



Variation in root traits of chickpea (*Cicer arietinum* L.) grown under terminal drought

R. Serraj*, L. Krishnamurthy, J. Kashiwagi, J. Kumar, S. Chandra, J.H. Crouch

International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru 502324, Andhra Pradesh, India

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Abstract

Deep and prolific root systems have been associated with enhanced avoidance of terminal drought stress in chickpea. This research evaluated the root traits of 257 recombinant inbred lines (RILs) derived from a cross between a breeding line with a large root system (ICC 4958) and an agronomically preferred variety (Annigeri) to assess the potential for identifying QTL for desirable root traits and to investigate the relationship between root traits, plant growth and seed yield under terminal drought stress. The root traits of field-grown chickpea RILs were measured using the monolith method during the 2001–2002 cropping season, while their shoot biomass and seed yield were evaluated during both 2000–2001 and 2001–2002 seasons. Significant genetic variation was observed amongst the RIL population for root length density, root dry weight and shoot dry weight at 35 days after sowing and for shoot biomass and seed yield at maturity. A linear relationship was observed between root dry weight and shoot dry weight at 35 days after sowing. The overall distribution of root length density and root dry weight among the RILs indicated that these traits are likely to be under polygenic control. The heritability of root dry weight was 0.27 and root length density was 0.23, compared to 0.49 for shoot dry weight at the same stage. The RILs exhibited a range of combinations of root size and seed yield, with a few RILs showing large root systems and high seed yield. However, there was no general correlation between seed yield and root size. High shoot biomass and harvest index contributed to high seed yield of the RILs. The implications for the molecular breeding of drought-avoidance root traits in chickpea are discussed.

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1. Introduction

Chickpea is the third most important pulse crop globally. Major producing countries include India, Pakistan and Iran (FAO, 2003), where the crop is generally planted after the main rainy season and grown on stored soil moisture making terminal drought stress a primary constraint to productivity. Early maturing varieties that escape terminal drought

and heat stress have been developed and adopted by farmers (Kumar and Abbo, 2001), but early maturity places a ceiling on the potential yield and limits the crops ability to exploit extended growing periods. Increasing the drought avoidance of the crop should help to stabilize yields at higher levels than possible with escape (Johansen et al., 1997).

Drought resistance is a generic term for highly complex phenomenon which has limited meaning without reference to a specific crop × environment situation. In chickpea, the focus of drought resistance is on the ability to sustain greater biomass production

* Corresponding author.

E-mail address: r.serraj@cgiar.org (R. Serraj).

and crop yield under a seasonally increasing water deficit, rather than the physiological aptitude for plant survival under extreme drought shock (Serraj and Sinclair, 2002). This has led to the focus on escape and avoidance strategies, such as early maturity (Kumar and Abbo, 2001) and large root systems (Saxena et al., 1995; Singh et al., 1995).

Breeding for enhanced yield stability and/or potential under drought stress has been successful in some crops (Fukai and Cooper, 1995; Schneider et al., 1997; White and Castillo, 1992; Banziger et al., 1999). However, the progress in breeding for drought resistance is generally considered to be slow due to the quantitative and temporal variability of available moisture across years, the low genotypic variance in yield under these conditions and inherent methodological difficulties in evaluating component traits (Ludlow and Muchow, 1990) together with the highly complex genetic basis of this character (Turner et al., 2001). The availability of genetically fixed RIL populations combined with DNA markers and rigorous phenotyping should improve the ability to study and manipulate drought resistance traits (Crouch and Serraj, 2002). In particular, introgression of simple component traits that contribute to yield under specific target drought environments appears to be within reach (Serraj et al., 2003).

Given the progressively receding moisture and (increasing temperature) of typical chickpea growing environments, the ability to maximize the extraction of water from the soil, particularly during pod filling, should provide an important advantage, making the root system an essential part of drought avoidance, along with early/appropriate maturity. Simulation studies in sorghum have confirmed the effect of deeper rooting systems across a number of years and environments in the USA (Sinclair, 1994). Similarly, a simulation model has been adapted for chickpea and used to predict crop yield potential and limitations in Iran (Soltani et al., 1999). The results showed that early maturity and increasing drought avoidance via deep roots, plus higher transpiration efficiency were the traits most likely to result in higher grain yield under terminal drought stress (Soltani et al., 2000). Field studies in various crops shown that both dense root systems extracting more of the water in upper soil layers and longer root systems extracting soil moisture from deeper soil layers are important for maintaining

yield under terminal drought stress (Ludlow and Muchow, 1990; Saxena and Johansen, 1990; Turner et al., 2001). In rice, traits such as deep root morphology and root thickness, have been associated with increased water extraction during progressive water stress (Fukai and Cooper, 1995; Kamoshita et al., 2002). A high ratio of deep root weight to shoot weight was also found to maintain higher plant water potentials and have a positive effect on yield under stress (Mambani and Lal, 1983). Current research on rice is focusing on the use of molecular markers for various root traits to improve drought avoidance in rice (Cui et al., 2002; Price, 2002).

Based on the above, chickpea germplasm accessions that possess large and deep root systems have been identified in an attempt to incorporate these traits into well-adapted crop backgrounds (Saxena et al., 1993). This has resulted in the identification of a germplasm accession ICC 4958 as one of the most drought-resistant breeding line and subsequent development of drought-avoidant lines with larger root systems (Singh et al., 1995; Saxena et al., 1995). However, the difficulties of concurrent selection for root traits and yield performance led to proposals for the development of mapping populations and the application of marker-assisted selection for drought-avoidance root traits in chickpea (Saxena, 2003). A recombinant inbred line population with 257 RILs was recently derived from a cross between a breeding line with a large root system (ICC 4958) and an agronomically preferred variety (Annigeri), for the purpose of mapping drought-avoidance root traits in chickpea. However, the success of this approach is dependent upon the development and appropriate phenotyping of mapping populations for drought-avoidance root traits.

Therefore, the objectives of this work were to quantify the genetic variation of root traits amongst the RIL population and the parental lines and to study the relationship between root traits, shoot biomass and seed yield.

