Early evaluation of root morphology of maize genotypes under phosphorus deficiency

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ABSTRACT

In Brazil savanna type of soils presents problems with phosphorus content. The selection and identification of maize genotypes to such environments is a high priority of Brazilian research. The purpose of this paper was to evaluate, in soils with different P concentrations, the dry mass attributes and characteristics of root morphology in eight maize lines with different genetic background and origins of the Breeding Program of the National Research Center for Maize and Sorghum. The experiment was carried out in plots prepared with two levels of phosphorus: high phosphorus (HP) and low phosphorus (LP). The experimental design was randomized blocks with three replications. The evaluation of the characteristics of the shoots and the root system morphology was performed 21 days after sowing. The WinRhizo program of images analysis was used for the root morphology. There were no differences between the phosphorus levels for the dry mass attributes. However, when we compared P levels, root morphology of L13.1.2 strain performed the highest surface area (SA) and total root length (RL), length of thin (TRL) and very thin (VTRL) roots in low P concentration. The root systems digital images analysis techniques allowed efficient discrimination of maize genotypes in environments with low P levels.

Keywords: phosphorus concentrations; *Zea mays*; root length; WinRhizo

The phosphorus (P) deficiency is considered as one of the greatest limitations in the productivity of crops, mainly in the tropics and subtropics (Ramaekers et al. 2010). According to Vance et al. (2003) about 30% of the crops production in the world is affected by the lack of macronutrients in the soil. Knowing that the use of fertilizers to correct the phosphorus concentration in the soil is expensive, the development of tolerant cultivars may represent an effective solution for the problem (Li et al. 2010).

However, further studies on the strategies for the P low levels tolerance are needed. Several papers report plants strategies for phosphorus low levels tolerance (Vance et al. 2003, Richardson et al. 2009, Ramaekers et al. 2010) and one of the main strategies that may be very useful for this tolerance is the root plasticity. The root morphology becomes very relevant in the efficient acquisition of phosphorus by the plants, as there is a relative P immobility that makes its acquisition dependent of greater soil exploitation by the roots (root greater length and surface area) (Li et al. 2007).

With the introduction of digital image analysis root system studies are nowaday less time consuming, allowing for more accurate and less subjective measures (Bouma et al. 2000). With this in mind we were interested to use digital image analysis for studies on the root development of maize at different P levels status. The digital image analysis has made the root system study less slow, allowing for more accurate and less subjective measures than the characteristics that the human eye can detect (Bouma et al. 2000). Therefore, considering the actual impact on the maize growth due to low concentrations of phosphorus (P) (Li et al. 2010), this study aimed to evaluate the root morphology of maize strains subjected to two concentrations of phosphorus (high and low) through the WinRhizo digital analysis system.

MATERIAL AND METHODS

The experiment was carried out in the National Research Center for Maize and Sorghum (EMBRAPA), in the town of Sete Lagoas, State of Minas Gerais, located at 19°28' of latitude S and longitude 44°15'08'' W at 732 m. The region climate is the type Aw (savanna climate with dry winter).

Eight lines with different genetic background and origins, of the National Research Center for Maize and Sorghum, were studied in experimental plots prepared separately for two different levels: low phosphorus (LP) (4 mg/dm^3 of soil) and high phosphorus (HP) (20 mg/dm^3 of soil) with soil classified as Red Latosol (Oxisol), average texture (Santos et al. 2006). P levels were determined based on previous research (Ribeiro et al. 1999). Soil content was performed by routine analysis at The National Maize and Sorghum Research Center. The experimental units were set in four lines of 1.2 × 0.70 m length and five plants per linear meter. The experimental design was randomized blocks, with three replications.

The evaluation of the shoots characteristics and the root system morphology was performed 21 days after sowing. Two plants per plot were collected, by replication. After the washing process, the plants were separated into root system and shoots at the crown region. The characteristics were evaluated as follows: shoots (SDM) and roots (RDM) dry mass and the ratio between them (RDM/SDM). The plant material was stored in paper bags and transported to a greenhouse with forced air circulation at 72°C until obtaining constant mass. The plants height (PH) was also evaluated.

For the root system morphology, the WinRhizo Pro 2007a (Régent Instr. Inc., Quebec, Canada) images analysis system was used, coupled with a professional scanner Epson XL 1000 equipped with additional light unit (TPU). For the images of root morphology (Bouma et al. 2000) the definition of 400 (dpi) was used. The roots were placed in a 20 cm wide and 30 cm long acrylic tank, with approximately one inch water depth and placed on the scanner. Then the root characteristics were determined as follows: total root length (RL) (cm), root surface area (SA) (cm^2), and root length per diameter (cm). The root length was classified according to three diameters, as described below: very thin roots (VTRL) (Ø lower than 0.5 mm), thin roots (TRL) (> 0.5 Ø < 2.0 mm) and thick roots (THRL) (Ø > 2.0 mm) (Bohm 1979). For the statistical analysis of the results, we applied the analysis of variance and the Scott-Knott test for the mean comparison at 0.05 significance, using the Sisvar program, version 5.0.

