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EVALUATION OF ADVANCED CHICKPEA (*CICER ARIETINUM* L.) ACCESSIONS BASED ON DROUGHT TOLERANCE INDICES AND SSR MARKERS AGAINST DIFFERENT WATER TREATMENTS

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Abstract

Chickpea is mainly grown on marginal lands and encounter the problem of erratic rainfall that causes lack of water availability especially at terminal growth stages. Forty advanced chickpea genotypes were grown under irrigated, rainfed and tunnel conditions for two years (2012-13 and 2013-14). Data were collected for seed yield and analyzed by analysis of variance. Highly significant differences among genotypes and water treatments were observed for seed yield. However, across the year differences were insignificant for seed yield of chickpea. Seed yield under rainfed was higher than under irrigated conditions. Forty genotypes were assembled in four distinct groups on the basis of PCA biplot for different drought tolerance indices. These four distinct groups were representative of genotypic performance under normal and stressed conditions. Twenty eight SSR primers were used for sortation of genotypes either as drought tolerant or susceptible and to find association with results of drought tolerance indices. Only nine SSR markers were found to be polymorphic while others were either monomorphic or not amplified. H3DO5 and TA8 with Group-I, TR19 and ICCM0035 with Group-II, ICCM0035 with Group-III and TA25 was strongly correlated with results of Group-IV. Genotypes of group-I were drought tolerant whereas, CH16/06, CH81/06 and D097-11 within this groups were more tolerant.

Key words: Water deficit, Pakistan, Irrigation, Tunnel, Rainfed, Marker assisted selection and PCA Biplot.

Introduction

Chickpea (*Cicer arietinum* L.) is currently cultivated in more than 50 countries across the globe. After dry beans and field peas; chickpea stands 3rd in ranking among legumes (Anon., 2012). Among top producers, Pakistan ranked 3rd for production and 2nd for total area under chickpea cultivation (Anon., 2012). In Pakistan, chickpea is leading legume crop which is occupying around 985 thousand hectares area, producing 673 thousand tons with average yield of 683 kg per ha. Punjab province is leading chickpea producer in Pakistan which is solely growing 82% of total crop. Thal (semi desert area) is producing 91% of chickpea whereas, it is also cultivated in Thal and Potohar regions of Pakistan. These regions have the problem of erratic rainfall and uncertainty of water availability poses the problems of water deficiency for crop. Pakistan has wide latitudinal (30° 00' N) and longitudinal (70° 00' E) range with southern regions are arid to hyper-arid whereas, Northern regions are semi-arid to humid. Chickpea cultivation is mostly restricted to rainfed areas due to overwhelming occupation of wheat in irrigated areas. Crops in rainfed areas are facing water deficit especially at the time of sowing and terminal growth stages (Anon., 2013).

Chickpea yield is far below than its genetic potential. Lower yield is attributed to biotic stresses i.e., *Ascochyta* blight, *Fusarium* wilt, *Botrytis* grey mold, *Helicoverpa* pod borer, and abiotic stresses i.e., drought and frost (Nayak, 2010). In global scenario, 40-50% reduction in chickpea yield is attributed to drought stress only (Ahmad *et al.,* 2005). Drought stress is described as water shortage due to non-availability of water, deficient rainfall, or inadequate water supply by external sources. Complexity of drought stress is attributed to duration of prevalence, timing of occurrence (growth stage), and intensity of stress (Serraj *et al*., 2003). Chickpea is facing terminal drought stress due to its restricted cultivation on marginal lands. Breaks-in rainfall concomitant with less rains in terminal growth stages poses the problem of intermittent and terminal drought stress (Toker *et al*., 2007).

