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BREEDING FOR DROUGHT TOLERANCE IN PROGNIES OF COWPEA

(*Vigna unguiculata* (L.) Walp)

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ABSTRACT

A field experiment was undertaken for two seasons viz spring and autumn of 2013 on farm of Department of field crop science, College of Agriculture university of Baghdad for genetic improvement and screening high yielding cultivars of cowpea under water stress condition. In this study total three progenies i.e. S1, S2, S3 were planted under two period of irrigation. All among the tested progenies S3 proved best in all tested parameters. Highest amount of chlorophyll content (65.20 & 56) was reported from the S3 progeny under period of 10 days irrigation in both seasons. In addition , same progeny have about 0.88 and 0.91 relative water content (RWC), 27 & 36 pod per branch, 13 & 12 seeds per pod and 79.39 & 109.29 gm/plant yield under the period of 10 days irrigation respectively for two seasons. The selection led to increase the effectiveness of physiological and morphological traits, which can be considered as effects of selection criteria on plant yield. Results of the study recommended the use selection methods to for developing cultivars of high vigor.

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1 Introduction

Drought is one of the major limiting factors in crop production; it can affect crop yield severely. Furthermore, it has reported that the availability of water for water agriculture purpose reduced day by day; and under present scenario it became limited in various agriculture zone of world (Carter, 1989). Drought affects various morphological and physiological traits associated with plant growth and development such as stomata closure, photosynthesis, respiration (Dulai et al., 2006). Furthermore, drought also affects the relative water content (RWC), CO₂ level by closing stomata to avoid water loss from leaf, and inhibited some enzymes activity such ribulose 1, 5-bisphosphate carboxylase this lead to reduce photosynthesis metabolism (Sumithra et al., 2007). Plant cells start accumulation of solutes such as sugar, amino acids (proline) and ion potassium (K⁺) for avoiding the drought condition. In addition, solutes accumulated in cytosol such as proline, glycinebetaine enhances of drought tolerance capacity of a plants (Yang et al., 1996). Various methods have been developed by the breeders for developing new drought resistance varieties. Selection method of variety development helps in developing new drought resistance varieties and also helped in screening of new drought resistance genotypes. Furthermore this method also induced resistance in available local cultivars (Hayatu & Mukhtar, 2010). Cowpea (*Vigna unguiculata* (L.) Wap) is an arid and semi-arid regions legumes crops which widely cultivated in all the arid and semi-arid regions of the world because it has ability to grow under severe water losses conditions and maintained a fix amount of water inside the cell (Kumar et al., 2008). Cowpea plants respond to drought in complex ways, and effects of drought on plants appeared through leaf abscission, reduced root growth in soil and by reducing leaf area. Under severe drought conditions, Cowpea decrease root growth in soil, closure the stomata and reduced gaseous exchange, thereby decreasing the water losses through transpiration (Anyia & Herzog, 2004). The balance water in plant will be happen when is absorption water from roots equal the water loss from stomata and water potential is negative (Stikic & Davies, 2000). The aim of this study was to identify the cowpea genotypes which have drought resistance

2 Material and Methods

A field experiment was conducted at college of agriculture– Abu-Graib in spring and autumn season of 2013, to screen the drought resistance and higher yielding progenies from local cultivars of cowpea. The study was conducted in randomized complete block design (RCBD) with two periods of irrigation (10 day and 15 day). Cowpea seeds were planted in five rows with 50 cm distance between holes and 75 cm between rows in 5m length. Area of each experiment unit was equal to 18.75 cm the parameters which taken randomly for assessing were chlorophyll content index (CCI), amount of amino acid proline, relative water content, number of pods per plant, number of seeds per pod, seed per pod, seed weight (g) and final yield (tan.h⁻¹)

2.1 Estimation of Chlorophyll Content Index (CCI)

Chlorophyll content was measured by chlorophyll meter SPAD at flowering stage in 10 leaves of each plant from 10 plants in experiment unit.

