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Scope for genetic manipulation of mineral acquisition in chickpea

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Abstract

Nutrient acquisition in chickpea needs to be efficient, because it is mainly grown as a post-rainy season, rainfed crop, and generally on soils inferior in physical characteristics and poor in fertility. Nutrient deficiencies have been reported to cause yield losses of varying magnitude in chickpea, e.g., 22–50% due to iron (Fe), around 10% due to sub-optimal nodulation and hence nitrogen (N) deficiency, 29–45% due to phosphorus (P), up to 100% due to boron (B), and 16-30% due to sulphur (S). Yield losses due to salinity are equally large but are difficult to estimate because of its heterogeneous occurrence. In chickpea, genotypic differences in morpho-physiological (including root size) and functional (exudates) root traits, and in nodulation capacity for increased nitrogen fixation have been identified. Genotypic differences in response to application of Fe, B and zinc (Zn) have also been found among chickpea genotypes. A drought tolerant chickpea genotype ICC 4958, which has a relatively large root system, acquired more P than other genotypes during the vegetative period in a pot experiment at ICRISAT. The recent thrust on identifying QTLs for root size should facilitate progress in incorporating useful root traits through marker assisted selection in desirable agronomic backgrounds. Selection for nodulation capacity in released cultivars has resulted in high nodulating chickpea genotypes that produced 10% higher yield than the control varieties. Information on targeted crop improvement for higher nutrient-use efficiency for P, S, Zn, B and Fe is not readily available. Methods to screen for tolerance to salinity are available, but sufficiently high levels of tolerance have not yet been found in germplasm or wild relatives of chickpea to warrant breeding for salinity tolerance. Use of alternative approaches, such as mutation to generate genetic diversity or introgression of alien genes from other crops (transgenic) are thus required, and these remain long-term objectives.

Introduction

Chickpea is an important food legume crop in many countries of the world. It is a source of high quality protein in food and feed. Since it is grown mostly as a rainfed, postrainy season crop (in winter in the subtropics and in spring in Mediterranean and temperate climates), it is often subjected to terminal drought stress. Being a legume, and having secondary status to cereals, it is often grown on land less preferred for cultivation of cereals, where soils are generally marginal in their soil physico-chemical characteristics. Thus, availability and interaction between soil moisture and nutrients are important considerations in chickpea management. The crop is efficient in biological nitrogen fixation (Peoples and Crasswell, 1992) and in accessing native soil P not utilized by crops such as cereals (Ae et al., 1991). These factors confer on it the characteristics for greater productivity under adverse environmental conditions and sustainability of the production systems in which it is grown. Mineral nutrient deficiencies commonly observed in major chickpea producing areas are: nitrogen (N) (due to sub-optimal nitrogen fixation), phosphorus (P), sulfur (S), iron (Fe), zinc (Zn) and boron (B). Salinity is another abiotic stress of economic importance: globally nearly 323 million ha of agricultural land is affected by saline or sodic soil conditions (Brinkman,

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1980). These soils occur mainly in arid and semi-arid regions where evaporation exceeds precipitation, coinciding with regions otherwise suitable for chickpea cultivation.

In this paper, recent findings on the chickpea root system, the nutrient-acquiring organ, are reviewed. The extent of genetic variation in chickpea is explored with respect to response to the major mineral limitations, and the scope for favorable genetic manipulation assessed, in terms of alternative management options. The scope for using emerging technologies in molecular genetics to enhance mineral acquisition of chickpea is considered.

The chickpea root system

The key to improving the ability to acquire often scarce resources of both water and nutrients lies in a plant's root system. Important factors are the ability of the root system to explore the available soil volume, to release substances which may enhance availability of nutrients and to host symbioses that favor nutrient acquisition (e.g. nitrogen fixation, mycorrhizae). Over recent years there have been several studies indicating genetic variation in key root traits. These studies are reviewed here in the context of recent studies of the chickpea root system in relation to nutrient and water acquisition.

Rooting depth

Rooting depth indicates the extent of soil that crop roots can exploit for nutrients and water. There are large variations in maximum rooting depth reported for chickpea (28 to 270 cm) (Krishnamurthy et al., 1996), mostly governed by growth duration (Aujla and Cheema, 1985; Sheldrake and Saxena, 1979; Subramanian et al., 1980) and the soil environment. Rooting depth is limited by soil bulk density, texture and moisture. In Vertisols at ICRISAT, Andhra Pradesh, India, maximum rooting depth is found to be 120–135 cm (Krishnamurthy et al., 1996; Sheldrake and Saxena, 1979). In clayey soils, very low or very high levels of soil moisture do not permit root penetration. When dry, these soils tend to harden by shrinking and impede root penetration. When wet, these soils expand but soil pores are filled with water resulting in anaerobic conditions and thus impeded root respiration (Hodgson and MacLeod, 1989; Okada et al., 1991), thereby limiting root penetration (Krishnamurthy et al., 1998). Limitation due to oxygen deficiency may not allow the intrinsic genetic potential for rooting depth to express in such soils. A classic example was provided by Gregory (1996). The average maximum rooting depth of lupin (*Lupinus* spp.), pea (*Pisum sativum* L.) and wheat (*Triticum aestivum*) on three deep sands (xeric psamments) in Western Australia was 190 cm, 65 cm and 113 cm, respectively, with significant differences between genotypes and species but not between sites (Hamblin and Hamblin, 1985). By contrast, when planted in a duplex soil in Western Australia, both lupin (*Lupinus angustifolius*) and wheat were able to extend roots only to 80 cm depth due to physical impediments to growth in both the sand and clay layers and restriction in depth of soil following re-wetting by rain (Dracup et al., 1993; Gregory and Eastham, 1996).

