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Traits for Screening and Selection of Cowpea Genotypes for Drought Tolerance at Early Stages of Breeding

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Abstract
The association of leaf water content with yield-attributes such as pod setting and number of pods/plant and seed yield in cowpea was examined using midday drop of leaf relative water content (RWC) determined from morning (800 h) and midday (1330 h) measurements of RWC. Midday drop of RWC was significantly correlated to pod setting ratio ($R^2 = 0.80$, $P < 0.01$), number of pods/plant ($R^2 = 0.87$, $P < 0.01$) and seed yield ($R^2 = 0.37$, $P < 0.05$). There was a significant genotypic variation for leaf water potential (LWP) at 800 and 1330 h and for RWC at 1330 h. Significant genotypic differences were also observed in pod setting ratio, number of pods/plant, number of seeds/pod, 1000-seed weight, biomass and seed yield. Pod setting ratio was significantly and positively correlated with number of pods/plant ($R^2 = 0.80$, $P < 0.01$) and seed yield ($R^2 = 0.38$, $P < 0.05$). These results showed that the genotypes with a smaller reduction in midday drop of RWC produced a larger number of pods/plant and consequently had higher seed yield as compared with a larger midday drop of RWC. The results also showed that there was a large genotypic variation in the midday drop of RWC, which was correlated with yield-attributes and seed yield. It may therefore be possible to use midday drop of RWC as a screening and selection trait for drought tolerance of cowpea genotypes.

Keywords: Cowpea, drought, pod setting, relative water content, screening trait, *Vigna unguiculata* L. Walp.

1 Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is one of the most important arid legumes cultivated for pulse and forage production in arid and semi-arid regions of the country. The crop grown under rainfed conditions often encounters drought during the pod formation period either due to long dry spells or early withdrawal of monsoon rains. Breeding improved genotypes for the arid and semiarid tropics by selection solely for seed yield

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is difficult, because of the variability in amount and temporal distribution of available moisture from year to year. The genotypic variation in yield is low under these conditions. Researchers (OMAE *et al.*, 2007; SINGH *et al.*, 2004, 2007; SHARMA *et al.*, 2007) now believe better adapted and high yielding genotypes could be bred more efficiently and effectively if traits that confer yield under drought conditions could be identified and used as selection criteria at the early stages of breeding programmes. However, there are examples where morpho-physiological traits have been used to identify drought tolerant genotypes in *Brassica* species (KUMAR and SINGH, 1998; SHARMA *et al.*, 2007), cowpea (MATSUI and SINGH, 2003) and snap bean (OMAE *et al.*, 2005b). The usefulness of selection for a trait depends on its correlations with seed yield in drought conditions. Midday drop in leaf relative water content in beans showed that with a limited reduction the genotypes displayed higher pod setting, number of pods/plant and finally higher seed yield in drought conditions (OMAE *et al.*, 2005a, 2007). The objective of this study was to evaluate the germplasm lines tested in initial varietal trials (IVT-I) for physiological traits such as leaf water potential, relative water content and midday drop in leaf relative water content during pod formation stage and correlate these differences with pod setting, number of pods and seed yield. This will aim at understanding the morphological and physiological traits that influence the productivity of cowpea, which may be helpful in the selection at early stages of breeding and further utilized as a trait in screening for drought tolerance.

2 Materials and Methods

The experiment was conducted in drought plots at CCS Haryana Agricultural University, Hisar, India (20 10' N, 75 45' E). The drought plots (30m in length, 6m in width and 2m in depth) filled with dune sand of 22±2.26% water holding capacity were constructed especially to screen large populations for drought tolerance. Twenty genotypes of cowpea (IVT-I received from Project Coordinator, Arid Legumes, CAZRI, Jodhpur, India; Table 1) were grown under drought conditions in a randomized block design with three replications of a plot size of 2.80×1.80 m, utilizing standard farming practices. The soil contained 14 cm of available water in the 195 cm soil depth at the time of seeding. Seeding was done on 24 July. During the growing season 13.51 cm rainfall was received. No post sowing irrigation was applied to the crop. Also no rainfall was received after 45 DAS. Soil moisture content (by Neutron Moisture Meter, Troxler, USA) was recorded at 60 DAS, i.e., on the day of measurement of the leaf water status. On the average, the values were 5.38+ 0.78% (w/w, mean ±SD) at the 0-15 cm, 6.49±0.43% at the 15-45 cm, 7.89±0.46 at the 45-75 cm, 9.35±0.26 at the 75-105 cm, 12.59±0.58 at the 105-135 cm, 13.21±0.24 at the 135-165 cm and 16.45±0.68% at the 165-195 cm soil depth.

