Journal of Plant Nutrition, xx, xxx-xxx

ISSN: 0190-4167 print / 1532-4087

Variation in root morphological and physiological traits and nutrient uptake of chickpea genotypes

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Key words: Abiotic stress; breeding; food legumes; rhizosphere pH; root exudates; root system

Abstract

Plant nutrients such as potassium (K), phosphorus (P), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) mostly remain fixed in soils and their bio-availability to plant roots is diffusion-limited. Hence, superior root traits that can enhance their dissolution and capture from the soils, can play a central role. We studied root morphological (root length, root hairs) and physiological traits (root exudation of protons and phosphatase enzymes) of ten selected varieties/breeding lines of chickpea (Bari-chhola-3, Bari-chhola-4, Bari-chhola-5, Bari-chhola-6, Bari-chhola-7, Bari-chhola-8, BGM-E7, ICCV-98926, ICCV-94924 and ICCV-98916) and related them to the uptake of the nutrients in a pot experiment. There were significant ($p < 0.05$) genotypic differences in root length (RL) and root hair length (RHL). The RL ranged between 70 m plant^{-1} and 140 m plant^{-1}. The variation in RHL was significant ($p < 0.05$) and it ranged between 0.58 ± 0.09 mm (Bari-chhola-5) and 0.26 ± 0.09 mm. The root hair density (RHD, number mm^{-1} root) varied between 13 ± 2 and 21 ± 3 among the genotypes. The presence of root hairs increased the effective root surface area (e.g. Bari-chhola-5) up to twelve times. The genotypes differed to acidify the rooting media in the laboratory agar studies, with Bari-chhola-5 inducing greatest acidification, followed by Bari-chhola-3. The ability of Bari-chhola-5 to acidify Rhizosphere could also be confirmed in the field by *in situ* agar studies. The genotypes did not differ to induce acid phosphatase activity (Aptase) in their rooting media. The genotypes inducing greater acidification and having prolific root hairs (Bari-chhola-3 and Bari-chhola-5) absorbed significantly

higher amounts of those nutrients (K, P, Fe, Mn and Zn) whose availability in soils is usually low. The results suggest that a collective effect of superior morphological and physiological root traits confers better nutrition of chickpea genotypes in low-nutrient soil environments.

Introduction

Chickpea is cultivated in Asia, Africa, south Europe, America, and Australia, under climatic conditions ranging from Mediterranean, sub-tropical and tropical. It is an important source of protein and calories for general population (Singh and Saxena, 1999), especially for vegetarians and low income groups. Chickpea is mostly grown without fertilizers by resource-poor farmers on marginal lands characterised as nutrient-poor. Superior root morphological (root length, root hairs) and physiological (exudation of protons and enzymes) traits facilitate efficient use of existing and fertilized nutrients in soils (Gahoonia and Nielsen, 2004). The physiological root traits of chickpea have been investigated (Serraj *et al*., 2004; Wouterlood *et al*., 2004), but only in few cases their importance has been addressed in relation to nutrient uptake (Ali *et al*., 2002; Saxena et al., 1990). The rhizosphere acidification of chickpea has been extensively demonstrated using agar-agar solution containing pH indicator dye *Bromocresol purple* under laboratory conditions (Marschner and Römheld, 1983; Marschner, 1995), but its validation under field conditions has not been done. This paper explores the genetic diversity in root morphological (root length, root hairs) and physiological traits (exudation of protons and phosphatase enzymes) among ten selected varieties/breeding lines and then them to their nutrients uptake.

Materials and Methods

Genotypes

Ten varieties/breeding lines (genotypes) of chickpea (*Cicer arietinum L.)* were selected for investigation; based on their popularity among Bangladeshi farmers and also their anticipated importance for breeding new improved varieties. Bari-chhola-3, Bari-chhola-4, Bari-chhola-5, Bari-chhola-6, Bari-chhola-7, Bari-chhola-8 are improved varieties. BGM-E7, ICCV-98926, ICCV-94924 and ICCV-98916 are breeding lines of potential importance. Bari-chhola-3 and B-chhola-5 are the popular varieties and therefore they were subjected to more detailed studies.