RESULTS AND DISCUSSION

Different P levels of soils – high (HP) and low (LP) – did not affect maize height characteristics (PH), shoots dry mass (SDM), roots dry mass (RDM) or the ratio between the roots dry mass and the shoots dry mass (RDM/SDM) (Table 1). However, there were differences among the genotypes. For instance, the genotypes L 29.1.1, L 2.3.2.1, L 13.1.2, and L1170 were the ones which presented the larger heights when cultivated in high P, whereas the genotypes L 2.3.2.1 and

Table 1. Average of plant height and dry mass attributes of shoots and roots in maize lines under high (HP) and low (LP) phosphorus content in the soil

Genotypes	HP	LP	HP	LP	HP	LP	HP	LP
	PH (g)		SDM (g)		RDM (g)		RDM/SDM	
L 29.1.1	10.15^{Aa}	10.28^{Ab}	9.33^{Aa}	8.06^{Ab}	7.58^{Ab}	6.46^{Aa}	0.81^{Aa}	0.80^{Aa}
L 14.1.1	8.58^{Ab}	8.60^{Ad}	9.02^{Ab}	9.31^{Aa}	6.90^{Ab}	8.28^{Aa}	0.77^{Aa}	0.91^{Aa}
L 31.2.1.2	7.62^{Ab}	8.17^{Ad}	8.25^{Ab}	8.15^{Ab}	5.87^{Ab}	5.51^{Aa}	0.72^{Aa}	0.68^{Aa}
L 2.3.2.1	10.95^{Aa}	11.52^{Aa}	9.67^{Aa}	8.48^{Ab}	8.31^{Aa}	6.94^{Aa}	0.87^{Aa}	0.81^{Aa}
L 13.1.2	10.75^{Aa}	12.05^{Aa}	8.42^{Ab}	8.57^{Ab}	6.26^{Ab}	6.05^{Aa}	0.74^{Aa}	0.71^{Aa}
L 6.1.1	7.90^{Ab}	8.02^{Ad}	9.96^{Aa}	10.41^{Aa}	8.95^{Aa}	7.66^{Aa}	0.90^{Aa}	0.74^{Aa}
L 1170	9.17^{Aa}	9.47^{Ac}	8.98^{Ab}	8.80^{Ab}	7.28^{Ab}	7.16^{Aa}	0.82^{Aa}	0.82^{Aa}
L 31.2.5	6.95^{Ab}	7.92^{Ad}	8.51^{Ab}	8.14^{Ab}	8.85^{Aa}	5.81^{Aa}	1.04^{Aa}	0.71^{Aa}

Average followed by the same low case letter in the columns for the genotypes and capital letters in the lines for the conditions (HP and LP), do not differ by the Scott-Knott test at 5% probability ($P \leq 0.05$). SDM – shoots dry mass; RDM – roots dry mass; PH – plant height; RDM/SDM – relationship between root dry mass and shoot dry mass

L 13.1.2 were superior in low P (Table 1). As far as shoots dry mass (SDM) is concerned, one may observe that from high P soil content three strains showed larger dry weight compared to the others: L 29.1.1, L 2.3.2.1, and L 6.1.1. On the other hand for low phosphorus content the lines L 2.3.2.1 and L 6.1.1 were the ones with the highest SDM means. There were no statistical differences for root dry mass (RDM) in low phosphorus concentration, (Table 1), however, in high phosphorus content the genotypes L 2.3.2.1, L 6.1.1, L 31.2.5 resulted in the highest root dry mass. There was no statistical difference in the RDM/SDM both in the high P and in the low P (Table 1).

Table 2 shows that there were differences between phosphorus levels for others characteristics evaluated. For example: for root total length (LR), the strains L 29.1.1, L 2.3.2.1, L 13.1.2, L 6.1.1, L 31.2.5 showed better performance in high P compared to the low phosphorus content.

The L 13.1.2 showed larger area of root surface (SA) in the low phosphorus content. Along with the L 13.1.2, the strains L 29.1.1, L 2.3.2.1, L 6.1.1, and 31.2.5 performed better in the low phosphorus content compared with its counterparts in high P.

For very thin roots length (VTRL) the strains L 2.3.2.1, L 13.1.2 and L 6.1.1 were superior in soil with low phosphorus content (LP), compared to high P (Table 2).

For thin roots length (TRL) most strains (L 2.3.2.1, L 13.1.2, L 6.1.1, L 1170, L 31.2.5) performed better in low P compared to high P, except for strain L 31.2.1.2 (Table 2).

No statistical difference was found between the genotypes and the two phosphorus contents in the soil just for the length of thick roots (THRL) (Table 2).