Measurements of leaf water potential, LWP (by Pressure Chamber Apparatus as described by SCHOLANDER *et al.* (1965) and leaf relative water content (RWC) were made 60 DAS (pod formation stage) at two times, i.e., between 730-800 h (referred as "800 h") and 1300-1330 h (referred as "1330 h"). A fully expanded youngest leaf from the top of the plant on the main shoot was used for the measurements.

Table 1: Leaf water potential (LWP), relative water content (RWC) and midday drop of RWC (ratio of RWC at 800 h to 1330 h) measured 60 DAS (pod formation period) in cowpea genotypes.

Genotypes	*LWP (MPa)*		*RWC (%)*		*Midday drop*
	800 h	*1330 h*	*800 h*	*1330 h*	*of RWC (%)*
CP 1	-0.57	-0.85	89.41	83.75	6.34
CP 2	-0.50	-0.73	95.72	86.74	9.38
CP 3	-0.57	-0.90	92.69	83.95	9.43
CP 4	-0.72	-0.75	93.40	85.96	7.96
CP 5	-0.63	-0.85	95.03	91.33	3.89
CP 6	-0.63	-1.12	95.11	94.16	1.00
CP 7	-0.68	-0.90	94.88	89.29	5.89
CP 8	-0.68	-0.90	93.39	87.78	6.01
CP 9	-0.73	-0.98	94.73	88.06	7.04
CP 10	-0.77	-0.83	94.44	91.32	3.31
CP 11	-0.68	-0.90	91.86	84.89	7.59
CP 12	-0.67	-0.90	89.18	78.80	11.63
CP 13	-0.67	-0.80	91.80	79.86	13.01
CP 14	-0.52	-0.88	91.47	79.70	12.86
CP 15	-0.72	-1.00	96.53	83.84	13.14
CP 16	-0.87	-0.78	90.59	79.75	11.97
CP 17	-0.72	-0.85	92.70	81.38	12.21
CP 18	-0.77	-0.82	90.77	80.73	11.06
CP 19	-0.75	-1.12	92.15	85.59	7.12
CP 20	-0.87	-1.12	99.22	86.41	12.91
LSD ($P < 0.05$)	0.08	0.12	NS	6.48	
CV	7.81	8.05	5.29	4.59	

RWC was estimated by using the following equation (Kumar and Elston, 1992):

$$RWC = (f.wt–d.wt)/(m.wt–d.wt)\,,$$

where $f.wt$, $d.wt$ and $m.wt$ are the fresh, oven-dry and fully-hydrated (maximum) weights of the leaf tissue. Midday drop of RWC was determined as the ratio of RWC at midday (1330 h) to that in the morning (800 h).

For the determination of pod setting ratio, 25 flowers per replication were tagged on the same day, i.e., on the day of measurement of plant water status. Only recently opened flowers were used for the study. Pod setting ratio was calculated as the ratio of the number of flowers tagged to the number of pods formed on the tagged flowers and expressed as per cent. All mature pods in each plot were harvested, and the number of pods/plant, biomass and seed yield/plot was recorded. Biomass and seed yield were converted to values per unit area. The number of seeds/pod (from 20 pods in each plot) and 1000-seed weight were measured.

3 Results and Discussion

Although severe drought seldom occurs during the monsoon season, a long dry spell during the reproductive period (15 September-15 October) of cowpea may cause plant water deficit severe enough to cause reduction in seed yield. The yield reduction is mainly caused by decrease in plant water status due to drought and or excessive transpiration in arid legumes including cowpea (GARG *et al.*, 2005). In this experiment, the water deficit seemed to have developed slowly as evident by narrow differences in LWP and RWC at 800 h (Table 1). However, the differences among the genotypes in LWP and RWC at 1330 h were substantially large and significant. At 1330 h, genotypes CP 6, CP 4 and CP 5 maintained highest (>90%) while genotypes CP 12, CP 14, CP 16 and CP 13 had the lowest RWC (<80%). The genotypic differences in midday drop of RWC were very large ranged from 1-13.14%. The midday drop of RWC was smallest in genotype CP 6 (1%) followed by genotypes CP 10 (3%), CP 5 (3.89%), CP 7 (5.89%) and the remaining genotypes in which the drop ranged from 6.01 to 13.14%. Higher RWC may be maintained either by developing a LWP gradient from soil to plant as displayed by genotypes CP 6, CP 7, CP 8, CP 9, CP 11 and CP 19 or by reduced water loss from the plant organs as displayed by genotypes CP 5, CP 10 and CP 4. The former genotypes had higher ability to extract moisture at low soil water content due to reduced LWP which contributed to the maintenance of higher RWC (OMAE *et al.*, 2005a). In cowpea, osmotic adjustment had also been found to be responsible in preventing the detrimental effects of drought in leaves (SUMITHRA *et al.*, 2007). On the other side, the latter genotypes maintained higher LWP as well as RWC perhaps due to reduced transpiration. The two types of mechanism suggests that the former genotypes may be better for soils where water is available in deeper layers due to their increased water extracting capacity whereas the latter genotypes maintained higher plant water status due to reduced water loss and therefore may perform better under conserved soil moisture conditions.

