Potential of new *Prunus cerasifera* based rootstocks for adapting under heavy and calcareous soil conditions

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Abstract

Five *Prunus cerasifera* (Myrobalan) based rootstocks, three already released (Adara, Ademir, and Myrobalan B) and two under selection (Miral 3278 AD, Myrobalan 713 AD), budded with two European plums (‘Reine Claude of Bavay’ and ‘Reine Claude Tardive of Chambourcy’) were assessed over sixteenth years at the Aula Dei Experimental Station under heavy and calcareous soil conditions. The adaptation variability of these rootstocks was expressed in tree survival, yield performance, leaf mineral concentration, fruit size and fruit quality traits. Myrobalan 713 AD and Myrobalan B rootstocks presented the highest tree mortality on both plum cultivars, whereas all trees budded on the rest of the rootstocks survived well exhibiting homogenous growth. For both plum cultivars, the less vigorous rootstock was Miral 3278 AD, having also the best agronomical performance (higher cumulative yield, yield efficiency and fruit size). Similar trend in terms of fruit production was also observed for the intermediate semi-vigorous rootstock Adara. Fruit quality traits such as flesh firmness, soluble solids content and titratable acidity were, in general, not significantly affected by rootstock. Independently of the cultivar, at the fifth year of planting all rootstocks induced optimum values of N, P, Ca, Mg, and Mn according to reference values, but lower Fe and higher K values than the optimum. However, at the sixteenth year of the study a different trend was observed, probably influenced by age and crop load of the trees. For both cultivars, rootstocks had optimum Mg, lower than the optimum N and Mn concentrations, while P was, in general, slightly higher than the optimum or close to the upper limit value in the optimum range. The results obtained in the present

work showed the hybrid Miral 3278 AD as a good alternative to Myrobalan rootstocks based on its best long-term tree survival and good agronomical performance. Further work should be done with Miral 3278 AD using more intensive orchard plum systems.

Introduction

Plum trees are grown worldwide, from cooler subtropical to temperate climates. The main producers of commercially grown plums are China, Romania, Serbia, and the United States (Igwe and Charlton, 2016). Spain is the seventh largest world plum producer (FAOSTAT, 2017).

The plum is a drupe fruit placed within the *Prunoideae* subfamily of the Rosaceae, *Prunus* genus, *Prunophora* subgenus, and section *Euprunus* for species (spp.) of plums originating in Europe and Asia, and section *Prunocerasus* for plums originating in America (Topp et al., 2012). There are between 19 and 40 different spp. of plums in the world that have originated in Europe, Asia and America (Topp et al., 2012). From this diversity, only two species predominate in modern commercial fresh fruit production: the hexaploid European plum (*Prunus domestica* L.) and the diploid Japanese plum (*P. salicina* Lindl and its hybrids). Outside of them, there is limited use of other *Prunus* species for that purpose. However, some of these plum species are widely used as rootstocks. That is the case of the diploids *P. cerasifera*, commonly known as the cherry plum or myrobalan plum, the different ploidy levels *P. cerasifera* × *P. munsoniana* (also called Marianna), and the hexaploids *P. insititia* (Damsons) (Topp et al., 2012).

Over the last decades, most worldwide new rootstocks released for plum production are clonally propagated and many of them are of interspecific origin. In particular, these rootstocks have their origins in private breeding programs (Agromillora Iberia S.L. from Spain; Gregory Bros. Nursery from USA), universities (University of California from USA) and research institutes (Aula Dei Experimental Station from Spain; East Malling from United Kingdom; Institut National de la Reserche Agronomique from France; Instituto Sperimentale per la Frutticoltura from Italy; Vavilov Research Institute from Russia) (Okie, 1987; Topp et al., 2012; Reig et al., 2018a). The Aula Dei Experimental Station has a long tradition of *Prunus* breeding aiming to select plum based rootstocks (mainly *P. cerasifera* Ehrh. and *Prunus insititia* L.) for plums and prunes, among other stone fruit species, well adapted to heavy and calcareous soils, typical of the Mediterranean area, where root asphyxia and iron-induced chlorosis are frequent problems (Moreno, 2004). In particular, a selection program of Myrobalan (*Prunus cerasifera*) was developed. The best performers were retained, and so far two rootstocks,

Ademir and Adara (Moreno et al., 1995 a,b), were already released since the 90's, and other promising rootstocks could be further released. The interest in Myrobalans as rootstocks for plum and other stone fruit species is due to their high tolerance to root asphyxia, iron-induced chlorosis and salinity; as well as their resistance to root-knot nematodes, soil borne pathogens (fungi and bacteria) and phytoplasms (Moreno, 2004). These tolerances suggest that they could be used as a gene source for obtaining new rootstocks. To the best of our knowledge, studies focused on physiological response and fruit quality properties of European plums budded on fast growing Myrobalan rootstocks and conducted under heavy and calcareous soil are very scarce (Reig et al., 2018a). Therefore, the goals of the present work were to: 1) evaluate the effect of five *P. cerasifera* plum based rootstocks on tree survival, yield performance, nutrition uptake, and fruit quality attributes of two scion plum cultivars 'Reine Claude of Bavay' and 'Reine Claude Tardive of Chambourcy'; 2) study the correlation among all traits evaluated; and 3) apply multivariate analysis to identify the most adapted rootstock in the soil conditions for both plum cultivars.

