

Genetic variation in root traits and nutrient acquisition of lentil (*Lens culinaris*, *Medikus*) 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 the nutrient poor soils. We investigated the root traits (root length, root hairs, root-induced acidification and phosphatase enzymes) of ten lentil genotypes (Barimasur-3, Barimasur-4, PLX-79542, GP-8407-5, GP-8403, BLX-79542, L-5x8704(2), L-107x87012, L-5x87272 and 8406-122) and related them to the plant uptake of twelve nutrients (P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B, Mo and Co) in the laboratory and pot experiments.

There were significant ($p < 0.05$) differences in root length (RL) and root hair density (number mm^{-1} root) of 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 RL *per se* 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 transport 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 carried out by including more root traits (N_2 fixation, organic acids, mycorrhizae) to find nutrient-efficient germplasm to promote lentil production.

Introduction

Lentil, a protein-rich staple pulse, complements the cereal-rich diet of the people in many developing countries, particularly those in the low-income groups. Lentil is often grown on nutrient-poor soils

without or little fertilizer applications. Superior morphological (root length, root hairs) and physiological (exudation of protons and enzymes) root traits facilitate efficient use of soil nutrients ^[1]. Identification of lentil germplasm with superior root traits may, therefore, help to sustain lentil production in nutrient poor soils of many developing countries including Indian subcontinent, Bangladesh, West Asia, North Africa, Sudan, Yemen, Ethiopia, Eritrea and South America ^[2]. The identified germplasm may be directly introduced or used for targeted breeding of nutrient-efficient and drought-tolerant varieties.

Longer root system may be expected to confer better capture of soil moisture and nutrients, but the knowledge on genetic diversity in root system of lentil is rudimentary ^[3]. Even less is known about whether genetic diversity in lentil root length *per se* can be related to better capture of soil nutrients. For higher plants the essentiality of 15 mineral elements (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B, Mo, Na, Cl, Co) mainly absorbed from soil by roots is well documented ^[4]. Cobalt (Co) is considered essential for nodule development and nitrogen fixation by legumes ^[5]. Lentil, a legume, may partially satisfy its need for nitrogen (N) by fixing atmospheric nitrogen with the help of root associated *Rhizobium*. Deficiency of any one of the other nutrient elements may limit plant growth and economic output ^[4]. Therefore, an integrated approach to explore genetic diversity in as many root traits and to assess their role in uptake of various nutrient elements may be more meaningful.

To understand the role of lentil root traits in uptake of the various nutrient elements and to explore the genetic diversity, we investigated the root morphological (root length, root hairs) and physiological (root exudation of protons and phosphatase enzymes) traits as related to the uptake of twelve mineral elements (except N, Cl and Na) by ten 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 the popularity among Bangladeshi farmers and the anticipated importance for breeding new improved varieties. Barimasur-3, Barimasur-4 are popular commercial varieties and PLX-79542, GP-8407-5, GP-8403, BLX-79542, L-5x8704(2), L-107x87012, L-5x87272 and 8406-122 are breeding lines of potential importance.

Soil properties

Some properties of soil used in the pot experiment are the following,

Soil pH 7.7 (0.01 M CaCl₂); organic matter 0.55 %; total N 0.029 %; major cations extracted with ammonium acetate and measured with flame photometer^[6] (meq/100ml), Ca = 12.0; Mg = 2.5; K = 0.25 and other nutrients (µg/g) P = 10.3^[7]; S = 20^[8]; B = 0.59 (hot water extract^[9]); Cu = 6.3; Fe = 11; Mn = 6; Zn = 1.7 (DTPA extracted and measured with atomic absorption spectroscopy^[10]).

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 two litre 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, 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 days 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 days 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 cut opened; the roots were washed out of soil and cleaned off debris. Pots were not inoculated, but minor nodulation was observed in all pots at 60 DAS. Visual assessment of the washed out roots gave an idea about that no differences in nodulation existed among the genotypes. One g 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, England) as described in Gahoonia et al.,^[11].

