

Comparison of pepper accessions acting as rootstocks: A case with low P inputs

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Abstract

Fertilization is essential for maintaining production in agriculture. Yet, in too high quantity it causes high impact in environment and in farmers economy. This is especially true in the case of phosphorus (P) fertilization. Finding genotypes adapted to low P conditions may help to reduce the problem. P efficiency depends to some extent on the ability of the roots to acquire this mineral, therefore using efficient rootstocks would provide a higher acquisition of P, maintaining the good characteristics of the scion varieties. In this study, twenty diverse pepper accessions (*Capsicum annuum* L.) have been evaluated as possible rootstocks to increase P acquisition and yield in pepper under no P fertilization. Plant production, biomass, P content and physiological phosphorous use efficiency were evaluated for 'Lobo' variety grafted onto different rootstocks. In addition, root traits, measured manually (shovelomics) and semi-automatically (Winrhizo) were studied. The results showed a great diversity in the root traits for the studied accessions. These root traits changed significantly when the accessions act as rootstock, indicating great rootstock/scion interactions. In general, all the rootstocks adapted their root size and shape to that displayed by 'Lobo' root system. Some accessions seemed to have some incompatibility whereas some others enhanced the scion performance. It was possible to identify some genotypes suitable to act as rootstocks for pepper with good performance under low P conditions. Root length, root weight, branching, and root angle were identified as key root traits for plant growth and P acquisition under low P conditions.

Keywords: breeding; grafting; incompatibility; low input systems; shovelomics; root architecture

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Introduction

In the last 10 years awareness of agriculture's impact on the environment has increased. The agricultural model based on optimizing the plant's environment (substrate, water, light, limited presence of pests and diseases, etc.) to obtain higher production regardless of the inputs or possible side effects is no longer valid. Especially in the context of degraded ecosystems, eutrophication of water bodies and loss of soil fertility (Howden *et al.*, 2007). A paramount example of this is the plant fertilization with phosphorus (P). This mineral is the second most limiting nutrient for plant growth after nitrogen. Many worlds' agricultural areas are rich in this element but, ironically, due to the soil high potential for phosphorus retention (Batjes, 2011; Lynch, 2011), this mineral is not easily available for the crops. Therefore, it is necessary to add P to the soil. This is usually done in mineral fertilization form. Unfortunately, P fertilization is not cheap for the farmers, nor clean for the environment. In one hand, P fertilizers are produced from the phosphoric rock deposits, which like oil, are finite, and unevenly distributed throughout the planet (Fafu *et al.*, 2021; Brownlie *et al.*, 2022). The period of exhaustion of this resource will depend on its use rate and the magnitude of the estimated reserves (the main ones are in North Africa and China), but the latest estimations indicate a reserve of approximately 72 billion metric tons (Statista, 2024). As it happens with other non-renewable resources, in latest years there has been an escalation in the price of phosphoric rock, which has increased the price of fertilizers. On the other hand, the fertilization is not always managed professionally. Mineral P fertilization disrupts the biological networks of the microorganism of the soil affecting in the long term the fertility of the soil (Zhang *et al.*, 2022; Morales-Manzo *et al.*, 2023). In addition, P over-fertilization leads to pollution and eutrophication of water bodies (Ge *et al.*, 2023). Fortunately, in the latest years several strategies to reduce the amounts of P added to the soil have been identified, in an effort to move from a high input agriculture to a low input one without losing yield (Brownlie *et al.*, 2022; Penuelas *et al.*, 2023). Some of the strategies are: i) improving the localization of the fertilizers and adjust them (Bindraban *et al.*, 2015), ii) improving the soil fertility (Möller, 2018), iii) using micorrryzaion (Wen *et al.*, 2019) and iv) taking advantage of the unexploited genetic potential of plants to acquire and use phosphorus (van de Wiel *et al.*, 2016) and improve their root development (Lynch, 2022) either by improving the crop root system directly (Ribeiro *et al.*, 2023) or improving the root system of a suitable rootstock and using grafting (Gao *et al.*, 2023).

Plant roots are directly in contact with the soil and therefore have a direct impact on the efficiency of the plant to acquire this mineral. Strategies identified as special adaptations of roots to low P systems can be divided in: i) those related to root architecture and morphology, ii) those related to Pi transporters, and iii) those related to mobilization of insoluble P through root exudates (Chen *et al.*, 2023). Regarding the adaptations in root architecture and morphology it has been described that shallow and branched root systems can explore the root layers rich in P (Williamson *et al.*, 2001; Lambers *et al.*, 2006; Lynch, 2019; Kirchgesser *et al.*, 2023). In legumes a growth of primary root and lateral roots are stimulated during P deficiency, in addition to an increase in the number and length of root hairs which facilitates greater Pi uptake (Chen *et al.*, 2023). This root plasticity is controlled genetically, therefore improving root architecture and morphology through phenotypic selection can be used to identify or generate low P input tolerant varieties.

Vegetable grafting techniques have been known for centuries and have been widely used since the 1920s. They have proven to be useful to avoid soil-borne diseases (biotic stress) (Louws *et al.*, 2010). But also, to avoid abiotic stresses, such as salinity, changes in temperatures, or lack of water or nutrients (Rouphael *et al.*, 2017; Ropokis *et al.*, 2019). The grafting potential is as broad as the genetic variability capable of being combined. However, rootstock/scion interactions determine the positive or negative influence of the rootstocks on the yield of the plant and the quality of the fruit of the grafted variety. Unfortunately, there is little information about the factors involved in the success of a certain combination (Tsaballa *et al.*, 2021). Therefore, it is difficult to know in advance which combination rootstock/scion are going to be optimal. One of the most discouraging

problems is the existence of incompatibilities between the rootstock and the variety of interest. Therefore, understanding the mechanisms of rootstock x variety interactions in different environments will contribute to developing rootstocks more suitable for the objectives of each moment.

Pepper (*Capsicum* spp.) is one of the most cultivated vegetables in the world. Its relevance has increased in recent decades and, consequently, its harvested area and its production volume have increased during that same period. Likewise, the average yield has been growing, because of the adoption of the latest technological advances, such as acclimatized greenhouses, higher inputs, and the introduction of improved varieties. In 2022, almost 37 million tons were produced in a dedicated area of 2 million hectares, with an average yield of 18.3 t ha⁻¹, although the most technified agricultures reach as much as 90 t ha⁻¹ (FAOSTAT, 2023). Recently several pepper varieties have been evaluated by its response to P deficiency (Pereira *et al.*, 2020). However, there is no information on their potential as rootstock or the interaction rootstock/graft. In this study, accessions of pepper are evaluated as possible rootstocks, to investigate the root characteristics more favourable to P acquisition, and the rootstock/scion interaction.

Materials and Methods

Plant material

For the study, 20 accessions of pepper (Table 1) were chosen according to criteria of diversity and better adaptation to phosphorus deficiencies, observed in previous studies (Pereira *et al.*, 2020). Among the varieties used there were traditional varieties, experimental lines and commercial hybrids. All these accessions were used as rootstocks for a commercial pepper called 'El lobo' (Zeraim Ibérica) to check their efficacy under conditions of low phosphorus fertilization.