2. Materials and methods

2.1. Plant material and field trial

Two European plum cultivars ('R.C. Bavay' and 'R.C.T. Chambourcy') were budded on different fast-growing plum rootstocks: the none yet released Myrobalan 713 AD, and the open-pollinated interspecific hybrid of Myrobalan parentage Miral 3278 AD, and the already released Myrobalans (*Prunus cerasifera*) such as Adara, Ademir and Myrobalan B (Table 1). Adara was originally released as a cherry rootstock, whereas Ademir was selected as rootstock for European and Japanese plum cultivars (Moreno et al., 1995 a,b). Myrobalan B was used as reference (Okie, 1987). Miral 3278 AD is a natural hybrid and it was considered to be of *P. cerasifera* × *P. amygdalus* parentage (Casas et al., 1999).

The trial was carried out at the Aula Dei Experimental Station (NE Spain; 41° 43' 42.7" N 0° 48' 44.1" W) on a heavy and calcareous soil (Table 2) and under a cold-semiarid Mediterranean climate (Bsk in the Köppen–Geiger climate classification system). The soil was strongly carbonated, with high limestone levels. Active limestone levels exceeded 7% in all profiles, and there was no evidence of salinity. The level of organic matter was low in all three depths. Phosphorus was high in the surface, with a descend trend in depth. The assimilable K was medium in the upper level and low in the rest. The Mg content of change was low in the surface and correct in the rest of depths.

One-year-old budded trees were established in the winter season of 1994-1995. The experiment was established in a randomized block design with five blocks, with the basic plot consisting of one tree per scion-rootstock combination. Trees were spaced at 4 m apart within rows and 6 m apart between rows amounting to a plant density of 417 trees ha⁻¹. The rows were oriented North-South and the trees were trained in a low density open-vase system to avoid vigour interferences between them. All trees were pruned to strengthen existing scaffold branches and eliminate vigorous shoots, inside and outside the vase, that would compete with selected scaffolds or shade fruiting wood. The plot was level-basin irrigated every 12 days during the summer. Standard pest and weed control, and fertilization practices were applied. Guard rows were used to preclude edge effects.

2.2. Growth measurement, yield and fruit sampling

Trunk girths were measured during bud dormancy phenological stage at 20 cm above the graft union, and the trunk cross-sectional area (TCSA, in cm²) was calculated. In the case of 'R.C.T. Chambourcy' trunk girth was measured from 1994 to 2010, and for 'R.C. Bavay' from 1994 to 2011. Dead trees were recorded each year at the time when growth measurements were taken.

The yield from all rootstock-scion combinations was weighed. Cumulative yield per tree and yield efficiency (cumulative yield in kg per final TCSA) were also computed from the harvest data. Fruit weight was calculated by sampling 50 fruits each year from 1999 to the end of the study. In addition, over the last years of the study (from 2009 to 2011 in the case of 'R.C. Bavay' and 2009-2010 in 'R.C.T. Chambourcy'), a sub-sample of 25 representative healthy fruits, free of visible defects, were randomly hand-picked at commercial maturity time for each scion-rootstock combination to determine fruit quality traits described in section 2.3. They were collected from shoots around the crown of the trees.

2.3. Fruit quality analysis

Fruits were assessed at harvest for the following attributes using standard techniques as previously described in Cantín et al. (2010) for cherries: Flesh firmness (FF), soluble solids content (SSC), and titratable acidity (TA). FF was measured with a durometer (Shore A, Durofel). SSC of juice extracted was determined with a digital refractometer (Atago PR-101, Tokyo, Japan), and TA by titrating the juice samples with 0.1 N NaOH to pH 8.2.

2.4. Chlorophyll and leaf mineral analysis

The chlorophyll (Chl) concentration per unit leaf area was estimated in the field, using a SPAD 502 meter (Minolta Co., Osaka, Japan), as described in other *Prunus* rootstocks studies (Jiménez et al., 2007; Mestre et al., 2015, 2017). Measurements were carried out at 115 days after full bloom (DAFB) in 2011 for ‘R.C. Bavay’ and in 2010 for ‘R.C.T. Chambourcy’.

Leaf mineral element concentrations were determined in 2000 and 2011 for ‘R.C. Bavay’, and 2000 and 2010 for ‘R.C.T. Chambourcy’, as described in different cherry and peach rootstock studies (Jiménez et al., 2007; Mestre et al., 2015, 2017). Leaf sampling was done at 115 DAFB for both plum cultivars. Total N was determined by Kjeldahl analysis (Gerhardt Vapodest); P was analyzed spectrophotometrically by the phospho-vanadate colorimetric method (Hewlett-Packard 8452A); K, Ca, and Mg by atomic emission spectroscopy (ICP, Horiba–Jobin Yvon, Aativa-M); and Fe, Mn, Cu and Zn by atomic absorption spectroscopy (PerkinElmer 1100).

