Root morphological and physiological traits and nutrient uptake of chickpea (*Cicer arietinum L.*) genotypes

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Abstract

Chickpeas are important source of protein in the diet of people in many developing countries of the world. They are often grown on nutrient-poor and dry soils; hence the role of root traits in capturing soil resources may be central for their economical outputs. Exploration of genetic diversity in root traits of chickpea germplasm will be beneficial for breeding of varieties able to withstand nutrient and water stresses. We investigated the morphological (root length, root hairs) and physiological (root exudation of protons and phosphatase enzymes) root traits and related them to the uptake of twelve nutrients by ten selected varieties/breeding lines of chickpea in a pot experiment. There were significant ($p< 0.05$) differences in root length (RL) among the genotypes. The genotypic variation in RL ranged between 70 m plant^{-1} and 140 m plant^{-1}. The range of variation in root hair length (RHL) was between 0.58 ± 0.09 mm 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 RL up to twelve times. The genotypes differed to induce changes in rhizosphere pH in the laboratory agar studies, which was confirmed by *in situ* field studies. Rhizosphere acid phosphatase activity (Aptase) did not differ among the genotypes. In the pot experiment, the genotypes differed ($P<0.05$) to acquire and to accumulate Potassium (K), Phosphorus (P), Calcium (Ca), Magnesium (Mg), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Boron (B), Molybdenum (Mo) in the shoot biomass (DM). The correlations between RL *per se* and the uptake of most nutrients (except for Mn and Zn) were non-significant. The genotypes inducing greater rhizosphere acidification and having prolific root hairs absorbed relatively higher amounts of the 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 traditionally cultivated in Asia, Africa, south Europe, America, and more recently also in Australia, under climatic conditions ranging from Mediterranean to sub-tropical and tropical, where it is an important source of protein and calories for general population (Singh & Saxena, 1999), especially for vegetarians and low income groups. It plays a vital role in many farming system, as it fixes atmospheric nitrogen through symbiosis with *Rhizobium* in the rhizosphere. There are two types of chickpeas: *desi*, with small angular blackish coloured seeds, is primarily grown in South Asia; and *kabuli*, with large, ram-head shaped and beige-coloured seeds, is predominately grown in West Asia and North Africa.

Chickpea is often grown by resource-poor farmers on marginal lands characterised as nutrient-poor and drought-prone, mostly without fertilizers and irrigation. Therefore, favourable root traits, facilitating the capture of soil nutrients and water, may play an important role to overcome the abiotic stresses and for enhancing its productivity. A combination of morphological (root length, root hairs) and physiological (exudation of protons and enzymes) root traits facilitate efficient use of existing and fertilized nutrients in soils (Gahoonia & Nielsen, 2004). The physiological root traits of chickpea have rarely been investigated (Wouterlood *et al*., 2004). The exceptional studies (Serraj *et al*., 2004) exploring the genetic diversity in morphological root traits of chickpea are uncommon and only in few cases their importance has been addressed in relation to nutrient uptake (Ali *et al*., 2002; Saxena, Malhotra & Singh, 1990). Advancement of knowledge of the chickpea root traits will support breeding of nutrient-efficient and drought-tolerant high-yielding varieties for marginal and dry areas.

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 by roots from soil is established (Marschner, 1995). Cobalt (Co) is considered essential for nodule development and nitrogen fixation by legumes (Dilworth & Bisseling, 1984). Chickpea, a legume, is able to satisfy its partial need for nitrogen (N) when it fixes atmospheric nitrogen with the help of root associated *Rhizobium.* Deficiency of any one of the other nutrient elements may limit its growth and economic output (Marschner, 1995). Therefore, breeding programmes for nutrient efficiency may be more feasible if integrated approach is made to understand the role of various morphological and physiological root traits in acquisition of as many nutrients.

To understand the role of chickpea root traits in uptake of the various nutrient elements and to explore the potential genetic diversity, we investigated the root morphological (root length, root hairs) and

physiological (exudation of protons and phosphatase enzymes) root traits as related to the uptake of twelve nutrient elements (except N, Cl and Na) by ten selected varieties/breeding lines of chickpea.