Plant analyses

Digestion of plant material

Shoots at flowering stage (60 DAS) was 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, USA) using a graphite-heating block (Mod Block, CPI International, Amsterdam, Holland). 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 ml of 35% HNO₃ (Instra analysed, Baker, Deventer, Holland) was added to the samples and the samples were boiled for approximately 15 minutes. After cooling 2.5 ml 70% HNO₃ was added and the samples were reheated. Twenty five minutes later samples was cooled and 1.5 ml H₂O₂ (Extra pure, Riedel-de Haën, Seelze, Germany) was applied. When the peroxide reaction ceased, 1 ml of H₂O₂ was added and samples were

reheated for approximately 40 minutes. 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, USA) 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% HNO₃) as standards and quantification was done by external calibration (P/N 4400 ICP-MS, Multi-elemental calibration standard, CPI-International, Amsterdam, Holland). Dilutions were performed in a class 100 laminar flow bench (KR-170s Biowizard, Kojair Tech Oy, Vilppula, Finland).

ICP-MS and IR-MS

Twelve elements (K, P, Ca, Mg, S, Fe, Zn, Mn, Cu, B, Mo, Co) were analysed by ICP-MS (Agilent 7500c, Agilent Technologies; Manchester, England). Nitrogen was not analysed, because lentil, a legume, can fix and make use of atmospheric N₂ and N uptake is less dependent on size of the root system ^[12].

Determination of root hairs

The soil was filled in 10-cm long test tubes of diameter 3 cm (soil bulk density 1.4 g cm⁻³, soil moisture 20 %, four replicates). One pre-germinated seed was planted in each tube. After 20 days, the tubes, after cutting the shoot, 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 for about 5-10 minutes removed remaining soil particles without damaging the root hairs. The root hairs were quantified using Quantimet 500+ Image Processing and Analysis System (Leica) at 10x magnification ^[13].

Determination of rhizosphere pH

The roots of 10 days old seedlings were embedded in agar containing pH indicator dye *Bromocresol purple* and adjusted to pH 6 ^[14]. The root-induced pH change, revealed by colour change, was recorded after one hour.

Rhizosphere phosphatase activity

The ability of the genotypes to release acid phosphatase (Aptase) in the rhizosphere was determined by the method of Dinkelaker & Marschner ^[15], which is based on enzymatic hydrolysis of 1-naphthylphosphate (substrate) by root released Aptase, yielding 1-naphtol, which produces a red complex with Fast Red TR (dye). The intact roots of 10 days old seedlings were sand-wiched between

two ashless filter papers, soaked in a mixture of the dye and the substrate. If roots release variable amounts of phosphatase enzymes, their activity is visible as reddish brown colour of variable intensity near the roots, because root-released phosphatase produces reddish brown complex with the dye Fast Red TR.

Statistical analyses were performed with Statistical Analysis System (SAS) Institute ^[16] and Microsoft Excel software as found appropriate. Statistical significant ($p < 0.05$) differences between the genotypes was analysed by 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). Higher amount of DM was produced by the commercial varieties (Barimasur-3 and Barimasur-4) and three breeding lines L-107x87012, L-5x87272 and 8406-122 (Figure 1). L-5x8704(2) produced the lowest amount of DM (Figure 1).

Root traits

There were significant ($p < 0.05$) differences in root length (RL) of the genotypes, both after 20 DAS and 60 DAS (Figure 2). The breeding line 8604-122 produced largest root system ($34.49 \text{ m plant}^{-1}$) at flowering (60 DAS), followed by L-107x87012, L-5x87272; but at 20 DAS, their RL was among the smallest. The improved variety Barimasur-4 produced 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 longest ($0.48 \pm 0.09 \text{ mm}$, Figure 3) and most dense root hairs, followed by Barimasur-3 ($0.38 \pm 0.10 \text{ mm}$). The average root hair length (RHL) of other genotypes was below $0.31 \pm 0.09 \text{ mm}$. Root hair density (RHD, number mm^{-1} root) on the roots of Barimasur-4 was 26 ± 3 as compared to about 17 ± 2 with 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 the presence of root hairs would increase the effective root lengths of Barimasur-4 by twelve times, Barimasur-3 by five times and that of other genotypes by four times. The application of colour 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).

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 longer root system covered with longer and denser root hairs (Figure 3).