2. Materials and methods

2.1. Crop management

A recombinant inbred line (RIL) population of 257 individuals was developed from a cross between ICC

4958 and Annigeri, for mapping QTLs for various root traits. Field evaluation of this RIL population (F_8 in 2000–2001 and F_9 in 2001–2002) and the parental genotypes was conducted during the post-rainy seasons of 2000–2001 and 2001–2002 on a Vertisol (fine montmorillonitic isohyperthermic typic pallustert) at ICRISAT, Patancheru ($17^{\circ}30'N$; $78^{\circ}16'E$; altitude 549 m) in peninsular India. The soil depth of the fields used in the two seasons was approximately 1.2 m and these soils retained about 190 mm of plant available water in the 120 cm soil profile (maximum rooting depth in this environment). The field used for the 2001–2002 post-rainy season was solarized using polythene mulch during the preceding summer to sanitize the field, particularly to eradicate *Fusarium* wilt causing fungi as chickpea was often grown in this field. The field chosen for 2000–2001 was not solarized as chickpea was raised in this field once in 4 years.

The field was prepared into 0.6 m ridges and furrows for the 2000–2001 experiment and as a flat seed bed for the 2001–2002 experiments. Surface application and incorporation of 18 kg N ha^{-1} and 20 kg P ha^{-1} as di-ammonium phosphate was carried out in both experiments. The plot size for the 2000–2001 experiment was $4 \text{ m} \times 1$ row and the 2001–2002 root experiment was $2 \text{ m} \times 3$ rows and the yield experiment was $4 \text{ m} \times 5$ rows. The experiments were conducted in an 11×24 alpha-lattice design (264 genotypes) with two replications during 2000–2001 and 7×40 alpha-lattice (280 genotypes) with two replications for root assessment and three replications for yield assessment during 2001–2002. In addition to the 257 RILs and their two parents, the experiments had five extra varieties during 2000–2001 and 21 during 2001–2002. Seeds were treated with 0.5% Benlate[®] (E.I. DuPont India Ltd., Gurgaon, India) + Thiram[®] (Sudhama Chemicals Pvt. Ltd. Gujarat, India) mixture in both the seasons. During the 2000–2001 season, the seed was drilled at 5 cm depth with rows 60 cm apart using a 4-cone planter on 17 October 2000 (as the first opportunity after the rains). About 45 seeds were used for each 4 m row and at 15 days after sowing the plants were thinned maintaining a plant-to-plant spacing of 10 cm. During 2001–2002, the seed was hand sown manually at a depth of 2–3 cm with 15 cm between plants within rows and 20 cm between rows on 2 November 2001 in

a dry seed bed. During both the seasons, the fields were inoculated with Rhizobium strain IC 59 using liquid inoculation method (Brockwell, 1982). A 20 mm irrigation was applied the next day to ensure complete emergence. Two seeds per hill were sown which was thinned to one at 12 days after sowing. Intensive protection against pod borer (*Helicoverpa armigera*) was provided and the plots were kept weed free by manual weeding. During 2001–2002 season a separate experiment was planted for the purpose of root and shoot extraction at 35 days after sowing. The crop management was the same except for the plot size ($2 \text{ m} \times 3$ rows) and replications (2). Two sets of entries were sown continuously without any alley in such a way that a common access pit could be dug in the middle to provide access for sampling two RILs one on each side of the pit. As all the root extraction could not be completed in a single day, the sowing was staggered over a period of 4 days (half a replication per day) thereby facilitating sampling at a consistent plant age.

By regular observation, the mean date at which more than 50% of the plants reached flowering was recorded as 50% flowering time and when 80% of the pods were dried was recorded as the physiological maturity for each plot.

2.2. Root sampling

Roots were extracted by a monolith method (Heerman and Juma, 1993) with minor modifications from a $30 \text{ cm} \times 20 \text{ cm}$ area at 35 DAS—about 5–10 days before 50% flowering for most RILs. Sampling time was chosen as 35 DAS in order to maximize the range of variation among RILs, as earlier studies showed that significant genetic variation in root dry weight was observable between ICC 4958 and Annigeri only up to flowering time (about 40 DAS) (Krishnamurthy et al., 1996). Our earlier studies also showed that the maximum rooting depth generally extends up to 60 cm at this stage, and in rare cases goes up to 75 cm (Krishnamurthy et al., 1996). An 80 cm wide and 1 m deep access trench, through the entire length of the field plot and extending 0.4 m on either side of the 2 m plot, was dug using a backhoe digger prior to extraction of soil with roots. This trench removed 0.4 m of likely adjacent-RIL-effect of the plot for root sampling. The soil blocks were sampled through the open side by driving in 15 cm deep specifically made ($30 \text{ cm} \times 20 \text{ cm}$)

steel templates (rectangular boxes open at the top and bottom) to ensure the constant size of the soil blocks and to prevent soil sliding. Sampling was carried out at 15 cm depth intervals up to 60 cm. The maximum rooting depth for sampling was determined by visual observations in the access trench and according to previous preliminary experiments. The soil samples containing roots were soaked in water overnight, soil was washed with tap water, and the roots were recovered by passing the soil-water suspension through a 2 mm wire mesh sieve. Chickpea roots were then separated from the organic debris and weed roots manually by floating the material on water in trays. Root length was measured using a scanner and the WINRHIZO software package (REGENT Instruments Inc., Que., Canada). The dry weights of the roots were recorded after oven drying for 3 days (to constant weight) at 80 °C.

As it was impractical to measure the root length of all the RILs in a short enough time, coefficients for root dry weight and root lengths were calculated using a random selection of 37 RILs and the two parents and for the roots extracted from all the four depth intervals in two replications. The root length to root dry weight ratios observed were 84.5 m g⁻¹ for the 0–15 cm soil depth, 162.5 m g⁻¹ for the 15–30 cm, 153.2 m g⁻¹ for the 30–45 cm and 155.8 m g⁻¹ for the 45–60 cm soil depth. No genotypic differences were observed among the RILs and the parents for this ratio (data not shown), and therefore the root length to weight ratios were averaged over RILs for each depth for further predictions. Similar absence of variation was also observed in a previous study in the same soil type and growing conditions with five genotypes including Annigeri and ICC 4958, indicating that this ratio is likely to be largely consistent across these two varieties and their progenies (Krishnamurthy et al., 1998). The root length to dry weight ratios specific to each depth interval were used to calculate root length density of all the RILs using the dry weight data for each depth separately.

2.3. Shoot sampling

Shoots from an area of 0.75 cm × 3 rows (15 hills) were sampled at the same time as that of the roots. The shoots were cut immediately above the cotyledons and were oven dried and the weights were recorded.