Genetic differences have been reported for effective rooting depth (75 to 150 cm), as estimated by soil moisture depletion, in spring-sown chickpeas in a Calcic Luvisol in Syria (Silim and Saxena, 1993). Wide varietal variation in chickpea was demonstrated in effective rooting depth in an alluvial sandy loam under nonirrigated conditions, and was positively correlated with water uptake (Nagarajrao et al., 1980).

Root distribution

Chickpea is generally grown on residual soil moisture and is exposed to varying degrees of terminal drought stress depending upon the soils, rainfall and evaporative demand. However, in several regions, chickpeas are grown with irrigation. Therefore, root proliferation is expected to vary across the soil profile influencing the timing and extent of nutrient and water uptake between irrigated and nonirrigated environments. In order to better understand this variation a field experiment was conducted in the 1998-99 postrainy season on a Vertisol (fine montmorillonitic isohyperthermic typic pallustert) having 230 mm available water to a soil depth of 150 cm at ICRISAT, Patancheru, India (17°30′ N, 78°16′ E, altitude 549 m) (Ali, 2000). Root distribution of chickpea genotypes ICC 4958 (a prolific rooting accession), Annigeri (a landrace well adapted to peninsular India) and ICCV 10 (a widely adapted variety) was compared with and without irrigation. Roots were extracted by the monolith method (Heeraman and Juma, 1993) and root length density (RLD) and dry weights measured.

The root distribution patterns of Annigeri and ICCV 10 were similar and thus only results for ICCV 10, for comparison with ICC 4958, are presented (Fig-

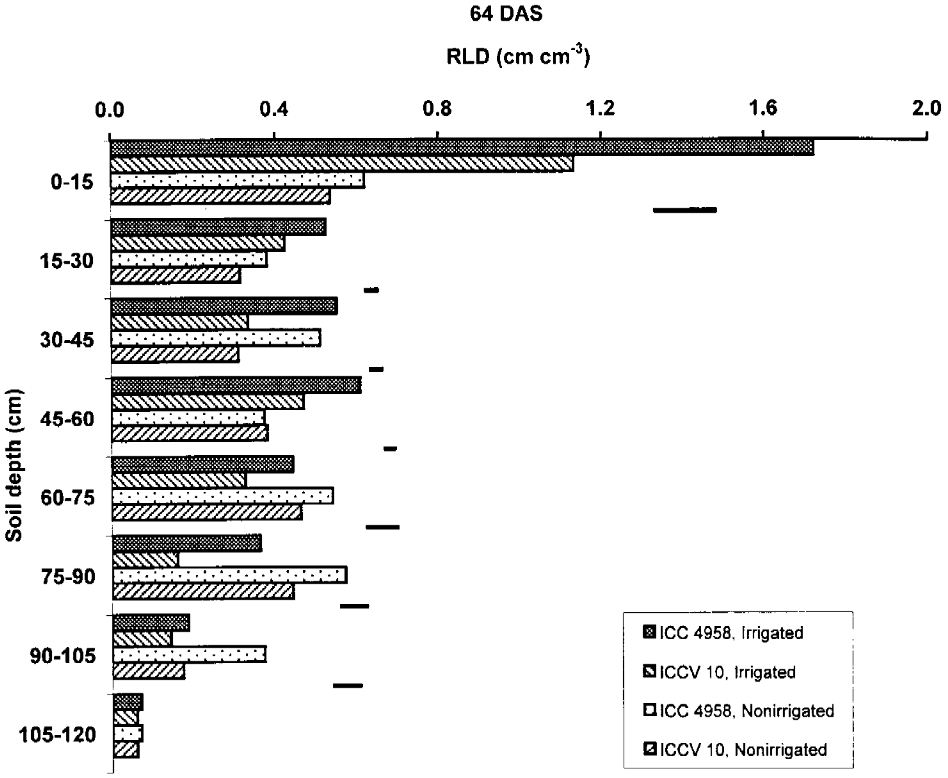

Figure 1. Root length density (RLD cm cm^{-3}) of two chickpea genotypes at 64 days after sowing under nonirrigated and optimum irrigated conditions in a Vertisol during the postrainy season 1998-99. (Values were means of 3 replicates. Horizontal bars denote SE of mean for irrigation × genotype interaction)