Soil properties

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

Soil pH 7.7 (0.01 *M* $CaCl_2$); organic matter 0.55 %; total N 0.029 %; K = 10.8 µg/g (K was extracted with ammonium acetate and measured with flame photometer, Doll and Lucas, 1973) and other

nutrients (µg/g) P = 10.3 (Olsen-P, Olsen *et al*., 1954); Fe = 11; Zn = 1.7; Mn = 6; Cu = 6.3 (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 varieties was studied in a pot experiment at Pulses Research Center, Ishurdi, Bangladesh. Pots were made by cutting two-litres transparent plastic bottles. They were filled with 2.2 kg of soil, at bulk density of 1.4 g cm^{-3} achieve by shaking. The soil column of each pot was 25 cm high. The pots were placed in the open, sides wrapped in black polythene to prevent exposure of roots to light. Soil moisture was maintained at 20 % 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 rest of the seedlings along with their roots. There were four replicates. At 60 days after sowing (60 DAS) the shoots were cut and stored in paper bags for drying 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 nodulation was observed in all pots. Visual assessment of the washed roots did not revealed noticeable differences in nodulation among the genotypes. One g of fresh root sample was uniformly spread between polythene transparencies and scanned using ScanJet IIcx (Gahoonia et al., 1999). The length of the one-g sample was measured using *Dt-Scan software* (Delta-T Devices, Cambridge, England). Dry weight of the one-g fresh sample and also of the dry weight of whole root mass per pot (three plants) was determined; for calculating the total length of the root system per plant.

Plant analyses

Shoots were dried at 60°C until constant weight was recorded. The whole shoot 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% HNO_3 (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% HNO_3 was added and the samples were reheated. Twenty five minutes later samples was 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 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% HNO_3) 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).

Nutrient elements (K, P, Fe, Zn, Mn and Cu) were analysed by ICP-MS (Agilent 7500c, Agilent Technologies; Manchester, England). Nitrogen was not analysed, because chickpea, a legume, can fix and make use of atmospheric N_2 and N uptake is less dependent on size of root system.

Determination of root hairs

The soil was filled in 10 cm long test tubes (painted black) of diameter 3 cm (soil bulk density 1.4 g cm^{-3}, soil moisture 20 %, four replicates). One pre-germinated seed was planted in soil of each tube. After 20 days the shoot was cut; the tubes were immersed in water overnight (to loosen the soil) in a dark room (to prevent mucilage formation). The content of the test tube (roots in loosen soil) were then poured in a water bath. Roots floated and all roots were removed carefully using a kitchen sieve and transferred into an Ultrasound water bath (Branson 5200). An ultrasound treatment (120W, 47k Hz) for about 5-10 minutes removed remaining soil particles without damaging the root hairs (Gahoonia and Nielsen, 1997). The root hairs were quantified using Quantimet 500_+ Image Processing and Analysis System (Leica) at 10x magnification (Gahoonia and Nielsen, 1997).

Determination of rhizosphere acidification

Seednings of the genotypes were raised in sand filled in 10 cm long test tubes (painted black) of diameter 3 cm (four replicates) by supplying a soil solution* (see blow). After 20 days, the sand was carefully washed out by flushing. The roots of the seelings remained in the tubes. The roots in test tubes were embedded in agar-agar containing pH indicator dye *Bromocresol purple* and adjusted to pH 6 (Marschner and Römheld, 1983). The root-induced pH change, revealed by color change, was recorded after one hour.

For *in situ* field studies of rhizosphere acidification, roots of field growing plants (B-chhola-5 growing in same field soil as used in the pot experiment) were uncovered, placed on soil bed and covered by pouring agar-agar solution (35°C) at pH 6 and containing pH indicator dye *Bromocresol purple*. Agar-

agar solution was prepared in soil extarct (soil solution*). To obtain the soil solution, one kg of soil was collected from the field and suspended in five litres of destilled water overnight and then filtered.