Table 1. Name, type and origin of each of the accessions tested in the experiments

Code	Variety	Type	Origin
204D	'204D Serrano'	Traditional variety	Mexico, Aguascalientes
96D	'96D-Ancho Poblano'	Traditional variety	Mexico, Aguascalientes
Serra	'Serrano criollo'	Experimental line	Mexico
Ají	'Ají Cacho Cabra'	Traditional variety	Peru
BGV1	'BGV-11814'	Traditional variety	Spain, León
BGV4	'BGV-4349'	Traditional variety	Spain, Cartagena (pimiento morro de vaca)
BGV6	'BGV-60'	Traditional variety	Spain, Zamora (morrón de bola)
Bola	'Bola'	Traditional variety	Spain, Murcia (ñora DOP Pimentón)
Piq	'Piquillo'	Traditional variety	Spain, Navarra
Doux	'Doux Long des Landes'	Traditional variety	France (INRA-GEVES, F. Jourdan)
Petit	'Petit Marsellaise'	Traditional variety	France (INRA-GEVES, F. Jourdan)
NuC	'Numex Conquistador'	Traditional variety	USA, New Mexico
NuG	'Numex Garnet'	Traditional variety	Mexico, Aguascalientes
NuS	'Numex Sandía'	Traditional variety	USA, New Mexico
Nu	'Numex'	Traditional variety	USA, New Mexico
Anc	'Ancares'	Commercial hybrid	Spain (Ramiro Arnedo)
Melchor	'Melchor'	Commercial hybrid	Spain (Ramiro Arnedo)
Oscos	'Oscos'	Commercial hybrid	Spain (Ramiro Arnedo)
Lobo	'El Lobo'	Commercial hybrid	Spain (Zeraim Ibérica)
Cat	'Catedral'	Commercial hybrid	Spain (Zeraim Ibérica)

Experimental design

Seeds were sterilized with a 30% bleach solution (v: v) for five minutes, followed by several rinses with water. Once seeds were disinfected, they began to germinate in Petri dishes with 2% thiram tetramethylthiuram disulphide (Adama agriculture España S.A, Spain) solution to avoid fungal growth. Seeds were incubated in a germination chamber at 70% humidity and 26 °C until two-cotyledon stage. Seedlings were then transferred to seedling trays and kept under nursery conditions until the five leaves stage. Some of them were directly transplanted to the mesh greenhouse whereas other were submitted to grafting. Lobo was used as variety grafted onto each of the varieties of the study (Table 1) in addition, a self-graft Lobo/Lobo was also performed. Graft was done with the splice grafting technique in which the scion is joined onto the stem of a rootstock. In splice grafting, both the stock and scion must be of the same diameter and stems are cut in a diagonal, then joined together and secured with rubber. Grafted plants were placed at dark and 90% humidity for 1 week and then acclimated as needed. Plants were grown in a mesh greenhouse, during the spring-summer cycle, on COMAV experimental area (Universitat Politècnica de València Vera Campus). All plants were drip irrigated with a solution without P as in Pereira *et al.*, 2020, where the concentrations for anions were: NO_3^- 15.55 mM; H_2PO_4^- 0 mM; SO_4^{2-} 2.45 mM; HCO_3^- 3.10 mM; Cl^- 1.61 mM; and for cations: NH_4^+ 1.5 mM; K^+ 6.5 mM; Ca^{2+} 4.75 mM, Mg^{2+} 1 mM.

Grafted plants were set in a randomized block design with two blocks of 3 plants per block. ungrafted plants were in a randomized design with 3 replicates. After 60 days of growth, all plants per genotype and treatment (6 plants for grafted and 3 for ungrafted) were harvested and evaluated. Fruits were harvested and weighted (fresh, Prod), aerial parts were cut at a 15 cm height over the ground and weighted (fresh, SFW and dry, SDW). To remove the roots from the soil, water supply was cut off 3 days prior to removal to avoid muddy soil which can hamper the extraction of the roots by breaking them. Then, with the help of a shovel, the roots were extracted from the soil in a cube area of 40×40×40 cm approximately. Finally, the soil at the rhizosphere was carefully removed by a soft shaking, trying to keep the roots intact. The remaining soil was washed out from the root system with tap water. Root systems were scored manually for their morphology, as in shovelomics approach (Trachsel *et al.*, 2011). Root angle (Ang, °) was measured as the maximum aperture of the root (Figure 1), maximum length (L_{max} , cm), was measured as the maximum length of the longest lateral root, total length (L_{total} , cm) was calculated as the sum of the four longest lateral roots, number of lateral roots with diameters higher than 0.2 mm ($N_{\text{lat}>0.2}$), D (Diameter, mm) was the average of the diameters of L_{max} , L1, L2 and L3, secondary density (SD) was evaluated as a scale from lowest (1) to highest (5) density of secondary roots, according to the distance between the lateral branches and tertiary density (TD) was measured according to a scale from lowest (1) to highest (4) (Figure 1). The root systems were separated into stump (Figure 1) and lateral roots, both were weighted in fresh: fresh stump weight (StFW) and lateral roots fresh weight (LRFW), and after drying them in an oven for 72 h at 60 °C, to obtain dry stump weight (StDW) and dry lateral weight (LRDW). Total fresh biomass of the plant was calculated as:

$$\text{BF (g)} = \text{Prod} + \text{SFW} + \text{StFW} + \text{LRFW},$$

whereas dry biomass was calculated omitting the fruit load, therefore:

$$\text{BD (g)} = \text{SDW} + \text{StDW} + \text{LRDW}.$$

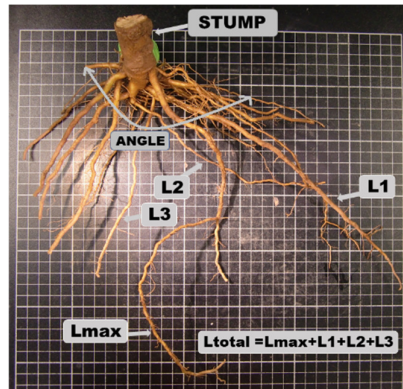


Figure 1. Manual measurements made on the roots

Before drying them, fresh lateral roots were scanned with Epson Expression 1640XL G650C scanner (Seiko Epson Corp., Suwa, Japan) and pictures were analysed with WinRIZHO™ Pro 2.3 software (Regent Instruments Inc., Canada). The parameters measured with WinRIZHO™ were: Root length (L, cm), root projected area (Pa, cm²), root volume (V, cm³), root average diameter (AD, mm), tips (T), forks (F), and length of roots with diameter under 1.5 mm ($L_{\leq 1.5}$, cm), length of roots with diameter between 1.5 mm and 3.5 ($L_{\leq 3.5}$, cm), and length of roots with diameter above ($L_{> 3.5}$, cm).

At the harvest time, leaves samples were taken to analyse the P content. Leaves' P-concentration, [P], was determined by colorimetric reaction (MAPA, 1994). This method is based on absorbance measurement at 430 nm of each sample in acid solution and on the presence of vanadium (V^{5+}) and molybdenum (Mo^{6+}) ions. Under these conditions, phosphoric acid forms a phosphomolybvanadate complex that gives yellow coloration. Hence, 5 mL of mineralized solution were pipetted into a new 25 mL volumetric flask, followed by addition of 5 mL of nitro-vanado-molybdic reagent. Volume was then brought up to 25 mL with distilled water. Prior to mineral concentration determination, a standard curve was constructed with standards 0, 2, 4, 6, 8, 10 y 12 μg of P mL⁻¹ prepared from an initial solution of 20 μg of P mL⁻¹. Sample P concentration was determined using a 6305 model UV/V spectrophotometer (Jenway, Gransmore Green, England, UK) at 430 nm against standard curve. Total amount of P in the plant was inferred as:

$$P_{\text{total}} = [P] \times \text{BD} \text{ (mg P)}$$

And physiological P use efficiency

$$\text{PPUE} \text{ (g}^2 \text{ DW mg}^{-1} \text{ P)} = \text{SDW} / [P]$$

Statistical analysis

One-way analysis of variance (ANOVA) was performed using individual plant values to assess accession differences. In addition, Student-Newman-Keuls post-hoc multiple range test ($p < 0.05$) was used to detect significant differences among accession means for all evaluated traits. Pearson correlations were calculated among different traits. A multivariate principal component analysis (PCA) was performed for standardized data of all root and plant traits using pairwise Euclidean distances among means. All statistical analysis were performed using Statgraphics Centurion XIX (StatPoint Technologies, Warrenton, VA, USA).