Betterment in drought tolerance is laggard due to uncertain water availability across the marginal lands, across the years, non-uniform evaluation methods, lower genotypic variance for seed yield under stressful conditions (Ludlow & Muchow, 1990) and complex genetic background of traits (Turner *et al*., 2001). So, betterment in drought tolerance by conventional breeding is a challenge due to reliance on improved yield under stressful condition, quantitative nature of traits and prevalence of linkage between desired and undesired genes (Richards, 1996; Yeo, 1998; Flowers *et al.*, 2000). For selection of relative drought tolerant genotypes, different drought tolerance indices are used extensively which compare the relative performance under normal and stress conditions (Maqbool *et al.,* 2015a). Genotype × environment interaction hinders the direct selection of genotypes for higher yield in association with ineffective control of stress level under field conditions (Maqbool *et al*., 2015b). However, manipulation of marker assisted selection (MAS) accelerates the breeding progression in more proficient and effective ways for betterment in drought tolerance in corroboration with conventional breeding (Varshney *et al*., 2005). Association of genotype and phenotype was established by QTL mapping based on SSR primers (Hüttel *et al*., 1999; Winter *et al*., 2000; Tar'an *et al*., 2007; Rehman, 2009; Varshney *et al*., 2009; Rehman *et al*., 2011). Among those mapped SSR primers selective primers were validated in current study for precise and robust sortation of chickpea germplasm. Diverse genetic markers have been used for

plant selection, genetic improvement and genomic characterization through genetic maps, gene tagging and MAS (Gupta & Varshney, 2000; Rafalski, 2002; Varshney *et al*., 2005). Simple sequence repeats (SSR) markers are known to be superior genetic markers due to co-dominant inheritance, chromosome-specific location, high throughput automation and multi-allelic in nature (see Nayak, 2010).

Different drought tolerance indices (DTIs) were used in current study for effective and precise sortation of relatively drought tolerant and susceptible chickpea genotypes to carryout breeding program for varietal development and to find its association with SSR markers.

Materials and Methods

Chickpea germplasm: Current experimental studies were carried out at research field of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad during cropping seasons 2012-2014 for two years. Faisalabad region is located in the longitudinal range of 73°-74° in East and latitudinal range of 30°-31.5° in North, with an elevation of 184 metres above sea level. Forty chickpea genotypes were used for this research study including Desi and Kabuli genotypes comprising of advanced lines developed through hybridization and induced mutation as listed in Table.1. Briefly Kabuli genotypes are also known as *Macrosperma* and characterized to bear white flowers, white or beige colored thin seed coat, stems are lacking anthocyanin pigments and seed head is owl shaped. Desi chickpea genotypes are known as *Microsperma* characterized to bear pink flowers, brown or black colored thick seed coat, stems have anthocyanin pigments and seed head is ram shaped.

Sowing method and water stress treatments: Land preparation was accomplished by standard agronomic practices and sowing was done using dibbling method. Row to row and plant to plant distance was maintained at 30cm and 15cm respectively. Ten seeds of single genotype were sown in each row and each genotype was repeated for three times (triplicated trial). Standard agronomic practices were followed strictly throughout life cycle of crop. At the time of sowing, moisture contents of field were estimated with gravimetric method (Schmugge *et al*., 1980). Evaluation of forty chickpea genotypes was done by using Split-Split Plot design with three replications of each accession. Genotypes were evaluated at three different water treatments.

(1) Irrigated Treatment (T_1; given irrigation at flowering + rainfall)

(2) Rain-fed Treatment (T_2; given no irrigation and only dependent on rainfall)

(3) Tunnel Treatment (T_3; given irrigation only at field bed preparation stage and prevented from rainfall by covering field with polythene tunnel).

Tunnel structure was developed to prevent the access of rainfall water to field plots whereas, temperature was maintained by regular removal of sheets and making door like structures for aeration. Data were recorded for seed yield in the form of yield per line. Five plants of each genotype were selected at random for data recording per replication and per treatment.

Table 1. List of 40 chickpea genotypes used for current research experiment.