2.4. Final harvest

At physiological maturity, plant aerial parts were harvested from an area of 2.4 m² in 2000–2001 and 3.45 m² in 2001–2002 in each plot, dried to constant weight in hot air dryers at 45 °C, and total shoot dry weights were recorded. Grain weights were recorded after threshing. Harvest index (%) was calculated as 100 × (seed yield/total shoot biomass at maturity).

Available soil moisture was predicted using a soil water balance model, WATBAL (Keig and McAlpine, 1976). The inputs required were maximum soil depth, weekly cumulative evaporation (provided from the meteorological observatory of ICRISAT situated about 2500 m from the 2000–2001 experiment and 100 m from the 2001–2002 experiment) and the weekly rainfall or irrigation (monitored through a total of 48 catch cans placed across the experimental area in rows of six between two laterals of perfo irrigation lines and in eight random places).

2.5. Statistical analysis

The data from any individual experiment were analyzed using the following linear additive mixed effects model:

$$Y_{ijk} = \mu + r_i + b_{ij} + g_k + e_{ijk}$$

where y_{ijk} is the observation recorded on genotype k in incomplete block j of replicate i , μ the general mean, r_i the effect of replicate i , b the effect of block j within replicate i , g_k the effect of genotype k , and e_{ijk} the effect of the plot. The general mean μ and replicate effect r_i were considered as fixed effects. The block effect b_{ij} , the RIL effect g_k , and the plot effect e_{ijk} , were assumed as random effects each with mean zero and constant variances σ_b^2 , σ_g^2 and σ_e^2 , respectively. Using the above model, the statistical procedure of residual maximum likelihood (ReML) was employed to obtain the unbiased estimates of the variance components σ_b^2 , σ_g^2 and σ_e^2 , and the best linear unbiased predictions (BLUPs) of the performance of the 257 RILs. Heritability was estimated as $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$. The significance of genetic variability among RILs was assessed from the standard error of the estimate of genetic variance σ_g^2 , assuming the ratio $\sigma_g^2/S.E. (\sigma_g^2)$ to follow normal distribution asymptotically.

The above model was extended for over-year analysis of traits recorded in both years, assuming year effect as fixed, with genotype \times environment interaction (GEI) effect being a random effect assumed to have a mean of zero and constant variance σ_{gE}^2 . The significance of GEI was assessed in a manner similar to that of σ_g^2 . The significance of the fixed effect of the year was assessed using the Wald statistic that asymptotically follows a χ^2 distribution and is akin to the F -test in the traditional ANOVA.

3. Results and discussion

3.1. Soil water extraction and extent of terminal drought

In general, up to the mid-post-flowering stage of the crop, the maximum temperature and evaporation were lower during the crop growing period of 2001–2002 compared to 2000–2001 (Fig. 1). The rainfall received during the first standard week in the 2000–2001 season and the second week in 2001–2002 had affected the crop differently. During the first season (2000–2001), rainfall (20 mm) occurred at the end of crop growth period, nearly at maturity stage; whereas for the second season (2001–2002), rainfall (22.4 mm) occurred during early pod-filling stage, which resulted in a slight and transient recovery of later duration RILs from terminal drought (Fig. 2).

Although the progressive decline of moisture in the soil profile was similar for both cropping seasons, the magnitude and the duration of terminal drought between the 2 years was different because of the difference in sowing time (Fig. 2). In order to grow the crop only as rainfed, the sowing was taken up immediately after the cessation of the rain during 2000–2001 season. As a result, most of the peak growth occurred relatively during cooler days compared to the 2001–2002 season and thus, the crop escaped from severe terminal drought stress and heat stress. In the second year, the crop was sown at the normal time in order to expose it to terminal drought stress. The drought stress progression experienced in the second year was moderately severe across the growing duration until standard weeks 1 and 2 (2000–2001 and 2001–2002, respectively). The rainfall (22.4 mm) on 8 January 2002 was received at the

early- to mid-pod fill stage in most of the RILs. This moisture was especially beneficial as it reached deeper soil layers, as there were deep soil cracks present at this stage of crop growth.

3.2. Phenology

Flowering time differed between the parents and among the RILs. ICC 4958 flowered at 38 and 35 DAS compared to Annigeri at 47 and 41 DAS in 2000–2001 and 2001–2002 seasons. The RIL predicted means ranged between 34–49 days in 2000–2001 season and 38–44 days in 2001–2002 (Table 1). The heritability of 50% flowering was relatively higher in 2001–2002 (0.84) than in 2000–2001 (0.51). ICC 4958 matured at 95 and 84 DAS compared to Annigeri at 104 and 84 DAS in 2000–2001 and 2001–2002 seasons, respectively. The RIL means ranged between 95–104 days in 2000–2001 season and 81–90 days in 2001–2002. The heritability of maturity time was 0.49 in 2000–2001 and 0.71 in 2001–2002. The crop phenology (flowering and maturity) was advanced in 2001–2002 compared to 2000–2001 because of relatively more severe drought and heat stress in the second season associated with the normal time sowing of the experiment in the second year. Both early flowering and early maturity in 2001–2002 ($P \leq 0.001$) and the RIL \times year interaction ($P \leq 0.001$) were significant. Plants, grown under progressively receding soil moisture conditions, tend to flower and mature earlier than nonstressed (optimally irrigated) plants (Saxena, 1984; Johansen et al., 1994).

Table 1

Trial means, range of best linear unbiased predicted means of RILs (BLUPs) and analysis of variance of phenology of the 257 RILs and their parents Annigeri and ICC 4958 in the field experiments during 2000–2001 and 2001–2002 seasons (Rep = 2 in 2000–2001 and Rep = 3 in 2001–2002)

Trait	Trial mean	Range of predicted means	σ_g^2 (S.E.)	Heritability (h^2)
Days to 50% flowering				
2000–2001	43.5	34.4–48.6	4.01 (0.56)	0.514
2001–2002	41.2	38.3–44.0	8.55 (0.78)	0.842
Days to maturity				
2000–2001	99.6	95.0–104.1	2.44 (0.36)	0.486
2001–2002	84.5	81.2–90.3	5.86 (0.58)	0.713