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 the 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-8 is *kabuli* type, which flowered after 40 days after sowing and others are *desi* type, which flowered at 55-60 days after sowing.

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 %; major cations extracted with ammonium acetate and measured with flame photometer (Doll & Lucas, 1973) (meq/100ml), Ca = 12.0; Mg = 2.5; K = 0.25 and other nutrients (µg/g) P = 10.3 (Olsen-P, Olsen *et al*., 1954); S = 20 (Tabatabai, 1982); B = 0.59 (hot water extract, Bingham (1982); Cu = 6.3; Fe = 11; Mn = 6; Zn = 1.7 (DTPA extracted and measured with atomic absorption spectroscopy, Lindsay & 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 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 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, Nielsen & Lyshede (1999).

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% 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).

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 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 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 (Gahoonia & Nielsen, 1997).

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 (Marschner & Römheld, 1983). The root-induced pH change, revealed by color change, was recorded after one hour. For *in situ* field studies of rhizosphere pH change, roots of field growing plants (B-chhola-5 growing in same field soil as used in the pot experiment) were uncovered and embedded in agar at pH 6 and containing pH indicator dye *Bromocresol purple*. Agar 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 & 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 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 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, (1989) and Microsoft Excel software as found appropriate. Statistical significance of the 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 *Kabuli* type genotype B-chhola-8 and the lowest by breeding line ICCV-98926 (Fig. 1) at 60 DAS. Among the *desi* type, B-chhola-5 and B-chhola-3 are popular among the Bangladeshi farmers and B-chhola-5 produced more biomass than B-chhola-3.

There were significant ($p < 0.05$) differences in root length (RL) of the genotypes, both at 20 DAS and at 60 DAS (Fig. 2). The breeding line BGM-E7 produced largest root system both at early (20 DAS) and at flowering (60 DAS). 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) at 60 DAS (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 0.26 ± 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. Using the average values of RHL and RHD, it was be calculated that the presence of root hairs on the roots will increase the effective root length of B-chhola-5 by 12 times and that of B-chhola-3 by 5 times.

B-chhola-5 possessed the extraordinary ability to acidify rooting medium at the early growth stage of 10 days, followed by B-chhola-3, as revealed by the yellow coloration of pH indicator dye *Bromocresol purple* in agar (Fig. 5A). Other genotypes did not change the pH of their rooting medium. The ability of B-chhola-5 to acidify its root zones was confirmed in *situ* under field conditions (Fig. 5C). 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 as indicated by the equal intensity of reddish brown colour near the roots (Fig. 5B). Aptase holds the potential to catalyse the conversion of soil organic P into inorganic P for plant uptake.

The investigated genotypes of chickpea differed in the ability to acquire and to accumulate K, P, Ca and Mg 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 linked 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). The concentration of P in the DM of B-chhola-3 was 2.00 g kg^{-1}. The variation in root length alone did not explain the variation in the P uptake of the genotypes ($R^2 = 0.004$). A combined effect of rhizosphere acidification, root size and root hairs mostly likely contributed to the higher uptake and accumulation of P. The *kabuli* type genotype B-chhola-8, which produced highest amount

of DM and had moderate size of root system, accumulated the lowest amount of P in its DM (1.55 g kg^{-1}) apparently due to the dilution effect.

The Ca uptake by the genotypes ranged between 14.13 g kg^{-1} (B-chhola-4) and 24.17 g kg^{-1} (B-chhola-7) (Table 1). The genotypic differences in Ca uptake could not be related to the root length (R^2 = 0.007) or any other root traits investigated here.

The uptake of Mg by B-chhola-5 ranked highest (1.67 g kg-1), followed by ICCV-94924 (1.64 g kg^{-1}) and ICCV-98926 (1.63 g kg^{-1}) (Table 1). The relationship between the Mg uptake and root length was weak (R^2 = 0.005). A combination of rhizosphere acidification and longer root hairs seems to have contributed to the highest Mg uptake of B-chhola-5.

The breeding line BGM-E7, producing largest root system, absorbed highest amount of S (1.88 g kg^{-1} DM), followed by ICCV-98926 (1.85 g kg^{-1}) (Table 1). However, no general relationship between the investigated root traits and S uptake of the genotypes (R^2 = 0.012) could be suggested.

