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RESEARCH ARTICLE

Influence of plum rootstocks on agronomic performance, leaf mineral nutrition and fruit quality of 'Catherina' peach cultivar in heavy-calcareous soil conditions

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Abstract

The agronomic performance and leaf mineral nutrition of the non-melting clingstone peach cv. 'Catherina' was evaluated on seven hexaploid plum rootstocks, as well as one *Prunus persica* seedling. They were assessed over a period of 15 years in a field trial at the Experimental Station of Aula Dei-CSIC (Zaragoza, Spain), located in the Ebro Valley (NE Spain). Growing conditions generated varying levels of tree mortality, the highest with Constantí 1, Monpol and Montizo, whereas all Adesoto, GF 655/2 and PM 105 AD trees survived well. GF 655/2 and P. Soto 67 AD proved to be the most dwarfing rootstocks, while Constantí 1 and Monpol were the most invigorating and generated greater cumulative yields. However, the highest yield efficiency was recorded on GF 655/2 and Montizo, although they did not differ significantly from Adesoto and P. Soto 67 AD. The highest average values for fruit weight were observed on PM 105 AD and the lowest on GF 655/2, but they did not differ significantly from the rest of the rootstocks. The highest average values for the soluble solids content were observed on the Pollizo rootstocks Adesoto and PM 105 AD, followed by P. Soto 67 AD. All rootstocks induced nitrogen deficiency, with the exception of Constantí 1, GF 655/2 and Montizo, and iron deficiency, except PM 105 AD. The invigorating rootstock Constantí 1 seemed to induce higher SPAD values. According to the ΣDOP index, Montizo presented the most suitable balanced nutritional index, but it did not differ significantly from the rest of the rootstocks except GF 655/2 and P. Soto 67 AD.

Additional key words: iron chlorosis; vigour; yield; SSC; SPAD.

Abbreviations used: a* (greenness/redness); b* (blueness/yellowness); C* (chroma); CY (cumulative yield); DAFB (days after full bloom); DOP (deviation from optimum percentage index); FF (flesh firmness); FW (fruit weight); H (lightness's angle); L* (brightness or lightness); RI (ripening index); SSC (soluble solids content); TA (titratable acidity); TCSA (trunk cross-sectional area); YE (yield efficiency).

Authors' contributions: MAM has designed and conducted the study since 1997. LM collected samples and performed the leaf mineral, SPAD and fruit quality analysis. JAB supervised the leaf mineral analysis. MAM supervised the research and guided data interpretation. LM, GR and MAM carried out statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

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Introduction

Peach [*Prunus persica* (L.) Batsch] is the most important temperate and deciduous fruit tree grown in Spain. It is mainly produced in the Ebro Valley (regions of Aragon and Catalonia) and other regions of Mediterranean climate (Spanish Ministry of Agriculture, http://www.magrama.es), where harvest season ranges from mid-April to November. Spain is the third-largest peach producer in the world, only surpassed by China and Italy, and the second-largest producer in the EU, after Italy in 2014 (FAOSTAT, http://www.fao.org/faostat/en/#data/QC).

Rootstocks are an essential component in modern fruit production because of their capability of adapting

scion cultivars to diverse environmental conditions and cultural practices. Among others, the main factors which determine the ideal rootstock are its compatibility with the scion cultivar (Zarrouk *et al.*, 2006), resistance and/or tolerance to soil pests and diseases, such as root-knot nematodes (Pinochet *et al.*, 1999), and adaptability to a wide range of soil types and climatic conditions (Reighard *et al*., 1997; Beckman & Lang, 2003; Giorgi *et al.*, 2005). Leaf mineral analysis is a useful tool for the assessment of the nutritional status of crops (Johnson & Uriu, 1989; Montañés *et al.*, 1993; Guo-yi *et al.*, 2015), and the use of tolerant rootstocks would prevent nutritional disorders that cause high economic losses for the fruit growers (Jiménez *et al.*, 2007; 2008). In addition, rootstocks should improve other scion characteristics as cold tolerance or low chilling requirements, harvest date, internal and external fruit quality, yield and post-harvest fruit quality (Castle, 1995; Remorini *et al.*, 2008; Tavarini *et al.*, 2011; Milošević *et al.*, 2015).

In the last decade, the fruit industry has changed in many ways. It increased the interest in fruit quality traits, including nutritional value as an important factor relevant to human health (Wolfe *et al.*, 2008; Byrne *et al.*, 2012) and in utilizing dwarfing plum rootstocks, since they may decrease management costs, such as harvesting and pruning, and improve production efficiency (Moreno *et al.,* 1994; Jiménez et al., 2011). However, soil properties and insufficient graft compatibility may limit the normal development of the cultivar, and consequently the agronomic and fruit quality characteristics (Moreno *et al.,* 2001; Milošević *et al.,* 2015). For this reason, the Experimental Station of Aula Dei (Zaragoza, Spain) started a breeding program of *Prunus* rootstocks aimed at obtaining new rootstocks best adapted to Mediterranean conditions. It included local Spanish indigenous plums commonly known as Pollizo (*P. insititia*) and other plum species (*P. domestica*, *P. cerasifera*) as multi-purpose rootstocks for different *Prunus* species (Moreno *et al.*, 1995; Moreno, 2004), but especially for peach trees grown in heavy and calcareous soil conditions. It is commonly assumed that Pollizo plums also reduce tree vigour and induce higher fruit quality than the most frequently used peach × almond hybrids.

