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Genetic Variation in Root Traits and Nutrient Acquisition of Lentil Genotypes

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ABSTRACT

Lentil (*Lens culinaris* L.), a pulse crop, is grown in nutrient-poor soils in many developing countries, often with little or no fertilization. Knowledge on root traits of lentil and the assessment of their role in nutrient capture would help to sustain its production in these nutrient-poor soils. Root traits (root length, root hairs, root-induced acidification, and phosphatase enzymes) of 10 lentil genotypes (Barimasur-3, Barimasur-4, PLX-79542, GP-8407-5, GP-8403, BLX-79542, L-5 × 8704(2), L-107 × 87012, L-5 × 87272 and 8406-122) were investigated and then related to the plant uptake of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), molybdenum (Mo), and cobalt (Co) in laboratory and pot experiments. There were significant ($p < 0.05$) differences in root length (RL) and root-hair density (number mm^{-1} root) among the genotypes. The genotypes did not differ to induce rhizosphere acidification and acid phosphatase activity (aptase). Uptake of most nutrients differed significantly ($p < 0.05$) among the genotypes, but root length (RL) was, in general, weakly correlated to the uptake of the most nutrients in the shoot dry matter (DM). The genotypes with prolific root-hair formation (Barimasur-4 and Barimasur-3) were particularly superior in uptake of those nutrients (K, P, Fe, Mn, Cu, Zn, Mo) whose availability in soils is usually low and whose transport

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to the roots is diffusion limited. The results of this investigation, though based on a small sample of lentil accessions/cultivars, suggest that genetic variation in lentil root traits and nutrient uptake can be pronounced. Screening of a large number of local and exotic cultivars or lines of lentil should be conducted by including more root traits (N_2 fixation, organic acids, mycorrhizae) to find nutrient-efficient germplasm to promote lentil production.

Keywords: abiotic stress, breeding, genetic diversity, rhizosphere, root hairs, root system

INTRODUCTION

Lentil, a protein-rich staple pulse, complements the cereal-rich diet of the people in many developing countries, particularly among low-income groups. Lentil is often grown on nutrient-poor soils with little or without fertilizer application. Superior morphological (root length, root hairs) and physiological (exudation of protons and enzymes) root traits facilitate efficient use of soil nutrients (Gahoonia and Nielsen, 2004). Identification of lentil germplasm with superior root traits may, therefore, help to sustain lentil production in nutrient-poor soils of many developing countries, including those on the Indian subcontinent and in Bangladesh, West Asia, North Africa, Sudan, Yemen, Ethiopia, Eritrea, and South America (Erskine and Saxena, 1993). The identified germplasm may be introduced directly or used for targeted breeding of nutrient-efficient and drought-tolerant varieties.

A longer root system may be expected to confer better capture of soil moisture and nutrients, but knowledge about genetic diversity in the root system of lentil is rudimentary (Sarker et al., 2005). Even less is known about whether genetic diversity in lentil root length can be related to better capture of soil nutrients.

For higher plants, the essentiality of 15 mineral elements absorbed from soil mainly by roots is well documented (Marschner, 1995). Cobalt (Co) is considered essential for nodule development and nitrogen (N) fixation by legumes (Dilworth and Bisseling, 1984). Lentil, a legume, may partially satisfy its need for N by fixing atmospheric N with the help of a root-associated *Rhizobium*. Deficiency of any one of the other nutrient elements may limit plant growth and economic output (Marschner, 1995). Therefore, an integrated approach to explore genetic diversity in root traits and to assess their role in uptake of various nutrient elements will be meaningful.

To understand the role of lentil root traits in uptake of the various nutrient elements and to explore their genetic diversity, the root morphological (root length, root hairs) and physiological (root exudation of protons and phosphatase enzymes) traits were investigated in relation to the uptake of 12 mineral elements [except N, chloride (Cl) and sodium (Na)] in 10 selected varieties/breeding lines of lentil.

MATERIALS AND METHODS

Genotypes

Ten varieties/breeding lines (genotypes) of lentil (*Lens culinaris, Medikus*) were selected for investigation, based on their popularity among Bangladeshi farmers and the anticipated importance for breeding new, improved varieties. Barimasur-3 and Barimasur-4 are popular commercial varieties and PLX-79542, GP-8407-5, GP-8403, BLX-79542, L-5 × 8704(2), L-107 × 87012, L-5 × 87272, and 8406-122 are breeding lines of potential importance.

