubes through the style, delayed ovule development at the prezygotic phase, and high fruit drop. This high fruit drop seemed to be due to the presence of many aborted fruits with an anomalous postzygotic development of the embryo sac, finally resulting in necrosis at the chalazal end and lack of endosperm cellularization. Therefore, data presented here indicate that the low or null fruit set observed after fertilization in highly inbred almond genotypes seems to be a consequence of inbreeding depression, and highlight the importance of avoiding successive self-fertilizations as strategy of breeding in this species.

1. Introduction

Gametophytic self-incompatibility is a genetic mechanism developed by most flowering plants to avoid self-fertilization or fertilization with genetically related pollen (de Nettancourt, 2001). The majority of *Prunus* fruit tree species (Rosaceae), including the cultivated almond [*Prunus dulcis* (Miller) D.A. Webb], exhibit this reproductive barrier (Kao and Tsukamoto, 2004). Although self-incompatibility appears to be the ancestral reproductive state in almond, some cultivars with a self-compatible phenotype have been identified, mainly in Apulia (Italy) (Godini, 2002).

Since most almond cultivars are self-incompatible, to obtain a commercial yield at least two cross-compatible cultivars with overlapping blooming times should be grown in the same orchard, what implies a number of economic disadvantages (Ortega and Dicenta, 2003). To overcome the inconveniences of self-incompatibility in almond production, for the last years, breeding programmes of this species have considered self-compatibility as one of their main objectives (Ortega and Dicenta, 2003). However, the use of a very limited germplasm base as source of self-compatibility has originated in most cases progenies with a highly similar gene pool. Therefore, the subsequent crossing of these individuals to obtain improved self-compatible selections is expected to greatly increase the degree of inbreeding in the new descendants.

The decrease in fitness of the inbred offspring, or inbreeding depression, it is known to have ecological and evolutionary consequences, and it has been attributed to the accumulation of lethal deleterious mutations that are recessive or partially recessive (partial dominance) or to the loss of the heterozygote advantage (overdominance) (Charlesworth and Charlesworth, 1987; Lande and Schemske, 1985). Moreover, it has been estimated that species with large outcrossing populations are affected to a greater extent by inbreeding depression and that they develop different barriers to prevent inbreeding, which could affect seed formation or growth and development of the seedlings, finally resulting in the elimination of most of the inbred descendants (Lande and Schemske, 1985). Similar barriers could be acting in almond, an obligate outcrossing

* Corresponding author. Tel.: +34 968 396368; fax: +34 968 396213.
E-mail address: eortega@cebas.csic.es (E. Ortega).

0304-4238/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2011.10.001

species in its origin, at different stages of the life cycle, including fertilization and seed development.

In almond, as in other *Prunus* species, development of the embryo sac (megagametogenesis) is of the Polygonum-type (Lersten, 2004). Initially two ovules are distinguished in each almond ovary, although usually one of them (named the secondary ovule) aborts, while the other (the primary ovule) may be fertilized. Before double fertilization occurs a mature embryo sac is generally observed. At this moment the synergids and the egg cell are distinguished at the micropylar pole, and the central cell (fused polar nuclei) and the antipodal cells appear at the chalazal position (Pimienta and Polito, 1983). After double fertilization a zygote is formed and the embryo sac elongates, extending into the nucellus and reaching the chalaza as a long narrow tube named the endosperm haustorium, which allows the transfer of nutrients to the embryo (Sarfatti, 1960).

A correct development of both embryo sac and zygote is essential to obtain a commercial yield. Thus, histological studies carried out in different *Prunus* fruit tree species, reported that aborted fruits presented different signs of degeneration such as embryo sac degeneration, disorders at the chalazal region or necrosis of the embryo, which were attributed to lack of pollination, lack of fertilization, or improper nutrition of the tree (Bradbury, 1929; Dorsey, 1919; Fukuda et al., 2006; Harrold, 1935). The first stages of haustorium development and divisions of the endosperm nuclei also seem to be important for fruit set, since alterations at these stages could result in fruit drop (Stösser and Schauz, 2000).

The effects of inbreeding depression in almond reproduction have been studied by several authors (Alonso and Socias i Company, 2005; Dicenta et al., 2002b; Grasselly and Olivier, 1988; Ortega and Dicenta, 2006; Ortega et al., 2010; Oukabli et al., 2000). However, in these studies the number of individuals, inbreeding levels, reproductive traits and/or ovule developmental stages considered were scarce.