The highest uptake Fe was observed with B-chhola-5 (491 mg kg^{-1}), fallowed by B-chhola-8 (487 mg kg^{-1}) and ICCV-98926 (481.19 mg kg^{-1}) (Table 2). The lowest uptake of Fe was observed with B-chhola-6 (413. 89 mg kg^{-1}). The relationship between root length and Fe uptake of the genotypes was non-significant (R^2 = 0.001). The greater rhizosphere acidification of B-chhola-5 seems to have supported its highest Fe uptake.

A combination of longer root size, rhizosphere acidification and root hairs seems to have contributed to the variation in Mn uptake of the genotypes. The correlation between the Mn uptake and root lengths of the genotypes was significant (R^2 = 0.48**). The genotype BGM-E7 absorbed highest amount of Mn (68.4 mg kg^{-1}) (Table 2). The Mn uptake of breeding lines (ICCV-98926, ICCV-94924 and ICCV-98916) was in general markedly less than other genotypes.

The Zn uptake was highest with those genotypes (Table 2), which either had largest root system (BGM-E7, 30.4 g kg^{-1}) or possessed longer root hairs and induced rhizosphere acidification (B-chhola-5, 29.3 mg kg^{-1}). The correlation between the Zn uptake and root lengths of the genotypes was significant (R^2 = 0.34*). Similar to Mn uptake, the Zn uptake of breeding lines (ICCV-98926, ICCV-94924 and ICCV-98916) was generally lower than other genotypes.

The Cu uptake of breeding lines (BGM-E7, ICCV-98926, ICCV-94924 and ICCV-98916) was generally higher than the improved commercial genotypes (Table 2). ICCV-98926 absorbed highest (9.4 mg kg^{-1}) amount of Cu, followed by BGM-E7 (8.4 mg kg^{-1}). In general, Cu uptake of the genotypes did not relate to their root length (R^2 = 0.06).

The highest amount of B was found in the DM of ICCV-98926 (16.3 mg kg^{-1}), followed by B-chhola-3 (15.5 mg kg^{-1}) (Table 2). The observed variation in B uptake among the genotypes could not be related to the variation in their root lengths (R^2 = 0.052) or other root traits.

The uptake Mo was lowest (0.26 mg kg^{-1}) in case of three genotypes, B-chhola-7, ICCV-98926, and ICCV-98916 (Table 2). Other genotypes absorbed about 0.36 mg kg^{-1} DM, which could not be related their root length (R^2 = 0.011) or other root traits.

The genotypes which had better root growth (BGM-E7 and B-chhola-8) accumulated high amount of Co in their DM (Table 2). However, the accumulation of Co was highest in case of B-chhola-5 (0.36 mg kg^{-1}) and B-chhola-3 (0.31 mg kg^{-1}), which possessed the ability to induce rhizosphere acidification in the rooting medium.

Discussion

The relative growth rate (RGR) of the genotypes was 0.11±0.02, indicating nutrient stress conditions in the pot experiment (Rakhmankulova *et al.,* 2001). 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). However, the root length *per se* was weakly correlated with the uptake of most nutrients, except for Mn and Zn. The strategies of efficient nutrient acquisition by plants are 1) the superior root morphology for exploring nutrients in soils through the development of longer roots covered with more root hairs and association with mycorrhizae especially for P acquisition; 2) better root physiology for dissolving soil nutrients through the exudation of protons, organic acids and phosphatase enzymes. The investigated genotypes differed in their ability to acquire and accumulate both macro- and micronutrients in their DM, but the variation could not be fully attributed to any single root traits. Root hairs (Gahoonia & Nielsen, 1998) and root induced rhizosphere acidification influence availability of soil inorganic phosphorus (Gahoonia & Nielsen, 1992), K (Jensen & Pedersen, 2003) and micronutrients to plants (Marschner & Römheld, 1996). Although all nitrogen fixing legumes possess the potential to induce rhizosphere acidification (Tang, McLay & Barton, 1997), it is interesting that B-chhola-3 and B-chhola-5 were able to induce rhizosphere acidification at early growth stage (10 days after germination, Fig. 5A) and in the absence of nodulation. The ability of B-chhola-5 to acidify rhizosphere could be demonstrated even under field conditions (Fig. 5C), offering it the advantage to overcome nutrient stress over the growth period.