ure 1). The time of this observation, 64 days after sowing, coincides with the mid-pod fill stage of the crop in nonirrigated and early pod fill stage in the irrigated conditions. Three major points are apparent in this figure. A significant increase in RLD with irrigation occurred in the 0–15 cm soil layer in both the genotypes. However, at depth there were more roots without irrigation, indicating the negative interaction between irrigation and rooting depth. There is an overall superiority of ICC 4958 over ICCV 10 in total root length per unit area of soil. These observations support the contention that genetic improvements to even well adapted genotypes are possible to increase root proliferation and thereby increase root surface area available for nutrient uptake from soil. This study also shows the advantage of irrigation in increasing root proliferation for nutrient uptake, as most nutrients are distributed in the surface soil layer.

Root activity

Uptake of nutrients such as P (Ae et al., 1991) and Fe (Ohwaki and Sugahara, 1997) is enhanced by root exudates. Chickpea produces more exudates compared to other legumes or cereals (Ae et al., 1991). A field study was conducted in a Vertisol at ICRISAT during the 1992–93 postrainy season in order to measure root respiration, as an estimate of root activity, over soil depth and time. The following chickpea genotypes/selections were grown under rainfed conditions (except for a post-sowing irrigation to ensure even crop establishment): Annigeri, ICC 4958, ICCV 94912, ICCV 94913 and ICCV 94916. The rate of respiration, measured after an hour of incubation of freshly extracted roots, did not differ among the genotypes/selections and therefore the means of genotypes are presented in Figure 2. The rate of respiration was the highest in the seedling stage (15 days after sowing) and this rate declined with the age of the crop. With the advance in crop growth and drying up of the surface soil layers the higher root activities moved to moist

