Journal of Food Legumes 23(2): 99-105, 2010

Significance and genetic diversity of SPAD chlorophyll meter reading in chickpea germplasm in the semi-arid environments

JUNICHI KASHIWAGI[1], HARI D. UPADHYAYA[2] and L. KRISHNAMURTHY[2]

[1]*Hokkaido University, Kita 9 Nishi 9, Sapporo 060-8589, Japan;* [2]*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India; E-mail: jkashi@res.agr.hokudai.ac.jp*

(Received: January, 2010; Accepted: August, 2010)

ABSTRACT

The SPAD chlorophyll meter reading is a measurement of the leaf chlorophyll contents, *viz.*, the nitrogen acquisition capability, and so it is often used to improve the yield through improved nitrogen status. The genetic diversity of the SCMR was investigated in the chickpea mini-core germplasm collection plus five control cultivars of chickpea (*Cicer arietinum* L.) (n = 216) of ICRISAT Genebank under field conditions during two consecutive post rainy seasons of 2005-06 and 2006-07. A large genetic variability for SCMR was observed among the 216 chickpea accessions. The SCMR at 62 days after sowing was positively correlated with the seed yield under drought environments. The SCMR at the earlier or later growth stages or under irrigated environment was not related to yield under drought environment, indicating that the selections for SCMR in chickpea need to be done at about mid pod-fill stage under drought stress conditions. A known drought avoidant chickpea genotype, 'ICC 4958' that has prolific and deep rooting system also showed the best SCMR performances among the 216 chickpea germplasm. 'ICC 4958' can be a potential donor parent for both root systems and SCMR advantages. In addition, few other outstanding genotypes such as 'ICC 1422', 'ICC 10945', 'ICC 16374' and 'ICC 16903', with the higher SCMR, were also identified in this study. This genetic variability for SCMR in the mini core provides valuable baseline knowledge in chickpea for further progress on the selection and breeding for drought tolerance through nitrogen acquisition capability.

Key words: Breeding, Chickpea (*Cicer arietinum* L.), Genetic diversity, Mini-core collection, SPAD chlorophyll meter reading (SCMR)

Chickpea (*Cicer arietinum* L.) is the third important food legume in terms of the cultivated area (11.7 million hectares) and in total annual production (9.3 million tons in 2007) (FAO Stat 2009). The major chickpea cultivation occurs in the developing countries that fall in the arid and semi-arid zones. The crop is largely grown rainfed, and therefore drought stress is one of the most serious constraints for the productivity (Ryan 1997).

In the last two decades, the chickpea yield under drought environments have been increased through improving some physiological, morphological and phenological characteristics that have been recognized to be significant in crop adaptation to drought stress during soil drying (Ludlow and Muchow 1990, Subbarao *et al*. 1995). Enhancing early maturity could lead the chickpea crops to escape from severe soil water depletion that generally occurs during the reproductive stage. 'ICCV 2', an early maturing chickpea variety, successfully brought in the yield stability in shorter duration drought-prone environments (Kumar *et al*. 1985). Recently, chickpea germplasm with deep and prolific root systems have attracted the attention as means to improve the drought tolerance through enhanced water uptake (Kashiwagi *et al*. 2006). During extensive characterization of the root traits, several chickpea genotypes with a prolific root system were identified, and brought into molecular marker assisted breeding programs (Chandra *et al*. 2004).

Under drought, the plants would also face difficulties in nutrient uptake for maintaining a proper growth in addition to soil water acquisition as nutrient absorption requires water. The chickpea acquires water soluble nitrogen contained in the soil via the roots, and also the nitrogen synthesized via biological nitrogen fixation in the nodules on their root systems. The biological nitrogen fixation is also influenced by drought as the rhizobial activities are adversely affected by heat as well as water deficit in the soil (Zahran *et al*. 1999). Thus, the leaf nitrogen concentration in chickpea is expected to be reduced under drought environments as both the nitrogen acquiring mechanisms are suppressed under such conditions, which would result in the serious yield reduction. Therefore, for a drought tolerance breeding program, it is important to characterize the chickpea germplasm and to identify sources of drought-tolerant chickpea germplasm that are efficient in nitrogen acquisition even under drought environments.