The per cent pod set, number of pods/plant, seeds/pod, 1000-seed weight, biomass and seed yield showed significant genotypic differences (Table 2). The pod setting was observed more than 50% in genotypes CP 5, CP 6, CP 1, CP 10, CP 4, CP 7, CP 9, CP 2, CP 19, CP 8 and CP 12 while it ranged between 36.7 to 46.7% in the remaining genotypes. Genotypes CP 6, CP 5, CP 10, CP 1, CP 8 and CP 9 produced >20 pods/plant. Most of the genotypes had >10 seeds/pod except genotypes CP 14, CP 11, CP 16 and CP 18 in which the number of seeds/pod was <10. Genotypes CP 14, CP 12, CP 3 and CP 9 displayed the boldest seeds (>150 g 1000-seed weight) while genotypes CP 5 and CP 20 the smallest seeds (<100 g 1000-seed weight). The biomass/m^2 was highest but statistically similar in genotypes CP 15, CP 9 and CP 1 which was significantly higher than the remaining genotypes. Genotypes CP 7, CP 5, CP 8, CP 1 and CP 20 produced seed yield >100 g/m^2, whereas genotypes CP 14, CP 13 and CP 17 <50 g/m^2 seed yield.

LWP or RWC either 800 h or 1330 h did not significantly correlate with either the pod setting ratio, number of pods/plant or the seed yield. However, the midday drop of RWC strongly and negatively correlated with pod setting ratio and number of pods/plant

Table 2: Pod setting ratio, yield-attributes and yield of cowpea genotypes.

Genotypes	*Pod setting ratio (%)*	*Pods/plant*	*Seeds/pod*	*1000 seed weight (g)*	*Biomass/ m^2 (g)*	*Seed yield/ m^2 (g)*
CP 1	66.67	22.78	15.89	109.83	619.05	114.44
CP 2	56.67	15.00	11.17	125.30	311.94	54.09
CP 3	43.33	11.89	10.33	155.85	519.60	60.49
CP 4	60.00	17.11	13.11	124.02	415.51	87.44
CP 5	80.00	27.00	11.89	74.46	371.87	134.32
CP 6	73.33	34.22	11.78	110.15	375.61	93.73
CP 7	60.00	17.89	11.44	138.37	628.70	153.39
CP 8	53.33	22.11	11.89	126.52	502.60	133.38
CP 9	60.00	20.78	11.56	152.94	470.99	128.72
CP 10	66.67	23.22	11.22	103.89	310.81	99.61
CP 11	56.67	16.00	8.89	132.22	307.91	76.13
CP 12	50.00	14.22	10.00	161.16	254.79	72.82
CP 13	40.00	8.11	10.44	147.14	182.13	43.26
CP 14	43.33	10.67	7.56	170.53	221.65	41.80
CP 15	36.67	11.44	11.44	148.02	679.68	90.41
CP 16	40.00	11.44	9.11	116.33	231.95	60.57
CP 17	43.33	11.00	10.22	126.65	305.40	47.10
CP 18	36.67	12.78	9.78	140.26	238.32	51.56
CP 19	56.67	17.11	11.11	107.12	276.91	63.65
CP 20	46.67	13.33	11.56	95.94	446.72	106.05
LSD ($P < 0.05$)	13.93	11.62	3.01	12.40	116.93	38.88
CV	15.69	42.04	16.75	5.82	18.37	27.36

(Fig. 1a,b) and poorly but significantly with seed yield (Fig. 1c). The relationships showed that the genotypes with a smaller midday drop of RWC set higher pods and produced larger number of pods/plant and consequently had higher seed yield as compared with the plants with a larger midday drop of RWC. There are reports that even short diurnal fluctuations in plant water status at the time of fertilization could adversely affect the development and function of reproductive organs (TSUKAGUCHI *et al.*, 2003). The results also showed that pod setting ratio was correlated with the number of pods/plant and seed yield (Fig. 2a,b).