Soil Properties

Some properties of soil used in the pot experiment were as follows: soil pH, 7.7 (0.01 *M* $CaCl_2$); organic matter, 0.55%; total N, 0.029%; major cations extracted with ammonium acetate and measured with a flame photometer (Doll and Lucas, 1973) (meq/100 mL), calcium (Ca) = 12.0; magnesium (Mg) = 2.5; potassium (K) = 0.25; other nutrients (μg/g), phosphorus (P) = 10.3 (Olsen et al., 1954); sulfur (S) = 20 (Tabatabai, 1982); boron (B) = 0.59 hot water extracted (Bingham, 1982); copper (Cu) = 6.3; iron (Fe) = 11; manganese (Mn) = 6; zinc (Zn) = 1.7 [DTPA extracted and measured with atomic absorption spectroscopy (Lindsay and Norvell, 1978)].

Determination of Root Growth and Length

The shoot growth and root length of the genotypes were studied in a pot experiment at Pulses Research Center, Ishurdi, Bangladesh. Pots were made by cutting 2 L transparent plastic bottles. They were filled with 2.2 kg of soil by shaking to achieve soil bulk density of 1.4 g cm^{-3}. The soil columns of all the pots were 25 cm high. The pots were placed in the open, with their sides wrapped in black polythene to prevent exposure of roots to light and maintained at 20% soil moisture by weighing and adding water. Six seeds were sown at 1 cm soil depth. At germination (3–4 d after sowing), three seedlings were left in each pot by removing the rest of the seedlings along with the roots. There were four replicates. At 20 and 60 d after sowing (20 DAS and 60 DAS, respectively), the shoots were cut and stored in paper bags for drying and determination of relative growth rate (RGR) and nutrient analyses. The plastic pots were then cut open and the plant roots were washed and cleaned of soil. Pots were not inoculated, but minor nodulation was observed in all pots at 60 DAS. Visual assessment of the washed roots gave no indication that differences in nodulation existed among the genotypes. One gram of fresh root sample was spread between polythene transparencies and scanned using ScanJet IIcx. The

total length of the root system was measured using Dt-Scan software (Delta-T Devices, Cambridge, UK) as described in Gahoonia et al. (1999).

Plant Analyses

Shoots at flowering stage (60 DAS) were dried at 60°C until constant weight was recorded. The whole plant material of each pot was ground using an Ultra centrifugal mill (Retsch ZM 100). Plant material (0.25 g) was digested in an open vessel system using 70 mL HD polyethylene vials (Capitol Vial Corp, Fulton Ville, NY) and a graphite heating block (Mod Block, CPI International, Amsterdam). The plant material was digested at 95°C using a slight modification of the EPA (Environmental Protection Agency, USA) Method 3050B, as described below. Five millileters of 35% HNO_3 (instra-analyzed, Baker, Deventer, Holland) was added to the samples, which were then boiled for approximately 15 min. After cooling, 2.5 mL of 70% HNO_3 was added and the samples were reheated. Twenty-five minutes later, samples were cooled and 1.5 mL H_2O_2 (extra pure, Riedel-de Haën, Seelze, Germany) was applied. When the peroxide reaction ceased, 1 mL of H_2O_2 was added and samples were reheated for approximately 40 min. During the digestion, vials were covered by watch glasses. Samples were cooled overnight and diluted to 50 mL with ultra-pure water. For each digestion, five blank samples were included. Furthermore, samples of a certified reference material (CRM) (apple leaf, standard reference material 1515; National Institute of Standards and Technology, Gaithersburg, MD) were digested to estimate the accuracy and precision of the analysis. Finally, an in-house barley reference material was included in order to keep a check of element concentrations in each individual run on the ICP-MS. Samples were diluted to the same acid concentration (1.75% HNO_3) as standards and quantification was performed by external calibration (P/N 4400 ICP-MS, multi-elemental calibration standard, CPI International, Amsterdam). Dilutions were performed on a class 100 laminar flow bench (KR-170s Biowizard, Kojair Tech Oy, Vilppula, Finland).

Twelve elements (K, P, Ca, Mg, S, Fe, Zn, Mn, Cu, B, Mo, Co) were analyzed by ICP-MS (Agilent 7500c, Agilent Technologies, Manchester, UK). Nitrogen was not analyzed, because lentil, a legume, can fix and make use of atmospheric N_2, and N uptake is less dependent on the size of the root system (Atkinson, 1991).

