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Long-term graft compatibility study of peach-almond hybrid and plum based rootstocks budded with European and Japanese plums

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ABSTRACT

The introduction of some new rootstocks requires knowledge of the potential 'translocated' and 'localized' graft incompatibilities before releasing these rootstocks into the market. Trials were established at the Aula Dei Experimental Station (Zaragoza, Spain) to assess graft compatibility between five European plum (*Prunus domestica* L.) and six Japanese plum (*Prunus salicina* L.) cultivars with thirty-eight rootstocks belonging to peach × almond hybrids, as well as slow-growing, fast-growing and interspecific hybrid plum groups. Some of these rootstocks are under process of selection and others are commercial rootstocks already introduced into the European market. External visual diagnosis of the scion-rootstock graft combinations was performed by observing symptoms of the 'translocated' incompatibility in the nursery. Visual symptoms of 'translocated' incompatibility were only found on the Japanese plum cv. 'Golden Japan' budded on the plum-apricot hybrid AP-45. The 'localized' graft incompatibility was assessed after internal macroscopically examination of graft unions. In the case of the European plum cvs., 'President' and 'Reine Claude Tardive of Chambourcy' exhibited good graft compatibility with all the tested rootstocks, with the exception of 'President' budded on the pentaploid plum hybrid rootstock Damas GF 1869. 'Reine Claude Verte' cv. showed 'localized' incompatibility with Myrobalan B and Myrobalan GF 3-1 since the second year after budding. 'Stanley' cv. showed 'localized' incompatibility with the six evaluated peach × almond hybrid rootstocks, although it was compatible with all plum based rootstocks. In the case of Japanese plums, four cultivars ('Angeleno', 'Black Amber',' Delbarazur' and 'Songold') did not show any graft incompatibility, but 'Friar' showed 'localized' incompatibility on Myrobalan B. In the case of peach-almond and *P. cerasifera* based rootstocks (Adarcias, GF 677, Damas GF 1869, Myrobalan B, Myrobalan 29 C, Myrobalan GF 3-1 and Marianna GF 8-1), 'Golden Japan' trees showed similar symptoms to those caused by TomRV in Myrobalan and peach rootstocks and 'localized' incompatibility. Finally, this study confirmed the interest of *P. insititia* specie as well as several interspecific *Prunus* hybrids as a good source of rootstocks for the plum industry.

1. Introduction

The orchard economic, longevity and environmental viability is linked to a proper cultivar and rootstock selection for a specific climate and soil conditions, together with its graft compatibility knowledge. The hexaploid European plums (*Prunus domestica* L.) and the diploid Japanese plums (*Prunus salicina* Lindl.) are among the most widely cultivated fruit species in temperate climates (Ferlito et al., 2015; Reig et al., 2018a). The commonly used rootstocks for European plum cultivars include different *Prunus* species such as *Prunus cerasifera* (Myrobalan or cherry plums, 2n = 16), *P. cerasifera* x *P. munsoniana* hybrids (Marianna), *P. spinosa* (Sloe or blackthorn plums, 2n = 32), *P. insititia* (Damson plums, 2n = 48), and *P. domestica* (common plums, 2n = 48). For Japanese plum cultivars, Myrobalan and Marianna are also commonly used, especially on orchards with poorly-drained soils and waterlogging conditions (Topp et al., 2012). To a lesser extent, interspecific hybrids of almond (*P. amygdalus*) and peach (*P. persica*) could be also used for European and Japanese plums to avoid iron chlorosis in calcareous soils and to have an adequate level of vigor on low fertility sites. Peach × almond hybrids have been successfully developed as rootstocks for almond and peach cultivars in Mediterranean countries (Mestre et al., 2015; Ben Yahmed et al., 2016). Nevertheless, they can

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exhibit graft incompatibility with some plum cultivars, especially with the European hexaploid plums (Moreno et al., 1995a). Most breeding programs, on diverse climate and soil conditions, aim to obtain commercial rootstocks with a wide range of cultivars compatibility (Moreno, 2004). However, the limiting factor for the widespread use of some *Prunus* spp. for plum production may be the occurrence of graft-incompatibility in the orchard.

Graft compatibility can be defined as the establishment of a successful graft union as well as extended survival and proper functioning of the composite grafted plant (Goldschmidt, 2014). Successful formation of graft depends on various complexes of biochemical and structural procedures, which includes callus formation, establishment of new vascular tissue, and formation of an active vascular system across the graft interface (Martínez-Ballesta et al., 2010; Pina et al., 2012, 2017). The grafted partners often belong to the same species or genus, but the use of genetically divergent genotypes is also common (Usenik et al., 2006). Therefore, graft incompatibility occurs frequently, especially in the inter-species combinations, mainly because of the differences in anatomy, morphology and physiology between the graft components (Darikova et al., 2011). This is often the case when pear is grafted on quince, and apricot and peach on other *Prunus* species (Ciobotari et al., 2010; Zarrouk et al., 2006, 2010). Likewise, late graft rejection in fruit trees has been limited by plasmodesmal coupling at the time of grafting within one of the partners (Pina et al., 2009).

To understand the mechanism responsible for graft incompatibility, Mosse (1962) described two types of graft incompatibility, ‘translocated’ and ‘localized’. The first one is characterized by visual symptoms in the tree (yellowing of leaves, which later became redder or more orange, premature defoliation, leaf wilting) and an earlier stop of tree growing and a radicular system not fully developed (Moreno et al., 1993; Hartmann et al., 2002; Zarrouk et al., 2006; Dogra et al., 2018). This type of incompatibility cannot be overcome by the use of an interstock (Hartmann et al., 2002). In contrast, ‘localized’ type is characterized by anatomical irregularities at the graft union interface accompanied by anatomical abnormalities of vascular tissue in the callus bridge (Hartmann et al., 2002) and by breaks in cambial and vascular continuity and poor vascular connections (Zarrouk et al., 2010). It can be overcome by the use of mutually compatible interstocks (Hartmann et al., 2002). These two types of incompatibility can present themselves jointly in a same graft combination, and neither of them is privative of determined species.