Barimasur-4 (3.95 g kg⁻¹) and Barimasur-3 (3.62 g kg⁻¹) were superior to acquire and accumulate P (Table 1). The concentration of P in the DM of other genotypes ranged between 2.63 g kg⁻¹ (L-5x8704-2) 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 with Barimasur-4 (20.14 g kg⁻¹). Other genotypes did not differ 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-8707-5 (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 highest amount of S. The correlation 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-5x87272, 8406-122) were among the genotypes absorbing most Fe, it was difficult to ascertain a clear relationship ($R^2 = 0.14$) between RL and Fe uptake.

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

The Zn uptake was highest with Barimasur-4 (35.2 mg kg⁻¹), followed by GP-8403 (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 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 ($R^2 = 0.31^*$).

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

The uptake Mo was highest with Barimasur-4 (1.96 mg kg^{-1}) and there was significant ($p < 0.05$) variation in Mo uptake of 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 it 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^[17] 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 *per se* was weakly correlated to the uptake of nutrients in DM (Table 1 and 2). Root architecture and placement of roots in the soil profiles play an important role in nutrient capture and plant productivity^[18]. The size and architecture of lentil root system may depend on the formation of lateral roots^[19]. 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 weak correlations between the root length and uptake of the nutrients. The enormous enlargement of effective root lengths of the genotypes (e.g. up to twelve times with Barimasur-4) due to the differential presence of root hairs might have masked the effect of RL *per se* 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 soils is usually low and transport to the roots is diffusion limited. The concentration range of the most elements (except Fe and Mn) lies close to lower limit of the critical deficiency levels^[20, 4] in DM of legumes.

Plant species, especially nodule forming and nitrogen fixing legumes, possess the potential to induce rhizosphere acidification^[21], but the differences in rhizosphere pH were not detected among the investigated lentil genotypes. In the present study, the ability of the genotypes to acidify rhizosphere was studied using 10 days old seedlings which have not yet formed nodules. Root induced rhizosphere

pH is known to influence availability of soil inorganic phosphorus^[22] and micronutrients to plants^[23]. The investigated lentil genotypes did not show differences in rhizosphere activity of phosphatase enzymes, suggesting that the observed variation in P uptake of the genotypes may not be due to the mobilisation of soil organic phosphorus^[24, 25]. A number of other factors like root-released organic acids^[26]; change in rhizosphere redox potential for Fe and Mn acquisition^[27]; rhizosphere microorganisms^[28] and mycorrhizae^[29, 30] 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, the studies with other legumes^[31] indicate that such variation may exist. The exploration of genetic variation in the ability of nitrogen fixation among the lentil genotypes/landraces through more detailed studies, therefore, deserves a special attention.

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Table 1 Concentration of macronutrients in shoot dry matter of ten lentil genotypes. (Mean \pm standard error of means, n= 4) and their correlation (R^2) with root length *per se*.

Genotypes	macronutrients (g kg ⁻¹)				
	K	P	Ca	Mg	S
B-masur-3	20.43 \pm 0.75	3.62 \pm 0.05	16.44 \pm 0.04	2.52 \pm 0.06	3.04 \pm 0.11
B-masur-4	28.12 \pm 1.03	3.95 \pm 0.06	20.14 \pm 0.57	3.06 \pm 0.10	3.05 \pm 0.20
PLX-79542	19.14 \pm 0.06	3.19 \pm 0.01	15.42 \pm 0.55	2.38 \pm 0.06	2.74 \pm 0.15
GP-8407-5	21.33 \pm 0.82	3.11 \pm 0.07	16.58 \pm 1.42	2.91 \pm 0.24	3.04 \pm 0.11
GP-8407	20.21 \pm 1.89	3.22 \pm 0.16	14.71 \pm 0.48	2.42 \pm 0.08	2.62 \pm 0.22
BLX-79542	22.81 \pm 1.61	2.87 \pm 0.09	14.39 \pm 0.66	1.66 \pm 0.21	2.62 \pm 0.14
L-5x8704(2)	20.14 \pm 0.20	2.63 \pm 0.03	14.36 \pm 0.18	2.00 \pm 0.04	2.05 \pm 0.12
L-107x87102	19.50 \pm 1.42	2.98 \pm 0.03	14.76 \pm 1.67	1.89 \pm 0.06	1.95 \pm 0.10
L-5x87272	19.98 \pm 0.89	3.14 \pm 0.15	14.12 \pm 0.16	2.25 \pm 0.16	1.86 \pm 0.13
8406-122	17.76 \pm 0.26	3.30 \pm 0.14	14.70 \pm 0.42	2.26 \pm 0.20	1.71 \pm 0.01
R^2	0.20*	0.23*	0.10	0.27*	0.51**

Table 2 Concentration of micronutrients in shoot dry matter of lentil genotypes. (Mean \pm standard error of means, n= 4) and their correlation (R^2) with root length *per se*.