Determination of Root Hairs

The soil was poured into 10 cm long test tubes 3 cm in diameter (soil bulk density 1.4 g cm^{-3}, soil moisture 20%, four replicates). One pre-germinated seed was planted in each tube. After 20 d, the shoot was cut and the tubes were immersed

in water overnight in a dark room to prevent mucilage formation. All roots were removed carefully using a kitchen sieve and transferred into an ultrasound water bath (Branson 5200, 120W, 47k Hz). The ultrasound treatment, lasting about 5–10 min, removed remaining soil particles without damaging the root hairs. The root hairs were quantified using the Quantimet 500_+ Image Processing and Analysis System (Leica) at $10\times$ magnification (Gahoonia and Nielsen, 1997).

Determination of Rhizosphere pH

The roots of 10-d-old seedlings were embedded in agar containing pH indicator dye Bromocresol purple and adjusted to pH 6.0 (Marschner and Römheld, 1983). The root-induced pH change, revealed by color change, was recorded after 1 h.

Rhizosphere Phosphatase Activity

The ability of the genotypes to release acid phosphatase (aptase) in the rhizosphere was determined by the method of Dinkelaker and Marschner (1992), which is based on enzymatic hydrolysis of 1-naphtylphosphate (substrate) by root-released aptase, yielding 1-naphtol, which produces a red complex with Fast Red TR (dye). The intact roots of 10-d-old seedlings were sandwiched between two ashless filter papers soaked in a mixture of the dye and the substrate. If roots released variable amounts of phosphatase enzymes, their activity was visible as a reddish-brown color of variable intensity near the roots, because root-released phosphatase produces a reddish-brown complex with the dye Fast Red TR.

Statistical Analyses

Statistical analyses were performed with Statistical Analysis System Institute (SAS, 1989) and Microsoft Excel software as appropriate. Statistically significant ($p < 0.05$) differences between the genotypes were determined using analysis of variance (ANOVA).

RESULTS

The growth patterns of the selected genotypes differed, and they produced significantly ($p < 0.05$) different amounts of shoot dry matter (DM). A higher amount of DM was produced by the commercial varieties (Barimasur-3 and Barimasur-4) and three breeding lines L-107 $\times$ 87012, L-5 $\times$ 87272, and

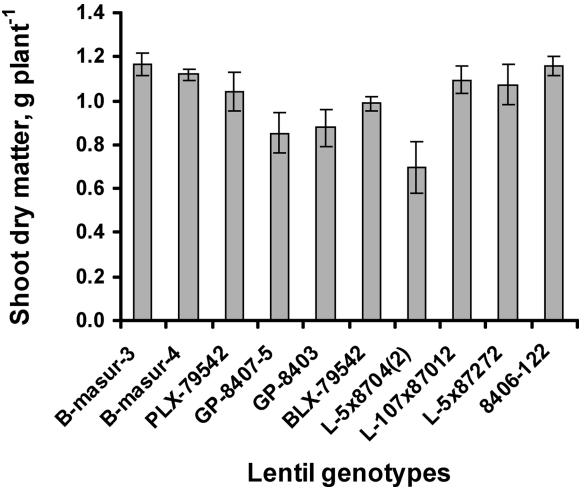

Figure 1. Shoot biomass of 10 lentil genotypes 60 d after sowing in a pot experiment. Bars are standard error of means (n = 4).

8406-122 (Figure 1). The L-5 × 8704(2) genotype produced the lowest amount of DM (Figure 1).

Root Traits

There were significant ($p < 0.05$) differences in root length (RL) of the genotypes, after both 20 DAS and 60 DAS (Figure 2). The breeding line 8604-122 produced the largest root system (34.49 m plant^{-1}) at flowering (60 DAS), followed by L-107 × 87012 and L-5 × 87272; but at 20 DAS, its RL was among the shortest. The improved variety Barimasur-4 produced the highest RL (4.8 m plant^{-1}) at early stage and maintained good root growth even at the flowering stage.

The roots of Barimasur-4 were covered with the longest (0.48 ± 0.09 mm, Figure 3) and most dense root hairs, followed by Barimasur-3 (0.38 ± 0.10 mm). The average root hair length (RHL) of other genotypes was below 0.31 ± 0.09 mm. Root hair density (RHD; number mm^{-1} root) on the roots of Barimasur-4 was 26 ± 3 as compared with about 17 ± 2 for Barimasur-3 and other genotypes. The differences in RHD were significant ($p < 0.05$), but not in RHL. Using the average values of RHD and RHL, it was calculated that the presence of root hairs would increase the effective root lengths of Barimasur-4 12 times, Barimasur-3 five times and those of other genotypes four times.