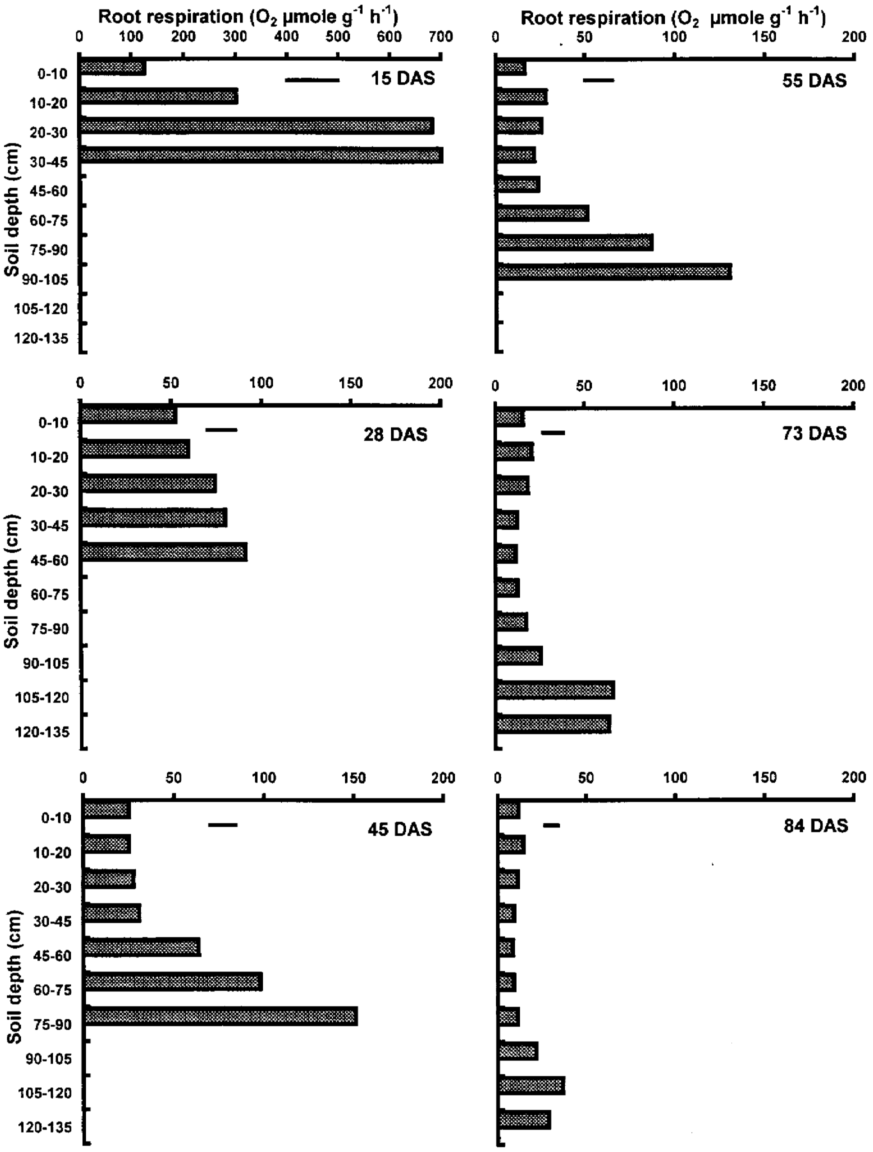

Figure 2. Rate of respiration of extracted roots as measured from various soil layers under receding soil moisture conditions of a Vertisol field, in 1992-93 season. (In this study, two genotypes, ICC 4958 and Annigeri, and three selections made from progenies of these genotypes were grown. The means of 3 replications and 5 genotypes are presented as the genotypic variation was nonsignificant. Horizontal bars denote SE of mean for irrigation × genotype interaction).

deeper soil layers over time. Therefore total root activity at deeper, moist soil layers is directly dependent on the amount of roots in those zones.

Molecular markers to select for root traits

Root studies in the field are laborious, fraught with errors and are limited in their interpretation because of normally variable soil conditions. Concerted efforts are being made to map quantitative trait loci (QTLs) for root traits in chickpea by several groups of researchers. Currently at ICRISAT, root volume and length of over 140 recombinant inbred lines of an interspecific *Cicer* cross are being measured. Molecular markers have been identified for these recombinant inbred lines (RILs). Studies on the segregation of molecular markers and root traits are expected to help tag root traits. This procedure should make it feasible to incorporate desirable root traits through marker assisted selection in disease resistant and high yielding genetic backgrounds of chickpea.

Scope for genetically enhancing mineral acquisition

Against the background of the accumulated knowledge of chickpea root behavior, the scope for enhancing acquisition of major mineral nutrient limitations to chickpea productivity is examined. Although chickpea may be considered efficient in this regard, in comparison with most other cultivated crop species, there is scope for further improving this efficiency to increase and stabilize yields of the crop in stress environments.

Nitrogen

Like other legumes chickpea can access soil mineral-N through its roots and atmospheric N through fixation in root nodules formed in symbiosis with rhizobia. Chickpea can potentially fix about 80% of its N-needs through N fixed by nodules (Peoples and Crasswell, 1992) under optimum growth conditions. Under these conditions, biomass yield and N_2-fixation traits correlated well in agronomic studies (Herridge et al., 1998). Also, several authors have reported a significant correlation in biomass yield and N_2-fixation traits, when a large number of legume cultivars (including those of chickpea) are screened (Rupela and Dart, 1982). Apparently, this led to the conclusion that breeding for high yield will result in high N_2-fixation as well. Therefore, in chickpea breeding programmes, the N_2-fixation trait is generally not considered in progeny selection (Bahl et al., 1990; Saxena and Singh, 1987). However, large plant-to-plant differences in N_2-fixation traits within particular cultivars of chickpea and pigeonpea (perhaps the only two legumes studied in this regard) speak differently (Rupela, 1994). Nodulation was assessed in two chickpea cultivars, G 130 (ICC 4948) and K 850 (ICC 5003). For each cultivar, plants were rated for nodulation on a ‘1’ (lowest nodulation) to ‘5’ (highest nodulation) visual rating scale. G 130, a selection from a landrace, has low nodulation capacity and K 850, a bred line, has high nodulation capacity. In G 130, about 70% plants had low nodulation (rating ‘1’), about 30% plants had medium nodulation (rating ‘2’ and ‘3’) and only 1% plants had high nodulation (rating ‘4’ and ‘5’). In K 850, on the other hand, almost 70% plants had high nodulation (rating ‘4’ and ‘5’), about 30% had medium nodulation (rating around ‘3’) and only 1% plants had low nodulation (rating ‘1’). Plants of contrasting nodulation capacities within each of the two cultivars were selected and advanced, by pure line selection. In initial verification at ICRISAT (Rupela, 1994) the contrasting pairs (HN: high nitrogen fixation, LN: low nitrogen fixation) within each cultivar and their parents (ICC 4948, ICC 5003) remained consistent for nodulation characteristics. In further studies, these were evaluated in seven experiments at five different locations in India (Dudeja et al., 1997) and one location each in Bangladesh (for two years), Nepal and Pakistan (Khanam et al., 1997). Relative differences for nodule number and nodule mass between the HN and LN selections within a cultivar were consistent across locations and years. The HN selections generally yielded higher (range 4–41% in ICC 5003 HN and 4–106% in ICC 4948 HN) than the LN selections of the same cultivar. However, the differences were significant ($P<0.05$) only in two of the seven experiments in India, one of the two experiments in Bangladesh and in one experiment in Pakistan.