The Σ DOP index (deviation from optimum percentage), a method used to determine the quantity and quality of each nutrient in plants: optimal (DOP = 0), deficiency (DOP < 0) or excess (DOP > 0) (Montañés et al., 1993), was also calculated as described in the previous studies above mentioned. The larger was the Σ DOP the greater was the intensity of imbalances among nutrients.

2.5. Analysis of data

Data were analyzed by one-way analysis of variance (ANOVA) using IBM SPSS Statistics 24.0 (USA). Means separation were conducted using a Duncan test at $P \leq 0.05$. Pearson correlation was performed among agronomic and fruit quality traits, and leaf mineral elements. A multivariate projection model (Principal Component Analysis, PCA) was performed to simultaneously analyse agronomic, leaf and fruit quality traits and to understand how these variables contribute to variability among rootstocks budded with ‘R.C. Bavay’ and ‘R.C.T. Chambourcy’ plum cultivars, using Unscrambler X 10.3 software (CamoAsa, 2001).

3. Results

3.1. Tree mortality

At the last year of the study (2011 for ‘R.C. Bavay’ and 2010 for ‘R.C.T. Chambourcy’), heavy and calcareous soil conditions generated varying levels of tree mortality among rootstocks, depending on the cultivar (Fig. 1). For ‘R.C. Bavay’ cultivar, tree mortality was observed only in one tree budded on Myrobalan 713 AD and another one on Myrobalan B (20% dead trees of total budded trees), whereas all trees budded on the rest

of the rootstocks survived well exhibiting homogenous growth. Similarly, for 'R.C.T. Chambourcy' cultivar, Myrobalan 713 AD and Myrobalan B experienced the highest tree mortality (80% and 100% dead trees, respectively). Because of this low survival rate, trees on these rootstocks were excluded from all subsequent analysis for 'R.C.T. Chambourcy'. The rest of the rootstocks showed lower tree mortality.

3.2. Vegetative growth, yield and fruit quality

As indicated in Figure 2, the vegetative growth of trees or vigour, expressed as TCSA, showed a great influence attributable to the rootstock since the seventh year of growth in the case of 'R.C. Bavay' and the eight year in 'R.C.T. Chambourcy'. At the end of the study (2011 for 'R.C. Bavay' and 2010 for 'R.C.T. Chambourcy') the significant influence of the rootstock on TCSA was highlighted (Table 3, Fig. 2). In the case of 'R.C. Bavay', lower values of TCSA were noted for Miral 3278 AD and Myrobalan B. Adara and Myrobalan 713 AD were intermediate in vigour, and TCSA was significantly greater on Ademir. Similar trend was observed in 'R.C.T. Chambourcy'. Miral 3278 AD had the lowest vigour whereas Ademir had the highest one, although differences were not significant when compared with Adara.

Regarding fruit production, rootstock effect was also statistically significant on both plum cultivars (Table 3). In 'R.C. Bavay', the lowest cumulative yield was recorded on Ademir and Myrobalan B. In contrast, Miral 3278 AD and Adara had the highest cumulative yield and yield efficiency values, although Adara was not statistically different from Myrobalan 713 AD and Myrobalan B concerning yield efficiency. Despite cumulative yield was not significantly affected by rootstock in the case of 'R.C.T. Chambourcy', Miral 3278 AD had the highest yield efficiency and Ademir had the lowest value, although differences were not significant with Adara. It is noteworthy that 'R.C. Bavay' trees came into production one year before (in the fourth leaf) than 'R.C.T. Chambourcy', but only on Adara, Myrobalan B and Miral 3278 AD (data not shown). Finally, rootstock effect was also observed on fruit size for both plum cultivars, being Miral 3278 AD the rootstock with the biggest fruits, although it did not differ significantly from Adara in 'R.C.T. Chambourcy'. Rootstock effect was not observed on the rest of fruit quality traits (Table 4), with the exception of FF in 2009 and SSC in 2010 for 'R.C. Bavay'. Miral 3278 AD rootstock had higher FF value than Myrobalan B in 2009 and lower SSC value in 2010, although it did not differ significantly from Ademir.

In terms of fruit production and fruit quality traits, significant positive correlations were found between cumulative yield and yield efficiency ($r = 0.74$, $P \leq 0.01$), annual yield

and cumulative yield ($r = 0.55$, $P \leq 0.01$), cumulative yield and fruit weight ($r = 0.51$, $P \leq 0.01$), TA and FF ($r = 0.48$, $P \leq 0.01$), and TCSA and annual yield ($r = 0.38$, $P \leq 0.01$). In contrast, negative correlations were shown between TCSA and yield efficiency ($r = -0.79$, $P \leq 0.01$), and between SSC and fruit weight ($r = -0.62$, $P \leq 0.01$).