Results

Root performance of different pepper genotypes

To explore differences at root level among the accessions assayed, the extracted roots from control plants (not used as rootstocks) were analysed. The ANOVA analysis over the shovelomic traits showed significant

differences among the studied accessions for all studied traits but root angle and tertiary density (Table 2). Some genotypes such as ‘204D Serrano’, ‘96D ancho poblano’, ‘Ají cacho cabra’, ‘Numex Garnet’, ‘Oscos’ and ‘Serrano criollo’ showed greater stumps, over 44 g in fresh weight and 12 g in dry weights, than the others (Supplementary table 1). For fresh lateral root weight (LRFW), the multiple range test separated genotypes in three groups, those having light lateral roots (aprox 12 g), such as ‘BGV1814’, ‘BGV4349’, ‘Numex conquistador’, ‘Numex sandía’, ‘Numex X’ or ‘Petit marsellaise’; those with intermediate LRFW which were the majority of genotypes, and then ‘Numex Garnet’ (38.72 g), ‘Serrano Criollo’ (57 g) and ‘Aji cacho cabra’ (51.8 g) which outstood because of their heavier lateral roots (Figure 2). This classification was similar for LRDW (Supplementary Table 1).

Great significant differences were observed for the number of laterals roots with diameter higher than 2 mm (Figure 2) In this case two varieties: ‘Petit Marsellaise’ and ‘Doux Long des Landes’, were the ones with lower number (4 and 5.5) then, the majority of genotyps had from 7 to 16 and finally two genotypes outstood, ‘BGV-60’ with 19 and ‘Ají Cacho Cabra’ with 22.67.

Finally, there were significant differences in terms of diameter. The ones with smaller diameters were ‘Bola’, ‘Numex Sandía’ and ‘Petit Marsellaise’ ranging from 2.17 mm to 2.63, followed by ‘BGV-4349’ and ‘Piquillo’. The genotypes displaying larger root diameters, were ‘Ají Cacho Cabra’, ‘Ancares’ and ‘Serrano Criollo’, ranging from 5.25 to 2.67 mm (Figure 2). In interestingly the L_{total} showed less differences among genotypes (Figure 2), probably due to the sampling effect, as the root was excavated to certain extend therefore the whole root system was not recovered. For L_{total} ‘BGV-11814’ and ‘Bola’ showed the shortest roots, ‘Ancares’ and ‘Ají cacho cabra’ showed the longest roots.

In summary, the varieties with more developed roots, greater weight, length, thickness and number of lateral roots were ‘Numex Garnet’, ‘Ancares’, ‘Ají Cacho Cabra’ and ‘Serrano Criollo’. Whereas the smallest roots were: ‘BGV-11814’ in length, ‘Bola’ in length and diameter, ‘Doux Long des Landes’ in number of lateral roots, ‘Petit Marsellaise’ in number of fine roots and diameter, and ‘Numex Sandía’ in diameter.

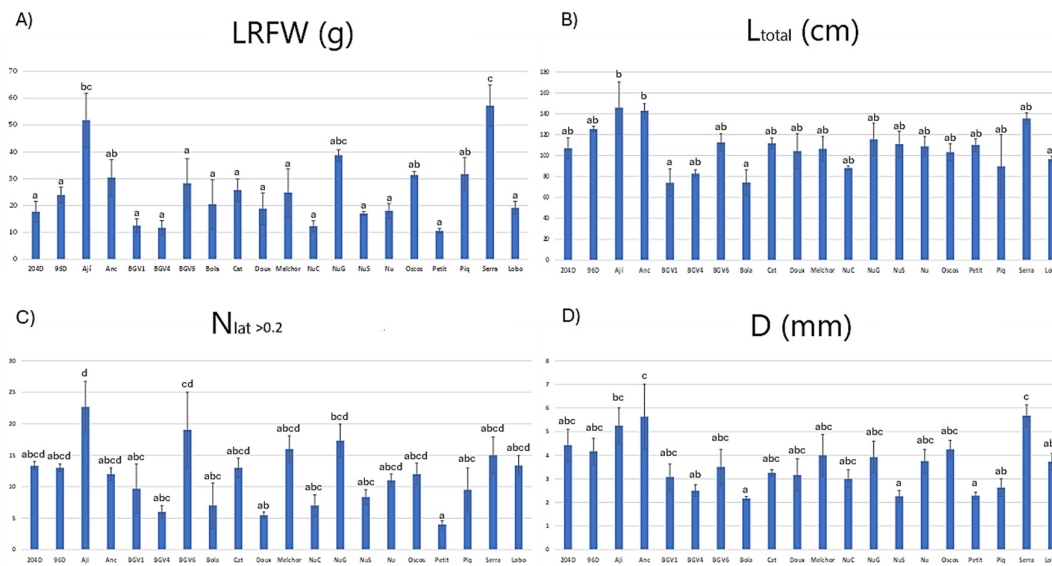


Figure 2. Variations in fresh lateral weight (FLW, g) (A), Total length of the root as the sum of the three longest roots (L_{total} , cm) (B), $N_{Lat>2}$ (C), D (D) Bars represent means (n = 3) with standard errors. Different letters indicate significant differences between genotypes according to the multiple range test with the Student-Newman-Keuls method at a p-value ≤ 0.05.

Table 2. Analysis of variance of root parameters of intact plants

MS											
Effect	df	StFW	StDW	LRFW	LRDW	Ang ²	L _{total} ³	N _{lat>2} ²	D	SD ²	TD ⁴
Genotype	19	605.9**	70.45**	477.9***	13.82***	741.7 ^{ns}	1130.2**	64.19***	3.12*	2.36**	0.32 ^{ns}
Residual	44	262.7	26.3	86.33	2.16	604.3	447.3	16.97	1.69	0.85	0.35

The columns show the mean square values (MS) for stump fresh weight (StFW, g); dry stump weight (StDW, g), lateral root fresh weight (LRFW, g), lateral root dry weight (LRDW, g), root angle (ANG, °); total root length (L_{total}, cm); number of lateral roots with diameter higher of 2 mm (N_{lat>2}), average diameter of the four longest roots (D, mm), secondary roots density (SD) and tertiary roots density (TD).

¹df, degrees of freedom, ^{ns}, *, **, and *** indicate, respectively, non-significant, and significant at P ≤ 0.05, ≤0.01, or ≤0.001, ^{2,3,4} in this cases the residual degrees for freedom were 41, 42 and 40 respectively.

The results of the ANOVA for the roots of the control plants analysed with WinrhizoTM were like those obtained with shovelomics. In this case, all studied traits: root length, root projected area, root average diameter, root volume, tips, forks, and root length of different diameters showed significant differences among the accessions (Table 3). When comparing the displayed values for root length (Figure 3) it was possible to observe accessions with short roots such as ‘Numex conquistador’ (1658 cm) and ‘BGV11814’ (1843 cm) and the majority of accessions, intermediate accessions as Ocoscos (5367 cm) and Catedral (4722 cm), accessions with high root length as ‘Aji cacho cabra’ (6168 cm) and ‘Numex Garnet’ (6485 cm) and very long roots systems as ‘Piquillo’ (8351 cm) and ‘Serrano criollo’ (9225 cm). This classification was similar for root projected area and root volume, tips and forks (Supplementary table 2).

The diameters were also variable among genotypes but with different pattern, the thinnest roots were displayed by ‘Petit Marsellaise’ (0.66 mm in average), whereas the genotypes with thickest roots displayed averages close to 1 mm (Figure 3). The partitioning of the root length regarding the different diameter classes revealed that most of the root length was due to fine roots (less than 1.5 mm in diameter), therefore the classification was the same for L than L_{<1.5} (Figure 3). The situation changed with the length of roots with diameter between 1.5 mm and 3.5 in this case ‘Aji cacho cabra’ (954 cm) outstood (Figure 3). In addition, ‘Aji cacho cabra’ also showed, along with ‘Serrano criollo’ a high length of thick roots (higher than 3.5 mm in diameter; Figure 3).

Table 3. Analysis of variance of root parameters of intact plants.