Leaf nitrogen content, *in situ*, could be estimated through SPAD chlorophyll meter reading (SCMR). The SPAD chlorophyll meter is a simple portable diagnostic tool that measures the greenness or relative chlorophyll content of leaves (Inada 1963, 1985; Richardson *et al*. 2002) and these readings are dispayed in Minolta Company (Konica-Minolta Inc. Japan) defined SPAD (soil plant analysis development) values. There has been a strong linear relationship between the SPAD values and weight-based leaf N concentration (N_w) but this relationship varies with crop growth stage and variety (Takebe and Yoneyama 1989; Turner and Jund 1994) mostly because of leaf thickness or specific leaf weight (Peng *et al.*

1993). Similarly, across crops also, the SCMR shows a linear correlation with extractable leaf chlorophyll (Yadava 1986). Particularly in chickpea, a significant close relationship between them ($r^2 = 0.81$) was obtained (Esechie and Al-Maskri 2006). The SCMR, therefore, could be taken as a good proxy for the chlorophyll contents in chickpea crop. The chlorophyll quantity in the plant leaves have good correlation with leaf nitrogen concentration since the leaf chloroplasts contain 70% of the leaf nitrogen (Bullock and Anderson 1998). Because of these, the SCMR is used to improve the yield via monitoring the nitrogen status.

Although it is desirable, it is practically not feasible to characterize/phenotype the whole chickpea germplasm collection for SCMR due to their large numbers (about 20, 000 at present). The genebank of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has developed a core collection of 1,956 germplasm accessions representing the diversity of entire collection (Upadhyaya *et al*. 2001) and from this core collection, a chickpea mini-core germplasm collection (211 accessions) has been developed (Upadhyaya and Ortiz 2001). In our previous studies, the characterization of the mini-core chickpea germplasm has led to the identification of sources of deep and prolific rooting known to assist enhanced drought tolerance (Kashiwagi *et al*. 2005) as well as sources of salinity tolerance (Vadez *et al*. 2007). It would also be valuable to characterize this mini-core set for other relevant drought related traits so that a comprehensive and integrated drought tolerance data base could be developed for supporting a drought tolerance breeding program in chickpea.

Thus, the main objective of this study was to i) characterize the chickpea germplasm for SCMR and to identify the superior chickpea germplasm in terms of the nitrogen acquisition capability, and ii) investigate the significance of SCMR for further plant breeding aimed towards improving the drought tolerance in chickpea.

MATERIALS AND METHODS

***Field trials*:** The measurements of the SPAD chlorophyll meter readings in chickpea mini-core collection were carried out in Vertisol fields (fine montmorillonitic isohyperthermic typic pallustert) at ICRISAT Center, Patancheru (17° 53' N; 78°27'E; altitude 545 m) in two crop seasons, 2005-06 and 2006-2007. The water holding capacity of these fields in lower limit: upper limit was 0.26: 0.40 $cm^3\ cm^{-3}$ for the 0-15 cm soil layer, and 0.30: 0.47 $cm^3\ cm^{-3}$ for the 105-120 cm soil layer. The available soil water up to 120 cm depth was 165 mm, and the bulk density was; 1.35 $g\ cm^{-3}$ for the 0-15 cm soil layer and 1.42 $g\ cm^{-3}$ for the 105-120 cm soil layer (El-Swaify *et al*. 1985).

A total of 216 chickpea genotypes comprising all of the chickpea mini-core germplasm collection of *C. arietinum* (211 accessions) plus 5 control cultivars ('Annigeri', 'ICC 4958', 'Chafa', 'ICCV 2', and 'ICC 898') were used. 'Annigeri' is an early-maturing *desi* cultivar grown in large areas of Peninsular India (Ali and Kumar 2003). 'ICC 4058' is drought avoidant *desi* germplasm lines with highly desirable root traits (Saxena *et al*. 1993, Kashiwagi *et al*. 2005). 'ICCV 2' ('ICC 12968') is an ICRISAT-bred early-maturing *kabuli* cultivar released in India (Kumar *et al*. 1985). 'Chafa' is the first variety of chickpea (*desi* type) released through selection in Wai at Niphad, in 1948 Maharashtra, and in 1960 in Gujarat, India (Dua *et al*. 2001). 'ICC 898' is a *desi* landrace from Rajasthan, India. The crop was sown on November 15 and November 2 in 2005 and 2006, respectively. The experimental design was an alpha lattice design (6 × 36 blocks) with three replications. The field managements were the same in both the seasons. Before sowing, the field was solarized with polythene mulch in both the seasons to prevent the incidence of *Fusarium* wilt, and then18 kg N/ha and 20 kg P/ha was applied as di-ammonium phosphate. A sprinkler irrigation (20 mm) was applied immediately after sowing to ensure uniform emergence. During both the seasons, the fields were inoculated with Rhizobium strain 'IC 59' using liquid inoculation method. The plots were kept weed free by hand weeding and intensive protection measures were taken against pod borer (*Helicoverpa armigera*).