3.3. Chlorophyll and leaf mineral analysis

Chlorophyll concentration, as determined by SPAD measurements, was significantly affected by rootstock on 'R.C. Bavay' (Table 5). For this cultivar, the rootstock Miral 3278 AD showed higher values than Myrobalan B and Myrobalan 713 AD, although it was not significantly different from Adara and Ademir. Myrobalan B had the lowest SPAD values, but it did not differ significantly from Myrobalan 713 AD.

Rootstock affected some leaf mineral concentrations of plum cultivars, both at the fifth year after planting (2000) and the last year of the study (2011 for 'R.C. Bavay' and 2010 for 'R.C.T. Chambourcy') (Table 6). Over the time, the concentration of leaf N, K, Mg, and Mn tended to decrease, whereas leaf Ca, P and Fe concentrations showed the contrary. In year 2000, Adara, Ademir and Miral 3278 AD trees budded with 'R.C. Bavay' had significantly higher concentrations of leaf N and P, but lower Mg compared to the rest of the rootstocks. These differences were not detected in 2011, when the rootstock effect was only significant on leaf Ca and Mn. Adara, Ademir and Myrobalan 713 AD induced the highest Mn values, although Adara and Myrobalan 713 AD were not statistically different from Miral 3278 AD. In the case of 'R.C.T. Chambourcy', rootstock effect was only observed in leaf Mn in 2000, being Adara the rootstock with the highest value, although it was not statistically different from Ademir. Nevertheless, in 2010 rootstock effect was only significant in leaf N and K. Adara presented the lowest values on both minerals, whereas Miral 3278 AD and Ademir had the highest N and the highest K mean values, respectively.

According to Σ DOP index, no statistically differences were found among rootstocks in both 2000 and 2011 years for 'R.C. Bavay' and in year 2000 for 'R.C.T. Chambourcy'. However, for the last cultivar, Ademir and Miral 3278 AD showed better balanced nutritional values compared to Adara in 2010.

Medium significant correlations were found between chlorophyll and leaf mineral values, and between them and yield and fruit quality parameters. Positive correlations were shown between N and Mg ($r = 0.60$, $P \leq 0.01$), N and FW ($r = 0.60$, $P \leq 0.01$), N and YE ($r = 0.56$, $P \leq 0.01$), SPAD and cumulative yield ($r = 0.51$, $P \leq 0.01$), Mg and FW ($r = 0.48$, $P \leq 0.01$), SPAD and N ($r = 0.47$, $P \leq 0.01$), and Mg and cumulative yield ($r = 0.47$,

$P \leq 0.01$). In contrast, negative correlations were found between Fe and SSC ($r = -0.65$, $P \leq 0.01$), N and K ($r = -0.56$, $P \leq 0.01$), SSC and SPAD ($r = -0.56$, $P \leq 0.01$), N and SSC ($r = -0.52$, $P \leq 0.01$), Mg and SSC ($r = -0.47$, $P \leq 0.01$), K and Mg ($r = -0.46$, $P \leq 0.01$), and Ca and Mg ($r = -0.46$, $P \leq 0.01$).

3.4. Principal Component Analysis

A broader view of rootstock–scion combination effect on the agronomic and fruit quality characteristics was obtained through PCA (Figure 3). The first two principal components (PC1 and PC2) were able to explain 72% of the overall variance, with PC1 accounting for 56% and PC2 for 16%. PC1 was positively correlated with FW, Ca, N, Mg, and tree survival (TS), and negatively correlated with SSC. PC2 was positively correlated with TCSA and P. Two groups were clearly observed: (1) A group clustered on the positive side of PC1 that included ‘R.C. Bavay’ on all rootstocks; (2) and a second group clustered on the negative side of PC1 encompassing ‘R.C.T. Chambourcy’ on all rootstocks.

4. Discussion

Good compatibility with the scion is a requisite before selecting suitable rootstocks for agronomic attributes such as resistance/tolerance to biotic and abiotic stress factors (Emmanouilidou and Kyriacou, 2017). In this study, there was not any sign of graft incompatibility for all scion–rootstock combinations, although graft-incompatibility with some ‘Reine Claude’ (Greengage) plums has been reported with Myrobalan B and other Myrobalan or Marianna selections (Okie, 1987; Moreno et al., 1995b).

In the experimental trial conditions (heavy soil and flooding irrigation), the Myrobalan B and Myrobalan 713 AD rootstocks experienced the highest tree mortality, especially when they were budded with ‘R.C.T. Chambourcy’. It appeared that tree losses could be caused mainly by a soil borne disease named *Phytophthora* spp., which is very common on waterlogged sites. In fact, *Phytophthora* was found after fungal isolation in necrotic roots and trunk bark samples from dead trees (Cambra, personal communication). In addition, a different susceptibility between cultivars was observed, also reported for two different cherry cultivars budded on several *Prunus* rootstocks in similar soil conditions (Moreno et al., 1996). Adara, Ademir and Miral 3278 AD, did not have any tree losses with ‘R.C. Bavay’. Higher tolerance to root asphyxia and/or associated diseases in cherry trees budded on Adara was also reported by Moreno et al. (1996).