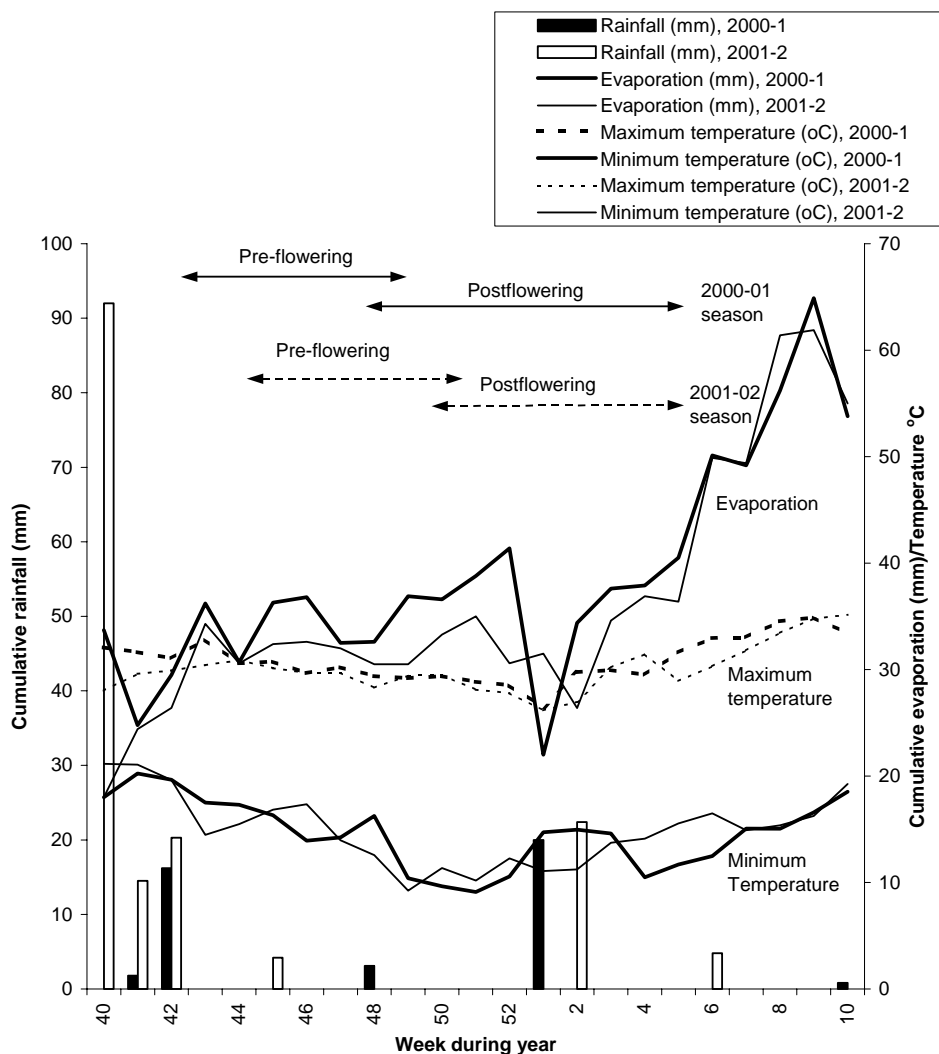


Fig. 1. Weather at experimental site (ICRISAT, Patancheru) during the crop growing seasons of the 2 years.

3.3. Root and shoot sampling at 35 days after sowing

The depth of rooting was confirmed visually in the access pit dug prior to sampling. In most RILs, the roots were present only up to the 45–60 cm depth. The mean root dry weight (per unit ground area) of RILs exhibited large variation (Table 2 and Fig. 3a) with a range of 7–14 g m⁻². The variation was skewed more towards Annigeri, the parental genotype with lower (9.4 g m⁻²) pre-flowering root growth. The overall distribution of the RILs indicates that root dry weight is likely to be under polygenic control. The parental

line ICC 4958 produced one of the highest (12.7 g m⁻²) root dry weights suggesting recessive inheritance. The heritability of this trait was 0.27. The combination of poor heritability, high G by E and largely recessive nature may explain the difficulty met by plant breeders to select for enhanced root traits in chickpea. This also indicates that DNA markers for root trait components would be particularly a powerful tool in the genetic enhancement of drought avoidance in chickpea.

The predicted root dry weights (BLUPs) at the deepest soil layer (45–60 cm), varied between 0.38

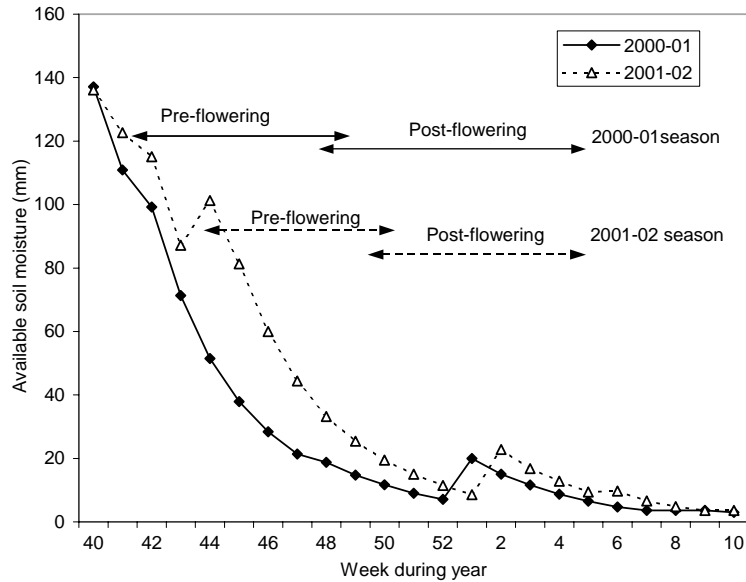


Fig. 2. Changes in total available soil water (mm) predicted using the soil water balance model (Keig and McAlpine, 1976) during the two seasons of crop growth.

and 0.79 g m^{-2} for RILs and these values were 0.39 and 0.77 g m^{-2} for Annigeri and ICC 4958, respectively (data not shown). The deep-layer root dry weights of RILs-8, -157, -211 and -234 were comparable to that of ICC 4958.

Root length density (RLD) varied between 0.16 and 0.27 cm cm^{-3} in the 0–60 soil layers (Fig. 3b), and the heritability of this trait was 0.23. However, depth-wise RLD ranged from 0.21 to 0.45, 0.27 to 0.36, 0.13 to 0.20 and 0.04 to 0.08 cm cm^{-3} in the 0–15, 15–30, 30–45 and 45–60 cm soil layers, respectively. RLD of RILs was generally lower than 0.5 cm cm^{-3} in all the depths including the well-colonized 15–30 cm soil depth, suggesting that these RLD values were sub-optimal for

maximum water extraction (Passioura, 1983). These values were also very low compared to the RLD of various cereal crops (Cooper et al., 1987; Siddique et al., 1990). However, the RLD values observed in this study and their distribution across various depths were close to the ones reported earlier in experiments at similar growth stage (Brown et al., 1989) and under similar environmental conditions (Gregory et al., 1994; Krishnamurthy et al., 1996). It was reported that root growth of chickpea attains its maximum at about 10–15 days before physiological maturity (Brown et al., 1989) and the RLD will increase in layers from 15 cm downwards. The RLD of ICC 4958 (0.249) was one of the largest, being 31% higher than that of Annigeri (Fig. 3b).