B-chhola-5, able to acidify rhizosphere, absorbed relatively higher amounts of diffusion-limited nutrients (K, P, Fe, Mn, Zn, Tables 1 and 2), which especially tend to get fixed as insoluble compounds in soils. Its longer root hairs might have further supported the uptake of the nutrients mobilised through the action of the acidification. 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 linked 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, Gahoonia & Nielsen, 1995; Li *et al.*, 2004). A number of other factors like root-released organic acids (Ryan, Delhaize & Jones, 2001); change in rhizosphere redox potential (Shiferaw, Shelton & So, 1992), microorganisms (Zaidi, Khan & Amil, 2003) and mycorrhizae (Alloush, Zeto & Clark, 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 & Pate, 2000).

The results suggest that the 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 trying to combine the superior root properties through crossing to generate progenies/germplasm for breeding of nutrient-efficient and drought-resistant chickpea.

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Table 1 Concentration of macronutrients in shoot dry matter of ten chickpea genotypes. (Mean ± standard error of means, n= 4).

Genotypes	macronutrients (g kg^{-1})				
	K	P	Ca	Mg	S
B-chhola-3	20.7±0.56	2.00±0.07	16.67±0.80	1.50±0.02	1.50±0.10
B-chhola-5	24.6±0.75	2.33±0.02	18.98 ±0.43	1.67±0.08	1.37±0.10
B-chhola-4	20.24±0.36	2.04±0.08	14.13±0.38	1.35±0.06	1.63±0.12
B-chhola-6	18.60±0.66	1.97±0.10	19.76±0.58	1.34±0.05	1.29±0.10
B-chhola-7	16.2±0.24	1.73±0.06	24.17±1.14	1.57±0.09	1.40±0.06
B-chhola-8	20.23 0.49	1.55±0.02	22.66±1.15	1.54±0.04	1.62±0.09
BGM-E7	23.9±0.22	2.04±0.06	20.96±0.06	1.52±0.03	1.88±0.13
ICCV-98926	20.24±1.01	1.76±0.10	22.20±1.04	1.63±0.03	1.85±0.08
ICCV-94924	16.98±0.28	1.88±0.04	23.65±0.99	1.64±0.19	1.55±0.02
ICCV-98916	19.11±0.80	1.90±0.08	20.20±0.39	1.39±0.11	1.77±0.11

Table 2 Concentration of micronutrients in shoot dry matter of chickpea genotypes. (Mean ± standard error of means, n= 4).

Genotypes	micronutrients (mg kg^{-1})						
	Fe	Mn	Zn	Cu	B	Mo	Co
B-chhola-3	443.6±5.0	47.0±1.6	25.9±0.3	6.2±0.2	15.5±0.4	0.36±0.01	0.31±0.01
B-chhola-5	490.8±16.6	57.8±2.1	29.3±0.7	7.4±0.2	11.8±0.1	0.35±0.02	0.36±0.01
B-chhola-4	443.5±5.0	50.5±1.4	26.1±0.9	6.7±0.4	12.5±0.7	0.34±0.04	0.25±0.03
B-chhola-6	413.5±20.3	49.7±1.4	25.6±1.7	6.9±0.1	11.5±0.2	0.37±0.03	0.25±0.01
B-chhola-7	452.5±7.7	58.2±1.7	26.2±0.4	6.2±0.1	11.7±0.2	0.26±0.02	0.24±0.01
B-chhola-8	487.2±4.5	59.5±0.8	27.5±1.1	5.7±0.3	8.9±0.1	0.37±0.02	0.29±0.01
BGM-E7	447.3±17.6	68.4±1.8	30.4±0.5	8.4±0.2	12.4±0.5	0.37±0.02	0.29±0.01
ICCV-98926	481.2±0.7	41.0±0.8	24.3±0.1	9.4±0.4	16.3±0.1	0.27±0.01	0.25±0.01
ICCV-94924	471.7±19.0	50.7±0.1	26.4±0.5	8.3±0.3	9.6±0.1	0.37±0.02	0.23±0.01
ICCV-98916	419.6±12.2	52.8±0.3	20.2±0.4	8.5±0.4	13.6±0.8	0.26±0.01	0.25±0.01

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Capture for Figures

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

Figure 2. Root length of ten chickpea genotypes 20 days after sowing (20 DAS) and 60 days after sowing (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 = 4).