MS										
Effect	df	L	Pa	AD	V	T	F	L<1.5	L≤3.5	L≥3.5
Genotype	19	1.29·10 ⁷ ***	9.16·10 ⁴ ***	0.018***	452***	2.59·10 ⁷ ***	1.20·10 ⁸ ***	1.04·10 ⁷ ***	1.62·10 ⁵ ***	996***
Residual	44	2.52·10 ⁶	1.73·10 ⁴	0.002	81	4.78·10 ⁶	2.45·10 ⁷	2.04·10 ⁶	3.1·10 ⁴	292

The columns show the mean square values (MS) for Root length (L, cm), root projected area (Pa, cm²), root volume (V, cm³), root average diameter (AD, mm), tips (T), forks (F), and length of roots with diameter under 1.5 mm (L_{<1.5}, cm), length of roots with diameter between 1.5 mm and 3.5 (L_{≤3.5}, mm), and length of roots with diameter above (L_{>3.5}, cm).

¹df, degrees of freedom, ^{ns}, *, **, and *** indicate, respectively, non-significant, and significant at P ≤ 0.05, ≤0.01, or ≤0.001.

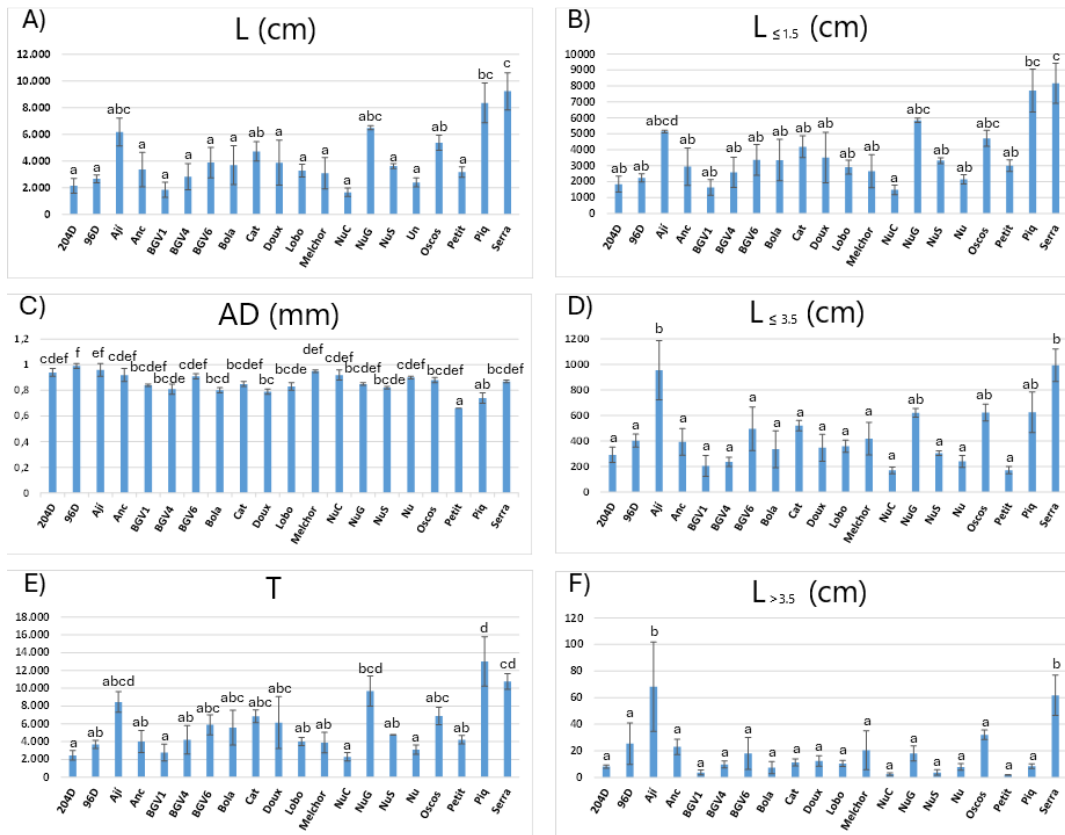


Figure 3. Variations in: A) root length (L, cm), B) Average root diameter (AD, mm), C) number of tips, D) length of roots of different diameter. Bars represent means (n = 3) with standard errors. Different letters indicate significant differences between genotypes according to the multiple range test with the Student-Newman-Keuls method at a p-value ≤ 0.05.

Rootstocks root architecture

To explore if the differences observed at root level among the accessions assayed were maintained when the accessions act as rootstock for ‘El Lobo’ variety, an ANOVA test was performed among rootstocks, and between root ‘use’, that is, comparing the accession root system without graft (control) and when it is acting as rootstock.

Again, the accessions displayed a wide range of values for root parameters when they act as rootstock (Supplementary Tables 1 and 2). Interestingly the results showed that, in some accession, root parameters undergo significant changes when they act as rootstocks in comparison to the same genotype as intact plant. For instance, some genotypes reduced their root size. That was the case of ‘Aji cacho cabra’ and ‘Serrano criollo’ which reduced FS_tW, DS_tW, LRFW, LRDW, D, L, Pa, T, and F, with the difference of ‘Serrano criollo’ also reducing Angle, L_{max}, and L_{total} but not the N>2 mm and ‘Aji cacho cabra’ just the contrary (Supplementary Tables 1 and 2). ‘96-D Ancho poblano’, ‘Doux Long des Landes’ and ‘Ocos’ also reduced FS_tW and DS_tW. In addition, ‘96-D Ancho poblano’ also reduced L_{max}, L_{total}, AD and D and ‘Doux Long des Landes’ also reduced L_{max}. Other genotypes change only few parameters when acting as rootstock, that was the case of ‘Numex Garnet’ reducing LRFW and AD, ‘Catedral’ only reducing root volume, ‘BGV 60’ reducing L_{total} and ‘Ancares’ reducing D (Supplementary Tables 1 and 2).

Interestingly other genotypes modify their root systems when acting as rootstock but increasing its size. That was the case of ‘Numex Conquistador’ which increased L_{max}, L_{total}, L, Pa, Tips, Forks and increased the number of thin roots (also decreased AD). Other effects observed were Bola increasing L_{total}, ‘Piquillo’

increasing the AD, 'Petit Marseallise' increasing LRFW and LRDW, $N_{\text{lat} > 2}$ and AD, and 'Numex Sandia' increasing D (Supplementary Tables 1 and 2).

To understand better the changes of the roots when acting as rootstock a principal component analysis was done. The first principal component explained 56.04% of the variation and was represented mainly by LRFW, LRDW, L, Pa, V, $L_{>3.5}$ (Table 4), the second principal component explained 16.95% of the variation and was represented by AD, D, F, $N_{\text{lat} > 2}$ (Table 4), whereas the third principal component explained only 6.13% of the variation and was represented negatively by Angle, $N_{\text{lat} > 2}$ and positively by L_{max} , L_{total} , and TD (Table 4). When projecting the accessions on the principal component space they were scattered. The intact roots were more disperse than the same root accessions acting as rootstocks which seemed to get close to 'Lobo' variety (Figure 4). As seen in the supplementary tables 1 and 2 the general trend was for the bigger genotypes to get reduced and more similar to the 'Lobo' root system, and for the smaller accessions increase its size to become also similar to 'Lobo' root system.