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 seedlins of the genotypes were grown for 20 days as described above in section "Determination of rhizosphere acidification". The intact roots of the seedlings were sand-wiched between two ashless filter papers, soaked in a mixture of the dye and the substrate. when roots released variable phosphatase enzymes, their activity was 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, (1989) and Microsoft Excel software as found appropriate. Statistical significant differences between the treatments was analysed by analysis of variance (ANOVA).

Results

The genotypes differed in growth pattern and production of shoot dry matter (DM). The highest amount of DM was produced by genotype B-chhola-8 and the lowest by breeding line ICCV-98926 (Fig. 1). B-chhola-5 and B-chhola-3 are popular among the Bangladeshi farmers and here B-chhola-5 produced more biomass than B-chhola-3 in the pot experiment (Fig. 1) and also under field conditions (Fig. 5D).

There were significant ($p < 0.05$) differences in root length (RL) of the genotypes at 60 DAS (Fig. 2). The breeding line BGM-E7 produced largest root system. The RL of the improved varieties B-chhola-3, B-chhola-5, B-chhola-4, B-chhola-6, B-chhola-7 and B-chhola-8) was generally greater than the other breeding lines (ICCV-98926, ICCV-94924 and ICCV-98916) (Fig. 2).

The roots of B-chhola-5 were covered with longest (0.58 ± 0.09 mm, Fig. 3) and most dense (Fig. 4) root hairs, followed by B-chhola-6 (0.46 ± 0.10 mm) and B-chhola-3 (0.38 ± 0.11mm). The average root hair length (RHL) of other genotypes was in the range of 0.29 ± 0.09 mm. Root hair density (RHD, number mm^{-1} root) on the roots of B-chhola-5 was 21 ± 3 compared to 13 ± 2 with B-chhola-3 and other genotypes (Fig. 4). Using the average values of RHL and RHD, it was calculated that the

presence of root hairs on the roots will increase the effective root surface area of B-chhola-5 by twelve times and that of B-chhola-3 by five times.

B-chhola-5 possessed the greater ability to acidify rooting medium, followed by B-chhola-3, as revealed by the increment in yellow coloration of pH indicator dye *Bromocresol purple* in agar-agar (Fig. 5A). Other genotypes did not change the pH of their rooting medium. The application of the agar technique to reveal rhizosphere pH change *in situ* in the field was tested using B-chhola-5. It seemed promising that of the technique can also be useful under field conditions (Fig. 5B). The rhizosphere acidification can enhance mobilization of soil nutrients for plant uptake, especially from alkaline soils as used here. The selected chickpea genotypes did not differ to induce acid phosphatase activity (Aptase) in the rhizosphere (data not shown).

The investigated genotypes of chickpea differed in the ability to acquire and to accumulate K and P in the shoot dry matter (DM). The concentration of K (Table 1) was highest in the DM of B-chhola-5 (24.6 g kg^{-1}) followed by BGM-E7 (23.9 g kg^{-1}) and the lowest in B-chhola-7 (16.2 g kg^{-1}). The ability of B-chhola-5 to acquire extra K is related to its ability to produce prolific root hairs (Fig. 3) and greater rhizosphere acidification (Fig. 5A). BGM-E7, which ranked second to acquire K, was able to explore more K due to its much larger root system as compared to other genotypes (Fig. 2).

B-chhola-5 (2.3 g kg^{-1}) and BGM-E7 (2.04 g kg^{-1}) remained superior to acquire and accumulate P (Table 1), followed by B-chhola-3 (2.00 g kg^{-1}).

The highest uptake of Fe was observed with B-chhola-5 (491 mg kg^{-1}), followed by B-chhola-8 (487 mg kg^{-1}) and ICCV-98926 (481.19 mg kg^{-1}) (Table 1). The lowest uptake of Fe was observed with B-chhola-6 (413. 89 mg kg^{-1}).

BGM-E7 absorbed highest amount of Mn (68.4 mg kg^{-1}) (Table 1). The Mn uptake of breeding lines (ICCV-98926, ICCV-94924 and ICCV-98916) was in general markedly less than other varieties.

The Zn uptake was highest with those genotypes (Table 1), which either possessed largest root system (BGM-E7, 30.4 g kg^{-1}) or longer root hairs and/or induced most rhizosphere acidification (B-chhola-5, 29.3 mg kg^{-1}). Similar to Mn uptake, the Zn uptake of breeding lines (ICCV-98926, ICCV-94924 and ICCV-98916) was generally lower than other varieties.