Another study examined a maximum of 353 plants derived from 86 entries including advanced breeding lines, recently released cultivars and germplasm accessions. Seed from plants selected for HN and LN at two soil-N levels (high = suppressive for BNF, low = control) were evaluated and advanced for 2–3 years following pure line selection. In the 1995/96 postrainy season, 84 entries involving HN and LN selections from 17 parent lines, each having 1–10 selections were evaluated in the field for N_2-fixation traits and yield at two soil N levels (Rupela, 1997). Three of the 17 advanced breeding lines (ICCV 91016, ICCV 91019 and ICCV 91026) had HN selections that were significantly ($P< 0.05$) better than parents. Some selections had superiority over parents in nodule mass at low soil N and others had superiority at high soil N. This superiority, however, was not necessarily associated with superiority in total dry matter yield. Some selections were, however, superior both for nodule mass and yield, suggesting the scope of simultaneous selection for these traits.

In another field study, the HN selection of ICC 4948 had greater (13%) overall N-yield in a chickpea-sorghum cropping system (Rupela et al., 1995). In this experiment, the soil health (measured as microbial biomass carbon and N) in the plots of the HN selection was superior (by 1.8 to 2.0 times) to those of the LN selection.

The studies confirmed that high N_2-fixing selections can be developed from advanced breeding chickpea lines having disease tolerance/resistance. It also demonstrated the scope of developing stress tolerant lines (high mineral-N tolerant symbiosis in this case) by pure line selection without resorting to a crossing program. The occurrence of large plant to plant differences in nodulation (including non-nodulation) within land races and advanced breeding lines (Rupela, 1992) was considered to be due to the ability of chickpea to effectively use both soil-N (through roots) and aerial-N (due to symbiotic N_2-fixation). As a result, both the HN and LN type plants within a line remain undetected from above ground traits, and are only detectable when nodulation or N_2-fixation are measured.

Phosphorus

Phosphorus is normally the most limiting nutrient for growth of leguminous crops in tropical and subtropical regions. The reports on the response of chickpea to applied P are highly inconsistent. Response of chickpea to P in the field can be 0–45% (Ae et al., 1991; Dadhich and Mali, 1991; Johansen and Sahrawat, 1991; Ninje, 1991), depending upon the agroclimatic environment. Phosphorus limitation is particularly severe in soils with high contents of Fe, Ca or Al oxides where P is strongly bound and is thus less available for uptake by crop plants (Ae et al., 1991). Chickpea is capable of P extraction from otherwise unavailable forms of P, particularly Ca-bound P, by acidifying alkaline calcareous Vertisols (Ae et al., 1991; Marschner and Romheld, 1983). Gas chromatographic analysis of the root exudates from the different crops showed that citric acid was the major component exuded and that chickpea exuded the most (Ae et al., 1991). The greater acid secretion by chickpea roots would imply a greater volume of rhizosphere acidification in chickpea than in the other crop species. This ability makes chickpea efficient in mining P from soils and converting it into organic forms, thus increasing its significance in recycling of soil P in cropping systems.

The availability of soil moisture plays a key role in P uptake. Drilling of P fertilizer below the soil surface compared to surface broadcasting and mixing was found to be superior for chickpea (Tandon, 1987). Later work demonstrated deep banding of P to be advantageous in chickpea as it enables more P uptake (Arihara et al., 1991).

Table 1. Root dry weight (g pot^{-1}) of five chickpea genotypes, as affected by levels of P fertilizer application, at 40 and 73 days after sowing[a]

40 days after sowing

Genotype	P levels (kg ha^{-1})				
	0	20	40	60	100
Annigeri	3.2abc	4.7ab	5.3ab	4.2bc	5.9a
ICC4958	4.6a	3.9bc	6.2a	7.0a	6.2a
ICCV10	2.5bc	2.8c	4.00bc	4.7b	4.9ab
ICCV94916-4	3.7ab	6.2a	5.0cb	7.4a	5.9a
ICC5680	2.0c	2.6c	3.0c	2.8c	3.6b

73 days after sowing

Genotype	P levels (kg ha^{-1})				
	0	20	40	60	100
Annigeri	11a	37a	28a	29ab	28a
ICC4958	22a	29ab	24a	24ab	20a
ICCV10	14a	25ab	27a	21b	26a
ICCV94916-4	16a	31ab	32a	34a	27a
ICC5680	12a	12a	23a	20b	23a

[a] Three plants were grown in 25-cm diameter, 120-cm tall pots during 1998/99 postrainy season at ICRISAT. Values are means of three replicates.

[b] In a column, means followed by a common letters are not significantly different at the 5% level – according to Duncan's multiple range test.

In an attempt to quantify differences between chickpea genotypes in their ability to acquire P, a pot culture study was conducted at ICRISAT to measure the effect of P application on extent and timing of root proliferation in different chickpea genotypes (Annigeri, ICC 4958, ICCV 94916-4, ICCV 10 and ICC 5680). Top soil (0–15 cm) from a P-deficient Vertisol (1 ppm Olsen's P) was uniformly packed into 120 cm tall polyvinyl chloride cylinders and different rates of P (0, 20, 40, 60, 100 kg P ha^{-1}, applied as $CaHPO_4.2H_2O$) were mixed in the top 30 cm of the soil column. The 3 plants grown in each cylinder were harvested at 20, 40 and 73 days after sowing (DAS) and the roots separated from the soil.