Two irrigation treatments, rainfed and optimally irrigated, were included as main plots. The rainfed treatment received no irrigation after the 20-mm post-sowing irrigation. The irrigated treatments, received three furrow irrigations besides the post-sowing one at 27 days after sowing (DAS), 50 DAS and 66 DAS in 2005-06 season, and 25 DAS, 48 DAS and 75 DAS in 2006-07 season.

An earlier preliminary survey showed a significant variation on the SCMR at different leaf positions. The SCMR of the top and the second top leaf was significantly lower than that of the other basal leaf positions, *viz.*, a stable SCMR was obtained below the third leaf position. Therefore, the third leaf from the top was used for SPAD evaluation in this study. In 2005-06, the SCMR was recorded at 62 and 90 DAS and at 40 and 62 DAS in 2006-07.

At final harvest, the shoot biomass, seed yield and other yield components were evaluated from an area of 1.5 × 2.5 m in both the seasons after removing the plot border on either end of the plot. The shoots were dried in hot air dryers at 45°C for three days, and the dry weights were recorded. Then, the shoots were threshed, and the extracted seeds were weighed.

Statistical analysis: The data from each trial were analyzed using a linear additive mixed effects model as described by Upadhyaya (2005). By using this model, the statistical procedure of residual maximum likelihood (ReML) was employed to obtain the unbiased estimates of the variance components $\sigma^2 b$, $\sigma^2 g$ and $\sigma^2 e$, and the best linear unbiased predictors (BLUPs) of the performance of the chickpea accessions. Heritability was estimated as $h^2 = \sigma^2 g / (\sigma^2 g + \sigma^2 e)$. As the block effects within each replication are separately worked out with ReML, the heritability values calculated are much more precise than the broad sense heritability and yet not that precise as that of the narrow sense heritability. In the phenotypic variability, which contain genetic as well as environmental variability, observed in the mini-core collection plus several entries, the significance of genetic variability was assessed from the standard error of the estimate of genetic variance σ^2g, assuming the ratio σ^2g /S.E. (σ^2g) to follow normal distribution asymptotically. The above model was extended for over-season analysis if traits recorded in both seasons, assuming season effect as fixed, with genotype by season interaction effect being a random effect assumed to have a mean of zero and constant variance σ^2gE. The significance of G × S was assessed in a manner similar to that of σ^2g. The significance of the fixed effect of the season was assessed using the Wald statistic that asymptotically follows a χ^2 distribution and is akin to the *F*-test in the traditional ANOVA.

RESULTS AND DISCUSSION

Genetic diversity of SCMR in chickpea mini-core germplasm: The chickpea cropping season was dry during both 2005-06 and 2006-07 (Fig 1). Total precipitation during the cropping season was only 3.1 mm and 17.2 mm, in 2005-06 and 2006-07 respectively, and the pattern and amount of evaporation was similar between the years. The dynamics of temperature was also almost the same between the years, but the minimum temperature across 2006-07 season was higher than that in 2005-06. In addition, the air was drier in 2006-07 than in 2005-06. It can be concluded that 2006-07 was more droughty year than 2005-06.

Irrespective of irrigation treatments, there was a significant difference on SCMR among the germplasm accessions at any measurement stages in both the years (Table 1). 'ICC 16374' was a noteworthy genotype as it showed the highest SCMR under rainfed condition at 62 DAS in 2005-06, and also under irrigated condition at 62 DAS in 2006-07. Under the rainfed (drought) conditions in 2006-07, the genotype 'ICC 4958' showed the highest SCMR at 62 DAS and 'ICC 7571' at 40 DAS. Genotypes 'ICC 12654' (62 DAS in 2005-06), 'ICC 4567' (40 DAS in 2006-07), 'ICC 11627' (62 DAS in 2006-07) showed the lowest SCMR under drought environments. The heritability values estimated under irrigated conditions ranged from 0.38 (at 90 DAS in 2005-06) to 0.56 (at 62 DAS in 2005-06), and were higher than that in drought stress conditions showing between 0.13 (at 90 DAS in 2006-07) and 0.24 (at 62 DAS in 2005-06) (Table 1). In one of our previous studies, shoot biomass at 35 DAS and root biomass at the same time possessed heritability values of more than 60% and 50%, respectively, which was seen to decline to 14% at 50 DAS (Kashiwagi *et al.* 2005). Irrespective of the irrigation treatments, the heritability of SCMR in 2006-07 did not show big reduction at 62 DAS compared to that of 40 DAS although the heritability under rainfed conditions were very low as 17% at 40 DAS and 13% at 62 DAS, respectively. Such poor heritability values indicate that larger populations would be required for