The Cu uptake of breeding lines (BGM-E7, ICCV-98926, ICCV-94924 and ICCV-98916) was generally higher than the improved commercial varieties (Table 1). ICCV-98926 absorbed highest (9.4 mg kg^{-1}) amount of Cu, followed by BGM-E7 (8.4 mg kg^{-1}).

Discussion

Relative growth rate (RGR) of the genotypes was 0.11±0.02, indicating nutrient stress conditions in the pot experiment (Rakhmankulova *et al.,* 2001). The strategies of efficient nutrient acquisition by plants are 1) the superior root morphology (root structure) for exploring nutrients in soils through the development of longer roots covered with more root hairs especially for P acquisition; 2) better root physiology for dissolving soil nutrients through the exudation of protons, organic acids and phosphatase enzymes (Gahoonia and Nielsen, 2004). Significant differences ($p<0.05$) in morphological (root length, root hairs) and physiological (rhizosphere acidification) root traits were found among the investigated chickpea genotypes and breeding lines (Figs. 2-5).The genotypes also differed in their ability to acquire and accumulate both macro- and micronutrients in their DM. Previous studies have demonstrated the significance of root hairs (Gahoonia and Nielsen, 1998) and root-induced acidification for P (Gahoonia and Nielsen, 1992), K (Jensen and Pedersen, 2003) and micronutrients uptake by plants (Marschner and Römheld, 1996). Although all nodulating and nitrogen-fixing legumes would possess the potential to induce rhizosphere acidification (Tang *et al.,* 1997), it is interesting that B-chhola-3 and B-chhola-5 were able to induce rhizosphere acidification at early growth stage (20 days after germination, Fig. 5A) and in the absence of nodulation. It is promising that agar-agar technique could be applied to demonstrate the ability of B-chhola-5 to acidify rhizosphere even under field conditions (Fig. 5C).

B-chhola-5, able to acidify rhizosphere, absorbed relatively higher amounts of the diffusion-limited nutrients (K, P, Fe, Mn, Zn, Table 1), which mostly tend to get fixed as insoluble compounds in soils. Its longer root hairs might have further supported the uptake of the nutrients dissolved through the action of the acidification in the alkaline soil used in the experiment. In case of common bean, prolific root hairs production was correlated with greater acid exudation (Yan *et al.,* 2004). It remains to be examined whether the ability of chickpea genotype B-chhola-5 to acidify its rhizosphere (Figs. 5A and 5C) is related to its denser root hairs (Fig. 4). The investigated chickpea 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 (Asmar *et al.,* 1995; Li *et al.,* 2004). A number of other factors like root-released organic acids (Ryan *et al.,* 2001); change in rhizosphere redox potential (Shiferaw *et al.,* 1992), microorganisms (Zaidi *et al.,* 2003) and mycorrhizae (Alloush *et al.,* 2000; Weber *et al.,* 1992) can 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 studies for identification of nutrient efficient chickpea germplasm would be beneficial. The

exploration of genetic variation in the ability of nitrogen fixation among the chickpea genotypes/landraces also deserves a special attention (Unkovich and Pate, 2000).

The results suggest that a combination of root traits (higher acid exudation and a greater root-hair density and length) might be synergistic for adaptation to low-fertility environments, highlighting the need to investigate genetic variation in morphological and physiological root traits in an integrated manner. The results show that B-chhola-5 possesses prolific root hairs (Fig. 4) and extra ability to induce rhizosphere acidification (Fig. 5), but relatively smaller root size (Fig. 2). BGM-E7 possesses extra large root system (Fig. 2). It may be worth to verify the superior root properties and then combine them through crossing to generate progenies/germplasm for breeding of nutrient-efficient chickpea.

Acknowledgements

The financial support of Danish Council for Development Research (RUF), Copenhagen, for conducting the here reported research, is thankfully acknowledged. Thanks to scientific assistant *Md. Saiful Islam Khan* for helping with the experimental work at Pulses Research Center, Ishurdi, Bangladesh.