The rootstock influence on tree growth, survival, yield, mineral uptake and fruit quality has been evaluated in some peach cultivars under Mediterranean conditions (Felipe *et al.,* 1997; Zarrouk *et al.,* 2005; Pinochet, 2010; Font i Forcada *et al.*, 2012, 2014a; Mestre *et al.,* 2015; Reig *et al.,* 2016). However, to the best of our knowledge, no study has been reported with the aim of determining the agronomic performance, leaf mineral nutrition and fruit quality properties all together of 'Catherina' budded on plum rootstocks under heavy-calcareous soil conditions. 'Catherina' is a clingstone peach cultivar of great interest in the Ebro Valley area for both fresh and processed markets, because of its maturity time and good fruit quality (Font i Forcada *et al.*, 2014a, 2014b). Plum rootstocks are more tolerant to compact soils and waterlogging than other species of *Prunus* L. They also provide greater tolerance to iron-chlorosis deficiency (Jiménez *et al.*, 2008) and to soil-borne pathogens, such as fungi and root-knot nematodes (Pinochet *et al.,* 1999), so common in many peach-growing regions of the Mediterranean area. Thus, the present study aims to evaluate the effect of seven plum rootstocks of different genetic backgrounds and origins and one peach rootstock, on agronomic, leaf mineral nutrition and fruit quality traits of 'Catherina' peaches grown over 15 years on a heavy and calcareous soil typical of the Mediterranean area.

Material and methods

Plant material and trial characteristics

Seven hexaploid plum rootstocks, including five Pollizo plums (*P. insititia*): Adesoto, Monpol, Montizo, P. Soto 67 AD and PM 105 AD; a St. Julien plum (*P. insititia*): GF 655/2, a common local plum (*P. domestica*): Constantí 1, and one *Prunus persica* rootstock (Benasque) were evaluated since the second (2001) to the fifteenth (2014) year after planting at the Experimental Station of Aula Dei-CSIC (Zaragoza, Spain) (Table 1). Adesoto (formerly Adesoto 101) and PM 105 AD (Moreno, 1990; Moreno *et al.*, 1995) were selected as polyvalent clonal rootstocks for different *Prunus* species, but especially for peaches to avoid waterlogging and iron chlorosis in heavy and calcareous soils. They also show good resistance or immunity to root-knot nematodes (Pinochet *et al.,* 1999). Constantí 1 is a local autochthonous plum that has shown a good performance as peach rootstock in field trials at the Experimental Station of Aula Dei (Moreno, 2004; Cantín *et al.,* 2006) and it is also resistant to root-knot nematodes (Pinochet *et al.*, 1999). Montizo and Monpol are also two Pollizo clonal selections from the CITA, Zaragoza, Spain. They were selected for their easy propagation, good graft compatibility with stone fruit species, resistance to nematodes and low rate of suckering (Felipe *et al.,* 1997). St. Julien GF 655/2 was a rootstock selection developed at the INRA, Bordeaux, France (Bernhard & Grasselly, 1959). It is fairly tolerant to calcareous, heavy, waterlogged soils and replant sickness (Reighard & Loreti, 2008). The Benasque peach seedling was used as a sensitive control to root asphyxia in field conditions.

Table 1. List of studied rootstocks, description and origin.

Rootstock	Species	Genetic background	Origin[a]	References
Adesoto[b]	*P. insititia*	op[d] Pollizo, clonal selection	CSIC, Spain	Moreno *et al.* (1995)
Benasque	*P. persica*	op[d], common local peach	CSIC, Spain	Font i Forcada *et al.* (2014b)
Monpol	*P. insititia*	op[d] Pollizo, clonal selection	CITA, Spain	Felipe *et al.* (1997)
Montizo	*P. insititia*	op[d] Pollizo, clonal selection	CITA, Spain	Felipe *et al*. (1997)
P. Soto 67 AD[c]	*P. insititia*	op[d] Pollizo, clonal selection	CSIC, Spain	Moreno (1990)
PM 105 AD[c]	*P. insititia*	op[d] Pollizo, clonal selection	CSIC, Spain	Moreno (1990)
GF 655/2	*P. insititia*	St. Julien clonal selection	INRA, France	Bernhard & Grasselly (1959)
Constantí 1[c]	*P. domestica*	op[d], common local plum	CSIC, Spain	Moreno (2004)

[a] CSIC = Consejo Superior de Investigaciones Científicas, Spain; CITA = Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain; INRA = Institut National de la Recherche Agronomique, France. [b] Protected grant by Community Plant Variety Office (CPVO). [c] non-released clones from the Aula Dei (Zaragoza) breeding program. [d] op: open-pollinated