The root dry weight harvested at 20 DAS showed no difference due to P application, possibly because of reliance on P derived from seed to this stage. However, at 40 DAS there was a significant increase in root dry

Table 2. Root:shoot ratio of five chickpea genotypes, as affected by levels of P fertilizer application, at 40 and 73 days after sowing[a]

40 days after sowing

Genotype	P levels (kg ha^{-1})					
	0	20	40	60	100	Mean
Annigeri	0.71	0.64	0.59	0.55	0.54	0.60
ICC 4958	0.73	0.52	0.45	0.50	0.41	0.52
ICCV 10	0.84	0.59	0.57	0.63	0.56	0.64
ICCV 94916-4	0.68	0.60	0.50	0.55	0.46	0.56
ICC 5680	0.60	0.49	0.48	0.52	0.44	0.51
Mean	0.71	0.57	0.52	0.55	0.48	
SE (±) for comparison of means of:						
Genotype	0.019***					
P level	0.019***					
Genotype × P level	0.043^{NS}					

73 days after sowing

Genotype	P levels (kg P ha^{-1})					
	0	20	40	60	100	Mean
Annigeri	0.59	0.80	0.65	0.64	0.52	0.64
ICC 4958	0.59	0.60	0.38	0.38	0.29	0.45
ICCV 10	0.74	0.65	0.62	0.63	0.45	0.62
ICCV 94916-4	0.54	0.59	0.57	0.47	0.36	0.51
ICC 5680	0.53	0.60	0.65	0.58	0.51	0.57
Mean	0.60	0.65	0.57	0.54	0.43	
SE ± for comparison of means of:						
Genotype	0.042**					
‘P’ level	0.042**					
Genotype × ‘P’ level	0.094^{NS}					

[a] Three plants were grown in each 25-cm diameter, 120-cm tall pots during 1998–99 postrainy season at ICRISAT. Values are means of three replicates.

weight up to 60 kg P ha^{-1} and at 73 DAS up to 20 kg P ha^{-1} (Table 1). Increased P application encouraged early shoot growth as well as root growth but a decrease in root:shoot ratio with P application indicated that a greater proportion of dry matter was diverted to the shoot (Table 2). Root:shoot ratio decreases with plant age (Krishnamurthy et al., 1996; Table 2) which implies that the bulk of plant P requirements would be acquired at early growth stages, when the top soil containing a higher P concentration is likely to be moist. The data of Tables 1 and 2 illustrate the strategy of chickpea in investing in a greater partitioning of dry matter to roots under P-limiting conditions.

At 40 and 73 DAS, there were significant genotypic differences in P response, with ICC 4958 and ICCV 94916-4 being least responsive with most root production at low P levels (Table 1). These two genotypes have been selected for more prolific root growth under drought stress. It therefore appears that they retain this characteristic under P stress, and thus have a comparative advantage over other genotypes in exploiting a greater soil volume and potentially extracting more P from the soil when soil P is in limiting supply. There is indeed a correlation between this root vigor and total P uptake by the plants at 40 and 73 DAS under conditions of low P supply, with more P uptake in the vigorous rooting ICC 4958 and ICCV 94916-4 and least in the poorly rooting ICC 5860 (Table 3). ICC 5680 was included in this study because of its poor rooting ability under drought conditions, and it also showed least root development among the genotypes at low soil P levels. Thus differences in root proliferation should reflect in differences in P acquisition ability. Exploration of a larger soil volume should permit greater acidification of that volume and consequent increased availability of P, in alkaline soil, for uptake by roots. It therefore seems feasible that P nutrition of chickpea can be improved by genetic improvement of root system proliferation.

Sulfur

Crops in general require as much S as they need P. Sulfur deficiency has been reported from over 70 countries worldwide including nearly 175 million hectares of cropped area in the Indian subcontinent (Tandon, 1991). Chickpea responds to S fertilization across locations in India with yield increases ranging from 16 to 30% in environments with a mean yield of 1851 kg ha^{-1} without S application (Tandon, 1991). Although genotypic differences in response to applied S have been documented for wheat and rapeseed mustard, we can find no reports of such genotypic comparisons for chickpea. In view of the fact that S availability generally tends to increase with soil depth, deeper rooting capability in chickpea may assist its acquisition of S, and deeper rooting genotypes would therefore have an advantage.