The aim of this work was to ascertain the causes of the very low productivity regularly observed in highly inbred self-compatible almond selections. For this, a comprehensive study of floral morphology, pollen viability, pollen tube growth through the pistil and fruit set was performed on four successive generations and a control cultivar. In those individuals with an extremely high fruit drop, particular attention was given to the histological analysis of embryo sac development from anthesis up to 40 days following pollination, thus considering both prezygotic and postzygotic stages.

2. Materials and methods

2.1. Plant material

Four generations of self-compatible almonds with an expected increasing degree of inbreeding were studied. The first generation (G1) consisted of the traditional cultivars 'Tuono' and 'Genco'. The second generation (G2) was composed of the pre-selections 'C1-010' and 'C1-050', obtained from crosses between 'Ferragnès' and the above mentioned cultivars. The pre-selections 'A1-536', 'A1-730', 'A2-198', 'A2-199' and 'A2-206' represented the third generation (G3), obtained after self-fertilization of individuals from G2. Finally, the pre-selections of the fourth generation (G4): 'D01-573', 'D01-574', 'D01-580', 'D01-582', 'D01-583', were obtained by self-fertilization of individuals from G3. In addition, the self-compatible cultivar 'Antoñeta' (Egea et al., 2000) was included as a control, and the self-incompatible cultivar 'Marcona' was used as pollinator. The trees of the indicated cultivars and pre-selections were grown under drip irrigation in the CEBAS-CSIC experimental field located in Santomera (Murcia, South East of Spain). In all cases the trees were at least seven years old and, since they had been subjected to preventive pesticide treatments, had a healthy phytosanitary status.

It should be pointed out here that in the original populations from which G3 and G4 individuals were selected, following open pollination nearly all the individuals had low productivity values (ranging between 0 and 2 in a 0–5 scale) for three consecutive years. Thus, the individuals included in this study were chosen to represent the detected productivity problem.

2.2. Floral morphology

To evaluate the presence or absence of floral abnormalities that could affect pollination, a sample of 10 flowers at 'F' stage, described as opened flower in which the petals spread out completely and the anthers dehisce releasing the pollen grains (Felipe, 1977), was collected from each genotype and taken to the laboratory. Once there, the flowers were dissected and then the number of pistils per flower was counted, the shape of each pistil was recorded as curved or straight, and pistil length was precisely measured using an electronic digital caliper (Starrett, 727 Series, Athol, New England, USA). The number of stamens and the position of the anthers with respect to the stigma (below, at the same level or above), were also determined.

2.3. Determination of pollen viability

For each genotype, pollen viability was *in vitro* tested after dusting pollen onto Petri dishes containing 15% sucrose and 1.2% agar medium (Remy, 1953). For this, anthers from 100 flower buds at 'D' stage, i.e. buds increased in volume and although still closed with a visible corolla (Felipe, 1977), were excised and desiccated under controlled conditions (22 °C and 35% RH) for 24 h. The pollen was then collected and applied using a paintbrush onto the agar–sucrose plates, which were incubated for 6 h at 22 °C, and then viewed at 40× magnification under an Olympus BH2 optical microscope. Pollen viability was expressed as the percentage of germinated pollen grains in the pollen sample. A pollen grain was considered as germinated when the length of the emerged pollen tube exceeded the diameter of the grain (Ducon, 1968).

2.4. Pollen tube growth through the pistil

Forty flower buds at 'D' stage were collected from each genotype and taken to the laboratory. Then, the flower buds were stripped of their corolla, stamens and part of the calyx, and placed on wet floral foam in plastic trays that were kept under controlled conditions in a growing chamber at 22 ± 2 °C and 80% relative humidity. The following day, coinciding with anthesis, 20 pistils from each genotype were self-pollinated and the other 20 were cross-pollinated with pollen of 'Marcona' (with 82% of germination rate). All pollinations were performed by hand using a paintbrush. Seventy-two hours after pollination, the pistils were collected, and then fixed and stained according to the procedures described in Ortega et al. (2002). The position of pollen tubes along the pistil and the percentage of pistils with pollen tubes in the ovary were determined using an Olympus BH2 microscope under epifluorescence from the UV light-adapted system BH2-RFL-T2.

2.5. Fruit set

For each genotype, 100 flowers at 'D' stage were emasculated and then 50 pistils were hand-self-pollinated and the other 50 were hand-cross-pollinated with pollen from 'Marcona'. The percentage of fruit set for each genotype and treatment was determined