The application of color-indicator dye methods did not reveal/detect differences in root-induced rhizosphere pH and acid phosphatase activity (aptase) in the rhizosphere of the selected genotypes (data not shown).

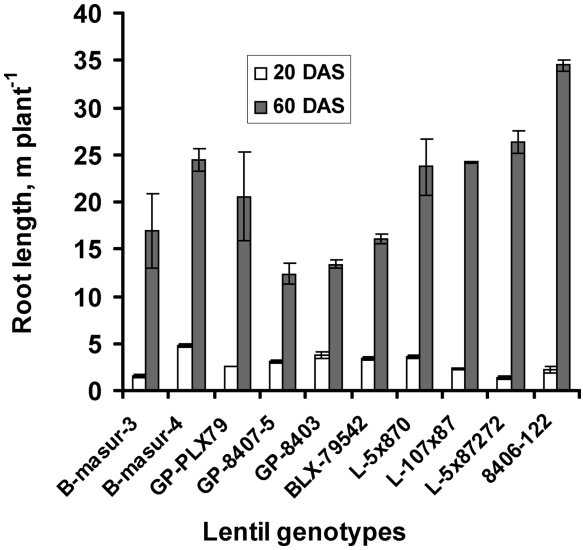

Figure 2. Root lengths of 10 lentil genotypes 20 d after sowing (20 DAS) and 60 d after sowing (60 DAS). Bars are standard error of means (n = 4).

Macronutrients

The concentration of K (Table 1) was highest in the DM of Barimasur-4 (28.12 g kg^{-1}). The variation in K uptake of the genotypes was significant ($p < 0.05$). The correlation between RL and K uptake was weak, though significant ($R^2 = 0.20^*$). The ability of Barimasur-4 to acquire extra K may be attributed to its ability to produce a longer root system covered with longer and denser root hairs (Figure 3).

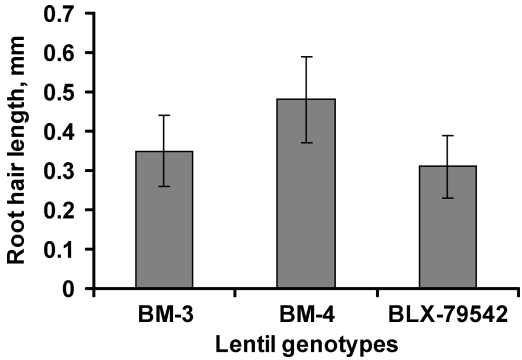

Figure 3. Average root-hair length of lentil genotypes, Barimasur-3 (BM-3), Barimasur-4 (BM-4), and BLX-79542. Bars are standard error of means (n = 4).

Table 1
Concentration of macronutrients in shoot dry matter of 10 lentil genotypes and their correlation (R^2) with root length

Genotypes	Macronutrients (g kg^{-1})				
	K	P	Ca	Mg	S
B-masur-3	20.43 ± 0.75	3.62 ± 0.05	16.44 ± 0.04	2.52 ± 0.06	3.04 ± 0.11
B-masur-4	28.12 ± 1.03	3.95 ± 0.06	20.14 ± 0.57	3.06 ± 0.10	3.05 ± 0.20
PLX-79542	19.14 ± 0.06	3.19 ± 0.01	15.42 ± 0.55	2.38 ± 0.06	2.74 ± 0.15
GP-8407-5	21.33 ± 0.82	3.11 ± 0.07	16.58 ± 1.42	2.91 ± 0.24	3.04 ± 0.11
GP-8407	20.21 ± 1.89	3.22 ± 0.16	14.71 ± 0.48	2.42 ± 0.08	2.62 ± 0.22
BLX-79542	22.81 ± 1.61	2.87 ± 0.09	14.39 ± 0.66	1.66 ± 0.21	2.62 ± 0.14
L-5 × 58704(2)	20.14 ± 0.20	2.63 ± 0.03	14.36 ± 0.18	2.00 ± 0.04	2.05 ± 0.12
L-107 × 87102	19.50 ± 1.42	2.98 ± 0.03	14.76 ± 1.67	1.89 ± 0.06	1.95 ± 0.10
L-5 × 87272	19.98 ± 0.89	3.14 ± 0.15	14.12 ± 0.16	2.25 ± 0.16	1.86 ± 0.13
8406-122	17.76 ± 0.26	3.30 ± 0.14	14.70 ± 0.42	2.26 ± 0.20	1.71 ± 0.01
R^2	0.20*	0.23*	0.10	0.27*	0.51**

(Mean ± standard error of means, n = 4).