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Table 1 Concentration of K, P (g kg^{-1}), Fe, Mn, Zn and Cu (mg kg^{-1}) in shoot dry matter of chickpea genotypes. (Mean ± standard error of means, n= 4).

Genotypes	K	P	Fe	Mn	Zn	Cu
B-chhola-3	20.7±0.56	2.00±0.07	443.6±5.0	47.0±1.6	25.9±0.3	6.2±0.2
B-chhola-5	24.6±0.75	2.33±0.02	490.8±16.6	57.8±2.1	29.3±0.7	7.4±0.2
B-chhola-4	20.24±0.36	2.04±0.08	443.5±5.0	50.5±1.4	26.1±0.9	6.7±0.4
B-chhola-6	18.60±0.66	1.97±0.10	413.5±20.3	49.7±1.4	25.6±1.7	6.9±0.1
B-chhola-7	16.2±0.24	1.73±0.06	452.5±7.7	58.2±1.7	26.2±0.4	6.2±0.1
B-chhola-8	20.23 0.49	1.55±0.02	487.2±4.5	59.5±0.8	27.5±1.1	5.7±0.3
BGM-E7	23.9±0.22	2.04±0.06	447.3±17.6	68.4±1.8	30.4±0.5	8.4±0.2
ICCV-98926	20.24±1.01	1.76±0.10	481.2±0.7	41.0±0.8	24.3±0.1	9.4±0.4
ICCV-94924	16.98±0.28	1.88±0.04	471.7±19.0	50.7±0.1	26.4±0.5	8.3±0.3
ICCV-98916	19.11±0.80	1.90±0.08	419.6±12.2	52.8±0.3	20.2±0.4	8.5±0.4

Capture for Figures

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

Figure 2. Root length of ten chickpea genotypes 60 days after sowing in a pot experiment (60 DAS). Bars are standard error of means (n = 4).

Figure 3. Average root hair length of chickpea genotypes. Bars are standard error of means (n = 60).

Figure 4. Variation in root hairs on the roots of two chickpea genotypes, B-chhola-3 and B-chhola-5.

Figure 5. Demonstration of chemical changes in the rhizosphere of chickpea genotypes B-chhola-3 (61), B-chhola-5 (68), B-chhola-6 (73) and BGM-E7 (85). (A) Visualisation of rhizosphere acidification by using pH indicator dye *Bromocresol purple* in agar; more yellow colour (lighter shade in black and white image) means more acidification; (B) Root induced acidification of chickpea genotype B-chhola-5 growing in the field at flowering stage (60 DAS); yellow colour (lighter shade in black and white image) near the roots indicates acidification; Performance of B-chhola-3 (C) and B-chhola-5 (D) in the field 60 days after sowing (60 DAS).

