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Immunolocalization and quantification of IAA after self- and free-pollination in *Olea europaea* L.

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Abstract

In order to characterise the self-compatibility in 'Pisa 9', that is an improved clone of cultivar Leccino (*Olea europaea* L.), biological, histological and biochemical analyses were carried out. Observations were also focused on 'Pendolino', the main pollinator for Italian cultivars considered as being self-incompatible. After self-pollination, in 'Pisa 9' the majority of the pollen tubes reached the distal part of the style, entered the ovule leading to a high fruit-set percentage. These same processes were also noted in the Pendolino cultivar, though in a lesser degree. The indole acetic acid (IAA) concentration and its immunocytochemical localization were determined in pollinated flowers after self- and free-pollination and in unpollinated flowers. In both cultivars the highest IAA levels and signal intensity were observed in free-pollinated pistils, whereas after self-pollination a different behaviour emerged. In 'Pendolino' the immunostaining was not uniformly distributed and stopped at the style level. This finding sustained the low percentage of fruit-set obtained in the cultivar. In 'Pisa 9' the auxin distribution was similar to the free-pollinated pistils where the immunolocalization staining reached the ovary. The correspondence between the IAA signal in the ovary and the high percentage of fruit-set in 'Pisa 9' self-pollinated pistils, confirms the role of this hormone in the induction and regulation of the fertilisation process.

Keywords: Incompatibility; Indole acetic acid; Immunocytochemical localization; *Olea europaea*; Pistils

1. Introduction

Many flowering plants (up to 70%) have a genetic system to ensure self-incompatibility (SI) and such a system allows the female pistil to recognize and then reject self or self-related pollen. SI is usually controlled by the S locus, one of the most polymorphic loci known, which has many alleles, denoted S_1, S_2 and so on (Hiscock, 2002). Classical genetic studies identified two distinct forms of SI, gametophytic and sporophytic, which can be distinguished by the site of pollen–pistil interaction. While in the sporophytic system the location of the incompatibility may occur on the stigmatic surface, in the gametophytic system the reaction may be localized on the stigma or even in the upper style (Van Marrewijk, 1989). This latter condition is typical of several fruit species, such as olive trees *Olea europaea* L., a preferential allogamous specie (Moutier, 2002; Tombesi, 2003). The incompatibility mechanism in olive tree is relatively complex with the possibility of the existence of two or more S gene loci as a consequence of polyploidy and gene segregation over the century (Bradley and Griggs, 1963). In this specie, self-incompatibility manifests as an incomplete impediment in pollen germ tube growth that cause a delay and lower level of fertilization (Bradley and Griggs, 1963; Cuevas, 1992). The world olive heritage is characterised by cultivars that, in most cases, are self-incompatible and, as consequence, require cross-pollination to obtain a good yield. Only few local cultivars are usually considered self-compatible, even if they benefit greatly from cross-pollination (Lavee and Dart, 1978; Androulakis and Loupassaki, 1990).

The cultivar Leccino, one of the most widespread oil olive cultivar in central Italy, is commonly classified as self-incompatible, as are most of Italian olive cultivars (Bellini et al., 2003). However, it has been found that in this cultivar, and in some of its clones, self-incompatibility mechanisms did not occur, proving it to be self-compatible, even if this ability may

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vary according to the year (Bartolini and Guerriero, 1995; Bartolini et al., 2002). This phenomenon, attributed to pseudo-compatibility, is well known in some fruit species, particularly in several apple and pear cultivars (Lombard et al., 1972; Williams and Maier, 1977; Dettori and Palombi, 1995), and was described for the first time by Bradley et al. (1961) in olive. This process is influenced by environmental factors such as temperature and nutritional status, which can inactivate the normally active locus S blocking the Rnase synthesis or the membrane receptors, and preventing the transcription products of gene S (Lewin, 1990).