Rootstocks induced significant differences in tree vigour and yield characteristics as also shown in other *Prunus* spp. studies (Hartman et al., 2007; Cantín et al., 2010; Font i Forcada et al., 2012; Mestre et al., 2015, 2017; Ben Yahmed et al., 2016). Under the heavy

and calcareous soil conditions prevailing in this trial, Ademir induced higher tree vigour. This made it more suitable for poor and replant soil conditions, where a higher level of vigour is very convenient (Mestre et al., 2017). In contrast, Miral 3278 AD resulted as one of the most dwarfing rootstocks, and Adara showed an intermediate level of vigour. The last two rootstocks profoundly affected the fruiting response, showing higher cumulative yield and yield efficiency compared to the other rootstocks. More dwarfing rootstocks usually induce better yield precocity and efficiency than more invigorating rootstocks, explained by the increased number and quality of floral buds produced on dwarfed trees, or less competition between vegetative and reproductive organs (Webster, 2002). In addition, Miral 3278 AD and Adara rootstocks could adapt well on high fertility sites, where some reduction in vigour is highly desirable to reduce pruning, thinning and picking costs. Consequently, it would be interesting to test them in semi-intensive plum commercial orchards with open vase trees (spacing: 4.5-5 m $\times$ 3.5-3 m).

As an added benefit, vigour reductions are often accompanied by increased fruit size (Beckman and Lang, 2003), in good agreement with our results. The most controlling vigorous rootstock, Miral 3278 AD, induced bigger fruit size on both plum cultivars. The highest fruit weight induced by Miral 3278 AD is a desirable marketable trait since thinning is not usually practised in plum production. Mass et al. (2014) reported that fruit weight is an important quality characteristic in plums, and accordingly high production efficiencies of rootstocks should not be accompanied by decreased fruit weights. None rootstock will succeed in the stone fruit industries without promoting superior horticultural performance of the scion, with premium quality fruit. Mean values of FF, SSC and TA were within the range of other plum studies (Rato et al., 2008; Milošević and Milošević, 2012; Sottile et al., 2012; Milošević et al., 2013).

Leaf chlorophyll concentration, measured by SPAD, was similar to that previously reported in other *Prunus* studies (Mestre et al., 2015, 2017). However, Myrobalan B and Myrobalan 713 AD budded with ‘R.C. Bavay’ had the lowest SPAD mean values, probably associated with their poor performance in the unfavourable soil conditions of the trial (calcareous soil, with high pH and prone to waterlogging).

Rootstock influenced the leaf mineral concentration of plum trees, in agreement with other *Prunus* rootstock studies (Jiménez et al., 2007; Mestre et al., 2015, 2017) as well as other fruit tree studies (Kucukyumuk and Erdal, 2011; İkinci et al., 2014; Reig et al., 2018b). At the fifth year of planting (2000), all rootstocks induced optimum values of N, P, Ca, Mg, and Mn according to reference values (Reuter et al., 1997), but lower Fe and

higher K values than the optimum. However, at the last years of the study a different trend was observed, probably influenced by age and crop load of the trees. For both cultivars, rootstocks induced optimum Mg, lower than the optimum N and Mn concentrations, and P slightly higher than the optimum or close to the upper limit value in the optimum range according to Reuter et al. (1997). In the case of 'R.C. Bavay', all rootstocks had optimum K and Fe values in 2011, but slightly higher Ca values than the optimum, with the exception of Miral 3278 AD and Myrobalan B. These two rootstocks induced leaf Ca values within the optimum range (Reuter et al., 1997).

Trees of 'R.C.T. Chambourcy', showed, in general, lower leaf Ca and Fe values, and higher K values than the optimum, especially with the Ademir rootstock. The excess of leaf K in 'R.C.T. Chambourcy' on Ademir could be due to their high-affinity to K⁺ influx from the soil to root cells and then to the leaves, probably due to the higher root volume in the soil caused by its higher vigour. That higher affinity could also explain the deficient concentration found for Ca and other mineral elements. Other rootstock studies with apple and pear orchards (Ikinci et al., 2014; Reig et al., 2018b) have reported that scion leaves of trees on more vigorous rootstocks exhibited higher K content than those on size-controlling rootstocks. In addition, plum-based rootstocks seem to increase K uptake more than other *Prunus* rootstocks (Mestre et al., 2015; Moreno et al., 1996).

Ranade-Malvi (2011) reported that an excess amount of P reduces uptake of cationic micronutrients like iron and manganese. In this study, that statement was especially observed on the combination 'R.C.T. Chambourcy'/Ademir. Therefore, and based on our results, Ademir could require less P and K fertilizer in this type of soils, meaning a reduction cost in terms of fertilizer needs.