Table 2

Means, range of best linear unbiased predicted means of RILs (BLUPs) and analysis of variance of root dry weight, root length density and shoot dry weight at 35 days after sowing of the 257 RILs and their parents Annigeri and ICC 4958 in the field experiment during 2001–2002 season

Trait	Trial mean	Range of predicted means	σ_g^2 (S.E.)	Heritability (h^2)
Root dry weight (g m^{-2})	10.1	7.9–14.4	3.074 (0.784)	0.267
Root length density (cm cm^{-3})	0.203	0.162–0.271	0.0012 (0.0004)	0.228
Shoot dry weight (g m^{-2})	57.5	52.1–63.0	37.88 (5.51)	0.489

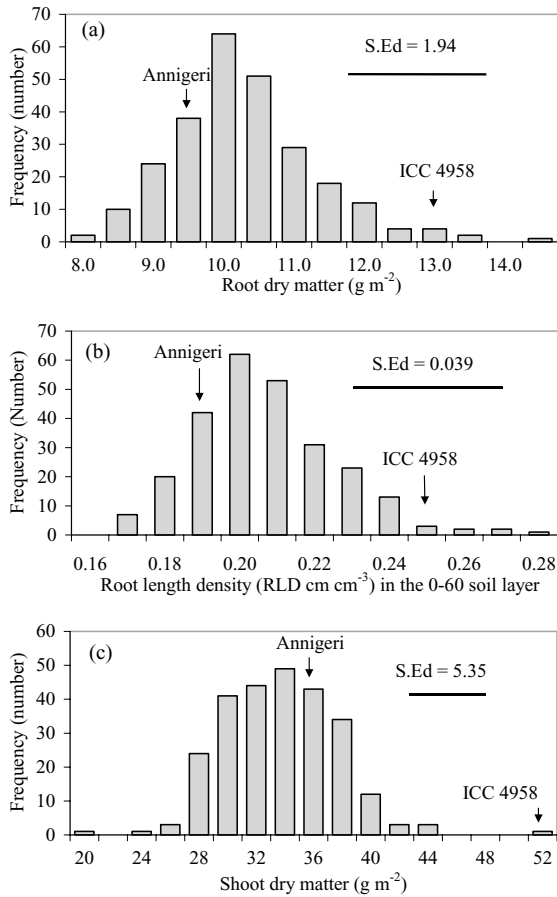


Fig. 3. Frequency distribution of: (a) total root dry weight (g m^{-2}), (b) root length density (cm cm^{-3}) in the 0–60 cm soil layer and (c) total shoot dry weight (g m^{-2}) at 35 DAS of 257 RILs along with their parents grown in a vertisol field during 2001–2002 season. The root dry weight values for Annigeri and ICC 4958 are 9.4 and 12.7 (g m^{-2}), root length density are 0.190 and 0.249 (cm cm^{-3}) and shoot dry weight are 34.4 and 50.5 (g m^{-2}) respectively; S.E.D. for comparison of means is: (a) 1.94 (g m^{-2}), (b) 0.039 (cm cm^{-3}) and (c) 5.35 (g m^{-2}).

Similar magnitude of difference between these two parents in RLD was reported (Saxena et al., 1993).

The distribution of mean shoot dry weight amongst the RILs and their parents at 35 DAS showed a similar pattern to that of the root dry weight. Again ICC 4958 had the largest shoot biomass, indicating a recessive basis to this trait (Table 2 and Fig. 3c). However, shoot biomass had a relatively high heritability of 0.49 compared to 0.26 for root biomass. The root and the shoot dry weights of the RILs were linearly correlated ($r^2 = 0.23$).

3.4. Shoot biomass and yield components at maturity

The pooled analysis of shoot biomass at maturity across years revealed that both the year and the RILs effects were significant, but the interaction year \times RIL was not significant. The mean and the range were different between the two seasons (Table 3), and shoot biomass varied among RILs in both years. Parent ICC 4958 produced less shoot biomass than Annigeri in 2000–2001 and both were equal in 2001–2002 despite the clear superiority of ICC 4958 in shoot dry matter production at 35 DAS. This has demonstrated that the post-flowering growth rate of Annigeri was higher compared to ICC 4958. The shoot dry matter of majority of the RILs in 2000–2001 season was distributed between 2600 and 3800 kg ha^{-1} compared to 2800 and 3700 kg ha^{-1} in the 2001–2002 season. Twenty-five RILs produced equal or more shoot biomass than the best parent ICC 4958 during 2001–2002, and the shoot biomass of some RILs (211, 40, 179 and 106) was clearly higher. The performance of these four RILs was also clearly higher than the trial mean during 2000–2001. However, the heritability of shoot biomass at maturity was relatively low (Table 3).

The seed yield across years exhibited that the year ($P \leq 0.001$) and the RILs effect were significant ($\sigma_g^2 = 6476$, S.E. = 3340), but the year \times RIL interaction was not significant ($\sigma_{gE}^2 = 7314$, S.E. = 4500). The mean and the range seed of yield were different between the years (Table 3) and there were differences among RILs in both years. During 2001–2002 season, it was observed that the majority of RILs recovered well after the rain received during the second week of January 2002, whereas a few of the relatively early duration RILs showed symptoms of forced maturity. Moreover, the improved nutrient status due to summer solarization and a following cowpea cover crop improved the yield levels in 2001–2002. The parent ICC 4958 had lower yield compared to Annigeri in 2000–2001 and there was no significant difference in 2001–2002. Some of the RILs that produced the highest seed yield (1878–1923 compared to the mean environmental yield of 1706 kg ha^{-1}), during relatively higher drought stressed 2001–2002 season, and above-average yield (1872–2010 compared to the mean environmental yield of 1965 kg ha^{-1}) during 2000–2001 are RILs-40, -106, -179 and -211. All these RILs produced a shoot biomass higher than