Zinc

Zinc deficient soils are common in both tropical and temperate climates (Graham et al., 1992; Sillanpaa and Vlek, 1985; Welch et al., 1991). In India, Zn deficiency is recognized as one of the most common micronutrient deficiencies, and about 50% of the Indian soils have been classified as Zn deficient (Katyal, 1985). Most arable land in Pakistan has inadequate levels of available soil Zn, and Zn deficiency is a common nutritional disorder in alkaline and calcareous soils (Kausar et al., 1979; Khattak and Perveen, 1986; Rashid et al., 1987). Chickpea, particularly, has been shown to be more sensitive to Zn deficiency (Ahlawat, 1990; Khan et al., 1998a; Sakal et al., 1988) than wheat and other cereals (Tiwari and Pathak, 1982). Therefore, chickpea production can be adversely affected by Zn deficiency as chickpea is mainly grown on Zn-deficient soils, although there are few recorded field responses of chickpea to Zn application.

Wide genotypic variation is available for sensitivity to Zn deficiency in chickpea (Khan et al., 1998b). Less increase in root:shoot ratio and transport of proportionally more Zn to the shoot (70%) under conditions of Zn deficiency were the salient characteristics of less sensitive genotypes. More studies are needed to understand the mechanisms underlying Zn efficiency in chickpea. However, it is feasible that production of more Zn-chelating exudates depends upon the extent of root system. Therefore, improving the root system should also improve Zn acquisition ability of chickpea plants. Inexpensive alleviation of Zn deficiency by fertilizer application is possible once the problem diagnosed. However, it is prudent to have genotypes efficient in Zn acquisition. The fact that breeding lines and newly released genotypes show improved Zn efficiency (Khan et al. 1998b) indicates an unconscious selection for this trait already. More conscious efforts are needed to select Zn-efficient genotypes of chickpea. Genetic transformation, by transferring rye chromosomes 1R and 7R into wheat, has increased the Zn-efficiency of wheat (Cakmak et al., 1997). Similar approaches could be tried for chickpea using genetic variation for Zn-efficiency from within the germplasm collection or wild species of *Cicer* or related legumes.

Table 3. Phosphorus uptake by whole plants (mg plant^{-1}) of five chickpea genotypes at 0 (P_0) and 100 (P_{100}) kg ha^{-1} levels of P fertilizer application, at 40 and 73 days after sowing (DAS)[a]

Genotype	40 DAS		73 DAS	
	P_0	P_{100}	P_0	P_{100}
Annigeri	4.4	12.8	12.0	47.0
ICC 4958	6.4	13.4	25.7	46.8
ICCV 10	2.4	11.0	17.0	55.4
ICCV 94916-4	4.3	13.4	18.3	51.3
ICC 5680	4.7	9.7	15.8	38.4
SE (genotype × P level)		0.62		1.77

[a] Three plants were grown in each of 25-cm diameter, 120-cm tall pots during 1998–99 postrainy season at ICRISAT. Values are means of three replicates.

Boron

Boron deficiency is a major cause of crop yield loss in China, India, Nepal and Bangladesh (Anantawiroon et al., 1997). Some of the major chickpea growing areas with soils diagnosed to be B deficient are found in the inner Terai region of Nepal, adjacent regions of Bihar state of India and in northern Bangladesh. The yield losses due to B deficiency can be as high as 100% in chickpea (Srivastava et al. 1997). Boron deficiency was noted to be more severe in soils containing a high sand fraction and low organic matter (Phetchawee and Ratanarat, 1989).

There has been little quantification of the extent of genetic variation in response of chickpea to B, except from the observation in Nepal that introduced (exotic) lines that are much more susceptible to the problem than local landraces, such as Dhanush. However, the grain yields of these landraces are also poor when compared with the yield potential of chickpea in other regions (Srivastava et al., 1997). A wide range of genetic variation in abilty to yield at low B supply was found in wheat (*Triticum aestivum*) (Anantawiroon et al., 1997) and an acceptable screening method (Rerkasem and Loneragan, 1994) has been developed and used. The backcross reciprocal monosomic method (Snape and Law, 1980) has been successfully employed to compare a particular monosomic chromosome from one variety with the same monosomic chromosome from another variety (Chantachume et al., 1997; Law et al., 1983) to identify the chromosome responsible for B efficiency. Similar efforts are needed to develop a screening method for selecting B-efficient genotypes in chickpea. It will be possible to choose an appropriate genetic enhancement method depending on the type and extent of variation available in chickpea.

Iron

Iron deficiency can occur in chickpea when it is grown in soils of high pH (8.0). Yield losses due to Fe-deficiency in susceptible chickpea genotypes can be in the range 22–50% (Saxena et al., 1994). Literature on the effects of alkaline soil conditions (high pH, calcareous soils), appearance of Fe-chlorosis, yield losses due to deficiency, genotypic differences in Fe-deficiency and its inheritance, screening methods, and criteria for selection have been reviewed by Saxena et al. (1994). Good progress has been made in understanding the physiological basis of development of Fe-deficiency. Present evidence suggests that the large genotypic differences in Fe response found may be related to differences in both root (Malewar et al., 1982) and shoot (Ohwaki and Sugahara, 1993) traits. Further work is warranted to develop this information and to use it in identifying tolerant sources in germplasm and in segregating populations derived from these sources. It does not seem necessary to develop a specific breeding program to produce Fe-efficient genotypes because the inefficient genotypes can easily be identified by the distinctive Fe deficiency symptoms and rejected in a breeding population. If necessary, these populations could be grown under conditions that favor induction of Fe-deficiency under natural conditions (wet, alkaline, clayey soils).