The eight rootstocks were budded with 'Catherina' peach cultivar during the summer of 1997, and trees were established in a trial during the winter of 1998-1999. The experiment was located in the Ebro Valley (NE Spain; 41º 43' 28.0" N 0º 48' 42.0" W), on a heavy and calcareous soil, with 30.5% total calcium carbonate, 8.8% active lime, water pH 7.7 and a clay-loam texture. Trees were trained to a low-density open-vase system (5 × 4 m). Cultural management practices, such as fertilization, winter pruning and spring thinning, were standard. The orchard was fertilized with 350 kg/ha N-P-K fertilizer 8-15-15 in November and 350 kg/ha N-P-K fertilizer 10-5-20 in May. No Fe chelates were used in the orchard. Formulations containing $Cu_2Cl(OH)_3$, were used as plant disease control chemicals at winter pruning and early-spring. Open vase trees were pruned to strengthen existing scaffold branches and vigorous shoots were removed, inside and outside the vase. Moderate-sized fruiting wood (0.3–0.6 m long) was selected. Trees were hand-thinned at 45–50 days after full bloom (DAFB) leaving approximately 20 cm between fruits. The plot was level-basin irrigated every 12 days during the summer. Guard rows were used to preclude edge effects. The experiment was established in a randomized block design with six replications for each scion-stock combination, except for Adesoto with five replications.

Tree survival and suckering

Tree health and survival were monitored throughout the trial. Dead trees were recorded each year when growth measurements were taken. The incidence of rootstock suckering (root and collar suckers) was also recorded during the study.

Growth measurements and yield characteristics

During all the cropping years, starting in 2002, trunk girth, yield and number of fruits per tree were recorded. Trunk girth was measured once a year after leaf fall at 20 cm above the graft union, and the trunk cross-sectional area (TCSA) was calculated. At harvest, all fruits from each tree were counted and weighted to determine total yield per tree (kg/tree). Fruit weight (FW) was calculated considering the total number of fruits and total yield per tree. Cumulative yield (CY) per tree and yield efficiency (YE) of each scion-stock combination were recorded from the harvest data. YE was calculated as the ratio between the cumulative yields (in kg/tree, from 2002 to 2014) and final TCSA (cm^2) determined in the winter of 2014-2015.

Fruit sampling and evaluation of agronomic traits

Over the last six years (2009-2014), twenty mature fruits of each tree were randomly selected at harvest to evaluate fruit quality. Fruits were considered ripe when they no longer grew and exhibited the ground color representative for 'Catherina' cultivar. However, in this study only the results from the last three years (2012-2014) are presented. Results from 2009 to 2011 were already reported by Font i Forcada *et al*. (2014a).

For individual fruits, values of L* (brightness or lightness), a* (−a* = greenness, +a* = redness), b* (−b* = blueness, +b* = yellowness), C* (chroma) and H (lightness's angle) were measured using a colorimeter (Chroma Meter, CR-400 Konica Minolta, Tokyo, Japan). Flesh firmness (FF) was measured on two paired sides of each fruit, after removing a 1-mm thick disk of skin from each side of the fruit, and using a penetrometer (Model FT-327, QA Supplies, Norfolk, VA, USA). After skin colour and flesh firmness determinations, the fruits of the sample were peeled, and a portion of the mesocarp was removed from each opposite face and cut into small pieces. A composite sample was built by mixing all pieces from all the selected fruits. In this composite sample, soluble solids content (SSC) of fruit juice was measured with a digital refractometer (Atago PR-101,

Tokyo, Japan) and expressed as °Brix. Titratable acidity (TA) was measured using 5 g of homogenized samples diluted in 45 mL of distilled water and microtitrated with 0.1 N NaOH. TA was expressed as g malic acid/100 g FW. Ripening index (RI) was calculated based on the SSC/TA ratio.

Leaf chlorophyll estimation

The chlorophyll concentration per unit leaf area was estimated in the field, using a SPAD 502 meter (Minolta Co., Osaka, Japan). Thirty leaves per tree, selected from the middle of bearing shoots located all around the crown, were measured to obtain an average concentration representative of the leaves belonging to the outer part of the tree canopy. Measurements were carried out 120 days after full bloom (DAFB) in 2012 and 2014.

Mineral analysis

Leaf mineral element concentrations were determined in 2014, *i.e.* in year 15 after planting, using trees with no asphyxia symptoms and/or associated diseases. Leaf sampling was carried out at 120 DAFB. Leaf samples (40 leaves per tree) were collected from shoots around the crowns of the trees. The mineral element composition of the dried tissue was determined using the methods of CII (1969) and CII *et al.* (1975), as previously reported by Mestre *et al.* (2015). Total N was determined by Kjeldahl analysis (Gerhardt Vapodest); P was analyzed spectrophotometrically by the phospho-vanadate colorimetric method (ThermoSpectronic Helios β); K, Ca, Mg and Na by atomic emission spectroscopy (ICP, Horiba-Jobin Yvon, Activa-M); and Fe, Mn, Cu and Zn by atomic absorption spectroscopy (PerkinElmer 2100).

The DOP index (deviation from optimum percentage) was estimated for the diagnosis of the nutrient status of the trees (Montañés *et al.*, 1993). This index provides similar information to the Diagnosis and Recommendation Integrated System (DRIS) (Sanz, 1999). The DOP index was calculated from the leaf analysis by the algorithm:

$$DOP = \frac{C \times 100}{C_{ref}} - 100$$

where C is the nutrient concentration in the sample to be studied and C_{ref} is the nutrient concentration considered as optimum, both values from dry matter tissues basis. The C_{ref} has been taken from the optimum values proposed by Leece (1975). The ΣDOP is obtained by adding the values of DOP index. The larger the ΣDOP the greater resultant imbalances among nutrients.