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 pH change by using pH indicator dye *Bromocresol purple* in agar; more yellow colour means more acidification; (B) Visualisation of rhizosphere acid phosphatase activity (Aptase), equal intensity of reddish brown colour near the roots means equal Aptase. (C) Root induced acidification of chickpea genotype B-chhola-5 growing in the field at flowering stage; yellow colour near the roots indicates acidification.

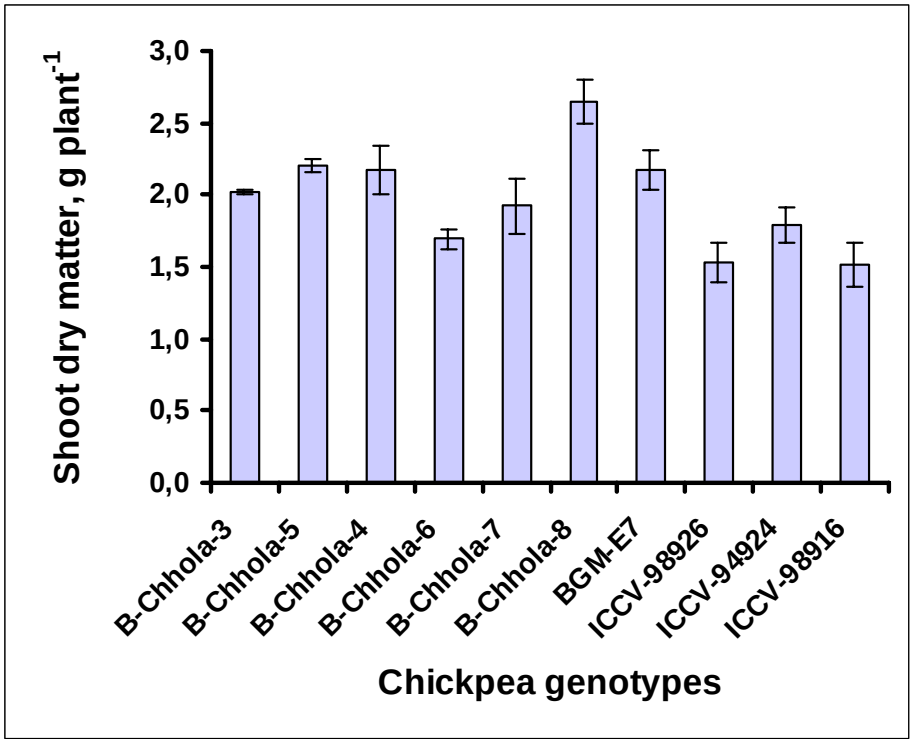

Figure 1
(Gahoonia et al.,)