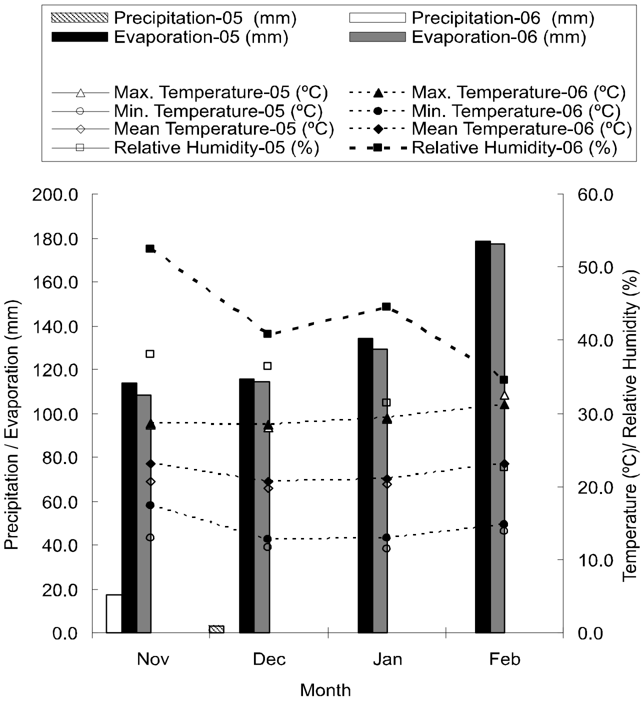

Fig 1. Weather at experimental site (ICRISAT, Patancheru) during the crop growing season of the years 2005-06 and 2006-07.

Table 1. Trial means, range of best linear unbiased predicted means (BLUPs) and analysis of variance of SCMR of the entries in the field trials in 2005-06 and 2006-07.

		Range of predicted means					Heritability	
SCMR	**Mean**	**Minimum**	**Maximum**	**Component**	**S.E.**	**Significance**	h^2	**S.E.**
2005-06								
Irrigated at 62DAS	47.4	40.1	53.1	5.07	0.63	**	0.56	0.070
Rainfed at 62DAS	57.6	52.0	61.4	6.08	1.32	**	0.24	0.052
Irrigated at 90DAS	53.4	49.1	58.6	5.21	0.82	**	0.38	0.060
2006-07								
Irrigated at 40DAS	57.5	52.8	62.0	4.97	0.74	**	0.42	0.062
Rainfed at 40DAS	58.6	56.2	60.8	1.92	0.55	**	0.17	0.049
Irrigated at 62DAS	49.2	43.6	54.4	5.01	0.77	**	0.40	0.061
Rainfed at 62DAS	64.0	61.6	66.4	2.67	0.97	*	0.13	0.047

DAS = days after sowing *, ** Significant at P = 0.05 and 0.01, respectively selections to improve the SCMR of chickpea thereby enhancing the drought tolerance.

The SCMR values in the drought environments were greater compared to the irrigated conditions (Table 1), which was similar to the SCMR response made in groundnut (Nigam and Aruna 2008). This phenomenon is an expected drought response in crop plants. Leaf area expansion gets more affected in response to drought with adversely affecting the specific leaf area (SLA), thereby reducing the leaf size so that the plants could minimize the water loss via the leaf surface. As a consequence of increased leaf thickness, the leaves could have greater concentration of the chlorophyll density in the leaves to maintain relatively better photosynthesis. Because of the denser chlorophyll content and thicker leaves, the SCMR is expected to be increased as repeatedly observed in groundnut (Nageswara Rao *et al.* 2001, Bindu Madhava *et al.* 2003, Upadhyaya 2005). In groundnut, a clear significant negative correlation between SLA and SCMR, and between the SLA and transpiration efficiency (TE) had been observed (Wright *et al.* 1994, Bindu Madhava *et al.* 2003) suggesting that SCMR could be used as an easily measurable surrogate for TE for improving the drought tolerance (Nigam and Aruna 2008). This approach of screening for TE via SCMR can be applicable also to chickpea to improve the drought tolerance. However, further studies with chickpea are needed to confirm the extent of clarity in such relationships as observed in groundnut.