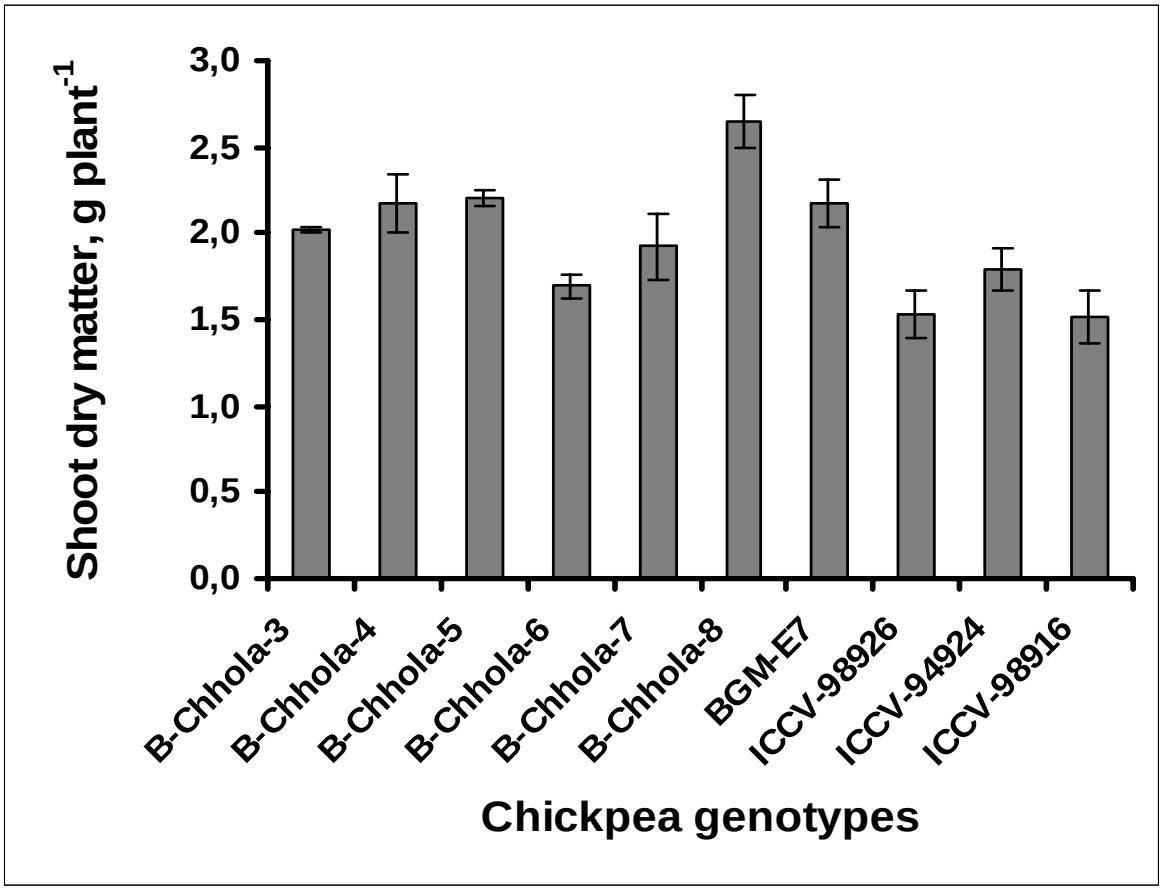
Shoot dry matter, g plant-1
3,0
2,5
2,0
1,5
1,0
0,5
0,0
B-Chhola-3
B-Chhola-4
B-Chhola-5
B-Chhola-6
B-Chhola-7
B-Chhola-8
BGM-E7
ICCV-98926
ICCV-94924
ICCV-98916
Chickpea genotypes