Data analysis

The data from all replicates were analyzed by ANOVA analysis using IBM SPSS Statistics 23.0 (USA). Significant differences among means were separated by Duncan's multiple range ($p \leq 0.05$). Pearson correlation was performed to study correlations among agronomic and fruit quality traits, and leaf mineral elements.

Results

Tree mortality, tree growth and yield characteristics

At the fifteenth year after budding, heavy and calcareous soil conditions generated varying levels of tree mortality. The peach seedling rootstock Benasque, initially included in the trial, experienced the highest

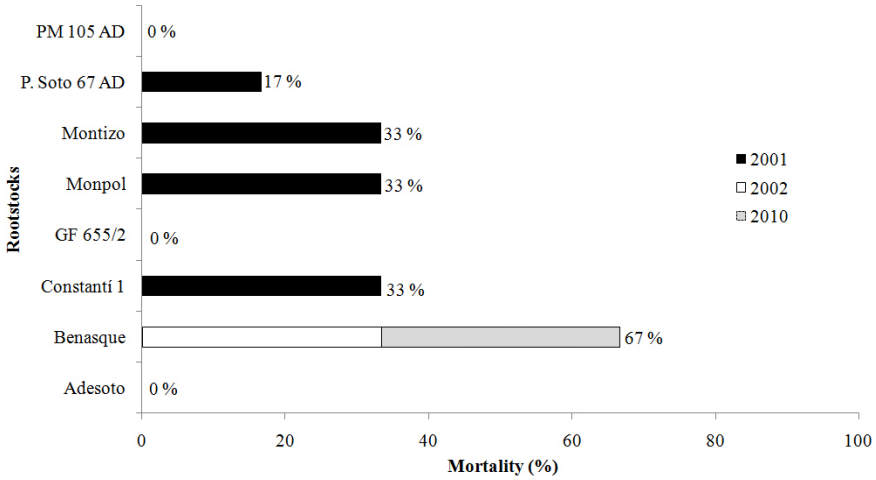

Figure 1. Tree mortality rate (%) from the second (2001) to the fifteen (2014) year after planting in the orchard trial. Percentages values right side of the bars indicated accumulated mortality rate at the end of the experiment.

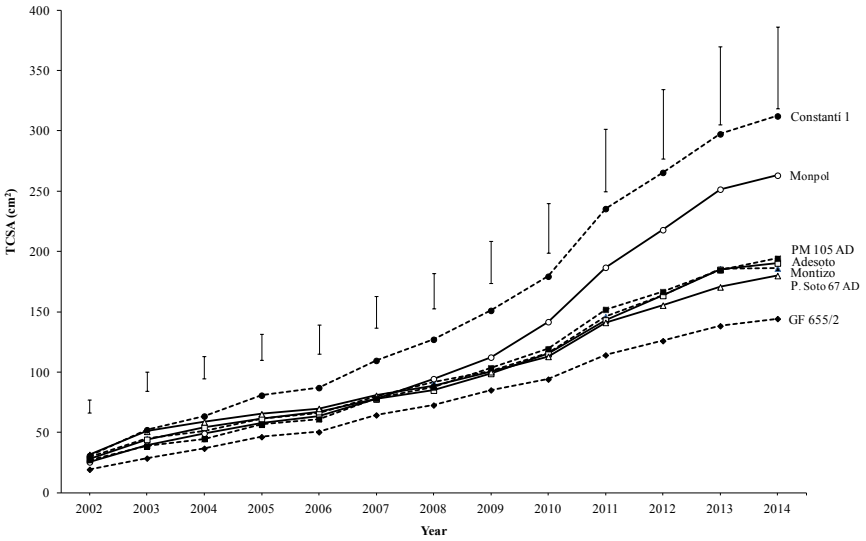

Figure 2. Effect of rootstock on trunk cross-sectional area (TCSA) of 'Catherina' peach cultivar during 15 years of study. Vertical lines indicate LSD ($p \leq 0.05$).

Table 2. Trunk cross-sectional area (TCSA), cumulative yield (CY), yield efficiency (YE) and root or crown suckering (SCK) of 'Catherina' budded on different plum rootstocks, at the fifteenth year after planting (2014).

Rootstock	TCSA (cm^2)	CY (kg/tree)	YE (kg/cm^2)	SCK (suckers/tree)
Adesoto	187.6 ab	269.8 ab	1.44 abc	1.8 a
Monpol	263.2 bc	303.3 bc	1.18 a	2.8 a
Montizo	190.4 ab	283.1 b	1.56 c	1.5 a
P. Soto 67 AD	180.0 a	261.5 ab	1.45 abc	1.6 a
PM 105 AD	194.3 ab	212.9 a	1.11 a	2.3 a
GF 655/2	144.3 a	241.2 ab	1.71 c	9.2 b
Constantí 1	312.3 c	354.6 c	1.22 ab	3.2 a

For each rootstock, means followed by the same letter in each column are not significantly different at $p \leq 0.05$ according to Duncan's Multiple Range Test.

tree mortality with more than 60% dead trees (Fig. 1). Because of this low survival rate, trees on this rootstock were excluded from all subsequent analysis. Lower mortality was found for P. Soto 67 AD with only a single dead tree, followed by Constantí 1, Montizo and Monpol. All those trees budded on Adesoto, GF 655/2 and PM 105 AD survived.