Breeding *Prunus* rootstocks studies are in progress at the Aula Dei Experimental Station for obtaining new *Prunus* rootstocks, with specific adaptation to Mediterranean environments (Font i Forcada et al., 2012; Mestre et al., 2015, 2017) and having a wide graft compatibility with different stone fruit species, including plum cultivars (Moreno, 2004; Reig et al., 2018b). Several graft compatibility studies were performed in plums budded on different plum based rootstocks (Mosse, 1960; Moreno et al., 1995b, 1995c). However, very few studies included peach × almond rootstocks (Moreno et al., 1995a; Duval, 2004) and new released rootstocks. Therefore, main objectives of the present study were: 1) to assess and determine the graft compatibility performance of European and Japanese plum cvs. budded on peach × almond hybrids, other interspecific hybrids and also on plum rootstocks recently released and/or in process of selection, and 2) to establish comparisons in terms of graft compatibility with commercial rootstocks of different origins, introduced into the European market.

2. Material and methods

2.1. Plant material

Five European plum cultivars (‘President’, ‘Reine Claude of Bavay’, ‘Reine Claude Tardive of Chamboucry’, ‘Reine Claude Verte’ and ‘Stanley’) and six Japanese plum cvs. (‘Angeleno’, ‘Black Amber’, ‘Delbarazur’, ‘Friar’, ‘Golden Japan’ and ‘Songold’) were T-budded *in situ* in summer and evaluated during 5 consecutive years on different *Prunus* rootstocks (Table 1). Approximately, at 15 cm from the ground, a T cut (approximately 1 cm width and 1.5 cm long) was made across the bark at each rootstock (one-year old tree, diameter ≈ 1 cm). For practical purposes, rootstock genotypes were divided into three different groups: (1) peach × almond hybrids (*P. amygdalus* × *P. persica*); (2) Slow-growing plums (*P. insititia* and *P. domestica*); and (3) Myrobalan-Marianna plums (*P. cerasifera* and interspecific hybrids having *P. cerasifera* as a parent). Among the thirty-eight rootstocks used in this study, the hexaploid plums ‘Pollizo de Murcia’ rootstocks and the interspecific hybrids Miral 3278 AD, PADAC 04-01 and PADAC 99-05 are under process of selection by the Aula Dei Experimental Station.

Rootstocks were propagated by hardwood cuttings treated with indole-3-butyric acid (indolebutyric acid) at 4 g L^{-1} for peach × almond hybrids, other peach and almond hybrids and slow-growing plums rootstocks (Moreno et al., 1995b), and at 0.5 g L^{-1} for Myrobalan-Marianna plum rootstocks (Moreno et al., 1995c). Stock-plants used for cutting production are routinely tested to maintain mother trees free of *Prunus* necrotic ringspot virus (PNRSV), Prune dwarf virus (PDV), Tomato ringspot virus (TomRV), Apple chlorotic leaf spot virus (ACLSV) and Plum pox virus, Sharka (PPV).

Trials were established in different nurseries at the Aula Dei Experimental Station (NE Spain; lat. 41° 43′ 42.7″N, long. 0° 48′ 44.1″ W), on heavy and calcareous soils with 29–30% total calcium carbonate, 7.4–7.6% active lime, water pH 8.0-8.5, and a clay-loam texture. The nurseries were level-basin irrigated every 12 days during the summer.

At each nursery, each scion/rootstock graft combination was replicated, in general, 10–30 times (trees) (10 trees per block) depending on the availability of plant material (previously rooted rootstocks). Consequently, not every scion/rootstock graft combination was available at each nursery. Ten replicates per combination were considered the minimum acceptable for assessment, although some combinations suffered field losses at each block after several years of field-testing, but mainly due to the initial grafting process failure.

2.2. ‘Translocated’ incompatibility evaluation

Firstly, the level of compatibility-incompatibility was mainly determined during the first two years after budding by visual diagnosis of the symptoms of the ‘translocated’ type of incompatibility in the nursery, *e.g.*, leaf and wood yellowing and reddening, defoliation, tree vigour reduction, and death (Moreno et al., 1993). Moreover, a determination of leaf chlorophyll concentration using SPAD 502 m (Minolta Co., Osaka, Japan) was made on 2-year-old trees from the end of June to the beginning of July. This procedure was used as a potential tool to estimate the rate of ‘translocated’ graft incompatibility (Fig. 1). Measurements were made on fully expanded leaves of 10 trees per combination selected from the middle of the tree.

2.3. ‘Localized’ incompatibility evaluation

Anatomic examination of each scion/rootstock union was carried out on 2–5 year-old budded trees. Each graft union was sawed by a radial-longitudinal plane, and each visual rating of ‘localized’ graft incompatibility (Fig. 2) was classified according to Mosse and Herrero (1951): 1) category A: Perfect unions (the line of the union between bark and wood is hardly visible); 2) category B: Good unions (the bark and wood are continuous although the line of union in the wood is often clearly distinguished by excessive ray formation); 3) category C: Unions with discontinuities in the bark (the bark tissues of rootstock and scion are separated by a dark brown layer of corky appearance); 4) category D: Unions showing vascular and wood discontinuities (the woody tissues of rootstock and scion are separated in many places by clusters of living, non-lignified parenchyma, whereas bark tissues are generally as category C); and 5) category E: Observed breakage of the tree at the