The leaf Mn and N deficiencies observed in this study were also reported for cherries and peaches growing in similar soil conditions (Jiménez et al., 2004; Zarrouk et al., 2005; Mestre et al., 2015, 2017). Indeed, Mn deficiency could be probably due to the insolubilization of this element in this type of soil. Furthermore, increased Ca in soil or an excess of phosphoric acid fertilization might decrease or block Mn uptake (Johnson and Uri, 1989). In this study, the positive correlation of N with SPAD, yield efficiency and fruit size, and its negative correlations with K and SSC agree with Guo-yi et al. (2015), who reported that N influences fruit size and composition, and higher N levels could decrease the amount of soluble solids in apples. The interaction between N and K is well known in crop growth, due to potassium affects nitrate absorption and reduction from the soil solution. In fact, improving N uptake and utilization with adequate K mean

improved N use and higher yields (Ranade-Malvi, 2011). It was observed that the crop response to applied nitrogen fertilizers decreases when the exchangeable potassium content of a soil is below the optimal level. In addition, as mentioned in the soil description, the soil K content in this study decreased accordingly to the soil level. That fact could help to explain the lower leaf N concentrations than the optimum found in this work. Despite the absence of Mg deficiency in leaves, Mg had similar significant correlations to N with the other traits evaluated, such as Ca, K, SPAD, cumulative yield, fruit size and SSC. In fact, Mg and N have a synergism relationship, whereas they have an antagonism relationship with K and Ca (Zarrouk et al., 2005; von Bennewitz et al., 2011).

Multivariate analysis through the PCA model showed a general overview of the two ‘Reine Claude’ plum cultivars budded on several *Prunus cerasifera* rootstocks in order to identify the most adapted rootstock in the soil conditions. The results obtained in the present work showed the hybrid Miral 3278 AD as a good candidate to replace some plum rootstocks based on its best long-term tree survival, higher yield efficiency and fruit quality performance.

5. Conclusions

This study revealed the significant effect of different *Prunus cerasifera* based rootstocks, with different degrees of vigour, on the horticultural performance of the two assessed European plum cultivars. It is worth underlining that scion had also an important effect, mostly reflected on the scion-rootstock susceptibility to waterlogging and/or associated diseases. Deleterious scion-rootstock combinations (‘R.C.T. Chambourcy’ / Myrobalan B’ and ‘R.C.T. Chambourcy’ / Myrobalan 713 AD) could be considered unequivocally incompatible for establishment on heavy and calcareous soils with waterlogging and associated fungal diseases. The less invigorating Miral 3278 AD rootstock showed a good yield and fruit quality performance on heavy and calcareous soil conditions. In addition, its lower vigour could result on reducing labor costs such as pruning and harvesting in commercial plum orchards. Considering its overall performance over sixteenth years (considered a normal orchard life for plum orchards) and its waterlogging tolerance, the hybrid Miral 3278 AD appears as a promising rootstock alternative to Myrobalan rootstocks. Further work should be done with Miral 3278 AD using more intensive orchard plum systems.

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Table 1. List of studied rootstocks.

Rootstock	Species	Origin	References
Adara	<i>P. cerasifera</i>	EEAD-CSIC, Spain	Moreno et al. (1995a)
Ademir	<i>P. cerasifera</i>	EEAD-CSIC, Spain	Moreno et al. (1995b)
Miral 3278 AD	<i>P. cerasifera</i> × <i>P. amygdalus</i> ?	EEAD-CSIC, Spain	EEAD ^a
Myrobalan B	<i>P. cerasifera</i>	East Malling, England	Okie (1987)
Myrobalan 713 AD	<i>P. cerasifera</i>	EEAD-CSIC, Spain	EEAD ^a

^aNon-released clones from the Aula Dei Experimental Station (EEAD) breeding program.

Table 2. Soil analysis description.

Depth (cm)	Texture	E.C. (1:5) (dS/m)	pH	Organic Matter ^a (%)	P ^b (ppm)	K ^c (ppm)	NO ₃ ^d (ppm)	Mg ^c (meq/100 g)	CaCO ₃ ^e (%)	Active Limestone ^f (%)
0-30	Silty-clay-loam	0.20	8.40	1.79	21.4	248	12.5	0.78	30.9	9.58
30-60	Silty-clay-loam	0.19	8.60	1.44	14.1	142	11.2	1.18	28.6	7.40
60-90	Silty-clay-loam	0.21	8.50	0.99	5.1	108	-	1.03	31.1	8.53

^aOrganic matter was measured using the Walkley-Black (1934) method.

^bThe assimilable phosphorus was measured using the Olsen and Watanabe (1957) method.

^cPostassium and magnesium were extracted by ammonium acetate 1N at pH = 7 and analyzed by atomic absorption spectrophotometry.

^dThe nitrogen was measured using Kjeldahl analysis (Gerhardt Vapodest).

^eThe carbonates were measured using Bernard calcimeter method (Gaucher, 1971).

^fThe active limestone was determined by Nijelson method.