Table 3

Trial means, range of best linear unbiased predicted means of RILs (BLUPs) and analysis of variance of shoot biomass and seed yield at maturity of the 257 RILs and their parents Annigeri and ICC 4958 in the field experiments during 2000–2001 and 2001–2002 seasons (Rep = 2 in 2000–2001 and Rep = 3 in 2001–2002)

Trait	Trial mean	Range of predicted means	σ_g^2 (S.E.)	Heritability (h^2)
Shoot dry matter (kg ha ⁻¹)				
2000–2001	3407	2613–3806	43257 (10737)	0.271
2001–2002	3216	2809–3667	47513 (13039)	0.167
Seed yield (kg ha ⁻¹)				
2000–2001	1958	1554–2156	13688 (3938)	0.234
2001–2002	1706	1507–1923	17757 (5746)	0.139
Harvest index (%)				
2000–2001	57.5	52.1–63.0	4.41 (0.61)	0.501
2001–2002	52.0	47.3–56.0	7.08 (1.21)	0.301
Pod number per square meter				
2001–2002	1001	795–1219	14941 (3309)	0.204
Seed number per pod				
2001–2002	0.98	0.92–1.04	0.0010 (0.0003)	0.166
Seed weight (mg per seed)				
2000–2001	220	136–285	1281.5 (123.9)	0.829
2001–2002	189	134–237	944.7 (100.7)	0.617

the environmental means. In contrast, some of the RILs that produced very poor seed yield and shoot biomass in both the years are RILs-112 and -133. The heritability of seed yield was lower than that of shoot biomass at maturity (0.234 in 2000–2001 and 0.139 in 2001–2002).

The harvest index of the parents did not differ in either year and the RILs varied (Table 3). In the pooled analysis of both years, the year effect ($P \leq 0.001$), the RILs effect ($\sigma_g^2 = 6.14$, S.E. = 0.81) were significant, but their interaction was nonsignificant ($\sigma_{gE}^2 = 0.00$, S.E. = 0.57). In general, the harvest index range and mean were lower in 2001–2002 compared to 2000–2001 (Table 1). The reduction in harvest index in 2001–2002 was associated with the increased severity of drought stress. The harvest index of RILs between the years was well correlated ($r = 0.38^{***}$). The heritability of this character was higher than those of shoot biomass and seed yield (0.501 in 2000–2001 and 0.301 in 2001–2002). It was previously reported that the partitioning efficiency plays a major role in contributing to higher and stable yield of chickpea under drought-prone conditions (Silim and Saxena, 1993; Krishnamurthy et al., 1999). Improvement in harvest index can be brought about in two ways. It can be

either through greater intrinsic ability for remobilizing the pre-flowering assimilates or through a shorter crop duration to escape severe terminal drought. In both seasons, the harvest index was linearly related to seed yield, with a stronger correlation in 2001–2002 (Fig. 4b). Some of the RILs leading to best yields were RILs-29, -37, -40, -56, -84, -102, -106, -137, -142, -155, -179, -192, -195, -197, -211, -231, -250 and ICC 4958 (selectively listed as these will be referred in 3.5) (data not shown). Few RILs with poor harvest index and poor yield were RIL-133 and -205.

The average seed weight of Annigeri was 171 mg per seed during 2000–2001 and 139 mg per seed during 2001–2002 and that of ICC 4958, 211 and 229 mg per seed. Seed weight range of RILs was 136–285 mg per seed in 2000–2001 and 134–237 mg per seed in 2001–2002 (Table 3). There was a significant reduction in seed weight during 2001–2002 compared to 2000–2001 due to the higher intensity of drought stress. However, a significant correlation was observed for seed weights between the 2 years ($r^2 = 0.67^{***}$) and the heritability was relatively high (0.829 in 2000–2001 and 0.617 in 2001–2002), indicating a good genetic control and that the character is more stable across seasons with relatively less G \times E interaction for this trait.

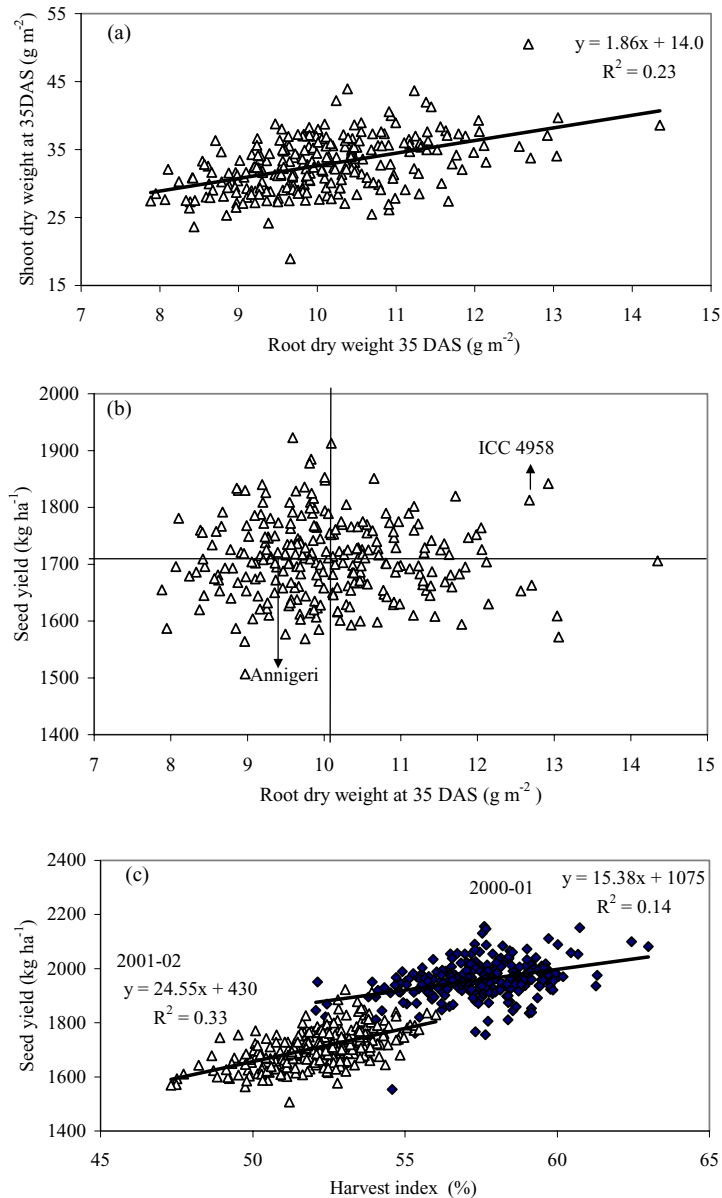


Fig. 4. Relationship of: (a) root dry weight (g m^{-2}) with shoot dry weight (g m^{-2}) at 35 days after sowing during the 2001–2002 season, (b) root dry weight (g m^{-2}) at 35 days after sowing with the seed yield at maturity (kg ha^{-1}) during the 2001–2002 season (vertical and horizontal lines represent mean root dry weight (10.1) and mean seed yield (1706)) and (c) harvest index (%) with seed yield (kg ha^{-1}) during 2000–2001 and 2001–2002 seasons across 257 RILs and their parents Annigeri and ICC 4958.