Table 4. Principal component loads for the evaluated root traits

Trait	PC1	PC2	PC3
L	0.26	-0.22	-0.09
Pa	0.28	-0.12	-0.09
AD	0.03	0.46	-0.11
V	0.27	0.01	0.00
T	0.25	-0.24	-0.10
F	0.23	-0.29	-0.04
$L_{\leq 1.5}$	0.25	-0.24	-0.09
$L_{\leq 3.5}$	0.28	0.04	-0.09
$L_{> 3.5}$	0.23	0.24	0.08
StFW	0.22	0.12	0.01
StDW	0.22	0.16	-0.02
LRFW	0.28	0.07	-0.02
LRDW	0.25	0.16	0.06
Ang	0.12	0.21	-0.40
L_{max}	0.19	0.03	0.46
L_{total}	0.18	0.17	0.44
$N_{\text{lat} > 2}$	0.16	0.25	-0.31
D	0.16	0.36	0.07
SD	0.23	-0.07	-0.04
TD	0.08	-0.04	0.51

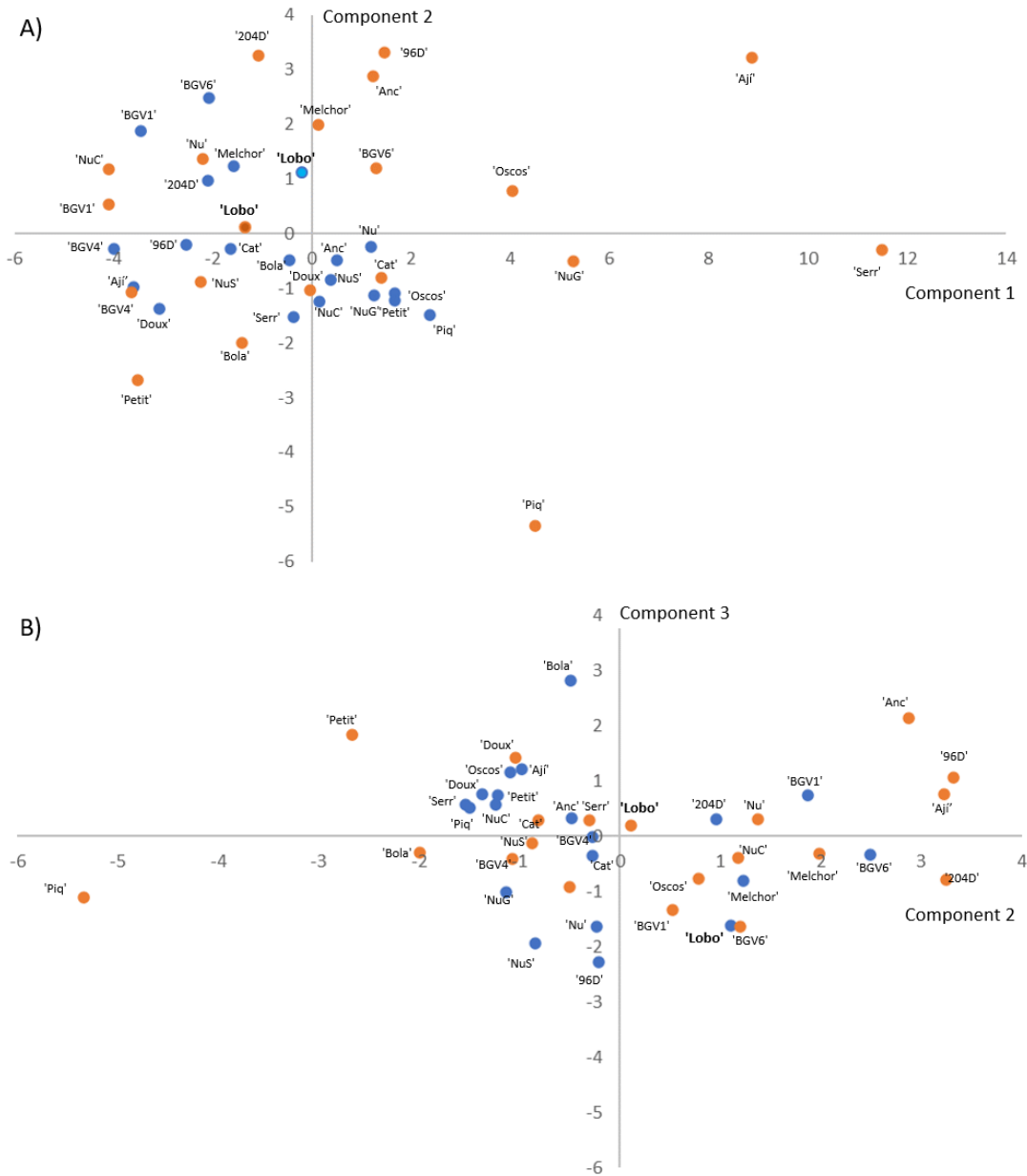


Figure 4. Principal component analysis of the root systems of different accessions of pepper as plants (control in orange) or acting as rootstock (blue) for ‘Lobo’ variety acting as scion. A) Projection principal component 1 and 2, B) projection principal component 2 and 3.

Rootstock effect onto the Lobo variety production and P content

Regarding the previous results, it was clear that there was diversity in terms of root development among accessions, both in intact plants and acting as rootstock. To study whether those differences accounted for differences in the P acquisition or plant production, a one-way ANOVA was performed to check the effect of each root accession acting as rootstock for ‘Lobo’ (Table 5).

Only few rootstocks affected the performance of ‘Lobo’ when it was grafted. The accession ‘BGV-4349’ produced a significant reduction in total production, dry aerial part, fresh biomass, dry biomass, and PPUE. Also, the accession ‘BGV-60’ produced a significative reduction of total production. On the contrary, the

accession ‘Numex X’ produced a significant increase in dry biomass and a very significant increase in the total P content in the plant, and showed a tendency to increase other ‘Lobo’ parameters despite the differences in this experiment were non-significant. ‘Oscos’ accession also produced a significant increase in the total P content in the plant. Interestingly, the concentration of P in the leaves was 488 mg P/100 g DW in ungrafted ‘Lobo’ and was not significantly different from the concentration when it was grafted, although ranging from 441 mg P/100 g DW when Doux long des landes acts as rootstock to 566 mg P/100 g DW when Oscos was the rootstock (Table 5).

Table 5. Averages and standard deviations for shoot evaluated traits of Lobo control plants (first row) and Lobo plants grafted in different rootstocks

Var	Rootstock	Prod	SFW	SDW	BF	BD	[P]	P _{total}	PPUE
‘Lobo’	--	777±109	308±46	46.1±6.2	1130±135	53.7±7.0	488±27	253 ± 28	10.07±1.74
	‘Lobo’	595a±162	293ab±60	44.8ab±7.7	936ab±215	54.6ab±8.6	474a±31	256a ± 41	9.78a±1.97
	‘204D’	637a ±122	278ab±40	39..2ab±6.1	958ab±147	47.5ab±7.1	460a±73	209a ± 40	9.95a±2.38
	‘96’	531a±134	182a±36	30.2ab±5.0	746ab±173	35.3a±6.1	563a±69	198a ± 35	5.73a±1.07
	‘Aji’	551a±154	246ab±36	37.0ab±5.6	816ab±154	41.0ab±6.0	513a±44	209a ± 32	7.51a±1.42
	‘Anc’	745a±92	278ab±20	42.2ab±4.1	1062ab±104	48.9ab±4.4	530a±54	253a ± 25	8.60a±1.53
	‘BGV1’	503a±94	240ab±40	32.4ab±5.2	770ab±111	36.9a±5.8	508a±43	194a±38	6.31a±0.92
	‘BGV4’	390a±35**	200a±17	28.3a±2.8*	615a±35***	32.5a±3.3*	536a±37	178a±27	5.31a±0.42*
	‘BGV6’	397a±80*	321ab±28	45.8ab±5.5	759ab±93	52.0ab±6.1	507a±24	266a±41	9.05a±0.92
	‘Bola’	545a±146	200a±30	27.3a±2.5	794ab±127	35.7a±3.1	520a±66	185a±26	5.56a±0.89
	‘Cat’	479a±121	266ab±65	38.3ab±9.2	783ab±187	44.9ab±10.4	499a±58	206a±35	9.01a±2.96
	‘Doux’	490a±83	289ab±51	41.0ab±6.8	817ab±133	47.5ab±8.0	441a±45	222a±54	9.24a±1.27
	‘Melchor’	735a±59	328ab±24	41.2ab±5.3	1106ab±67	48.8ab±5.8	508a±34	245a±30	8.45a±1.54
	‘NuC’	671a±136	366ab±85	51.5ab±12.9	1092ab±194	60.4ab±15.2	532a±32	322a±91	9.91a±2.44
	‘NuG’	876a±154	337ab±66	46.3ab±9.3	1266ab±190	56.1ab±11.7	504a±26	294a±75	8.99a±1.47
	‘NuS’	921a±221	291ab±31	42.0ab±4.1	1266ab±217	52.2ab±6.5	443a±28	230a±28	9.64a±1.09
	‘Nu’	969a±259	462b±76	63.8b±7.1	1492b±314	76.7b±6.7*	503a±29	387a±43**	12.94a±1.83
	‘Oscos’	725a±57	373ab±10	51.7ab±3.3	1158ab±64	61.4ab±2.7	566a±30	346a±18*	9.30a±0.88
	‘Petit’	782a±93	326ab±27	46.7ab±3.6	1162ab±74	56.5ab±4.0	513a±32	290a±29	9.19a±0.64
	‘Piq’	509a±140	346ab±72	46.2ab±10.7	906ab±211	54.0ab±11.9	514a±41	263a±49	9.69a±2.59
‘Serra’	512a±67	249ab±36	36.3ab±4.7	807ab±88	43.1ab±5.2	536a±33	236a±35	6.73a±0.74	