The final test of utilization for a genotype with drought tolerance would be the enhancement of yield performance. Pod setting ratio showed large significant genotypic differences displaying that similar differences may exist in transfer of assimilates to flowers necessary for the development and function of reproductive organs (OMAE *et al.*, 2005a). It is interesting to note that midday drop of RWC showed a strong significant association with pod setting ratio. Genotypes with a smaller reduction in midday RWC set more pods and vice versa.

Figure 1: Relationship between midday drop of leaf relative water content (RWC) and (a) pod setting ratio, (b) number of pods plant^{-1} and (c) seed yield in cowpea. * and ** indicate significance at 1 and 5% level, respectively.

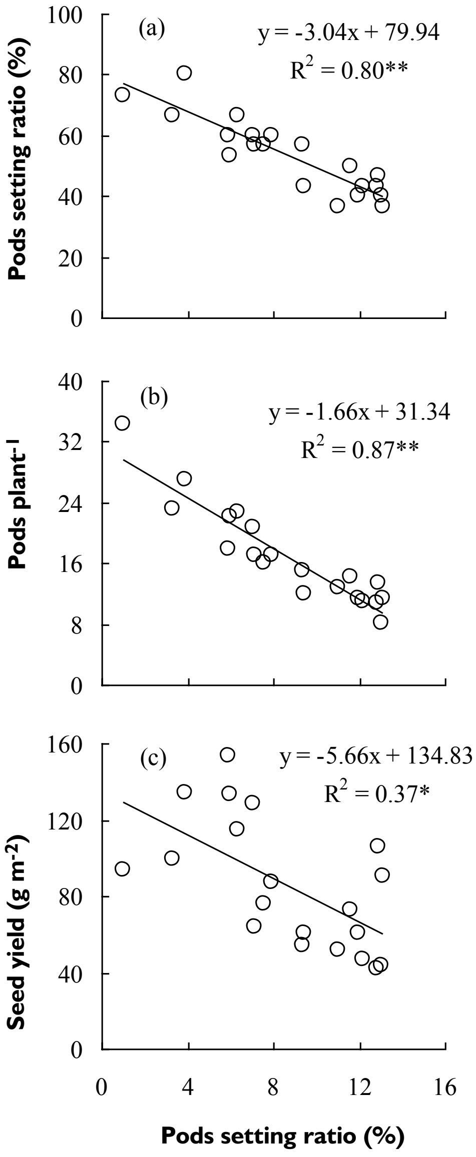
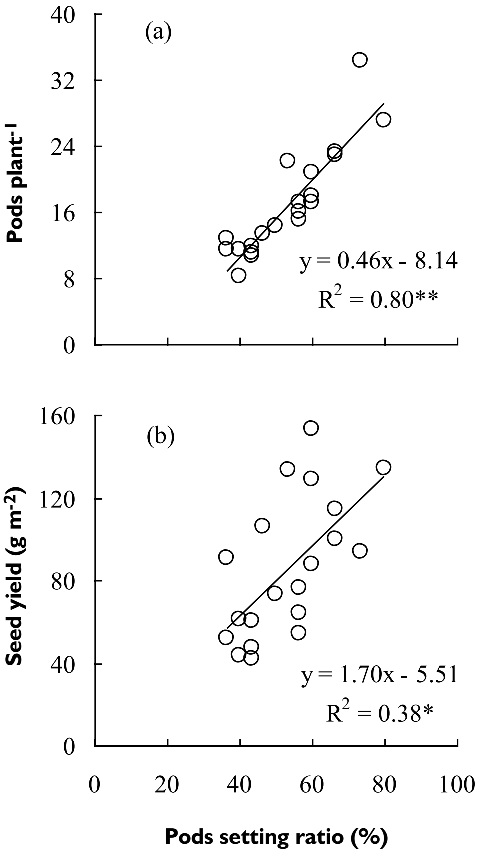


Figure 2: Relationship between pod setting ratio and (a) number of pods plant^{-1} and (b) seed yield in cowpea. * and ** indicate significance at 1 and 5% level, respectively.



Osmotic adjustment and cell wall elasticity enable the plants to maintain higher RWC, turgor and turgor related processes during water deficit (Morgan *et al.*, 1986; Kumar and Singh, 1998; Sumithra *et al.*, 2007). This allowed more pod setting and their survival longer in drought tolerant than susceptible genotypes. In this study, significant genotypic differences were observed in LWP, RWC and midday drop of RWC. But the plants made similar recovery in RWC overnight (as shown by non significant differences in RWC at 800 h), however, the water loss during the day time showed very large differences (1-13%). Therefore, selection for smaller midday drop of RWC in cowpea may be desirable under drought conditions occurring especially during pod formation. The use of midday drop of RWC as a physiological trait to screen cowpea germplasm needs further research particularly on inheritance.

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