Table 3. Rootstock effect on trunk cross-sectional area (TCSA), cumulative yield, yield efficiency and fruit weight of ‘R.C. Bavay’ in the sixteenth year after budding (2011) and ‘R.C.T. Chambourcy’ in the fifteenth year after budding (2010).

Cultivar	Rootstock	TCSA (cm ²)	C.V. (%)	Cumulative yield (kg)	C.V. (%)	Yield efficiency (kg cm ⁻²)	C.V. (%)	Fruit weight ^a (g)	C.V. (%)
‘R.C. Bavay’	Adara	372.7 b	14.4	422.5 b	11.7	1.13 bc	21.7	44.1 a	18.2
	Ademir	485.2 c	13.5	300.2 a	9.3	0.62 a	13.0	44.3 a	18.6
	Mirobolán 713 AD	360.1 b	19.4	381.9 b	13.4	1.06 b	27.4	44.7 a	7.8
	Mirobolán B	257.6 a	6.3	276.4 a	17.5	1.07 b	21.2	43.8 a	34.8
	Miral 3278 AD	268.8 a	25.4	390.9 b	16.9	1.45 c	16.2	47.1 b	11.7
‘R.C.T. Chambourcy’	Adara	418.3 ab	10.8	265.5 a	16.9	0.65 a	20.2	38.5 ab	17.5
	Ademir	453.9 b	17.4	218.9 a	22.1	0.47 a	11.0	37.7 a	11.1
	Miral 3278 AD	321.2 a	16.2	304.5 a	30.7	0.98 a	40.2	40.3 b	4.56

Duncan test ($P \leq 0.05$) was performed. For the same cultivar, data followed by the same letter within a column are not significantly different.

^a Mean value obtained from 1999 to 2011 for ‘R.C. Bavay’ and from 1999 to 2010 for ‘R.C.T. Chambourcy’.

Abbreviation: C.V., coefficient of variance.

Table 4. Rootstock effect on fruit quality of the ‘R.C. Bavay’ and ‘R.C.T. Chambourcy’ plum cultivars.

Cultivar	Trait	Rootstock	2009	2010	2011	Average	C.V. (%)
‘R.C. Bavay’	FF	Adara	50.6 ab	74.8 a	59.8 a	61.7 a	17.3
		Ademir	49.5 ab	75.7 a	60.7 a	61.9 a	23.5
		Miral	51.1 b	76.6 a	61.6 a	63.1 a	17.9
		Myrobolan B	48.7 a	75.3 a	60.3 a	61.4 a	18.7
		Myrobalan 713	50.2 ab	75.2 a	60.2 a	61.9 a	17.5
	SSC	Adara	21.1 a	25.5 b	19.9 a	22.4 a	12.8
		Ademir	21.1 a	24.3 ab	20.2 a	21.9 a	9.7
		Miral	20.5 a	23.3 a	18.6 a	20.8 a	11.1
		Myrobolan B	20.9 a	25.5 b	15.4 a	20.9 a	29.1
		Myrobalan 713	20.3 a	26.2 b	20.6 a	22.4 a	13.8
	TA	Adara	0.57 a	0.68 a	0.60 a	0.61 a	11.6
		Ademir	0.58 a	0.66 a	0.62 a	0.62 a	8.9
		Miral	0.57 a	0.68 a	0.66 a	0.64 a	10.9
		Myrobolan B	0.58 a	0.69 a	0.63 a	0.63 a	11.1
		Myrobalan 713	0.57 a	0.65 a	0.63 a	0.62 a	11.5
‘R.C. Chambourcy’	FF	Adara	49.3 a	73.5 a	-	59.7 a	22.0
		Ademir	48.9 a	69.4 a	-	59.4 a	20.1
		Miral 3278 AD	50.3 a	75.2 a	-	62.7 a	22.5
	SSC	Adara	22.6 a	26.4 a	-	24.2 a	11.8
		Ademir	23.0 a	26.7 a	-	24.5 a	11.3
		Miral 3278 AD	20.5 a	25.6 a	-	22.7 a	15.1
	TA	Adara	0.57 a	0.64 a	-	0.59 a	8.3
		Ademir	0.56 a	0.64 a	-	0.60 a	10.0
		Miral 3278 AD	0.53 a	0.65 a	-	0.59 a	13.3

Duncan test ($P \leq 0.05$) was performed. For the same cultivar, data followed by the same letter within a column are not significantly different. *Abbreviations:* C.V., coefficient of variance (obtained from the average values); FF, flesh firmness; SSC, soluble solids content; TA, titratable acidity.

Table 5. Effect of rootstock on leaf chlorophyll concentration, measured as SPAD values, of ‘R.C. Bavay’ in the sixteenth year after budding (2011) and ‘R.C.T. Chambourcy’ in the fifteenth year after budding (2010).