Sr. No.	Genotypes	Type	Origin	Sr. No.	Genotypes	Type	Origin
1	CH53/07	Kabuli	NIAB	21	K008-11	Kabuli	AARI
2	CM1529/03	Kabuli	NIAB	22	K0032-11	Kabuli	AARI
3	CH46/07	Kabuli	NIAB	23	K0034-11	Kabuli	AARI
4	CH48/07	Kabuli	NIAB	24	K0041-11	Kabuli	AARI
5	CM1592/08	Kabuli	NIAB	25	K0048-11	Kabuli	AARI
6	CM98/05	Desi	NIAB	26	K0063-11	Kabuli	AARI
7	CH104/06	Desi	NIAB	27	K0065-11	Kabuli	AARI
8	CH107/06	Desi	NIAB	28	K0070-11	Kabuli	AARI
9	CM510/06	Desi	NIAB	29	D088-11	Desi	AARI
10	CM526/05	Desi	NIAB	30	D089-11	Desi	AARI
11	CM562/05	Desi	NIAB	31	D090-11	Desi	AARI
12	CH16/06	Desi	NIAB	32	K051-11	Kabuli	AARI
13	CH30/06	Desi	NIAB	33	K055-11	Kabuli	AARI
14	CH36/06	Desi	NIAB	34	K064-11	Kabuli	AARI
15	CH70/06	Desi	NIAB	35	D094-11	Desi	AARI
16	CH81/06	Desi	NIAB	36	D097-11	Desi	AARI
17	CH84/06	Desi	NIAB	37	D098-11	Desi	AARI
18	CH85/06	Desi	NIAB	38	D072-11	Desi	AARI
19	11K113	Kabuli	AZRI	39	D078-11	Desi	AARI
20	TGDX201	Desi	AZRI	40	D086-11	Desi	AARI

Note: NIAB = Nuclear Institute for Agriculture and Biology, AARI = Ayub Agriculture Research Institute, AZRI = Arid Zone Research institute, Bakhar

Drought tolerance indices (DTIs): Data for seed yield of studied chickpea genotypes were collected from three treatments after crop maturity and subjected to different drought tolerance indices. Several drought tolerance indices (DTIs) were calculated i.e., Geometric mean productivity (GMP; Fernandez, 1992), Stress tolerance index (STI; Fernandez, 1992), Mean productivity (MP; Rosielle & Hamblin, 1981), Yield index (YI; Gavuzzi *et al.*, 1997), Stress susceptibility index (SSI; Fischer & Maurer, 1978) and Yield stability index (YSI; Bouslama & Schapaugh, 1984).

GMP, STI, MP, SSI and YSI were calculated in three different ways. GMP(T_1&T_2), STI (T_1&T_2), MP(T_1&T_2), SSI(T_1&T_2) and YSI(T_1&T_2) showed that T_1 (irrigated) was considered as normal and T_2 (rainfed) as stressed condition. GMP(T_1&T_3), STI (T_1&T_3), MP(T_1&T_3), SSI(T_1&T_3) and YSI(T_1&T_3) were calculated by regarding T_1 (irrigated) as normal and T_3 (tunnel) as stress condition. GMP(T_2&T_3), STI (T_2&T_3), MP(T_2&T_3), SSI(T_2&T_3) and YSI(T_2&T_3) were estimated by considering T_2 (rainfed) as normal and T_3 (tunnel) as stress environment. Yield index (YI) was also measured in three different ways. YI (T_1) means that YI for T_1 (irrigated). YI (T_2) showed that YI at T_2 (rainfed). YI (T_3) means YI at T_3 (tunnel).

Statistical data analysis: Data for seed yield were collected after maturity and subjected to analysis of variance (ANOVA) devised by Steel *et al.* (1997). Tukey's HSD (honest significant difference) test was used for mean comparison of genotypes across the years and across the treatments. Principle component (PCA) biplot analysis was used for evaluation of genotypes on the basis of drought tolerance indices (Gabriel, 1971). PCA Biplot analysis was used for estimation of association among different DTIs and seed yield under different stress treatments. Correlation between DTIs is elaborated in terms of angle between vectors; acute angle (<90 ^{o}) showed positive correlation, acute angle (<45^o) showed strong positive correlation, right angle (=90^o) showed independence or no correlation, obtuse angle (>90^o) showed negative correlation and obtuse angle of >135^o&<180^o showed strong negative correlation.