The vegetative growth of trees or vigour, expressed as TCSA, showed important differences attributable to the rootstock from the eighth year of planting until the fifteenth (Fig. 2). Trees on Constantí 1 were the most vigorous and had the highest cumulative yield (Table 2), but differences were not significant from Monpol. On the contrary, trees on GF 655/2 and P. Soto 67 AD showed the lowest TCSA, although they did not differ significantly from Adesoto, Montizo and PM 105 AD at the fifteenth year after planting (2014). On P. Soto 67 AD and GF 655/2, the reduction in TCSA was 42% and 54% compared to Constantí 1, and 32% and 45% compared to Monpol. The rest of rootstocks showed intermediate tree growth (Fig. 2). The lowest cumulative yield was recorded on PM 105 AD, although it did not differ from Adesoto, GF 655/2 and P. Soto 67 AD (Table 2). Yield was generally proportional to growth or tree size. Thus, positive correlations were found between rootstock vigour and annual yield ($r=0.67$; $p \leq 0.01$) or cumulative yield in 2014 ($r=0.66$; $p \leq 0.01$).

In this trial, trees on GF 655/2 and Montizo produced higher yield efficiency, although they did not significantly differ from Adesoto and P. Soto 67 AD (Table 2). Yield efficiency was negatively correlated with rootstock vigour ($r=-0.70$; $p \leq 0.01$).

The number of suckers per tree was also determined. On average, the GF 655/2 rootstock showed the

Table 3. Rootstock effects on fruit quality traits of 'Catherina' peach cultivar over three years (2012-2014).

Rootstock	FW	SSC	TA	FF	RI	L*	a*	b*	C*	H
Adesoto	172.1 ab	13.9 c	0.59 a	23.8 ab	23.0 d	66.3 a	22.1 ab	57.0 a	61.5 a	68.5 a
Monpol	173.8 ab	12.9 ab	0.62 a	23.6 ab	21.0 bcd	65.5 a	22.1 ab	56.6 a	61.2 a	68.3 a
Montizo	163.1 ab	12.6 a	0.63 ab	25.1 b	20.0 ab	66.0 a	20.6 a	56.9 a	61.0 a	69.8 a
P. Soto 67 D	168.7 ab	13.6 bc	0.62 a	23.8 ab	22.3 cd	65.8 a	22.2 b	56.9 a	61.5 a	68.3 a
PM 105 AD	178.6 b	13.8 c	0.68 c	25.1 b	20.3 bc	66.2 a	21.5 ab	57.2 a	61.6 a	69.1 a
GF 655/2	156.4 a	12.3 a	0.63 ab	22.0 a	19.5 ab	65.9 a	20.8 ab	56.7 a	60.9 a	69.4 a
Constantí 1	171.9 ab	12.3 a	0.67 c	25.8 b	18.3 a	65.8 a	20.8 ab	56.3 a	60.4 a	69.5 a

For each trait, means followed by the same letter in each column are not significantly different at $p \leq 0.05$ according to Duncan's Multiple Range Test. Abbreviations: FW, fruit weight; SSC, soluble solids content; TA, titratable acidity; FF, flesh firmness; RI, ripening index; L*, lightness; a*, greenness/redness; b*, blueness/yellowness; C*, chroma; H, hue.

highest number of root suckers when compared with the other evaluated rootstocks, which had few, if any, root suckers.

Fruit quality

Fruit quality evaluated during the last three cropping years (2012-2014) was significantly affected by rootstocks (Table 3). The average of the three years' data showed that PM 105 AD induced the highest fruit weight to 'Catherina' fruits, although no significant differences were found with the other rootstocks, except for GF 655/2 that showed lower values. Regarding SSC (°Brix), fruits of 'Catherina' on Adesoto and PM 105 AD showed the highest average values, although no significant differences were found with P. Soto 67 AD. The lowest SSC values were induced by Constantí 1, GF 655/2 and Montizo, but they did not significantly differ from Monpol. Fruits on PM 105 AD and Constantí 1 showed the highest TA, while the lowest TA values were found on Adesoto, Monpol, and P. Soto 67 AD, although they did not differ from GF 655/2 and Montizo. Regarding FF, fruits of 'Catherina' on Constantí 1, Montizo and PM 105 AD showed the highest average values, although no significant differences were found with Adesoto, Monpol and P. Soto 67 AD. The lowest FF values resulted from GF 655/2, but it did not significantly differ from the last three rootstocks. Firmness was significantly positive correlated with TA ($r=0.63$; $p \leq 0.01$), reflecting the decrease of acidity with fruit softening. Fruits of 'Catherina' on Adesoto showed the highest RI mean value, although no significant differences were found with Monpol and P. Soto 67 AD. The lowest value resulted from Constantí 1; however it did not significantly differ from GF 655/2, Montizo and PM 105 AD.