Significance of SCMR in chickpea to the yield under drought conditions: At 62 DAS, under drought environments, there was a significant positive correlation between the SCMR and the seed yield in both the cropping seasons, whereas under irrigated conditions, only in one season, 2006-07, such relationship was observed between the SCMR and yield but not in 2005-06 (Table 2). In many crops, such as groundnut (Nigam and Aruna 2008), sorghum (Xu *et al.* 2008), wheat (Silva *et al.* 2007), and maize (Zaidi *et al.* 2008), this strong correlation was observed between SCMR and seed yield under drought environments. Interestingly, the SCMR at the 62 DAS under drought environments also showed significant positive relationship with the shoot biomass and harvest index in chickpea (Table 2). Thus, the SCMR could be considered as one of the traits that should be incorporated into breeding programs aimed at improving the drought tolerance in chickpea. On the other hand, at earlier growth stage, 40 DAS, the SCMR did not show any such significant relationship with the shoot biomass, harvest index, and seed yield, but a significant relationship between the SCMR and yield was observed at 90 DAS. It is that the SCMR of chickpea accessions is an adaptive trait and some of the genotypes are capable of adjusting their leaf thickness/leaf nitrogen content under drought stress as seen in the current case at 62 DAS, and that could reflect in a maximized vegetative as well as reproductive growth particularly under drought stress. Similar behavior of SCMR had been reported in groundnut mapping population derived out of a high (‘ICGV 86031’) and a low TE (‘TAG 24’) parents (Krishnamurthy *et al.* 2007).

Table 2. Correlation coefficient between SCMR and the yield and yield components

SCMR	Shoot biomass	Harvest index	Yield
Rainfed			
Rainfed at 40DAS-2006	0.053	0.091	0.087
Rainfed at 62DAS-2005	0.342**	0.230**	0.341**
Rainfed at 62DAS-2006	0.161*	0.301**	0.329**
Irrigated			
Irrigated at 40DAS-2006	0.034	-0.109	-0.098
Irrigated at 62DAS-2005	0.000	0.056	0.042
Irrigated at 62DAS-2006	-0.069	0.209**	0.138*
Irrigated at 90DAS-2005	0.171*	0.237**	0.389**

DAS = days after sowing

*, ** Significant at P = 0.05 and 0.01, respectively

The SCMR at 62 DAS under drought conditions alone exhibited its contribution to the seed yield (Table 2). A significant linear relationship of the SCMR at 62 DAS in rainfed conditions was observed between 2005-06 and 2006-07, although the G × E interaction was not significant (F prediction = 0.732 ns). The regression coefficient, however, was low (r^2 = 0.202, P<0.01) (Fig 2), as an indicator of the heritability presented in Table 1. Thus, the promising genotypes which showed constantly higher SCMR in both years were identified among 216 accessions on a biplot chart (Fig 2). The top 20 accessions with the best SCMR in each year are presented in Table 3 (the best 10% of the total 216 accessions). Five genotypes were the common ones that appeared on the lists of both the years. The genotype ‘ICC 4958’ that originated in India happened to be the most outstanding, showing the highest SCMR of 66.4 in 2006-07 and 60.4 with the fourth rank in 2005-06. In our previous study, the same chickpea mini-

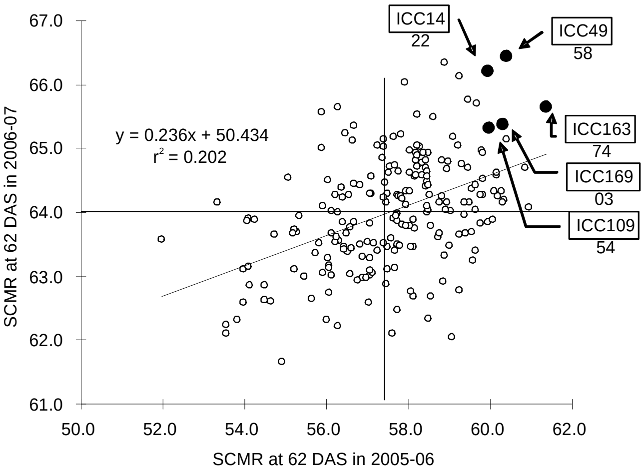

Fig 2. Relationship in the SCMR at 62 DAS under rainfed conditions between the year 2005-06 and 2006-07. The vertical and horizontal lines indicate the mean of SCMR in 2005-06 and 2006-07, respectively.