Fruit production (Prod, g), shoot fresh weight (SFW, g), shoot dry weight (SDW, g), Biomass fresh weight (BF), Biomass dry weight (BD), shoot phosphorous concentration ([P], mg P/100 g DW), total phosphorous in the plant (P_{total}, mg) and physiological phosphorous use efficiency (PPUE, g²DW mg⁻¹ P).

Mean values in the columns followed by different letter are significantly different according to the Student-Newman-Keuls multiple range analysis with P-value < 0.05. * Significantly different from ungrafted Lobo control at p-value 0.05, ** at p-value 0.001, *** at p-value according to the Dunnett test.

Identification of possible root parameters affecting plant performance and P acquisition

To check if there is any root parameter specially involved in the acquisition of P, Pearson correlations were calculated among the root parameters and the productivity and phosphorus related traits. Root length, root projected area, root forks, root length of all roots under 3.5 mm in diameter, stump dry weight and lateral root dry weight were significantly correlated (values ranging from 0.49 to 0.79) with fruit production, shoot weight, dry biomass, P total and PPUE (Table 6). Other parameters such root volume, tips, SD were correlated only with SDW, BD and P_{total}. Interestingly any of the root parameters were correlated with the concentration of P in the leaves. Winrhizo parameters seemed to be better as predictor for the plant’s performance than

manual measurements on the roots, nevertheless root angle was correlated with fruit production and $N_{lat>2}$ was correlate to BD and P total. Surprisingly TD was negatively correlated with production.

Table 6.- Statistical correlations and significance shoot and root parameters

	L	Pa	AD	V	T	F	L_{≤1.5}	L_{≤3.5}	L_{>3.5}
Prod	0.70***	0.65**	-0.29	0.21	0.65**	0.61**	0.70***	0.49*	0.08
SDW	0.63**	0.63**	-0.12	0.55*	0.54*	0.56*	0.62**	0.60**	0.32
BD	0.68***	0.69***	-0.14	0.56*	0.58**	0.60**	0.67**	0.65**	0.33
[P]	0.06	0.06	-0.12	0.4	0.08	-0.03	0.05	0.1	-0.14
P _{total}	0.69***	0.68***	-0.18	0.65**	0.60**	0.59**	0.68***	0.64**	0.29
PPUE	0.53*	0.55*	-0.07	0.38	0.43	0.48*	0.53*	0.52*	0.29
	StDW	LRDW	L_{max}	L_{total}	D	Ang	N_{lat>0.2}	SD	TD
Prod	0.75***	0.61**	0.34	0.38	0.27	0.49*	0.35	0.36	-0.45*
SDW	0.70***	0.71***	0.27	0.24	0.22	0.33	0.49	0.43*	-0.1
BD	0.79***	0.76***	0.3	0.27	0.23	0.36	0.52*	0.49*	-0.1
[P]	-0.25	-0.1	0.22	0.27	-0.36	0.03	0.2	0.17	0.24
P _{total}	0.71***	0.70***	0.35	0.35	0.15	0.3	0.55*	0.53*	-0.02
PPUE	0.71***	0.68***	0.19	0.1	0.3	0.35	0.38	0.32	-0.19

Root length (L, m), root projected area (Pa, cm²), root volume (V, cm³), root average diameter (AD, mm), tips (T), forks (F), and length of roots with diameter under 1.5 mm (L_{≤1.5}, cm), length of roots with diameter between 1.5 mm and 3.5 (L_{≤3.5}, cm), and length of roots with diameter above (L_{>3.5}, cm). Fruit production (Prod, g), shoot fresh weight (SFW, g), shoot dry weight (SDW, g), Biomass fresh weight (BF), Biomass dry weight (BD), shoot phosphorous concentration ([P], mg P/100 g DW), total phosphorous in the plant (P_{total}, mg) and physiological phosphorous use efficiency (PPUE, g²DW mg⁻¹ P).

Discussion

Pepper displays a broad range of root morphologies useful for breeding.

The twenty genotypes of pepper assayed displayed a great diversity of root morphologies as shown in Figures 3, 4 and 5 and supplementary Tables 1 and 2. It was possible to find accessions with big and branched root systems, such as ‘Aji cacho cabra’, ‘Serrano criollo’ or ‘Numex Garnet’, as well as genotypes with long and thin lateral roots with good branching such as ‘Piquillo’, but also accessions with small root systems but with thick lateral roots such as ‘204D Serrano’ or accessions with small root systems and thin lateral roots such as ‘Petit Marsellais’. Root diversity has been described in many other crops such as wheat (Nakhforoosh *et al.*, 2014), legumes (Ye *et al.*, 2018), melon (Fita *et al.*, 2008; Fita *et al.*, 2011) or tomato (Alaguero-Cordovilla *et al.*, 2018). This means that there exists a potential for breeding elite varieties with improved and combined root traits to fit different purposes. However, finding the appropriate ideotype to each purpose is not an easy task. Recently, investigations on roots have resulted in a clearer understanding on the best root ideotypes depending on the breeding objective (Lynch, 2022). The wide range of combination of root traits observed in pepper and the independence among them creates a great basis to improve the root systems towards more efficient plants.

Apart from the difficulty on setting the root ideotype, the diversity at the root level have been largely neglected by breeders due to the difficulties in accessing the roots. Here two root traits’ evaluation methods have been used, the manual in field scoring, also known as shovelomics (Trachsel *et al.*, 2011) and the semi-automated scoring with image analysis (Arsenault *et al.*, 1995). Our results indicate that both systems are valid to identify root diversity and that they provide complementary information. In general, many parameters obtained with Winrhizo (Length, Projected area, Tips...) correlated a lot with root weight but that was not the case or average diameter or length of lateral roots of certain diameters. On the contrary, other easy manual evaluated traits, as root angle, L_{total}, N_{lat >2} have shown to be able to provide important and non-redundant

information. Shovelomics approach has been used successfully for genome wide association mapping in brassica (Arifuzzaman *et al.*, 2019) and other species.

The root system adapts to the scion

Fifteen accessions out of twenty changed significantly some of their root traits means when they act as rootstock for 'Lobo'. This change in the performance was in some cases a downsizing, reducing root length, and root diameter, but other times implied the extension of the root to longer and heavier root systems as in the case of 'Numex Conquistador'. The plasticity of the rootstock depending on the scion has been reported previously. For example, when using 'Pat81', a wild relative of melon to a cultivated variety, the root of 'Pat 81' increased its size due to the support of the photoassimilates of the scion (Fita *et al.*, 2007). This can be the case of 'Numex Conquistador', 'Petit Masellaise' or 'Bola'. On the contrary, the cases of reducing the root system can be explained by either an adaptation of the root to the size of the scion (photoassimilates and hormonal support) or to incompatibilities in the graft. In the case of 'BGV-60', it seems that there was some kind of incompatibility as not only the root showed a poorer performance but the scion, which shown significantly lower production than the control. Similar incompatibility effects have been described previously in 'Serrano' peppers acting as rootstock (Leal-Fernández *et al.*, 2013). The cases of 'Catedral', 'Piquillo', 'Serrano' and 'Ají caco cabra' were no so clear, as the roots were affected but the production and/or the biomass of the scion was not significantly affected (although sometimes a tendency of biomass reduction was clearly observed). In similar way, 'BGV-4349', significantly affected the production of the scion but did not show important changes in its root morphology.