Throughout the study, significant differences for fruit chromatic parameters were only found among rootstocks for a* (Table 3). Average a* values of the three years (2012-2014) were higher on P. Soto 67 AD and lower on Montizo, although they did not differ from the other rootstocks.

Leaf chlorophyll concentration

Chlorophyll concentration, as determined by SPAD, was significantly affected by rootstocks the years it was measured (Table 4). SPAD readings were higher for Constantí 1, although it did not differ from Monpol in 2012 and PM 105 AD in 2014. In

Table 4. Effect of rootstock on leaf chlorophyll concentration measured as SPAD values, of 'Catherina' peach cultivar at the thirteen (2012) and fifteenth (2014) year after planting.

Rootstock	2012	2014
Adesoto	42.63 ab	34.3 ab
Monpol	48.1 bc	31.2 a
Montizo	42.0 ab	35.5 b
P. Soto 67 AD	42.1 ab	34.2 ab
PM 105 AD	44.9 ab	37.6 bc
GF 655/2	37.7 a	34.3 ab
Constantí 1	53.9 c	39.7 c

Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ according to Duncan's Multiple Range Test.

Table 5. Rootstock effects on leaf mineral element concentrations of 'Catherina' at 120 days after full bloom, by the fifteenth year after planting (2014). Results for N, P, K, Ca and Mg are expressed as percentage of dry matter, and for Fe, Mn, Cu and Zn, as mg/kg.

Rootstock	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn	∑ DOP
Adesoto	2.8 b	0.22 a	2.67 a	1.97 b	0.35 a	83.0 a	6.8 d	16.8 a	30.4 a	- 236.9 abc
Monpol	2.9 bc	0.21 a	2.67 a	1.89 b	0.43 d	89.3 a	5.9 b	16.6 a	33.8 a	- 219.7 ab
Montizo	3.0 c	0.22 a	2.76 a	2.06 b	0.42 cd	84.7 a	5.8 b	23.0 b	38.0 a	- 201.5 a
P. Soto 67 AD	2.7 a	0.20 a	2.74 a	1.94 b	0.33 a	70.2 a	6.1 bc	17.0 a	23.8 a	- 276.3 bc
PM 105 AD	2.9 bc	0.21 a	2.70 a	2.00 b	0.39 bc	108.8 a	6.6 cd	21.5 b	39.3 a	- 215.9 ab
GF 655/2	3.1 c	0.18 a	2.92 a	1.61 a	0.36 ab	70.7 a	5.0 a	21.0 ab	31.5 a	- 278.0 c
Constantí 1	3.0 c	0.20 a	2.73 a	2.04 b	0.33 a	85.8 a	6.0 bc	23.9 b	28.1 a	- 243.5 abc

Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ according to Duncan's Multiple Range Test.

contrast, lower values were found on GF 655/2 in 2012, although differences were not significant from the other rootstocks, with the exception of Constantí 1 and Monpol. In 2014, lower values were found on Monpol when compared with Constantí 1, Montizo and PM 105 AD. On the remaining rootstocks, values were intermediate.

Leaf mineral nutrients and DOP index

Some minerals content of 'Catherina' leaves were significantly affected by rootstocks (Table 5). Constantí 1, GF 655/2 and Montizo had significantly higher leaf N concentration than did the other rootstocks, but they did not differ from Monpol and PM 105 AD. Significant negative correlations between leaf N concentration and fruit SSC (r=-0.62; $p \leq 0.05$) and RI (r=-0.45; $p \leq 0.05$) were found in 2014, meaning that decreasing the leaf N concentration will increase these fruit quality parameters. The P and K concentrations did not differ when compared among rootstocks. Leaf Ca concentration of 'Catherina' peach was smaller on the lowest vigorous rootstock GF 655/2, whereas the rest of the rootstocks did not differ among them. The Mg concentration was higher on Monpol, although not significantly different from Montizo, and lower on Adesoto, P. Soto 67 AD and Constantí 1, but not different from GF 655/2.

Visual symptoms of leaf iron chlorosis appeared for several seasons, being more severe for GF 655/2. In 2014, the Fe concentration was not significantly different between rootstocks, although a slight positive correlation was found between SPAD and leaf Fe content (r=0.42; $p \leq 0.05$). The Cu concentration was higher on Adesoto, although it did not differ from PM 105 AD, and lower on GF 655/2. A positive correlation was found between Cu and SSC (r=0.59; $p \leq 0.05$). The highest Mn concentration was observed on Constantí 1, Montizo and PM 105 AD, although they did not differ from GF 655/2.

According to the ΣDOP index, GF 655/2 showed wider imbalanced nutritional values, whereas Montizo showed the best balance in nutritional values, although this rootstock did not differ significantly from Adesoto, Constantí 1, Monpol and PM 105 AD.

Discussion

Tree mortality, tree growth and yield characteristics

The soil conditions of the trial (heavy and calcareous soil with clay-loam texture) and the use of flooding irrigation make the peach trees more prone to waterlogging and root asphyxia, as previously reported in similar soil conditions (Zarrouk *et al.*, 2005; Mestre *et al.*, 2015). In addition, on waterlogged sites, *Phytophthora* spp. is often involved in tree decline and death. In certain cases, tree mortality can also be caused by graft incompatibility between rootstock and scion. However, no graft incompatibility has been observed in death trees over the years, suggesting that root asphyxia and/or the root rot pathogen *Phytophthora* could be the main cause of tree death in this study, being the peach seedling Benasque the most susceptible rootstock, followed by Constantí 1, Montizo and Monpol. In contrast, all trees budded on Adesoto, GF 655/2 and PM 105 AD survived well to the end of the experiment.