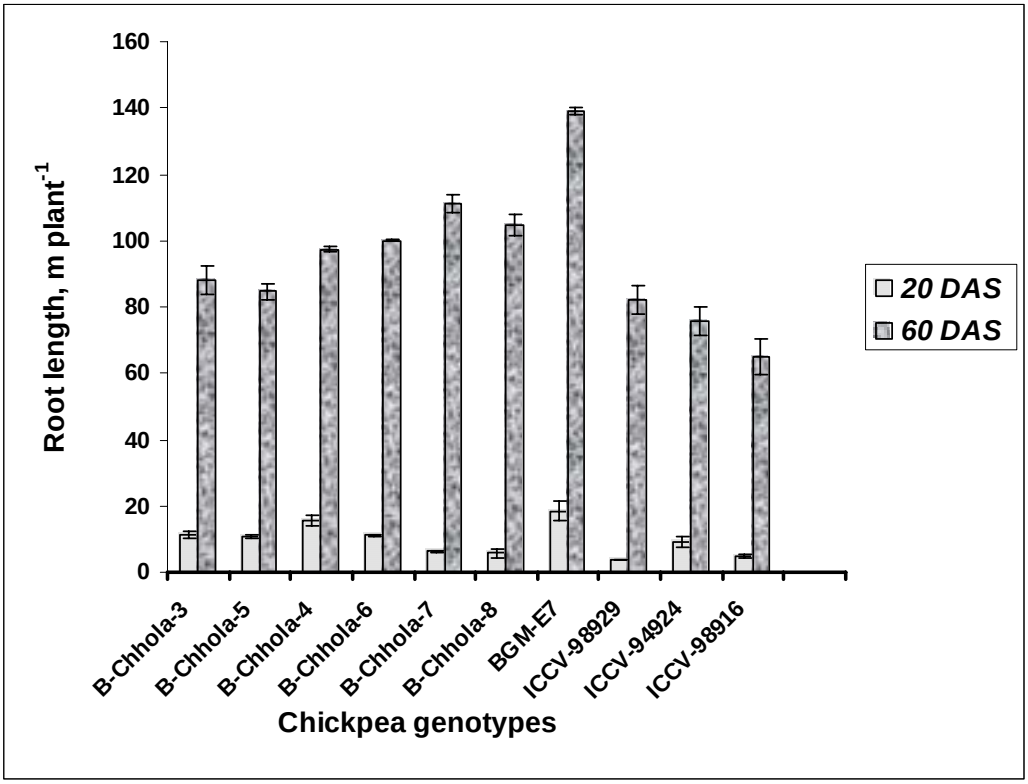

Figure 2

(Gahoonia et al.,)

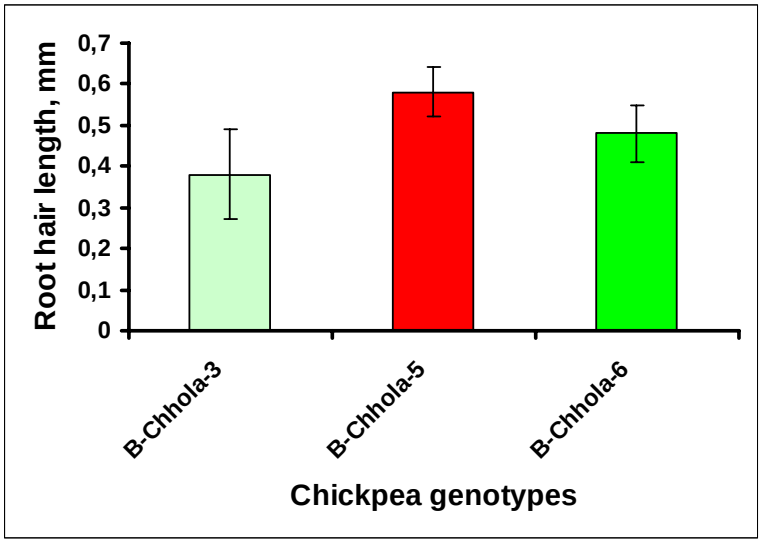

Figure 3

(Gahoonia et al.,)

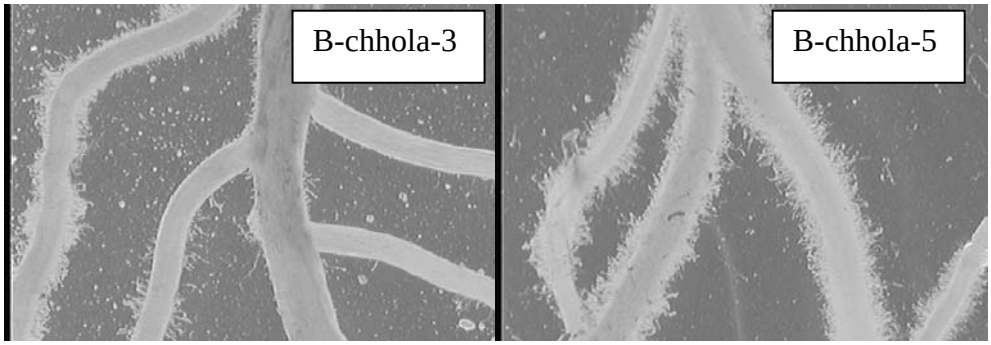

Figure 4

(Gahoonia et al.,)