Figure 1

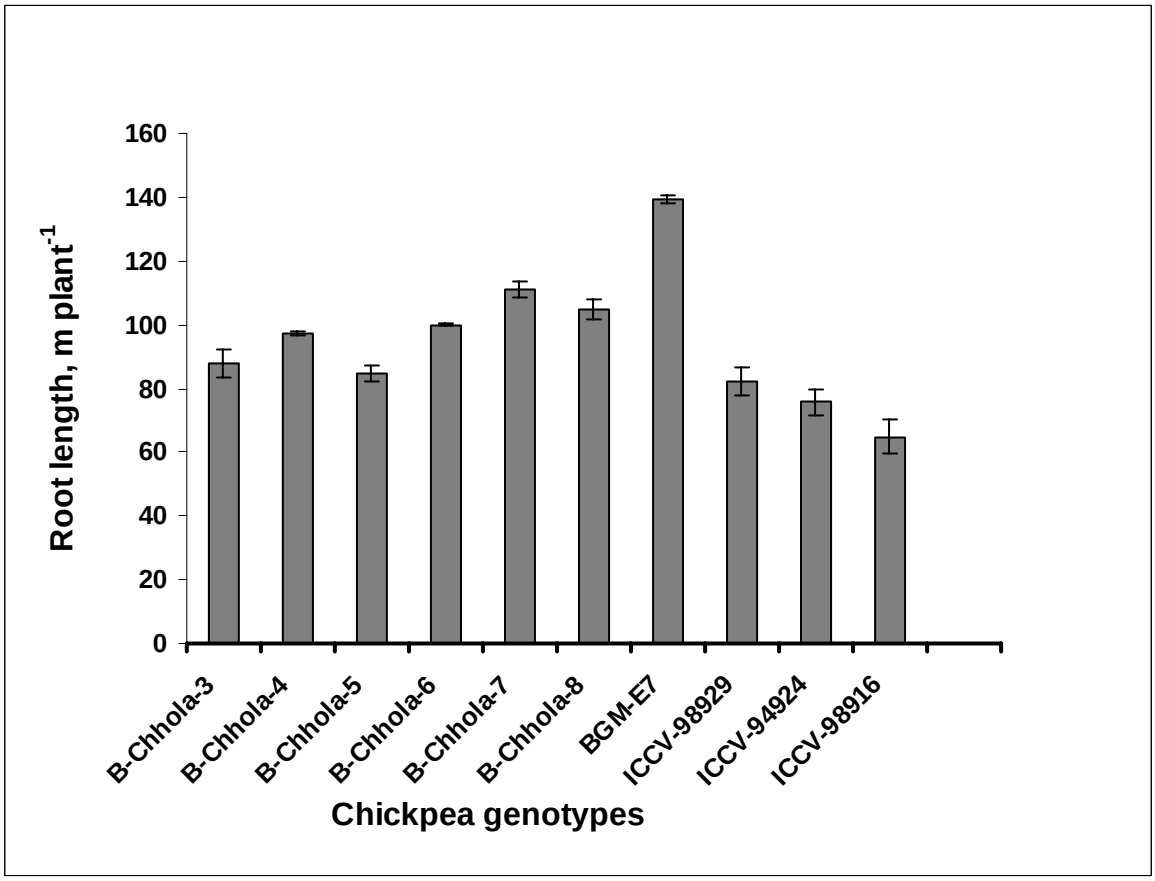
160
140
120
100
80
60
40
20
0
Root length, m plant-1
B-Chhola-3
B-Chhola-4
B-Chhola-5
B-Chhola-6
B-Chhola-7
B-Chhola-8
BGM-E7
ICCV-98929
ICCV-94924
ICCV-98916
Chickpea genotypes

Figure 2

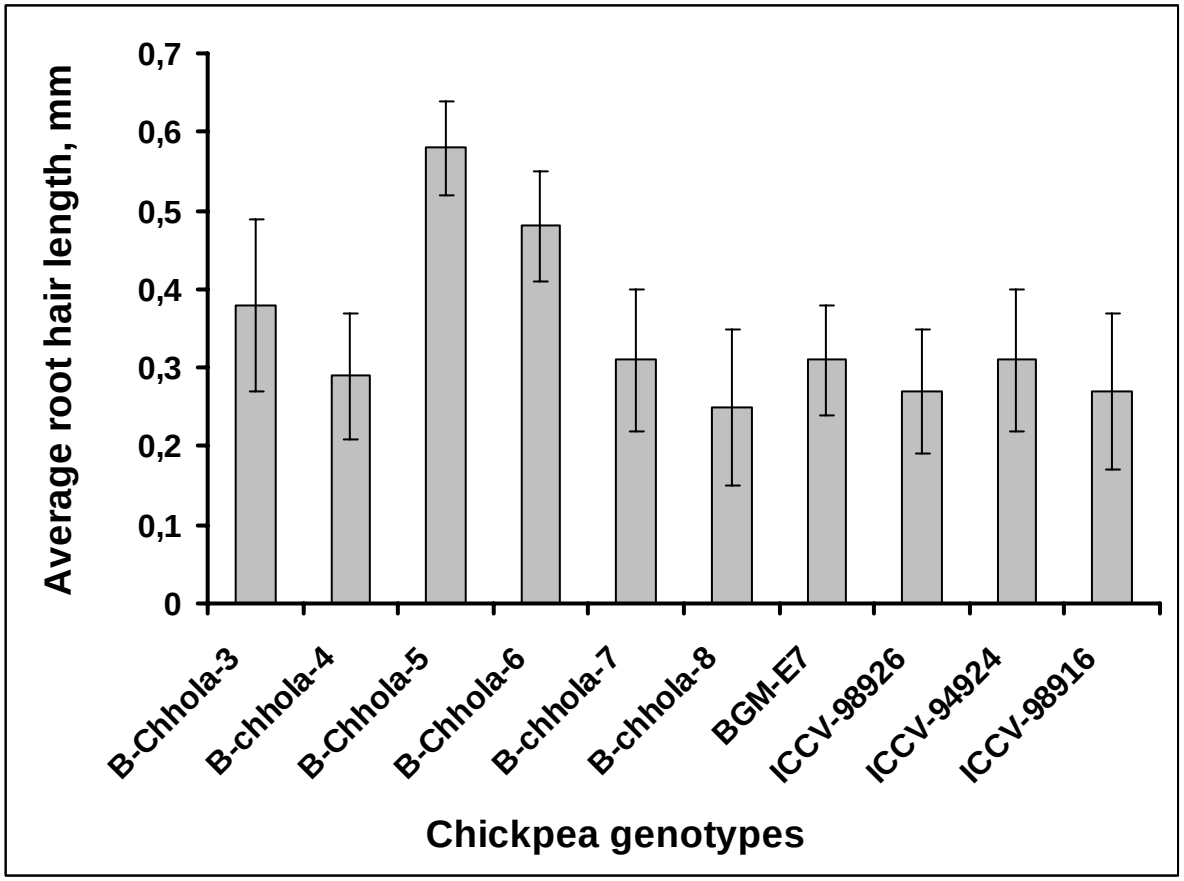
Average root hair length, mm
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
B-Chhola-3
B-chhola-4
B-Chhola-5
B-Chhola-6
B-chhola-7
B-chhola-8
BGM-E7
ICCV-98926
ICCV-94924
ICCV-98916
Chickpea genotypes