Barimasur-4 (3.95 g kg^{-1}) and Barimasur-3 (3.62 g kg^{-1}) were superior in acquiring and accumulating P (Table 1). The concentration of P in the DM of other genotypes ranged between 2.63 g kg^{-1} (L-5 × 8704-2) and 3.30 g kg^{-1} (8406-122) and the genotypic differences were significant ($p < 0.05$). The correlation between RL and P uptake was weak, but significant ($R^2 = 0.23^*$).

The Ca uptake was highest in Barimasur-4 (20.14 g kg^{-1}). Other genotypes did not differ much in ability to absorb Ca (Table 1), despite differences in their RL (Figure 2).

The uptake of Mg by Barimasur-4 (3.06 g kg^{-1}) and GP-8407-5 (2.91 g kg^{-1}) ranked highest. The lowest amount of Mg was absorbed by BLX-79542 (1.66 g kg^{-1}). The correlation between the Mg uptake and RL was weak ($R^2 = 0.27^*$).

Barimasur-3 and Barimasur-4 together with GP-8707-5 absorbed the highest amount of S. The correlation between RL and S uptake of the genotypes was significant ($R^2 = 0.51^{**}$).

Micronutrients

There was a significant ($p < 0.05$) variation among the genotypes in Fe uptake (Table 2). Although some genotypes with larger root system (Barimasur-4, L-5 × 87272, 8406-122) were among the genotypes absorbing the most Fe, it was difficult to ascertain a clear relationship ($R^2 = 0.14$) between RL and Fe uptake.

Table 2
Concentration of micronutrients in shoot dry matter of lentil genotypes and their correlation (R^2) with root length

Genotypes	Macronutrients (g kg^{-1})						
	Fe	Mn	Zn	Cu	B	Mo	Co
B-masur-3	376 ± 5	49.1 ± 0.2	25.7 ± 1.8	15.6 ± 0.5	14.7 ± 0.1	1.12 ± 0.02	0.26 ± 0.03
B-masur-4	400 ± 7	57.4 ± 1.8	35.2 ± 1.5	20.2 ± 1.5	16.0 ± 0.7	1.96 ± 0.04	0.26 ± 0.02
PLX-79542	338 ± 11	41.4 ± 4.0	29.3 ± 0.9	16.5 ± 0.4	15.6 ± 0.2	0.97 ± 0.04	0.26 ± 0.04
GP-8407-5	315 ± 6	48.6 ± 2.4	33.0 ± 1.4	19.6 ± 0.3	8.5 ± 0.25	0.97 ± 0.01	0.26 ± 0.05
GP-8407	390 ± 4	43.4 ± 0.9	34.4 ± 1.4	18.4 ± 0.7	9.8 ± 0.33	1.36 ± 0.00	0.27 ± 0.03
BLX-79542	352 ± 12	44.1 ± 1.2	26.1 ± 0.2	18.0 ± 0.4	10.7 ± 0.5	1.21 ± 0.10	0.28 ± 0.01
L-5 × 58704(2)	297 ± 10	47.5 ± 1.2	29.0 ± 0.5	12.7 ± 0.9	7.3 ± 0.35	1.45 ± 0.06	0.23 ± 0.01
L-107 × 87102	305 ± 14	40.8 ± 0.3	29.2 ± 0.4	18.1 ± 1.8	7.4 ± 0.33	0.87 ± 0.01	0.23 ± 0.04
L-5 × 87272	390 ± 1	38.3 ± 0.5	27.8 ± 1.3	15.4 ± 0.4	12.2 ± 1.0	1.20 ± 0.11	0.21 ± 0.00
8406-122	373 ± 9	53.4 ± 2.8	27.2 ± 0.2	16.9 ± 0.3	16.3 ± 1.0	0.92 ± 0.01	0.22 ± 0.03
R^2	0.14	0.20*	0.31*	0.31*	0.10	0.10	0.08

(Mean ± standard error of means, n = 4).