3.5. Relationship between root biomass and seed yield

A linear (or higher order polynomial) regression of root dry weight at 35 DAS with the final seed yield at

crop maturity showed no significant correlation (Fig. 4a). The progenies of a self-pollinated population are expected to segregate in all combination of the parental characters. However, root traits can have a major influence on yield only when root-mediated

water absorption becomes the major limitation under drought stress conditions. But in the case of 2001–2002, a 2 cm rain during mid-reproductive stage obliterated the drought development and favored a selected set of RILs. Previous reports have showed that large root length delayed leaf senescence in upland rice and resulted in improved seed yield only under water-limited conditions but not under nonstress or one cycle of intermittent drought (Mambani and Lal, 1983; Lilley and Fukai, 1994). Seed yield and shoot dry weight of common bean under drought stress were also reported to be more influenced by ‘root effects’ compared to ‘shoot effects’ (White and Castillo, 1992). For chickpea grown under terminal drought in highly cracking Vertisols, it was found that maximizing transpiration over evaporation through early growth vigor and optimum duration was beneficial to shoot biomass production and seed yield (Johansen et al., 1994).

Some of the extreme RILs (outliers) showed different combination of characters (Fig. 4b). RILs-169, -231, -56 and -142 can be grouped as the ones with poor root system and high yields and high shoot biomass. RILs-106, -211, -40, -179 and -29 with moderate root system and RILs-102 and -137 with large root system possessed high and stable yields. These RILs also had above-average shoot biomass production at maturity and above-average harvest indices. Notably, these RILs showed very less reduction in seed size during 2001–2002 when most other RILs showed a decline, indicating better maintenance of water status during seed filling. The low yielding examples with poor roots are RILs-112 and -113 and with large roots are RILs-229, -157, -187 and -255.

4. Conclusions

The variations observed for root traits, shoot biomass at flowering, and seed yield between the parental genotypes and among the RIL population was considerable. The RILs showed different possible combinations of root dry matter at 35 DAS and final seed yield expression, although there was no general correlation between root traits and seed yield. Seed yield was more related to shoot biomass at maturity, harvest index and seed size. It is suggested that efforts need to be focused on the devel-

opment of molecular markers, not only for root traits but also for the shoot biomass production and harvest index to improve the yield stability under terminal drought stress. The existence of large variability among RILs justifies efforts towards the identification of molecular markers for root traits. Further, the creation of a stable field drought-screening environment, with reproducible stress occurrence and intensity for the root trait’s expression and to test the large number of RILs required for mapping studies, is labor and cost intensive and requires tremendous efforts. Alternate simple methods need to be developed that have precise control over the natural development of drought in field condition that are more economic and time saving for effectively characterizing the populations in molecular studies.

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References

- Banziger, M., Edmeades, G.O., Lafitte, H.R., 1999. Selection for drought tolerance increases maize yields across a range of nitrogen levels. *Crop Sci.* 39, 1035–1040.
- Brockwell, J., 1982. Inoculation methods for field experimenters and farmers. In: Vincent, J.M. (Ed.), *Nitrogen Fixation in Legumes*. Academic Press, New York, pp. 211–221.
- Brown, S.C., Gregory, P.J., Cooper, P.J.M., Keatinge, J.D.H., 1989. Root and shoot growth and water use of chickpea (*Cicer arietinum*) grown in dryland conditions: effect of sowing date and genotype. *J. Agric. Sci. Camb.* 113, 41–49.
- Cooper, P.J.M., Gregory, P.J., Tully, D., Harris, H.C., 1987. Improving water use efficiency of annual crops in the rainfed farming systems of west Asia and north Africa. *Exp. Agric.* 23, 113–158.
- Crouch, J.H., Serraj, R., 2002. DNA marker technology as a tool for genetic enhancement of drought tolerance at ICRISAT. In:

- Saxena, N.P. (Ed.). Proceedings of the International Workshop on Field screening for Drought Tolerance in Rice. International Crops Research Institute for the Semi-arid Tropics, Patancheru, India.
- Cui, K.H., Peng, S.B., Xing, Y.Z., Xu, C.G., Yu, S.B., Zhang, Q., 2002. Molecular dissection of seedling-vigor and associated physiological traits in rice. *Theor. Appl. Genet.* 105, 745–753.
- FAO, 2003. Production Year Book, 2002. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. <http://apps.fao.org>.
- Fukai, S., Cooper, M., 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. *Field Crops Res.* 40, 67–87.
- Gregory, P.J., Saxena, N.P., Arihara, J., Ito, O., 1994. Root form and function in relation to crop productivity in cool season food legumes. In: Muehlbauer, F.J., Kaiser, W.J. (Eds.), *Expanding the Production and Use of Cool Season Food Legumes*. Kluwer Academic Publishers, Netherlands, pp. 809–820.
- Heeraman, D.A., Juma, N.G., 1993. A comparison of minirhizotron, core and monolith methods for quantifying barley (*Hordeum vulgare* L.) and faba bean (*Vicia faba* L.) root distribution. *Plant Soil* 148, 29–41.
- Johansen, C., Krishnamurthy, L., Saxena, N.P., Sethi, S.C., 1994. Genotypic variation in moisture response of chickpea grown under line-source sprinklers in a semi-arid tropical environment. *Field Crops Res.* 37, 103–112.
- Johansen, C., Singh, D.N., Krishnamurthy, L., Saxena, N.P., Chauhan, Y.S., Kumar Rao, J.V.D.K., 1997. Options for alleviating moisture stress in pulse crops. In: Asthana, A.N., Masood, A. (Eds.), *Recent Advances in Pulses Research*. Indian Society of Pulses Research and Development, IIPR, Kanpur, India, pp. 425–442.
- Kamoshita, A., Wade, L.J., Ali, M.L., Pathan, M.S., Zhang, Z., Sarkarung, S., Nguyen, H.T., 2002. Mapping QTLs for root morphology of a rice population adapted to rainfed lowland conditions. *Theor. Appl. Genet.* 104, 880–893.
- Keig, G., McAlpine, J.R., 1976. WATBAL: A Computer System for the Estimation and Analysis of Soil Moisture Regimes from Simple Climatic Data, 2nd ed. Technical Memorandum 74/4. CSIRO Division of Land Use Research, Canberra, Australia, 45 pp.
- Krishnamurthy, L., Ito, O., Johansen, C., 1996. Genotypic differences in root growth dynamics and its implications for drought resistance in chickpea. In: Ito, O., Johansen, C., Adu Gyamfi, J.J., Katayama, K., Kumar Rao, J.V.D.K., Rego, T.J. (Eds.), *Dynamics of Roots and Nitrogen in Cropping Systems of the Semi-arid Tropics*. JIRCAS Agriculture Series No. 3. Japan International Research Center for Agricultural Sciences, Tsukuba, Japan, pp. 235–250.
- Krishnamurthy, L., Ito, O., Johansen, C., Saxena, N.P., 1998. Length to weight ratio of chickpea roots under progressively receding soil moisture conditions in a vertisol. *Field Crops Res.* 58, 177–185.
- Krishnamurthy, L., Johansen, C., Sethi, S.C., 1999. Investigation of factors determining genotypic differences in seed yield of non-irrigated and irrigated chickpeas using a physiological model of yield determination. *J. Agron. Crop Sci.* 183, 9–17.
- Kumar, J., Abbo, S., 2001. Genetics of flowering time in chickpea and its bearing on productivity in semiarid environments. *Adv. Agron.* 72, 107–138.
- Lilley, J.M., Fukai, S., 1994. Effect of timing and severity of water deficit on four diverse rice cultivars. I. Rooting patterns and soil water extraction. *Field Crops Res.* 37, 205–214.
- Ludlow, M.M., Muchow, R.C., 1990. A critical evaluation of traits for improving crop yields in water-limited environments. *Adv. Agron.* 43, 107–153.
- Mambani, B., Lal, R., 1983. Response of upland rice varieties to drought stress. I. Relation between root system development and leaf water potential. *Plant Soil* 73, 59–72.
- Passioura, J.B., 1983. Roots and drought resistance. *Agric. Water Manage.* 7, 265–280.
- Price, A., 2002. QTLs for root growth and drought resistance in rice. In: Mohan, S., Brar, D.S., Ahloowalia, B.S. (Eds.), *Molecular Techniques in Crop Improvement*. Kluwer Academic Publishers, Dordrecht, 616 pp.
- Saxena, N.P., 1984. The chickpea. In: Goldsworthy, P.R., Fisher, N.M. (Eds.), *The Physiology of Tropical Field Crops*. Wiley, UK, pp. 419–452.
- Saxena, N.P., 2003. Management of drought in chickpea—a holistic approach. In: Saxena, N.P. (Ed.), *Management of Agricultural drought. Agronomic and Genetic Options*. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 103–122.
- Saxena, N.P., Johansen, C., 1990. Functional ideotypes for increasing and stabilizing chickpea yield. In: Proceedings of the Fifth Australian Agronomy Conference, September 24–29, 1989. The University of Western Australia, Perth, Western Australia, p. 657.
- Saxena, N.P., Krishnamurthy, L., Johansen, C., 1993. Registration of a drought-resistant chickpea germplasm. *Crop Sci.* 33, 1424.
- Saxena, N.P., Sethi, S.C., Krishnamurthy, L., Johansen, C., Haware, M.P., 1995. Physiological approaches to genetic improvement of drought resistance in chickpea. In: Proceedings of the InterDrought 1995, Génétique et Amélioration des Plantes. INRA Montpellier, France, pp. 1–6.
- Schneider, K.A., Rosales-Serna, R., Ibarra-Perez, F., Cazares-Enriquez, B., Acosta-Gallegos, J.A., Ramirez-Vallejo, P., Wassimi, N., Kelly, J.D., 1997. Improving common bean performance under drought stress. *Crop Sci.* 37, 43–50.
- Serraj, R., Bidinger, F.R., Chauhan, Y.S., Seetharama, N., Nigam, S.N., Saxena, N.P., 2003. Management of drought in ICRISAT cereal and legume mandate crops. In: Kijne, J.W., Barker R., Molden D. (Eds.), *Water Productivity in Agriculture: Limits and Opportunities for Improvement*. CABI, Wallingford, UK, 332 pp.
- Serraj, R., Sinclair, T.R., 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions? *Plant Cell Environ.* 25, 333–341.
- Siddique, K.H.M., Belford, R.K., Tennant, D., 1990. Root:shoot ratios of old and modern, tall and semi-dwarf wheats in a Mediterranean environment. *Plant Soil* 121, 89–98.
- Silim, S.N., Saxena, M.C., 1993. Adaptation of spring-sown chickpea to the Mediterranean basin. II. Factors influencing yield under drought. *Field Crops Res.* 34, 137–146.

- Sinclair, T.R., 1994. Limits to crop yield? In: Boote, K.J. (Ed.), *Physiology and Determination of Crop Yield*. ASA, CSSA and SSSA, Madison, WI, pp. 509–532.
- Singh, K.B., Bejiga, G., Saxena, M.C., Singh, M., 1995. Transferability of chickpea selection indices from normal to drought-prone growing conditions in a Mediterranean environment. *J. Agron. Crop Sci.* 36, 472–479.
- Soltani, A., Ghassemi-Golezani, K., Khoorie, F.R., Moghaddam, M., 1999. A simple model for chickpea growth and yield. *Field Crops Res.* 62, 213–224.
- Soltani, A., Khoorie, F.R., Ghassemi-Golezani, K., Moghaddam, M., 2000. Thresholds for chickpea leaf expansion and transpiration response to soil water deficit. *Field Crops Res.* 68, 205–210.
- Turner, N.C., Wright, G.C., Siddique, K.H.M., 2001. Adaptation of grain legumes (pulses) to water limited environments. *Adv. Agron.* 71, 193–231.
- White, J.W., Castillo, J.A., 1992. Evaluation of diverse shoot genotypes on selected root genotypes of common bean under soil water deficits. *Crop Sci.* 32, 762–765.