Table 3. Twenty top ranking chickpea germplasm on SCMR among 216 accessions in each year

		2005-06		2006-07	
Ranking	Accession	Origin	SCMR	Accession	SCMR
1	'ICC 4958'	India	66.4	'ICC 16374'	61.4
2	'ICC 1882'	India	66.3	'ICC 4872'	60.9
3	'ICC 1422'	India	66.2	'ICC 4495'	60.8
4	'ICC 5383'	India	66.1	'ICC4958'	60.4
5	'ICC 283'	India	66.0	'ICC 14402'	60.4
6	'ICC 15868'	India	65.8	'ICC 2580'	60.3
7	'ICC 13124'	India	65.7	'ICC 13461'	60.3
8	'ICC 7441'	India	65.7	'ICC 16903'	60.3
9	'ICC 16374'	Unknown	65.7	'ICC 12155'	60.3
10	'ICC 15618'	India	65.6	'ICC 15435'	60.2
11	'ICC 8318'	India	65.5	'ICC 7308'	60.2
12	'ICCV2'	India	65.5	'ICC 7272'	60.1
13	'ICC 16903'	India	65.4	'ICC 4463'	60.1
14	'ICC 13863'	Ethiopia	65.4	'ICC 15802'	60.1
15	'ICC 10945'	India	65.3	'ICC 10945'	60.0
16	'ICC 10399'	India	65.2	'ICC 1422'	60.0
17	'ICC 7571'	Israel	65.2	'ICC 1431'	59.9
18	'ICC 14778'	India	65.2	'ICC 2884'	59.8
19	'ICC 8855'	Afghanistan	65.2	'ICC 6263'	59.8
20	'ICC 14669'	India	65.1	'ICC 2990'	59.8
		Mean (n=216)	57.6		64.0
		S.E.	1.32		0.97

DAS = days after sowing

*, ** Significant at P = 0.05 and 0.01, respectively

core collection plus 5 popular varieties were characterized for the root traits (Kashiwagi *et al.* 2005). Interestingly, 'ICC 4958', a genotype identified to possess one of the most prolific and deep root systems, showed the best SCMR performance among the 216 chickpea germplasm in the present study. This genotype happens to be one of the most promising breeding material for improving the drought tolerance of chickpea in terms of not only the soil water acquisition but also nitrogen acquisition. The accessions 'ICC 16374' (origin unknown), 'ICC 1422' (India), 'ICC 16903' (India) and 'ICC 10945' (India) were also noteworthy as they exhibited high and repeatable SCMR values.

Investigations on the existence of any association between our previous results on various root traits and the SCMR did not exhibit any relationship (root length density and SCMR: r = 0.102 ns, and rooting depth and SCMR: r = 0.094 ns). In maize, however, the genotypes with extensive and deep root systems had been shown to have the advantage of acquiring greater amount of nitrogen under drought conditions (Banziger *et al.* 1999, Kamara *et al.* 2001). It would be because the extensive roots in the surface soil layer allowed the crops to use the soil inorganic nitrogen effectively, while the deeper roots were able to extract nitrate leached to deeper soil layers. However, in our current study on chickpea, it showed that the soil nitrogen acquisition of chickpea is independent of the root systems. This could more likely be due to the nitrogen compensation provided by the biological nitrogen fixation in chickpea in addition to the root system acquisition advantage. Interactions between the rhizobial activities and the chickpea genotype-rhizobium affinity under drought condition would influence the nitrogen acquisition. Our results suggest that the use of two different genetic sources, i.e. one for the root system advantage (*viz.*, water uptake) and the other for the SCMR advantage (nitrogen acquisition ability) could be a more beneficial strategy for genetic improvement of drought tolerance in chickpea. Alternatively, a single genotype 'ICC 4958' also can be the source for the twin alleles such as the best root system and the best SCMR. The accessions/genotypes listed on Table 3 would be valuable sources of nitrogen acquisition capability for further breeding programs to improve the drought tolerance in chickpea.