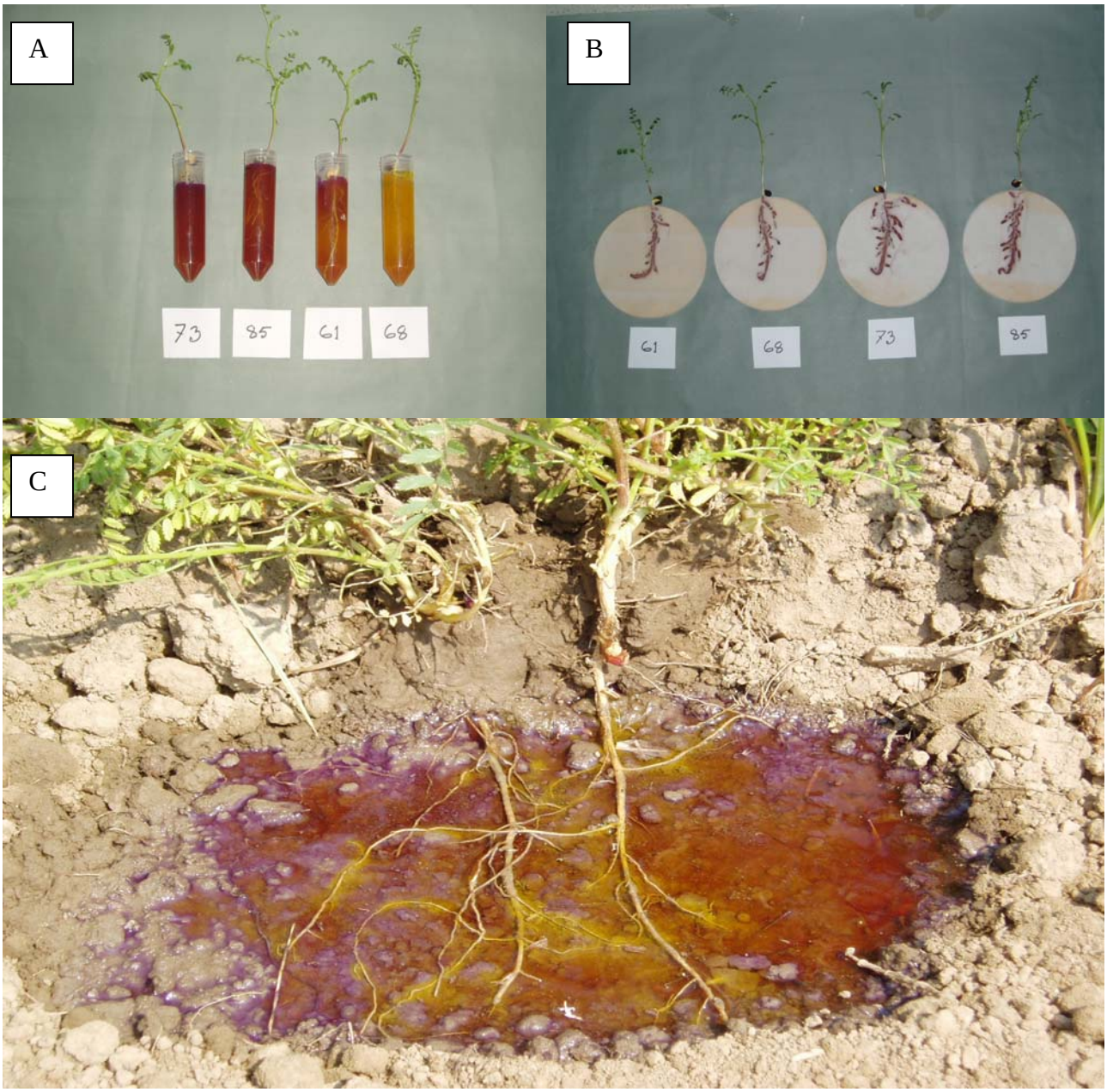

Figure 5.

(Gahoonia et al.,)