Genotypes	micronutrients (mg kg ⁻¹)						
	Fe	Mn	Zn	Cu	B	Mo	Co
B-masur-3	376 \pm 5	49.1 \pm 0.2	25.7 \pm 1.8	15.6 \pm 0.5	14.7 \pm 0.1	1.12 \pm 0.02	0.26 \pm 0.03
B-masur-4	400 \pm 7	57.4 \pm 1.8	35.2 \pm 1.5	20.2 \pm 1.5	16.0 \pm 0.7	1.96 \pm 0.04	0.26 \pm 0.02
PLX-79542	338 \pm 11	41.4 \pm 4.0	29.3 \pm 0.9	16.5 \pm 0.4	15.6 \pm 0.2	0.97 \pm 0.04	0.26 \pm 0.04
GP-8407-5	315 \pm 6	48.6 \pm 2.4	33.0 \pm 1.4	19.6 \pm 0.3	8.5 \pm 0.25	0.97 \pm 0.01	0.26 \pm 0.05
GP-8407	390 \pm 4	43.4 \pm 0.9	34.4 \pm 1.4	18.4 \pm 0.7	9.8 \pm 0.33	1.36 \pm 0.00	0.27 \pm 0.03
BLX-79542	352 \pm 12	44.1 \pm 1.2	26.1 \pm 0.2	18.0 \pm 0.4	10.7 \pm 0.5	1.21 \pm 0.10	0.28 \pm 0.01
L-5x8704(2)	297 \pm 10	47.5 \pm 1.2	29.0 \pm 0.5	12.7 \pm 0.9	7.3 \pm 0.35	1.45 \pm 0.06	0.23 \pm 0.01
L-107x87102	305 \pm 14	40.8 \pm 0.3	29.2 \pm 0.4	18.1 \pm 1.8	7.4 \pm 0.33	0.87 \pm 0.01	0.23 \pm 0.04
L-5x87272	390 \pm 1	38.3 \pm 0.5	27.8 \pm 1.3	15.4 \pm 0.4	12.2 \pm 1.0	1.20 \pm 0.11	0.21 \pm 0.00
8406-122	373 \pm 9	53.4 \pm 2.8	27.2 \pm 0.2	16.9 \pm 0.3	16.3 \pm 1.0	0.92 \pm 0.01	0.22 \pm 0.03
R^2	0.14	0.20*	0.31*	0.31*	0.10	0.10	0.08

Capture for Figures

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

Figure 2. Root length of ten lentil genotypes 20 days after sowing (20 DAS) and 60 days after sowing (60 DAS). Bars are standard error of means ($n = 4$).

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 ($n = 4$).