It has been shown that endogenous factors, such as phytohormones, play an important role in ovary and fruit development before, during, and after pollination. In some cases, applications of exogenous hormones can replace fertilization and allow fruit development (Goodwin, 1978). Recent studies reported the involvement of IAA, abscissic acid (ABA), cytokinins and ethylene in compatible pollination (Kovaleva et al., 2002). Among these hormones, IAA appeared to be a key substance for fruit set by regulating the postpollination phenomena (Strauss and Arditti, 1982). An increase in IAA content was detected in concurrence with pollen tube growth in the stylar tissues, as well as in the placenta and ovary of netted muskmelon (Hayata et al., 2002). In order to localise plant hormones, when these are effectively fixed in situ, the immunocytochemical technique is one of the most useful tools (Ohmiya and Hayashi, 1992).

The purpose of this study was to investigate the biological and biochemical aspects of the floral biology in two olive cultivars (*O. europaea* L.) characterized by a different compatibility degree. In particular, the IAA phytohormone level was explored during the period just before and just after self- and free-pollination.

2. Materials and methods

2.1. Plant material

Our research, conducted in 2002, was carried out on 8-year-old own-rooted trees of two Tuscan oil olive cultivars: 'Pisa 9', a pseudo-compatible clone of 'Leccino' (Bartolini et al., 2003), and 'Pendolino', the main pollinator considered as being self-incompatible (Bellini et al., 2003). The trees, trained to bush and planted according to a randomised block plane, were growing on an experimental field under the climatic conditions of the Tuscan coastal area (IVALSA, National Research Council, Follonica, Grosseto, Italy; latitude 42°55′38″, longitude 10°45′45″). Uniformly distributed branches were tagged throughout the canopy (four per trees) and used for the following pollination plane:

(a) passive self-pollination: prior to anthesis at the 'white button' phenological stage (second decade of May), flowers (16,000–18,000 per cultivar) were bagged, using paper pollination bags, to prevent contamination by foreign pollens. Since flowers started to open the enclosed branches were hand-shaken to insure pollination. The bags were removed 10 days after flowering (first decade of June);
(b) free-pollination: the same number of flowers were left to free-pollination, in order to act as controls.

In this experimental trial, bagged-cross pollination treatment, which is usually performed for compatibility studies in olive (Bartolini et al., 2002), was deliberately eliminated for the need to emasculate a very high number of flowers (>15,000/thesis) prior to making controlled pollination.

2.2. Evaluation of self-compatibility pollen tube growth assessment

Samples of 100 pistils from self- and free-pollinated flowers were collected 10 days after anthesis in order to monitor the *in vivo* pollen tube growth. Pistils were temporarily fixed in Carnoy: ethanol and glacial acetic acid (3:1, v/v). Subsequently, pistils were softened, stained with aniline blue, squashed and then examined using a standard microscope (Nikon-Fluophot) under fluorescent light, according to Martin's (1959) protocol, appropriately modified (Viti et al., 1990). The pollen tube growth progress was assessed by counting the number of tubes along the length of the pistil, from the stigma down to the ovary, according to the schematic representation shown in Fig. 1.

2.3. Evaluation of fruit-set under field conditions

The number of clusters and the mean number of flowers per cluster were recorded on the tagged branches to determine the fruit-set percentage (60 days after full bloom) and, at harvest time, the fruit quantity yielded by self- and free-pollination.

During the period from the inflorescence emission to fruit-set, minimum and maximum daily temperatures, relative humidity and rainfall levels were recorded.

2.4. IAA immunocytochemical localisation

Samples of 20 self- and free-pollinated pistils, collected 10 days after anthesis corresponding to the G phenological stage

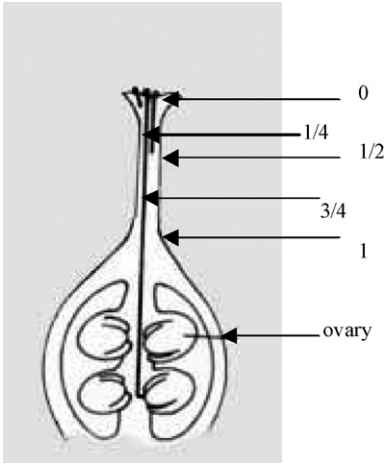

Fig. 1. Schematic representation of pollen tubes growth in the pistil from the stigmatic surface (0), to the bottom of the style (1), down to the ovary (modified from D'Arcy et al., 2001).