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Rapid and reliable screening technique for root traits in chickpea

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ABSTRACT

A set of 257 chickpea recombinant inbred lines (RILs) were derived from a cross of a prolific root system (ICC 4958) and an agronomically preferred variety (Annigeri). The RILs were developed for identifying QTLs associating with desirable root traits and were grown in tall PVC cylinders (120 cm height) filled with the vertisol and sand mixture under two initial soil water contents, 100% field capacity (irrigated) and 70% field capacity (stressed) during 2001 post-rainy season. The same chickpea RILs were also grown in an experimental field under rainfed condition. At 35 days after sowing (DAS) in cylinder cultures, there were significant variability in shoot and root dry weights and root length density among the RILs. ICC 4958 showed larger shoot and root growth than Annigeri in field and water stressed cylinder systems but not in irrigated cylinder. A significant positive correlation was observed in the root growth between the field and cylinder conditions and significant G x E interaction in the root dry weight and length density was also detected in irrigated cylinder. This cylinder system with 70% field capacity can be used as powerful and rapid phenotyping tool for root traits in large scale screening of germplasm or mapping populations.

Key words: Chickpea, Root dry weight, Root length density, Screening method

Chickpea (*Cicer arietinum* L.) is grown largely under rainfed conditions with progressively receding soil moisture and, as a consequence, suffers varying degrees of terminal drought stress. Therefore, development of chickpea varieties that can cope well with terminal drought is needed to improve yield and stability under drought. In the last decade, breeding efforts for drought tolerance in chickpea have mainly focused on early maturity, *viz.*, drought escape mechanism. Through these efforts, chickpea breeders have successfully released some popular varieties, *e.g.* ICCV 2, and KAK 2 which are well adopted by the farmers. However, short duration varieties have relatively low seed yield and biomass because of a shortened total photosynthetic duration. It is, therefore, important to identify the relevant traits that confer improved drought tolerance and then to develop a trait-based breeding strategy. Based on previous studies, root traits such as root biomass and depth have been shown to be the foremost among traits that contribute to drought tolerance in chickpea (Silim and Saxena 1993, Johansen *et al.* 1994).

In chickpea, the comparative advantage of prolific root system at the early stages of crop growth was well demonstrated with the performance of ICC 4958, which was selected as a drought tolerant variety (Saxena *et al.* 1993, Johansen *et al.* 1994, Kamoshita *et al.* 2002). Recently, the existence of a clear and positive relationship between root length density and seed yield under terminal drought environment has been shown in chickpea (Kashiwagi *et al.* 2006). In addition, the importance of rooting depth has also been shown when the drought intensity was severe.

However, the progress of breeding programmes aimed at improving root system is very slow because it is quite difficult to conduct large scale field based conventional root research which is too laborious and time consuming. The molecular breeding, especially marker assisted selection (MAS), is one of the breakthrough techniques that can promote the breeding on root traits. This requires not only dense and well distributed linkage map but also a rapid and reliable phenotyping for the trait under consideration (Kamoshita *et al.* 2002). In chickpea, a dense molecular linkage map is being developed (Teresa *et al.* 2006). Therefore, the objective of this study was to establish alternative rapid and reliable screening method to phenotype the root traits in large population for MAS in chickpea.

MATERIALS AND METHODS

A set of 257 recombinant inbred lines (RILs) in F_8 generation derived from a cross between ICC 4958 (male parent, with prolific rooting and drought tolerant) and Annigeri (female parent well adapted to peninsular India and high yielding) along with the parental lines were used. The experiments were conducted in experimental field as well as in tall PVC cylinder systems at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru India (17°30'N; 78°16'E; altitude 549 m).

In 2001 post-rainy season, a field trial was conducted on a vertisol field (fine montmorillontitic isohyperthermic typic pallustert) at ICRISAT centre. This soil retains approximately 200 mm of plant extractable water in the upper 1.5 m of the soil profile (Singh and Sri Rama 1989), and average bulk density was 1.43 g/cm^3. The field was solarized using polyethylene mulch during the preceding summer to sanitize the field, then carried out surface application of 18 kg N/ha and 20 kg P/ha as Di-ammonium phosphate. Each plot was prepared into a flat seedbed with 20 cm row distance and 15 cm plant distance with alpha design in two replications. Two sets of entries were sown continuously without any alley in such a way that a common access trench could be dug by heavy digging machine in the middle to provide access for sampling two RILs one on each side of the pit. As all root extraction could not be completed in a single day, the sowing was staggered over a period of four days starting 29 October 2001 thereby facilitating sampling at a constant plant age. At 35 days after sowing (DAS), the shoots were sampled in each plot, then after that the soil blocks on two plant rows were sampled through the open side by driving in 15 cm deep specifically made (30 cm x 20 cm) steel templates (rectangular boxes open at the top and bottom) to ensure the constant size of the soil blocks and prevent soil sliding. The sampling was carried out at 15 cm interval up to 60 cm. The soil blocks including roots were immersed into water overnight, and then the soil was washed gently under running water on a 2 mm wire mesh sieve till the soil adhering to the roots was thoroughly removed. To estimate the ratio of the root dry weight to length, randomly selected root samples were scanned and the image analyzed using an image analysis device (WINRHIZO, REGENT Instruments Inc., Que., Canada). The root and shoot dry weights were recorded after drying them in a hot air oven at 80 °C for 72 hours. Based on the ratio of root dry weight to length, the root length of the rest of all RILs was estimated.