In this trial, GF 655/2 was the lesser vigorous rootstock when compared with other plum-based rootstocks, in agreement with results reported by Glucina *et al.* (1992), while Constantí 1 and Monpol

were the most vigorous ones. In contrast, Felipe *et al.* (1997) reported that Montizo and Monpol, budded with 'Catherina' peach, had similar TCSA to Adesoto, both being rootstocks of slightly more vigor than GF 655/2 by the tenth year after planting. In our study, Monpol was about 30% more vigorous than Adesoto after fifteen years.

In the first three years of this trial, impact of rootstock on yield was similar and there were no differences between rootstocks. However, in the following cropping years, differences among rootstocks became evident (Mestre, 2014), Constantí 1 being the rootstock that presented the greatest cumulative yield, although not different from Monpol. The highest vigour, yield and cumulative yield of Constantí 1 were already mentioned by Cantín *et al.* (2006) when compared with two other hexaploid plum rootstocks, budded with the late-season 'Miraflores' peach cultivar. Under harsh replant conditions or in poor and calcareous soils that might otherwise be unfavourable for growing peach, vigorous rootstocks appear suitable for peach production (Moreno *et al*., 1994, 1996; Pinochet, 2010). However, on high fertility sites with vigorous scion cultivars, some reduction in vigour is highly desirable for reduced pruning, thinning and picking costs. Similarly, lower vigour and increased tree density in the orchard (Moreno *et al.*, 1995) allows the possibility of establishing pedestrian orchards with the benefits of reducing labour costs (Jiménez *et al.*, 2011). In the present work, YE was negatively correlated with rootstock vigour. Thus, less vigorous rootstocks such as Adesoto, GF 655/2, Montizo and P. Soto 67 AD, seem to induce higher YE.

Excessive suckering is a problem in different species of rootstocks (apple, apricot, cherry, peach, and plum). Too many suckers means more labour to get rid of them, and at the end of the season more cost for the grower, because there is no technique capable of eliminating them forever. Sucker production is a genetic predisposition of the rootstock. In this trial, GF 655/2 produced at least four times more suckers than the rest of the rootstocks, which is a disadvantage observed with this plum rootstock, even more if propagated by in vitro techniques (Glucina *et al.*, 1992; Reighard & Loreti, 2008). Reighard *et al.* (1997, 2008) also reported that rootstock suckering is a common drawback inherent with some plums.

Fruit quality

This study demonstrated that fruit quality of a given peach cultivar varies depending on the rootstock, in agreement with other *Prunus* rootstock studies (Orazem *et al.*, 2011; Font i Forcada *et al.*, 2012, 2014a; Reig *et al*., 2016). Generally, fruit weight is affected by crop load, and there is a correspondence between low yield and large fruit weight (Egea *et al.*, 2004) and vice versa. However, in our study, no significant correlation was found between fruit weight and yield.

The effect of different *Prunus* rootstocks on SSC, TA, RI and FF was also found significant by other authors. Font i Forcada *et al.* (2014a) also reported higher SSC and individual sugars as sucrose, the sugar present at the highest concentration in peaches, and higher RI on Adesoto and PM 105 AD. Orazem *et al.* (2011) found that Adesoto rootstock induced higher values on SSC and fruit weight to 'Redhaven' fruits when compared to five different plums and five peach-based rootstocks. It is noteworthy that despite the higher acidity of fruits on PM 105 AD, they exhibited higher SSC and lower or intermediate RI values. High sugar contents and, to a lower extent, high acid contents seem to increase fruit quality as evaluated by consumers (Crisosto & Crisosto, 2005).

Regarding to color parameters at harvest (L*, a*, b*, C and H), poor rootstock effects were observed. In addition, no significant correlations were observed between the color measurements and fruit quality traits, probably because fruit samples were harvested according to commercial color standards, similar for each scion-rootstock combination.

Leaf chlorophyll concentration

SPAD values were in the same range as previously reported in peaches (Zarrouk *et al.*, 2005; Jiménez *et al.*, 2011; El-Jendoubi *et al.*, 2012; Mestre *et al.*, 2015). They have been used as an indicator of iron chlorosis tolerance in different *Prunus* trees (Jiménez *et al.*, 2004, 2008; El-Jendoubi *et al.*, 2012). Based on these results, GF 655/2 and Monpol appear to be more sensitive to iron deficiency in compact and calcareous soils.