Salinity

A large potentially arable area, nearly 323 million ha on a global basis, is estimated to be saline or sodic (Brinkman, 1980). In some countries, e.g. Pakistan, saline land areas are substantial and cover nearly 40% of the cultivated area (Mohammad, 1978). Excessive and injudicious application of irrigation water, among various factors, have contributed to recent increases in saline land area. Legumes, in general, have been found to be more sensitive to saline soil conditions than other crop species (Maas and Hoffman, 1977). Among legumes, cool season food legumes – chickpea, lentil and faba bean – are more sensitive to soil salinity (Lauchli, 1984; Maas and Hoffman, 1977).

Responses of cool season food legumes (including chickpea), to salinity, methods to screen for tolerance to saline conditions, inheritance of the trait, and genotypic differences in tolerance, have been reviewed (Saxena et al., 1993, 1994). The levels of tolerance to soil salinity are indeed low in chickpea as seen in a 50% reduction in shoot biomass at an electrical conductivity (EC) of 5–6 dS m^{-1}. At this relatively low level of tolerance, L 550, a Kabuli chickpea variety from India was found to be most tolerant, and this has been confirmed in many experiments grown in pots and field (Johansen et al., 1990; Saxena, 1987). However, it is disappointing to find that the wild relatives of chickpea are even more sensitive to salinity than the cultivated species (Johansen et al., 1990).

Some progress has been made in generating understanding of mechanisms of salinity tolerance, e.g., Na^+ accumulation in the tops in chickpea (Lauter and Munns, 1986). It is likely that other mechanisms, e.g., selective retention of Na^+ in the roots and stems of soybean (Jacoby, 1964; Salim, 1987), may also be present in the germplasm and are worth investigating. Many studies have shown that *in vitro* selection of cells for high salinity levels does not lead to development of salt tolerant plants regenerated from those single cells (Rowland et al., 1989; Watad et al., 1991). This is because expression of tolerance at the cellular level does not necessarily translate into tolerance at the whole plant level. It does not seem that empirical methods of breeding for yield are likely to be effective in selecting tolerant plants until sources of greater salinity tolerance are found in cultivated accessions of chickpea or its wild relatives. A systematic evaluation of genotypes that have evolved in naturally saline soils (e.g. as in Iran or Iraq) would be a first step in this regard. Research efforts should also be directed towards characterization of the physiological mechanisms or traits conferring salinity tolerance to the point of identifying genes controlling them. Then enhancement of salinity tolerance in chickpea by transgenic methods may become feasible.

In the short term, it appears that use of high yielding varieties developed for rainfed cultivation should increase production on saline lands as well, because salinity occurs heterogeneously across a field. These varieties would contribute more from non-saline or less saline patches, compared to other varieties. Increasing production from the non-saline patches should therefore lead to increased productivity. Some even question whether increasing salinity tolerance, with mechanisms such as Na/K discrimination, would indeed lead to increased productivity of these species in dry saline soils. Evidence in barley and wheat (Richards, 1993) suggests that it may not be so.

Conclusion

With a shrinking area of quality arable land for agriculture and its increasing allocation to staple cereal or high value crops, it is unlikely that in future chickpea will be grown on lands which are better endowed with nutrients than on which it is grown at present. Mineral nutrient deficiency will therefore continue to be a major and increasing constraint to chickpea production. It is encouraging that in recent years substantial progress has been made in generating information and knowledge on the crop. Also conventional and recently-developed tools are available to impart increased nutrient acquisition ability for important nutrient elements. It seems, therefore, realistic to expect some recovery of losses in yield due to nutrient deficiency through genetic improvement for increased nutrient acquisition. Mechanisms of efficient uptake of nutrients, mediated through either physical root size, microbial symbioses or surface chemical characteristics of roots (exudates) are now better understood. Tools of modern molecular biology, for identification, mapping and tagging of the genes are now being investigated to develop flanking markers for QTLs indicating the large root trait in chickpea. Once this is done on a set of RILs, laborious root measurements on a large scale need not be necessary and marker-assisted selection for improvement of root size, and hopefully also for root exudates could be carried out in the near future. Outputs of transgenic research in other crops, in due course of time, could be extended for enhancement of nutrient uptake mechanisms and salinity tolerance in chickpea. Increased emphasis on better understanding of mechanisms of nutrient uptake and their genetic control are required for chickpea if the crop is to maintain production under seemingly inevitable decreasing soil fertility conditions.

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