A large genetic variability for SPAD chlorophyll meter reading (SCMR), as a proxy to the nitrogen acquisition capability, was observed among the 211 mini-core chickpea germplasm accessions plus 5 cultivars from the ICRISAT genebank. The SCMR seemed to be an adaptive trait. A significant relationship between the SCMR and seed yield under drought environment was observed only at 62 DAS, a stage when the crop had already experienced considerable drought stress, while this relationship could not be noticed in early growth stages and soil moisture environments. Therefore, selections for SCMR need to be made at a stage when the crop has been adequately subjected to drought stress and at later stages of crop growth such as mid pod-fill stage. A known drought-avoidant genotype with the most prolific and deep root system 'ICC 4958' also showed the best SCMR performances among the 216 chickpea germplasm accessions. This genotype will remain to be a unique promising breeding material for improving not only the soil water but also soil nitrogen acquisition. In addition, some other outstanding genotypes such as 'ICC 1422', 'ICC 10945', 'ICC 16374' and 'ICC 16903' with the best SCMR were also identified. This can be used as valuable baseline information in future breeding programs to improve the drought tolerance and QTL mapping of nitrogen acquisition capability in chickpea to develop high yielding cultivars for drought environments.

ACKNOWLEDGMENT

This research was partly supported by the unrestricted funds from the Japanese Government earmarked for drought tolerance research and breeding in ICRISAT.

REFERENCES

Ali M and Kumar S. 2003. Chickpea research in India. An over view. In: Masood Ali, Shiv Kumar, Singh NB (Eds), Chickpea research in India. Pp. 1-13. Indian Institute of Pulses Research, Kanpur, India.

Bänziger M, Edmeades GO and Lafitte HR. 1999. Selection for Drought Tolerance Increases Maize Yields across a Range of Nitrogen Levels. Crop Science **39:** 1035-1040.

Bindu Madhava H, Sheshshayee MS, Shankar AG, Prasad TG and Udayakunar M. 2003. Use of SPAD chlorophyll meter to assess transpiration efficiency of peanut. In: Cruickshank AW, Rachaputi NC, Wright GC, Nigam SN, eds. Breeding of drought resistant peanuts. ACIAR Proceeding No. 112, Pp. 3-9. Australian Centre for International Agricultural Research, Canberra.

Bullock DG and Anderson DS. 1998. Evaluation of Minolta SPAD-502 chlorophyll meter for nitrogen management in corn. Journal of Plant Nutrition **21:** 741-755.

Chandra S, Buhariwalla HK, Kashiwagi J, Harikrishna S, Rupa Sridevi K, Krishnamurthy L and Serraj R. 2004. Identifying QTL-linked markers in marker-deficient crops. 4th International Crop Science Congress, Brisbane, Australia.

Dua RP, Chaturvedi SK and Sewak S. 2001. Reference varieties of chickpea for IPR regime, Pp. 7. Indian Institute of Pulses Research, Kanpur, India.

Esechie HA and Al-Maskri AY. 2006. Relationship between SPAD-502 meter values and extractable chlorophyll in chickpea (*Cicer arietinum* L.). Research on Crops **7:** 313-317.

El-Swaify SA, Pathak P, Rego TJ and Singh S. 1985. Soil management for optimized productivity under rainfed conditions in the semi-arid tropics. Advances in Soil Science **1:** 1-64.

Food and Agricultural Organization of the United Nations. 2009. FAO Statistical Databases. Available at http://faostat.fao.org/ FAO, Rome.

Inada K. 1963. Studies on a method for determining deepness of green color and chlorophyll content of intact crop leaves and its practical applications. 1. Principle for estimating the deepness of green color and chlorophyll content of whole leaves. Proceedings of Crop Science Society of Japan **32:** 157-162.

Inada K. 1985. Spectral ratio of reflectance for estimating chlorophyll content of leaf. Japanese Journal of Crop Science **54:** 261-265.

Kamara AY, Kling JG, Ajala SO and Menkir A. 2001. Vertical root-pulling resistance in maize is related to nitrogen uptake and yield. Pp. 228-232. 7th Eastern and Southern African regional Maize Conference. Feb 11-15 2001.

Kashiwagi J, Krishnamurthy L, Upadhyaya HD, Krishna H, Chandra S, Vadez V and Serraj R. 2005. Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). Euphytica **146:** 213-222.

Kashiwagi, J, Krishnamurthy L, Crouch JH and Serraj R. 2006. Variability of root characteristics and their contributions to seed yield in chickpea (*Cicer arietinum* L) under terminal drought stress. Field Crops Research **95:** 171-181.