Figure 3

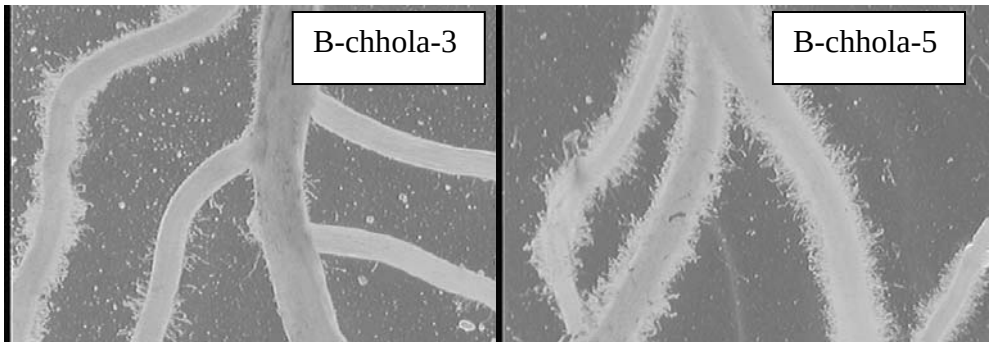

Figure 4


A
B
C
D
73
85
61
68
BREEDER SEED PRODUCTION
BARI CHHOLA-3
AREA :- 59mX15m.
D/S :- 17.11.2003.
BREEDER SEED PRODUCTION
BARI CHHOLA-5
AREA :- 96mX 20m.
D/S :- 17.11.2003.

Figure 5.

Filnavn: Chickpea-Gahoonia.doc
Bibliotek: C:\Documents and Settings\tsg.TARA\Desktop
Skabelon: C:\Documents and Settings\tsg.TARA\Application Data\Microsoft\Skabeloner\Normal.dot
Titel: Root traits and nutrient uptake efficiency of Lentil (Lens culinaris), Grasspea (Lathyrus sativus) and Chickpea (Cicer arietin
Emne:
Forfatter: tsg
Nøgleord:
Kommentarer:
Oprettelsesdato: 24-11-2004 21:24:00
Versionsnummer: 82
Senest gemt: 26-06-2006 10:20:00
Senest gemt af: Tara Singh Gahoonia
Redigeringstid: 720 minutter
Senest udskrevet: 26-06-2006 10:21:00
Ved seneste fulde udskrift
Sider: 16
Ord: 3.954 (ca.)
Tegn: 24.125 (ca.)