Here, it has been demonstrated that even when using the same species as rootstock, the success of the graft is not only dependent on the vigour or characteristics of the accession acting as rootstock but on the scion x rootstock interactions. The scion not only provides with photoassimilates to the rootstock needed for its development but also with hormones and other signals that modify the gene expression at rootstock level (Perez-Cordoba *et al.*, 2018; Gautier *et al.*, 2020; Tsaballa *et al.*, 2021). Recent studies in vegetable grafting have shown that incompatibility in melon and pumpkin was driven by a healing of the graft union with undifferentiated cells and lower rootstock content of trans-zeatin type cytokinins (Camalle *et al.*, 2021). These rootstock-scion interactions are unique and difficult to be predicted, therefore any potential rootstock should be tested as such for each possible scion to maximize the positive effect of the technique (Falik *et al.*, 2020).

Some rootstocks improve yield and P acquisition

Although many of the rootstocks did not significantly change the performance of the scion, and apart from 'BG-60' and 'BGV-4349' which negatively affected the production and other traits, there were some genotypes which improve the biomass and total P content of the scion. Considering these results, the best rootstock candidate by far under our experimental conditions would be 'Numex', since it is the one which improved the majority of the scion parameters. Other good rootstock candidates would be 'Numex Garnet' and 'Petit Marsellaise', and to a lesser extent 'Oscos', 'Numex Sandía', 'Numex Conquistador' and 'Piquillo'. It is important to note that if the criteria for selection of the genotypes was only their intact roots systems Numex would never have been selected because was not one of the genotypes that were especially different from the others.

Root length, branching and root angle are key factors for plant growth under low P conditions

Different root architectures have been associated with specific adaptations previously. For instance, shallow roots with dense branching of thin roots have been associated to higher absorption to P which usually is more abundant in the top-soil layer (Lynch, 2022). Long deep roots on the contrary have been correlated with drought tolerant genotypes or low N adaptation, as N leaks easily with water (Lynch, 2022).

In our experiment some root traits influenced positively the yield, biomass and/or P total content and PPUE (Table 6). Those were L, V, T, F, $L_{\leq 1.5}$, $L_{> 3.5}$, StDW, LRDW and Ang. Shallow and longer roots have been correlated with higher acquisition of P in pepper (Pereira *et al.*, 2020). Interestingly no correlation was found for the diameter in any of the ways as it was measured D and AD and the yield and P-parameters. Other authors have found that thinner roots are able to explore more intensively the soil and then increase P acquisition.

The fact that any of the root parameters evaluated correlated with the concentration of P in the leaves indicate certain independence on this parameter. This buffering effect of the scion genotype can be explained by the ability of the aerial part to maintain the P levels constant. This has been found in other studies such in tomato were the growth reduction under low-P was only explained by decreased root P export rather than by low foliar P concentrations (Martínez-Andujar *et al.*, 2017). It is important to note that under our experimental conditions although no mineral P fertilization was applied the P reserves of the soil were enough to prevent for a severe deficit of this mineral in the leaves, and in all cases the P acquired from the root and translocated to the aerial part was enough to keep P concentration in leaves.

Conclusions

According to our results, the diversity found in our pepper collection could be used to improve the performance of elite cultivars of pepper by breeding their root systems. In addition, some of the varieties could be useful for developing rootstocks with improved performance under P deficiency. The root traits more important to scion productivity were root length, root angle, number of lateral roots and root density. However, when selecting for rootstock it is necessary to test the scion-rootstock interaction, as unexpected changes in both scion and rootstock may occur. Therefore, more studies on the rootstock-scion interaction must be done in the future.

Authors' Contributions

Conceptualization: ARB, AF, VC; Data curation: MJPC, AF; Formal analysis: MDD, AL, AF; Funding acquisition: AF; Investigation: MJPC; Methodology: MDD, AL, AF; Resources: VC, ARB, AF; Supervision: MDD, AL, AF; Writing - original draft: MJPC; Writing - review and editing: MJPC, MDD, AL, VC, ARB, AF. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Alaguero-Cordovilla A, Gran-Gómez FJ, Tormos-Moltó S, Pérez-Pérez JM (2018). Morphological characterization of root system architecture in diverse tomato genotypes during early growth. *International Journal of Molecular Sciences* 19(12):3888. <https://doi.org/10.3390/ijms19123888>
- Arifuzzaman M, Oladzadabbasabadi A, McClean P, Rahman M (2019). Shovelomics for phenotyping root architectural traits of rapeseed/canola (*Brassica napus* L.) and genome-wide association mapping. *Molecular Genetics and Genomics* 294:985-1000. <https://doi.org/10.1007/s00438-019-01563-x>
- Arsenault JL, Poulcur S, Messier C, Guay R (1995). WinRHIZO™, a root-measuring system with a unique overlap correction method. *HortScience* 30(4):906D-906. <https://doi.org/10.21273/HORTSCI.30.4.906D>
- Batjes NH (2011). Global distribution of soil phosphorus retention potential (No. 2011/06). ISRIC-World Soil Information.
- Bindraban PS, Dimkpa C, Nagarajan L, Roy A, Rabbinge R (2015). Revisiting fertilisers and fertilisation strategies for improved nutrient uptake by plants. *Biology and Fertility of Soils* 51(8):897-911. <https://doi.org/10.1007/s00374-015-1039-7>
- Brownlie WJ, Sutton MA, Heal KV, Reay DS, Spears B (2022). Our phosphorus future: towards global phosphorus sustainability. Published by UK Centre for Ecology & Hydrology (UKCEH), on behalf of the Our Phosphorus Future Network.
- Camalle MD, Sikron N, Zurgil U, Khadka J, Pivonia S, Pěňčík A, ... Tel-Zur N (2021). Does scion–rootstock compatibility modulate photoassimilate and hormone trafficking through the graft junction in melon–pumpkin graft combinations? *Plant Science* 306:110852. <https://doi.org/10.1016/j.plantsci.2021.110852>
- Chen Z, Wang L, Cardoso JA, Zhu S, Liu G, Rao IM, Lin Y (2023). Improving phosphorus acquisition efficiency through modification of root growth responses to phosphate starvation in legumes. *Frontiers in Plant Science* 14:1094157. <https://doi.org/10.3389/fpls.2023.1094157>
- Fafu W, Jianxiong W, Jiangtao L, Guoping Z, Peng X, Peng H, Wenshuai X (2021). Distribution, geology and development status of phosphate resources. *Geology in China* 48(1):82-101. <https://dx.doi.org/10.12029/gc20210106>
- Fallik E, Ziv C (2020). How rootstock/scion combinations affect watermelon fruit quality after harvest? *Journal of the Science of Food and Agriculture* 100(8):3275-3282. <https://doi.org/10.1002/jsfa.10325>
- FAOSTAT (2023). Retrieved 2024 March 1st from: <https://www.fao.org/faostat/es/#home>.
- Fita A, Nuez F, Picó B (2011). Diversity in root architecture and response to P deficiency in seedlings of *Cucumis melo* L. *Euphytica* 181:323-339. <https://doi.org/10.1007/s10681-011-0432-z>
- Fita A, Picó B, Dias RCS, Nuez F (2008). Effects of root architecture on response to melon vine decline. *The Journal of Horticultural Science and Biotechnology* 83(5):616-623. <https://doi.org/10.1080/14620316.2008.11512432>
- Fita A, Pico B, Roig C, Nuez F (2007). Performance of *Cucumis melo* ssp. *agrestis* as a rootstock for melon. *The Journal of Horticultural Science and Biotechnology* 82(2):184-190. <http://dx.doi.org/10.1080/14620316.2007.11512218>
- Gao ZY, Hu JA, Zhang BB, Gong B (2023). Screening and comprehensive evaluation of tomato rootstocks with high efficiency of phosphorus utilization. *Scientia Agricultura Sinica* 56:2761-2775. <https://doi.org/10.3864/j.issn.0578-1752.2023.14.011>
- Gautier AT, Cochetel N, Merlin I, Hevin C, Lauvergeat V, Vivin P, ... Cookson SJ (2020). Scion genotypes exert long distance control over rootstock transcriptome responses to low phosphate in grafted grapevine. *BMC Plant Biology* 20:1-15. <https://doi.org/10.1186/s12870-020-02578-y>
- Ge X, Chen X, Liu M, Wang C, Zhang Y, Wang Y, ... Zhang T (2023). Toward a better understanding of phosphorus nonpoint source pollution from soil to water and the application of amendment materials: Research trends. *Water* 15:1531. <https://doi.org/10.3390/w15081531>