Cultivar	Rootstock	SPAD	C.V. (%)
‘R.C. Bavay’	Adara	36.4 bc	9.3
	Ademir	35.2 bc	4.2
	Miral 3278 AD	37.8 c	15.9
	Myrobolan 713 AD	32.7 ab	5.3
	Myrobolan B	29.3 a	7.4
‘R.C.T. Chambourcy’	Adara	27.3 a	14.4
	Ademir	30.1 a	3.3
	Miral 3278 AD	31.2 a	5.6

Duncan test ($P \leq 0.05$) was performed. For the same cultivar, data followed by the same letter within a column are not significantly different. *Abbreviation:* C.V.: coefficient of variance.

Table 6. Rootstock effect on leaf mineral concentrations at 115 days after full bloom for ‘R.C. Bavay’ at the fifth (2000) and sixteenth year (2011) after budding and for ‘R.C.T. Chambourcy’ at the fifth (2000) and fifteenth year (2010).

Cultivar	Rootstock	N		P		K		Ca	
		2000	2011	2000	2011	2000	2011	2000	2011
‘R.C. Bavay’	Adara	2.8 b	2.0 a	0.17 ab	0.30 a	3.5 a	2.6 a	2.6 a	3.3 b
	Ademir	2.7 b	2.0 a	0.19 b	0.29 a	3.2 a	2.6 a	2.5 a	3.2 ab
	Miral 3278 AD	2.9 b	2.1 a	0.19 b	0.23 a	3.0 a	2.6 a	2.3 a	2.7 ab
	Myrobalan B	2.4 a	1.9 a	0.15 a	0.27 a	2.3 a	2.4 a	2.8 a	2.3 a
	Myrobalan 713	2.5 a	1.9 a	0.17 ab	0.20 a	3.1 a	2.5 a	2.9 a	3.1 ab
	<i>Optimum values^a</i>	2.4 - 3.0		0.14 - 0.25		1.6 - 3.0		1.5-3.0	
		Mg		Fe		Mn		Σ DOP	
		2000	2011	2000	2011	2000	2011	2000	2011
	Adara	0.60 a	0.49 a	90.0 a	120.4 a	66.4 a	35.8	-54.8 a	-42.0 a
	Ademir	0.61 a	0.54 a	67.8 a	109.0 a	61.0 a	39.0 b	-71.2 a	-63.5 a
	Miral 3278 AD	0.62 a	0.49 a	77.8 a	119.8 a	53.8 a	30.2 a	-77.5 a	-104.7
	Myrobalan B	0.78 b	0.49 a	77.8 a	173.6 a	74.8 a	30.2 a	-62.1 a	-82.8 a
	Myrobalan 713	0.71	0.42 a	70.5 a	145.8	59.8 a	34.8	-43.6 a	-99.6 a
	<i>Optimum values^a</i>	0.3 - 0.8		100 - 250		40 - 160		0	
Cultivar	Rootstock	N		P		K		Ca	
		2000	2010	2000	2010	2000	2010	2000	2010
‘R.C.T. Chambourcy’	Adara	2.6 a	1.5 a	0.15 a	0.22 a	3.0 a	2.1 a	1.9 a	1.1 a
	Ademir	2.6 a	1.6 ab	0.17 a	0.27 a	3.2 a	4.5 b	2.4 a	0.6 a
	Miral 3278 AD	2.7 a	1.7 b	0.16 a	0.24 a	2.8 a	3.1 ab	1.9 a	1.7 a
	<i>Optimum values^a</i>	2.4 - 3.0		0.14 - 0.25		1.6 - 3.0		1.5-3.0	
		Mg		Fe		Mn		Σ DOP	
		2000	2010	2000	2010	2000	2010	2000	2010
	Adara	0.46 a	0.37 a	78.8 a	96.6 a	60.5 b	32.8 a	-144.1	-211.8
	Ademir	0.56 a	0.29 a	76.5 a	75.7 a	55.3	32.2 a	-97.2 a	-161.1
	Miral 3278 AD	0.47 a	0.39 a	78.8 a	101.1 a	49.3 a	43.7 a	-154.3	-118.3
	<i>Optimum values^a</i>	0.3 - 0.8		100 - 250		40 - 160		0	

Duncan test ($P \leq 0.05$) was performed. For the same cultivar, data followed by the same letter within a column are not significantly different. ^a Reuter et al. (1997).

Figure 1. Tree mortality rate (%) from the fourth (1999) to the last year after planting in the orchard trial (2011 for 'R.C. Bavay' and 2010 for 'R.C.T. Chambourcy').

Figure 2. Effect of rootstock on trunk cross-sectional area (TCSA) of 'R.C. Bavay' (A) and 'R.C.T. Chambourcy' (B) plum cultivars during 16 and 15 years of study, respectively. Vertical lines indicate LSD ($p \leq 0.05$).

Figure 3. Principal component biplot for agronomic and fruit quality characteristics of 'R.C. Bavay' and 'R.C.T. Chambourcy' budded on Adara, Ademir, Miral 3278 AD, Myrobalan B and Myrobalan 713 AD rootstocks. *Abbreviations:* CY, cumulative yield; FF, flesh firmness; FW, fruit weight; SSC, soluble solids content; TA, titratable acidity; TCSA, trunk cross sectional area; TS, tree survival.