2.2 Estimation of Leaf Relative Water Content (RWC)

For estimated of RWC, 20 discs of 10 mm were randomly collected from selected plant leaves. RWC for collected leaves were calculated according to formula given by Kumar & Elston (1992).

$$RWC = [(FM-DM)/(TM-DM)]$$

Where FM is fresh matter, TM is turgid matter (water saturated discs for 24 hour at 4°C), DM is dry matter (oven dried leaves 80 °C)

2.3 Estimation of Amino acid proline amount

0.5gm of fresh leaf samples was collected and ground well in the mortar in 10 ml from sulphosalicylic (3%) acid. The extract was filtered and 2ml of the filtrate was used for proline estimation. Collected filtrate was mixture with acid nihydrin (2ml), glacial acetic acid and boiled on water bath for one hour, after this the test tube was transferred to ice water bath for cooling, at the cooling stage 6ml of toluene was mixed with mixture and shaken for 15-20 minutes to separate of solution for two layer: lower layer was discarded and upper layer was taken into test tube. The optical density of color complex was calculated for estimated proline (Bates et al., 1973)

$$\text{Proline (g/gm fresh wt.)} = 36.2311 \times OD \times V \times d / 2 \times f$$

Where,

OD=optical density at 520 nm

V=Total volume of the extract in ml

d=fresh weight/dry weigh ratio

f=Milligrams of fresh sample taken for proline estimation

2=Volume of extract taken for proline estimation

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3 Results and Discussnoi

3.1 Chlorophyll content index (CCI)

Chlorophyll content is parameters which is responsible for keeping greens to the photosynthetically active leaves, and that leads to delay the leaf senescence and longevity of photosynthetic that make increasing of metabolic transport from source to sink (Thomas & Smart, 1993). The results in table (1) showed there is a significant differences among the progenies selected in chlorophyll content (cc) measurement planted at the periods of irrigation. Among the various tested progenies, S3 progeny planted in both season of 2013 showed highest CCI value which was followed by S2 and S1 progenies

respectively. In the spring season of 2013, S3 progeny shows 65.2 and 60.1 CCI on the irrigation of 10 and 15 days respectively. Though similar type of trends was reported in the autumn season but the value of CCI was slightly lesser than the spring season. The stay green characteristic can be increasing with water quantity, which prevented the leaves from rolling, and then increase cell division and expansion (Ismail et al, 2004). The performance at long period of irrigation make increasing water stress, that will be slumping cell division and lack photosynthetic capacity due to decrease of CO₂ in the leaves (Blum & Pnuel, 1990).

3.2 Proline concentration (mg/gm fresh weight)

Results presented in table (2) revealed a significant difference in proline concentration among various progenies selected for study periods of irrigation. At 10 days irrigation, S1 progeny shows highest proline content (4.12 mg/gm fresh leaves) this was followed by progeny S2 and S3 (3.43 & 3.21 mg/gm fresh weight respectively), while at 15 days irrigation, the S3 progeny shows highest proline content in spring season (7.30 mg/gm fresh weight). Similar types of trends was reported in autumn season also. Increase in proline leads to increase to osmosis of cells and then pull water from plant roots (Rashmi & Agarwal, 1998). The increasing in water quantity, lead to stability of plasma membranes and proteins in the cells (Chinnusamy et al., 2005).

3.3 Relative Water Content (RWC)

The relative water content technique is formerly known as relative turgidity to describe capacity of leaf for expansion or extension (Valaire & Thomas, 1995). The results of table (3) showed a significant difference among all of progenies were selected. The S3 progeny planted in spring season at 10 days of irrigation shows 0.88 RWC and it is significantly differing than S1 progeny in which RWC have been reported only 0.82%. Also the progeny S3 planted in autumn season gave high mean of RWC (58%) compared with the S1 progeny that gave 85%.

The high relative water content resulted from transpiration low rate from stomata and water absorption from soil, that leads to increase ribulose1, 5-bisphosphate (RUBP), ATP synthetase activity in phosphorylation (Chaves, 1991). While in second period of irrigation (15 days) the S3 progeny gave high mean (78%) compared with the S1 progeny planted in spring season 2013 at the same period of irrigation. Decreasing of RWC in plant resulted from low transpiration high rate from stomata and low absorption water from soil, decrease water in the leaves leads to impair of ATP regeneration of RUBP carboxylase and losing of pigment of chlorophyll in plants (Holady et al., 1992).