The experiment in cylinder system was carried out in alpha design with two replications. Four seeds of each RIL were sown on a 18 cm diameter PVC cylindrical pipe with 120 cm height filled with vertisol sand mixture (1:1 ratio, w/w) with two different initial soil water contents *i.e.*, 100 and 70% of field capacity (FC) on November 2001. The vertisol-sand mixture was packed into the cylinders with approximately 1.0 of bulk density up to 15 cm from the rim. In the top 15 cm, the vertisol sand mixture containing Di-ammonium phosphate at the rate of 100 kg/ha was filled. The sowing was staggered with one half of a replication per day and all in four days staggered scheme on each soil water treatment. Four cylinders were erected in a cluster with the support of a central wooden pillar. Immediately after sowing, a rhizobial inoculum (strain IC 59) as a water suspension was applied on all cylinders (Brockwell 1982). All cylinders were given 150 ml of supplemental irrigation on alternate days till emergence. After emergence, the plants were thinned to two seedlings per cylinder. At 35 DAS, the soil water content was measured by gravimetric method in some extra cylinders with parental genotypes. In the rest of the cylinders, the shoots were sampled, and immediately after the shoot sampling, the cylinders were placed horizontally and the vertisol-sand mixture was washed from both sides of the cylinder with running water. When approximately three quarter of filled soil medium was washed out, the cylinder was erected carefully on a sieve so that the entire intact root system could be easily slipped down. After removing the debris from the roots, the root and shoot dry weights, and root length were measured in the same way as in the field experiment.

RESULTS AND DISCUSSION

In the field experiment during the first 35 days, the maximum air temperature remained around 30 °C and the open pan evaporation was around 4.6 mm/day with small variation (Fig 1). There was a minor rainfall (4.2 mm) immediately after sowing. The soil water loss in the field was normal and no soil cracking was evident at the time of root sampling at 35 DAS. Between the two cylinder culture experiments with 70% and 100% of the field capacity (FC) soil moisture, there were large differences in soil moisture use. Soil moisture loss occurred at all soil depths but the amount of water lost was higher in 100% compared to the 70% FC cylinders. However, the amount of soil moisture still remained in the soil was considerably higher in the FC cylinders (Fig 2). The wilting point of this vertisol sand mixture used was about 8% of soil water content (El-Swaify *et al.* 1985). At 35 DAS, therefore, most of the available soil water up to a soil depth of 75 cm was used by the plants in the 70% FC soil moisture treatment. The water that remained at depths 75-120 cm was also marginal (Fig 2b). In contrast, in the 100% FC cylinders, major part of the soil water up to a soil depth of 45 cm was used by the plants and a large portion of the water still remained at soil depths below 45 cm (Fig 2a). In this cylinder culture, root sampling could be done much easier than in the field. In the field screening, large