Barimasur-4 and 8406-122, both producing relatively larger RL, absorbed significantly ($p < 0.05$) higher amounts of Mn (57.4 mg kg^{-1}mg and 53.4 mg kg^{-1}, respectively) than did other genotypes. Only a weak correlation ($R^2 = 0.20^*$) between RL and Mn uptake of the genotypes could be found (Table 2).

The Zn uptake was highest in Barimasur-4 (35.2 mg kg^{-1}), followed by GP-8407 (34.4 mg kg^{-1}) and GP-8407-5 (33.0 mg kg^{-1}). The genotypic differences were significant ($p < 0.05$). However, there was a weak, though significant, correlation ($R^2 = 0.30^*$) between RL and Zn uptake of the genotypes (Table 2).

The Cu uptake of the investigated genotypes varied significantly (Table 2), but the variation was weakly correlated to RL ($R^2 = .31^*$).

The genotypes that had better root growth (e.g., Barimasur-4 and 8406-122) accumulated a higher amount of B in their shoot biomass (Table 2). As not all genotypes with larger root systems had superior uptake ($R^2 = 0.10$), other soil-based factors may have been involved in B uptake.

The uptake of Mo was highest in Barimasur-4 (1.96 mg kg^{-1}) and there was significant ($p < 0.05$) variation in Mo uptake in other genotypes (Table 2). The Mo uptake of the genotypes was not correlated to their RL ($R^2 = 0.10$).

Most of the genotypes absorbed Co in the range of 0.25 mg kg^{-1} and the results did not differ with genotype (Table 2).

DISCUSSION

The relative growth rate (RGR) of the genotypes was 0.13 ± 0.03, indicating moderate nutrient-stress conditions (Rakhmankulova et al., 2001) in the pot experiment. The investigated lentil genotypes differed significantly ($p < 0.05$) in root length (RL) and in uptake of most plant nutrients. However, in most cases, RL was weakly correlated to the uptake of nutrients in DM (Tables 1 and 2). Root architecture and placement of roots in the soil profiles play an important role in nutrient capture and plant productivity (Lynch, 1995). The size and architecture of lentil root system may depend on the formation of lateral roots (Mia et al., 1996). In the pot experiment, the genotypic variation in lateral spread of the roots in soil profiles could not be determined, which might have contributed to the week correlations between the root lengths and the uptake of nutrients. The enormous enlargement of effective root lengths of the genotypes (e.g., up to 12 times in Barimasur-4) due to the differential presence of root hairs might have masked the effect of RL on the uptake of the nutrients. The lentil genotypes with prolific root-hair formation (Bari-masur-4 and Bari-masur-3) were particularly superior in uptake of those nutrients (K, P, Fe, Mn, Cu, Zn, Mo) whose availability in soil is usually low and whose transport to the roots is diffusion limited. The concentration range of most elements (except Fe and Mn) lies close to the lower limit of the critical deficiency levels (Bergmann, 1992; Marschner, 1995) in DM of legumes.

Plant species, especially nodule-forming and nitrogen-fixing legumes, possess the potential to induce rhizosphere acidification (Tang et al., 1997), but differences in rhizosphere pH were not detected among the lentil genotypes investigated. In the present study, the ability of the genotypes to acidify the rhizosphere was studied using 10-d-old seedlings, which had not yet formed nodules. Root-induced rhizosphere pH is known to influence availability of soil inorganic P (Gahoonia and Nielsen, 1992) and micronutrients to plants (Marschner and Römheld, 1996). The lentil genotypes investigated did not show differences in rhizosphere activity of phosphatase enzymes, suggesting that the observed variation in P uptake of these genotypes may not be due to the mobilization of soil organic P (Asmar et al., 1995; Li et al., 2004). A number of other factors such as root-released organic acids (Ryan et al., 2001), change in rhizosphere redox potential for Fe and Mn acquisition (Shiferaw et al., 1992), rhizosphere microorganisms (Zaidi et al., 2003), and mycorrhizae (Alloush et al., 2000; Weber et al., 1992) may play a role in acquisition of soil nutrients. In the present study, the potential role of these factors was not investigated. Integration of these factors in future studies for identification of nutrient-efficient lentil germplasm would be beneficial. Although in the present study the genotypes did not appear to differ in nodulation, studies with other legumes (Unkovich and Pate, 2000) have indicated that such variation may exist. Therefore, the exploration of genetic variation in the ability to fix N among the lentil genotypes/landraces deserves special attention through more detailed studies.

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