Table 1 Chlorophyll content Index (CCI) measured by SPAD in tested progenies of cowpea selected.

Genotypes	Spring season			Autumn season		
	Period of irrigation 10 days	Period of irrigation 15 days	Mean	Period of irrigation 10 days	Period of irrigation 15 days	mean
S1	58.30	54.50	56.40	54.60	41.30	47.95
S2	61.00	58.30	59.15	55.50	58.40	56.95
S3	65.20	60.10	62.65	56.00	45.80	50.90
mean	61.50	57.63	59.4	55.37	48.50	51.93
L.S.D 5%	P=3.30 G=2.60 PG=3.58			P=4.22 G=4.34 PG=5.01		

Data given in table are the mean of ten replicate

Table 2 The effect of two periods of irrigation on proline concentration(mg/gm fresh weight) of cowpea progenies

Genotypes	Spring season			Autumn season		
	Period of irrigation 10 days	Period of irrigation 15 days	Mean	Period of irrigation 10 days	Period of irrigation 15 days	Mean
S1	4.12	6.13	5.13	5.44	7.15	6.29
S2	3.43	4.84	4.14	2.88	6.33	4.60
S3	3.21	7.30	5.26	2.14	9.16	5.65
Mean	3.59	6.09	4.84	3.49	7.55	5.51
L.S.D 5%	P=2.62 G=1.32 PG=3.60			P=2.04 G=1.08 PG=4.54		

Table 3 The effect of two periods of irrigation on (RWC) of cowpea progenies

Genotypes	Spring season			Autumn season		
	Period of irrigation 10 days	Period of irrigation 15 days	Mean	Period of irrigation 10 days	Period of irrigation 15 days	Mean
S1	0.82	0.73	0.77	0.85	0.79	0.82
S2	0.85	0.75	0.80	0.87	0.82	0.84
S3	0.88	0.78	0.83	0.91	0.85	0.88
Mean	0.85	0.75	0.80	0.88	0.82	0.85
L.S.D 5%	P=0.02	G=0.053	PG=0.056	P=0.01	G=0.021	PG=0.034

Data given in table are the mean of ten replicate

Table 4 The effect of two periods of irrigation on number of pods of cowpea progenies

Genotypes	Spring season			Autumn season		
	Period of irrigation 10 days	Period of irrigation 15 days	Mean	Period of irrigation 10 days	Period of irrigation 15 days	Mean
S1	20.00	17.00	18.5	30.00	24.00	27.00
S2	23.00	19.00	21.0	33.00	27.00	30.00
S3	27.00	23.00	25.0	36.00	31.00	33.50
Mean	23.33	19.66	21.5	33.00	27.33	30.17
L.S.D 5%	P=2.54	G=2.61	PG=4.55	P=3.55	G=2.45	PG=5.63

Table 5 The effect of two periods of irrigation on number of seeds per pods of cowpea progenies

Genotypes	Spring season			Autumn season		
	Period of irrigation 10 days	Period of irrigation 15 days	Mean	Period of irrigation 10 days	Period of irrigation 15 days	Mean
S1	8.00	6.00	7.00	10.00	8.00	9.00
S2	11.00	7.00	9.00	12.00	9.00	10.50
S3	13.00	10.00	11.50	12.00	9.00	10.50
Mean	10.66	7.66	9.17	11.33	8.60	10.00
L.S.D 5%	P=2.02	G=1.30	PG=3.03	P=1.12	G=0.53	PG=2.02

3.4 Number of pods per plant

Like other parameters here also S3 progenies shows superiority over the other two progenies i.e. S1 & S2 in both cropping seasons and at both time of irrigation. In case of cropping seasons, crops grown in autumn seasons shows higher number of pods per plant than the crop grown in spring seasons (Table 4). Highest number of pods per plant was reported in S3 genotypes at ten days of irrigation. Number of pods trait can be increasing with water quantity for level limited, which prevented the clusters in plants from inhibition, and then increase number of seeds (Tsukaguchi et al., 2003).The performance at long period of irrigation make for increasing in water stress, that will be decreasing in flowers formatting and loss of pods per plants (Garg et al., 2005)

3.5 Number of seeds per pod

The number of seeds per pod also shows similar trends of pods per plants and highest number of seeds per pods was reported in S3 in both cropping seasons and irrigation period. These numbers was immediately followed by the S2 genotypes and in case of autumn season it is almost at par to the S3 genotypes and are not significantly differ than the S3. The number of seeds per pod can be increasing with water quantity , which increase metabolic product of leaves ,and then increase number of seeds (Niinemates, 2002).The performance at long period of irrigation make for increasing in water stress, that will be reduce number of seeds per pod(Winter et al.,1988; Ortize-Lopez, et al.,1991) .