Krishnamurthy L, Vadez V, Jyotsna Devi M, Serraj R, Nigam SN, Sheshshayee MS, Chandra S and Aruna R. 2007. Variation in transpiration efficiency and its related traits in a groundnut (*Arachis hypogaea* L.) mapping population. Field Crops Research **103:** 189-197.

Kumar J, Haware MP and Simthson JB. 1985. Registration of four short-duration fusarium wil-resistant *kabuli* (garbanzo) chickpea germplasm. Crop Science **25:** 576-577.

Ludlow MM and Muchow RC. 1990. Critical evaluation of traits for improving crop yields in water-limited environments. Advances in Agronomy **43:** 107-153.

Nigam SN and Aruna R. 2008. Stability of soil analytical development (SPAD) chlorophyll meter reading (SCMR) and specific leaf area (SLA) and their association across varying soil moisture stress conditions in groundnut (*Arachis hypogaea* L.). Euphytica **160:** 111-117.

Nageswara Rao RC, Talwar HS and Wright GC. 2001. Rapid assessment of specific leaf area and leaf N in peanut (*Arachis hypogaea* L.) using chlorophyll meter. Journal of Agronomy and Crop Science **189:** 175-182.

Peng S, Garcia FC, Laza RC and cassmann KG. 1993. Adjustment for specific leaf weight improves chlorophyll meter's estimation of rice leaf nitrogen concentration. Agronomy Journal **85:** 987-990.

Richardson AD, Duigan SP and Berlyn GP. 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytol **153:** 185-194.

Ryan JG. 1997. A global perspective on pigeonpea and chickpea sustainable production systems: Present status and future potential. In: Asthana AN, Ali M, eds. Recent Advantages in Pulses Research. Indian Society of Pulses Research and Development. Pp. 1-31. Indian Institute of Pulses Research (IIPR), Kanpur, India.

Saxena NP, Krishnamurthy L and Johansen C. 1993. Registration of a drought-resistant chickpea germplasm. Crop Science **33:** 1424.

Silva MA, Jifon JL, Silva JAG and Sharma V. 2007. Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane. Brazilian Journal Plant Physiology **19:** 193-201.

Subbarao GV, Johansen C, Slinkard AE, Rao RCN, Saxena NP and Chauhan YS. 1995. Strategies for improving drought resistance in grain legumes. Critical Reviews in Plant Science **14:** 469-523.

Takebe M and Yoneyama T. 1989. Measurement of leaf color scores and its implication to nitrogen nutrition of rice plants. Japan Agricultural Research **23:** 86-93.

Turner FT and Jund MF. 1994. Assessing the nitrogen requirements of rice crops with a chlorophyll meter method. Australian Journal of Experimental Agriculture **34:** 1001-1005.

Upadhyaya HD, Bramel PJ and Singh S. 2001. Development of a chickpea core subset using geographic distribution and quantitative traits. Crop Science **41:** 206-210.

Upadhyaya HD and Ortiz R. 2001. A mini core subset for capturing diversity and promoting utilization of chickpea genetic resources in crop improvement. Theoretical and Applied Genetics **102:** 1292-1298.

Upadhyaya HD. 2005. Variability of drought resistance related traits in the mini core collection of peanut. Crop Science **45:** 1432-1440.

Vadez V, Krishnamurthy L, Serraj R, Gaur PM, Upadhyaya HD, Hoisington DA, Varshney RK,Turner NC and Siddique KHM. 2007. Large variation in salinity tolerance in chickpea is explained by differences in sensitivity at the reproductive stage. Field Crops Research **104:** 123-129.

Wright GC, Nageswara Rao RC and Farquhar GD. 1994. Water use efficiency and carbon isotope discrimination under water deficit conditions. Crop Science **34:** 92-97.

Xu W, Rosenow DT and Nguyen HT. 2008. Stay green trait in grain sorghum: relationship between visual rating and leaf chlorophyll concentration. Plant Breeding **119:** 365 – 367.

Yadava UL. 1986. A rapid and nondestructive method to determine chlorophyll in interact leaves. Horticultural Science **21:** 1449-1450.

Zahran HH. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and Molecular Biology Review **63:** 968-89.

Zaidi PH, Mamata Yadav, Singh DK and Singh RP. 2008. Relationship between drought and excess moisture tolerance in tropical maize (*Zea mays* L.). Australian Jornal of Crop Science **1:** 78-96