- Howden SM, Soussana JF, Tubiello FN, Chhetri N, Dunlop M, Meinke H (2007). Adapting agriculture to climate change. *Proceedings of the National Academy of Sciences* 104(50):19691-19696. <http://dx.doi.org/10.1073/pnas.0701890104>
- Kirchgesser J, Hazarika M, Bachmann-Pfabe S (2023). Phenotypic variation of root-system architecture under high P and low P conditions in potato (*Solanum tuberosum* L.). *BMC Plant Biology* 23:68. <https://doi.org/10.1186/s12870-023-04070-9>
- Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ (2006). Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. *Annals of Botany* 98(4):693-713. <https://doi.org/10.1093/aob/mcl114>
- Leal-Fernández C, Godoy-Hernández H, Núñez-Colín CA, Anaya-López JL, Villalobos-Reyes S, Castellanos JZ (2013). Morphological response and fruit yield of sweet pepper (*Capsicum annuum* L.) grafted onto different commercial rootstocks. *Biological Agriculture & Horticulture* 29(1):1-11. <https://doi.org/10.1080/01448765.2012.746063>
- Louws FJ, Rivard CL, Kubota C (2010). Grafting fruiting vegetables to manage soilborne-pathogens, foliar pathogens, arthropods and weeds. *Scientia Horticulturae* 127:127-146. <https://doi.org/10.1016/j.scienta.2010.09.023>
- Lynch J (2011) Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. *Plant Physiology* 156(3):1041-1049. <https://doi.org/10.1104/pp.111.175414>
- Lynch J (2022). Harnessing root architecture to address global challenges. *The Plant Journal* 109(2):415-431. <https://doi.org/10.1111/tpj.15560>
- Lynch JP (2019) Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. *New Phytologist* 223:548-564. <https://doi.org/10.1111/nph.15738>
- Martínez-Andújar C, Ruiz-Lozano JM, Dodd IC, Albacete A, Pérez-Alfocea F (2017). Hormonal and nutritional features in contrasting rootstock-mediated tomato growth under low-phosphorus nutrition. *Frontiers in Plant Science* 8:533. <https://doi.org/10.3389/fpls.2017.00533>
- Möller K (2018). Soil fertility status and nutrient input–output flows of specialised organic cropping systems: A review. *Nutrient Cycling in Agroecosystems* 112(2):147-164. <https://link.springer.com/article/10.1007/s10705-018-9946-2>
- Morales-Manzo II, Ribes-Moya AM, Pallotti C, Jimenez-Belenguer A, Moro CP, Raigón MD, ... Fita A (2023). Root–soil interactions for pepper accessions grown under organic and conventional farming. *Plants* 12(9):1873. <https://doi.org/10.3390/plants12091873>
- Nakhforoosh A, Grausgruber H, Kaul HP, Bodner G (2014). Wheat root diversity and root functional characterization. *Plant and Soil* 380:211-229. <https://doi.org/10.1007/s11104-014-2082-0>
- Penuelas J, Coello F, Sardans J (2023). A better use of fertilizers is needed for global food security and environmental sustainability. *Agriculture & Food Security* 12(1):1-9. <https://doi.org/10.1186/s40066-023-00409-5>
- Pereira-Dias L, Gil-Villar D, Castell-Zeising V, Quiñones A, Calatayud Á, Rodríguez-Burruezo A, Fita A (2020). Main root adaptations in pepper germplasm (*Capsicum* spp.) to phosphorus low-input conditions. *Agronomy* 10(5):637. <https://doi.org/10.3390/agronomy10050637>
- Perez-Cordoba MJ, Rodríguez Burruezo A, Díez Díaz M, De Luis A, Fita A (2018). Crosstalk scion-rootstock modifies root architecture in pepper rootstocks. *Journal of Biotechnology* 280:S86. <http://dx.doi.org/10.1016/j.jbiotec.2018.06.284>
- Ribeiro CAG, de Sousa Tinoco SM, de Souza VF, Negri BF, Gault CM, Pastina MM, ... Guimaraes CT (2023). Genome-wide association study for root morphology and phosphorus acquisition efficiency in diverse maize panels. *International Journal of Molecular Sciences* 24(7):6233. <https://doi.org/10.3390/ijms24076233>
- Ropokis A, Ntatsi G, Kittas C, Katsoulas N, Savvas D (2019). Effects of temperature and grafting on yield, nutrient uptake, and water use efficiency of a hydroponic sweet pepper crop. *Agronomy* 9:110. <https://doi.org/10.3390/agronomy9020110>
- Rouphael Y, Venema JH, Edelstein M, Savvas D, Colla G, Ntatsi G, Ben-Hur M, Kumar P, Schwarz D (2017). Grafting as a tool for tolerance of abiotic stress. In: Colla G, Pérez-Alfocea F, Schwarz D (Eds). *Vegetable Grafting: Principles and Practices*. CABI: Oxfordshire, UK.
- Statista (2024). US Geological Survey. (January 29, 2024). Reserves of phosphate rock worldwide in 2023, by country (in million metric tons) [Graph]. In: Statista. Retrieved 2024 April 3 from: <https://www.statista.com/statistics/681747/phosphate-rock-reserves-by-country/>

- Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2011). Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant and Soil* 341:75-87. <http://dx.doi.org/10.1007/s11104-010-0623-8>
- Tsaballa A, Xanthopoulou A, Madesis P, Tsaftaris A, Nianiou-Obeidat I (2021). Vegetable grafting from a molecular point of view: the involvement of epigenetics in rootstock-scion interactions. *Frontiers in Plant Science* 11:621999. <https://doi.org/10.3389/fpls.2020.621999>
- van de Wiel CC, van der Linden CG, Scholten OE (2016). Improving phosphorus use efficiency in agriculture: opportunities for breeding. *Euphytica* 207:1-22. <https://doi.org/10.1007/s10681-015-1572-3>
- Wen Z, Li H, Shen Q, Tang X, Xiong C, Li H, ... Shen J (2019). Tradeoffs among root morphology, exudation and mycorrhizal symbioses for phosphorus-acquisition strategies of 16 crop species. *New Phytologist* 223(2):882-895. <https://doi.org/10.1111/nph.15833>
- Williamson LC, Ribrioux SPCP, Fitter AH, Leyser HMO (2001). Phosphate availability regulates root system architecture in *Arabidopsis*. *Plant Physiology* 126:875-882. <https://doi.org/10.1104/pp.126.2.875>
- Ye H, Roorkiwal M, Valliyodan B, Zhou L, Chen P, Varshney RK, Nguyen HT (2018). Genetic diversity of root system architecture in response to drought stress in grain legumes. *Journal of Experimental Botany* 69(13):3267-3277. <https://doi.org/10.1093/jxb/ery082>
- Zhang C, Zhang S, Li C, Li Q, Zhang J, Wang J, ... Guo L (2022). Long-term fertilization altered microbial community structure in an aeolian sandy soil in northeast China. *Frontiers in Microbiology* 13:979759. <https://doi.org/10.3389/fmicb.2022.979759>



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