It can be noted that there were no differences between the dry mass attributes in both phosphorus levels (Table 1). In contrast, significant differences were reported between genotypes in the total dry matter accumulation, in response to the P deficiency, for the maize crop (Liu et al. 2004, 2007). Besides, the increase of the roots dry mass by the shoots dry mass was noted as a major strategy in the tolerance to phosphorus stress (Nielsen et al. 2001).

This study noted clearly that the strain L 13.1.2 showed higher values for the root morphology characteristics in the soil with phosphorus deficiency (low phosphorus content-LP) and the strain L 31.2.1.2 in the high phosphorus content (HP) (Table 2). One of the main changes in plants for the acquisition of phosphorus in deficient soils is the increase of soil exploration through the exploitation of the soil through greater root growth and proliferation of the roots mainly responsible for this metabolic function (roots of smaller diameter) (Lynch and Ho 2005). The greater root growth, mainly of thin roots and very thin roots, as noted in the strain L 13.1.2, may be connected to this root exploitation in the search of greater acquisition of phosphorus. Imada et al. (2008) say that the root surface area is more related to the nutrients absorption. A larger surface area was noted in the strain L 13.1.2, which can help the plant to obtain

Table 2. Average of the roots morphology characteristics in maize lines under high (HP) and low (LP) phosphorus content in the soil

Genotypes	RL		SA		VTRL		TRL		THRL	
	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP
L 29.1.1	544.22^{Bb}	611.40^{Ab}	70.34^{Bb}	83.37^{Ab}	409.70^{Aa}	457.15^{Ab}	131.38^{Ab}	150.31^{Ab}	1.99^{Aa}	2.72^{Aa}
L 14.1.1	365.21^{Ac}	276.51^{Bd}	53.64^{Ac}	40.79^{Bc}	243.08^{Ab}	174.84^{Bd}	118.99^{Ab}	99.81^{Ab}	2.43^{Aa}	1.32^{Aa}
L 31.2.1.2	672.84^{Aa}	493.56^{Bc}	92.92^{Aa}	71.21^{Bc}	482.67^{Aa}	347.50^{Bc}	186.15^{Aa}	142.47^{Bb}	2.60^{Aa}	1.91^{Aa}
L 2.3.2.1	367.49^{Bc}	445.70^{Ac}	50.01^{Bc}	63.89^{Ac}	271.52^{Bb}	330.19^{Ac}	93.32^{Bc}	111.96^{Ab}	1.56^{Aa}	2.36^{Aa}
L 13.1.2	495.12^{Bb}	795.28^{Aa}	74.64^{Bb}	110.03^{Aa}	355.64^{Ba}	588.10^{Aa}	136.29^{Bb}	202.07^{Aa}	2.05^{Aa}	3.84^{Aa}
L 6.1.1	309.67^{Bc}	403.68^{Ac}	46.76^{Bc}	58.90^{Ac}	208.04^{Bb}	272.95^{Ac}	98.38^{Bc}	127.53^{Ab}	2.21^{Aa}	1.90^{Aa}
L 1170	530.08^{Ab}	529.78^{Ac}	64.52^{Ab}	67.98^{Ac}	407.68^{Aa}	397.27^{Ab}	119.09^{Bb}	129.17^{Ab}	1.96^{Aa}	1.93^{Aa}
L 31.2.5	308.74^{Bc}	343.57^{Ad}	37.26^{Bc}	53.68^{Ac}	227.40^{Ab}	220.33^{Ab}	79.05^{Bc}	120.05^{Ab}	1.70^{Aa}	2.20^{Aa}

Average followed by the same low case letter in the columns for the genotypes and capital letters in the lines for the conditions (HP and LP), do not differ by the Scott-Knott test at 5% probability ($P \leq 0.05$). RL – total root length (cm); SA – root surface area (cm^2); VTRL – very thin roots length (cm); TRL – thin roots length; THRL – thick roots length (cm) sources for the deficient nutrients. For instance, Gaume et al. (2001) reported a greater root system that resulted in maize genotypes phosphorus deficiency-tolerant in their research.

One of the poorly studied maize characteristics is the root morphology, which is justified by the difficulty is sampling and evaluating the roots. Until recently, there were no equipments or even appropriate and reliable methodology able to characterize, analyze and quantify the maize roots. With the aid of the WinRhizo digital images analysis system, this scenario tends to change, especially for the early assessment of the roots system. Furthermore, the data we have brought show great efficiency in the early discrimination of phosphorus deficiency-tolerant genotypes, thus contributing to the improvement of plants by roots screening, making it more accurate and faster.

In conclusion one may say that the root systems digital images analysis techniques allowed efficient discrimination of maize genotypes in environments with low P levels.

The strain L 13.1.2 showed the best root development under the phosphorus stress condition.

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