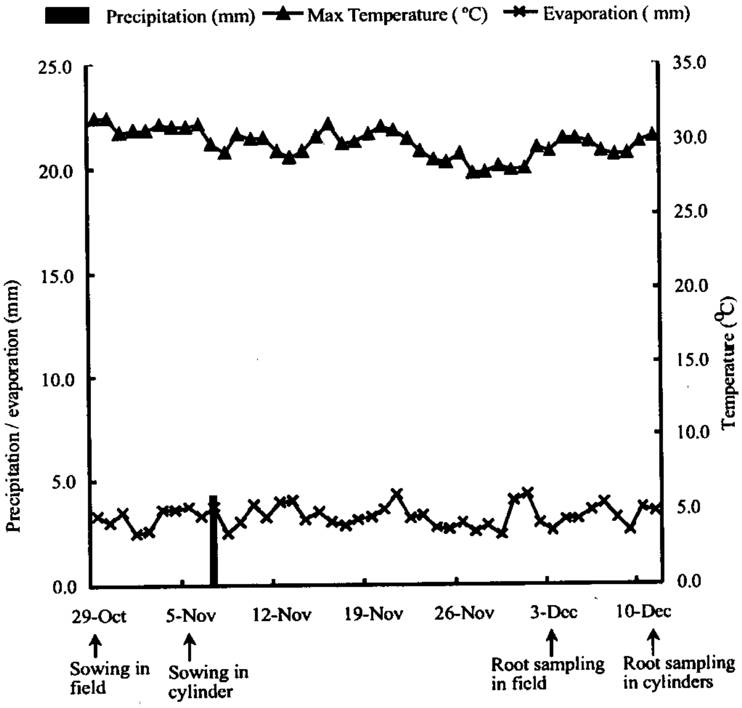

Fig 1. Climatic conditions during chickpea season in 2001

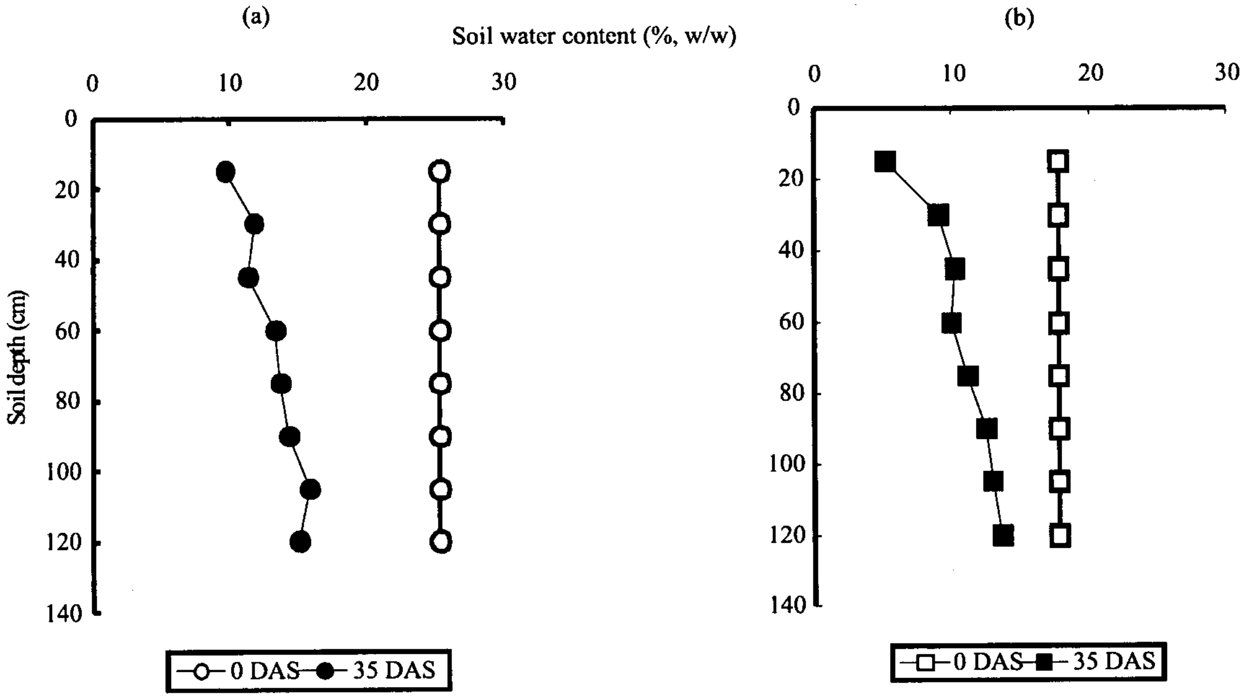

Fig 2. Soil water profile at sowing time and 35 days after sowing in cylinder systems. (a) 100% field capacity initial soil water treatment, and (b) 70% field capacity initial soil water treatment

manpower (about 50 workers/day for 15 root profiles) and heavy digging machines are needed. On the other hand, this root screening method was quite easier and quicker than the field root screening. It allowed to complete more than 100 chickpea root profiles/day with 20 workers.