Leaf mineral nutrients and DOP index

Ten essential macro- and micronutrients were determined in peach leaves at 120 DAFB, and a significant effect of the rootstock was found. Concerning macronutrients (N, P, K, Ca and Mg), all rootstocks had correct P, K, Mg and Ca concentrations, except GF 655/2 which presented a marginal value according to Leece (1975). In the case of potassium and magnesium, values were slightly higher than the multi-year means obtained in commercial peach orchards located in the Ebro river basin area (El-Jendoubi *et al.*, 2012). In contrast, other studies reported K deficiency on peaches budded on peach-based rootstocks growing in similar soil conditions (Zarrouk *et al.*, 2005; Mestre *et al.*, 2015). Thus, plum rootstocks might present better uptake of this element in this type of soil. In contrast,

N concentrations were slightly lower than optimum (marginal values), except in the case of GF 655/2, Constantí 1 and Montizo rootstocks in which values were not marginal. Comparable N concentrations have been reported in similar growing conditions for different peach cultivars (Zarrouk *et al.*, 2005; Mestre *et al.*, 2015). Guo-yi *et al*. (2015) reported that N influenced fruit size and composition, and higher N levels could decrease the amount of soluble solids and increase the titratable acid level in apples, which agrees with the negative correlations found in the present study between leaf N concentration and SSC and RI.

All rootstocks presented lower Fe concentrations than the optimum according to Leece (1975), except PM 105 AD with adequate values. In absence of significant correlation between the annual yield and Fe concentration in 2014, the tendency of PM 105 AD to induce higher Fe concentration shows the interest of this rootstock in heavy-calcareous soils where iron chlorosis is commonly observed. Low iron bioavailability is mainly the result of its insolubility at higher pH values, especially in calcareous soils, where roots of some species are unable to acquire Fe (Hell & Stephan, 2003). Iron deficiency could be also more severe when soil aeration is poor because of a high water table or compact soils. Most tolerant rootstocks to iron chlorosis are, in general, *P. amygdalus* × *P. persica* hybrids, probably because of the influence of its chlorosis tolerant almond pedigree. Nevertheless, several hexaploid plum rootstocks (Adesoto and Tetra) also appear to be more tolerant to iron-chlorosis than other peach-based hybrids (*P. persica* × *P. davidiana*) commonly used for peach growing (Jiménez *et al.*, 2008; Mestre *et al.*, 2015). Tolerance to Fe-induced chlorosis is an important selection criterion for *Prunus* rootstocks in Mediterranean environmental conditions.

In terms of leaf Cu concentration, all rootstocks had adequate leaf Cu values, according to Leece (1975). However, El-Jendoubi *et al.* (2012, 2013) reported higher Cu values in commercial peach orchards located in the Ebro river basin area. All rootstocks had marginal Mn values according to reference values (Leece, 1975), probably due to the insolubilization of this element in this type of soils. Furthermore, increased Ca in soil or an excess of phosphoric acid fertilization might decrease or block Mn uptake (Johnson & Uriu, 1989; Moreno *et al.*, 2001; Jiménez *et al.*, 2007). Nevertheless, lower values were even shown when compared to other works in similar compact and calcareous soils (Moreno *et al.*, 1996; Jiménez *et al.*, 2004; Zarrouk *et al.*, 2005; El-Jendoubi *et al.*, 2012, 2013; Mestre *et al.*, 2015). The SPAD of leaves was positively correlated with Mn ($r=0.47$, $p\leq0.05$), as reported by Zarrouk *et al.* (2005) and El-Jendoubi *et al.* (2012). The performance of Constantí 1 presenting higher SPAD and higher Mn values than most rootstocks could be related to its greater capacity to uptake this element in this type of soil and, consequently, the role that Mn plays in the photosynthesis process. The Zn concentration was found not significantly different among rootstocks, having all of them adequate values (Leece, 1975).

The imbalance of nutrients negatively affected tree growth, fruit quality and yield (Marschner, 1995), and according to the ΣDOP index, GF 655/2 had more imbalanced nutritional values than most rootstocks. In general, lower leaf nutrients concentration were observed on less vigorous rootstocks as GF 655/2 and P. Soto 67 AD, except for leaf N concentration in GF 655/2. This suggests that dwarfing rootstocks could be less efficient in the absorption of some nutrients from the soil. A similar pattern has been found in other studies for peach (Zarrouk *et al.*, 2005; Mestre *et al.*, 2015), cherry (Moreno *et al.*, 2001) and apricot rootstocks (Rosati *et al.*, 1997).

In summary, performance of 'Catherina' peach was influenced by *Prunus* rootstock's capacity to adapt to growing conditions. The largest vigour and yield, as well as higher N and Mn leaf content and SPAD values induced, in general, by Constantí 1, makes it more suitable for heavy and calcareous soils with lower fertility, where a higher vigour is convenient. The medium-vigour rootstocks Adesoto and PM 105 AD showed the tendency to induce a better organoleptic quality of the fruit, based on a higher concentration of soluble solids and fruit size, demonstrating their commercial interests as rootstocks for peach. The smaller vigour induced by GF 655/2 makes this rootstock the most interesting in intensive peach plantations. However, the slightly smaller fruit, compared with the other rootstocks, and its greater tendency to sucker are strong drawbacks of this rootstock. Unless suckering can be readily controlled, size-controlling rootstocks such as GF 655/2 are unlikely to be used in commercial orchards. The mineral elements foliar analysis showed that Montizo presented, in general, the most suitable balanced nutritional index, mainly compared with GF 655/2, which showed the more unbalanced nutritional index, especially when compared to Monpol and PM 105 AD.

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