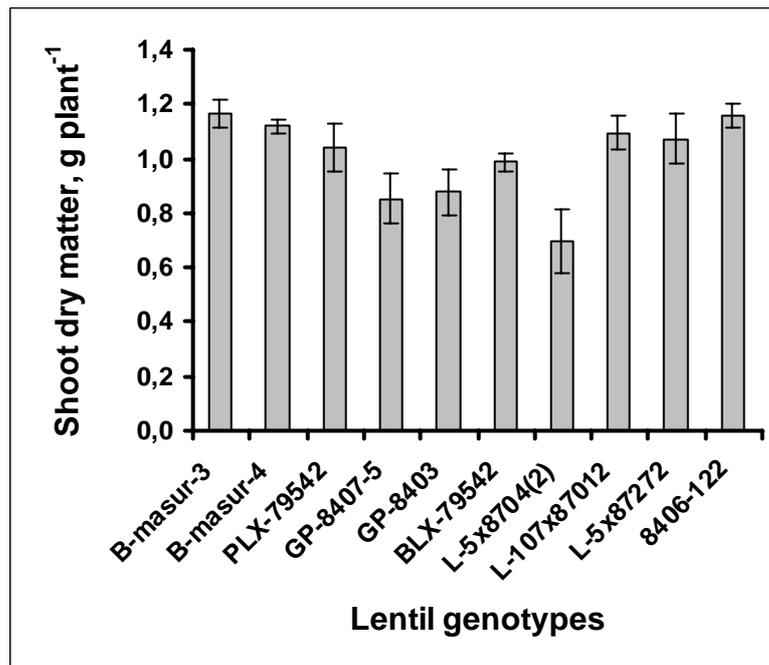


Figure 1

Gahoonia et al.,

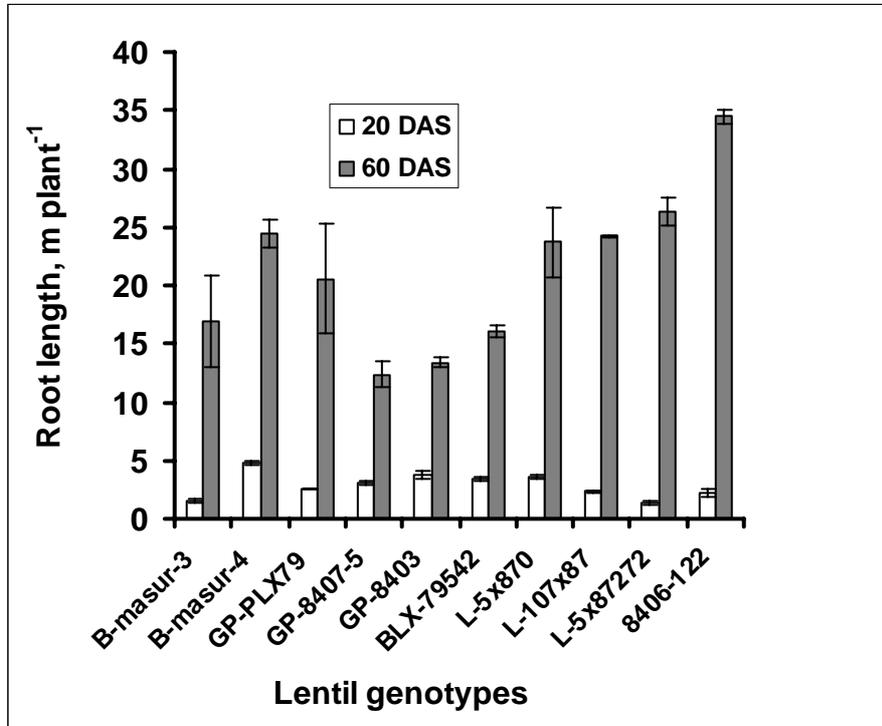


Figure 2

Gahoonia et al.,

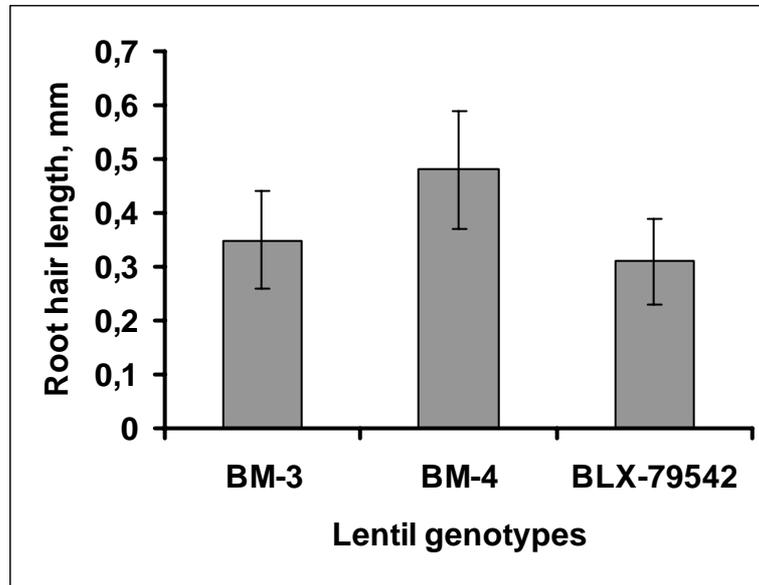


Figure 3

Gahoonia et al.,