The comparison of best linear unbiased predictions (BLUPs) estimated through the statistical procedure of residual maximum likelihood (ReML) showed that in the field conditions at 35 DAS, there was a large variability for root dry weight (RDW), root length density (RLD) and shoot dry weight (SDW) among the RILs, but not in relative biomass allocation to root system indicated by the ratio of root dry weight to total plant dry weight (R/T) (Table 1). The root growth as well as shoot growth of ICC 4958 was larger than that of Annigeri. In the cylinder culture also, a large variability was observed on the shoot and root growths in both 100 and 70% FC treatments but not R/T (Table 1). In FC cylinders, however, the root growth of Annigeri was larger than that of ICC 4958 unlike in the field conditions, an indication of the existence of genotype by environment interaction (G x E). The difference between the lowest and the best root growth indicated by RDW as well as RLD in 100% FC cylinders was slightly larger than in 70% FC cylinders. This indicates that relatively severe soil water stress imposition restricted over all plant growth in 70% FC cylinder. The average root growth in field conditions was less than that in the cylinder conditions (Table 1). Iijima *et al.* (1991) reported that root growth of rice and maize was significantly reduced when the plants were grown in compacted soil. In this experiment, the cylinders were compacted with soil to arrive at a bulk density of 1.1 in both 100% and 70% FC soil water treatments, whereas the bulk density of field soils below 30 cm soil depth was reported to be about 1.43 g/cm^3 (El-Swaify *et al.* 1985). This was likely to be one of the reasons for increased root growth in the cylinder systems.

Table 1. Range, mean, and S.E. of shoot and root traits of parental lines and RILs of a chickpea cross Annigeri x ICC 4958

Treatment/ Trait	Variety		RIL		
	Annigeri	ICC 4958	Range	Mean	SE
Field-Rainfed					
RDW (g/plant)	0.284	0.358	0.253-0.396	0.300	0.012
RLD (cm/cm)	0.376	0.468	0.335-0.481	0.396	0.019
R/T ratio (%)	22.3	22.8	21.1-26.4	23.3	0.725
SDW (g/plant)	1.018	1.369	0.788-1.232	0.979	0.018
Cylinder-100% field capacity					
RDW (g/plant)	0.970	0.954	0.601-1.159	0.821	0.090
RLD (cm/cm)	0.633	0.625	0.457-0.724	0.56	0.046
R/T ratio (%)	57.4	57.3	57.2-57.6	57.40	2.101
SDW (g/plant)	0.698	0.734	0.450-0.759	0.602	0.021
Cylinder-70% field capacity					
RDW (g/plant)	0.565	0.606	0.507-0.668	0.580	0.070
RLD (cm/cm) .	0.393	0.413	0.365-0.44	0.400	0.035
R/T ratio (%)	56.6	55.7	54.8-57.6	56.4	1.693
SDW (g/plant)	0.407	0.506	0.344-0.542	0.435	0.029

RDW = Root Dry Weight, RLD = Root Length Density, T/R ratio = Ratio of Root Dry Weight to Total Dry Weight, SDW = Shoot Dry Weight

In a pooled analysis with two environments, there were significant G x E interactions for RDW and RLD between field and FC cylinder conditions ($p<0.01$; $p<0.05$, respectively) whereas no such interaction was observed with the root growth between field and 70% FC cylinder conditions (Table 2). This indicates that difference of soil water content preferentially influence the root growth of some of the RILs but not in others. Similar observation was also made in several root morphological traits in rice (Kamoshita *et al.* 2002). A significant positive correlation was found to exist for the RDW and RLD between field conditions and 70% FC cylinder ($r = 0.350$, $p<0.01$; $r = 0.375$, $p<0.01$, respectively). Though such relationships were significant, the magnitude of the closeness between field and irrigated cylinder conditions were relatively low ($r = 0.201$, $p<0.01$; $r = 0.199$, $p<0.01$, respectively). These results suggest that root growth of the RILs and their parents in the field was relatively closer to the growth in 70% FC cylinders and therefore, root growth in cylinders packed with an initial soil moisture of 70% field capacity can largely represent the performance under field conditions.

Table 2. Variance components of genotype by environment interaction (G x E) in root and shoot traits between the field and cylinder

Treatment/	G x E interaction		
Trait	Variance	S.E.	Significance
Field *vs* Cylinder (100% field capacity)			
RDW	0.007	0.002	**
RLD	0.002	0.001	*
R/T ratio	0.300	1.490	NS
SDW	0.003	0.002	NS
Field *vs* Cylinder (70% field capacity)			
RDW	0.0002	0.0014	NS
RLD	0.0003	0.007	NS
R/T ratio	2.720	2.220	NS
SDW	0.003	0.002	NS

RDW = Root Dry Weight, RLD = Root Length Density, R/T ratio = Ratio of Root Dry Weight to Total Dry Weight, SDW = Shoot Dry Weight

This screening method also presents fairly rapid and reliable estimations and a powerful tool for phenotyping of root growth in chickpea mapping populations that are large enough in size for molecular marker assisted breeding programme as well as for selection of outstanding genotypes for root traits in germplasm collections. However, further modification, especially in the soil compaction, is expected to improve the estimation of the rooting depth in this system as the rooting depth is critically important as a drought avoidance mechanism.

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