Table 6 The effect of two periods of irrigation on 100 seed weight of cowpea progenies.

Genotypes	Spring season			Autumn season		
	Period of irrigation 10 days	Period of irrigation 15 days	Mean	Period of irrigation 10 days	Period of irrigation 15 days	Mean
S1	21.30	18.50	19.90	22.1	17.4	19.75
S2	25.12	20.44	22.78	25.24	20.12	22.68
S3	22.62	18.62	20.62	25.30	23.34	24.32
Mean	23.01	19.14	21.10	24.21	20.28	22.25
L.S.D 5%	P=2.13 G=1.33 PG=2.54			P=2.33 G=2.03 PG=2.76		

Table 7 The effect of two periods of irrigation on grain yield (gm/plant) of cowpea progenies.

Genotypes	Spring season			Autumn season		
	Period of irrigation 10 days	Period of irrigation 15 days	Mean	Period of irrigation 10 days	Period of irrigation 15 days	Mean
S1	34.08	18.87	26.47	66.30	33.40	49.85
S2	63.55	27.18	45.36	99.94	48.89	74.41
S3	79.39	42.82	61.10	109.29	65.18	87.27
Mean	59.00	29.62	44.31	91.84	49.15	70.51
L.S.D 5%	P=6.23 G=3.54 PG=8.89			P=12.23 G=4.56 PG=10.13		

3.6 Seed weight

The average seed weight was calculated for the hundred seeds and a significant differ was reported in both cropping seasons and at both periods of irrigation (Table 6). In spring seasons S2 genotypes (25.12g and 20.44g) proved superior over the S3 (22.62g and 18.62g) and S1 (21.30g and 18.50g) at both irrigation period respectively. There is no significant difference among S3 and S1 while S2 genotypes are significantly different than these two. Seed weight trait can be increasing with water quantity for level limited, which makes the cell division and cell size, and then increase of seed weight (El-Naim et al; 2011). While, In autumn season also S2 progeny proved best for tenth day irrigation while in case of fifteenth day interval, S3 progeny gives high mean of seed weight as compared with the S1 and S2 progeny. The performance at long period of irrigation make for increasing in water stress, that will be photosynthesis rate and loss metabolites from source to sink and became a little seed weight (El-Naim et al.,2011)

3.7 Grain yield (gm/plant)

In grain yield a significant difference was reported among the all tested progenies. Maximum yield was reported in the S3 genotypes grown in autumn season and irrigated on the tenth days. At this irrigation period, S3 genotypes had average yield 109.29g/plant. This treatment was followed by the S2 genotype (99.94g/plant) of the same cropping season and same irrigation periods. The lowest yield was reported in S1 genotype (18.87g/plant) of spring season at fifteenth day irrigation. Here

also autumn season favor the final production of the selected genotypes and it is significantly differ than the crop grown in spring season (Table 7). grain yield trait can be increased with water quantity up to a limited level, at this level increasing of water increased number of pods per plant, number of seeds per pods and seed weight (Tsukaguchi et al.,2003).The negative impact of water deficit stress for traits, leads to decline in number of pods per plant, seeds per pods ,seed weight ,relative water content in leaves, photosynthesis assimilation rate ,and imbalance between plant hormone and biological process in plants (Dulai et al.,2006; Sumithra et al.,2007)

Plant breeders made crop improvement programs to develop genotypes and which are adapted to range wide of circumstances such as salt stress, drought stress and temperature stress. In addition, phenotypic performance of genotypes under various climate conditions are different and it depends on the interaction of genotypes and environmental factors. Results of the present study are in agreement with Sharma et al. (2010) and Eberhart & Russel (1996).

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