SSR markers: DNA extraction was carried out using CTAB extraction method (Doyle & Doyle, 1987) with little modifications as per requirement (Khan *et al.,* 2004). Previously reported 28 SSR markers (Hüttel *et al*., 1999; Winter *et al*., 2000; Tar'an *et al*., 2007; Rehman, 2009; Varshney *et al*., 2009; Rehman *et al*., 2011) were used for evaluation of chickpea genotypes under drought stress. PCR reaction mixture was of 20µl, containing 10X PCR buffer, 0.2 mM dNTPs mixture, 1.5mM $MgCl_2$, 0.35µM of each primer, 1 unit of Taq DNA polymerase (Fermentas, Germany) and 50ng template DNA. PCR reaction was carried out by using an Infinigen, CIVIC^{TM} thermal cycler. Initial denaturation step was of 2 mints at 94^{o}C followed by repeated 35 cycles each consisted of denaturation step for 20 sec at 94^{o}C, annealing step for 20 sec at 54^{o}C and extension for 50 sec at 72^{o}C. 3.5% agarose gel was used for band differentiation and gel pictures were captured using UVP photoDoc-itTM U.K., Imaging System. The Gel results were analyzed through "Gel Analyzer 2010a" software (Lazar, 2010) for the estimation of base pair size of bands.

Results

Analysis of variance and mean comparison: Analysis of variance (ANOVA) for seed yield of chickpea was elaborated in Table (2) which showed that genotypes were significantly different and treatment effects were also highly significantly different but across the year effects were insignificant. In this experiment there were three factors i.e. years (Y), water treatments (T) and chickpea genotypes (G) whereas, these were subjected to split-split plot design. Y×T, Y×G and Y×G×T interactions were insignificant whereas G×T interaction was highly significant (Table 2). Water treatments were significantly different in their effects and Tukey's HSD test categorized water treatments into three distinct groups. Seed yield of chickpea genotypes was observed in subsequent order under three water treatments; Rainfed >Irrigated >Tunnel with mean values of 339.33g, 285.41g and 35.16g respectively (Table 3). Genotype 11K113 was early in maturity because it harbored flowers 75 days after sowing whereas genotype CM510/06 took 100 days for flowering which were maximum days in subjected germplasm. Genotype 11K113 had lowest seed yield whereas, genotype CM510/06 was high yielder among studied germplasm. So, it was found that early maturity was negatively correlated seed yield that was attributed to shortening of duration for full reproductive growth.

Table 2. Split-Split Plot analysis of variance of 40 chickpea genotypes for seed yield.

Source of variation	DF	SS	MS	F
Replications (R)	2	83246.4	41623	
Years (Y)	1	3136.72	3137	1.81ns
Error R×Y	2	3460.74	1730	
Treatments (T)	2	1.264E+07	6322050	1367.12***
Y×T	2	1052.58	526	0.11ns
Error R×Y×T	8	36994.9	4624	
Genotypes (G)	39	2395097	61413	14.94***
Y×G	39	2283.29	59	0.01ns
T×G	78	1498690	19214	4.67***
Y×T×G	78	3604.62	46	0.01ns
Error R×Y×T×G	468	1923784	4111	
Total	**719**	**1.860E+07**		

* = Significant at 5%, ** = Highly significant at 1%, *** = Highly significant at 0.1%, ns = Non-significant

Table 3. Tukey's HSD All-Pairwise Comparisons Test of seed yield for years and treatments

Year	Mean	Homogenous group	Treatments	Mean	Homogenous group
2012-13	222.06	A	Rainfed	339.33	A
2013-14	217.88	A	Irrigated	285.41	B
			Tunnel	35.16	C
Critical value for comparison 9.36			Critical value for comparison 13.71		

Alpha = 0.05

Table 4. Eigen values and variability contribution of different factors of principle components.

	F1	F2	F3	F4	F5	F6
Eigenvalue	12.82	5.704	1.44	0.84	0.12	0.04
Variability (%)	61.07	27.16	6.88	3.98	0.57	0.17
Cumulative %	61.07	88.23	95.11	99.09	99.66	99.84
	F7	F8	F9	F10	F11	F12
Eigenvalue	0.02	0.014	0.004	0.001	0.0	0.0
Variability (%)	0.07	0.07	0.02	0.004	0.001	0.0
Cumulative %	99.91	99.98	99.99	99.9	100.0	100.0

PCA biplot analysis for drought tolerance indices (DTIs): Mathematical formulae for indices were reviewed from literature and used for derivation of drought tolerance indices (DTIs) for seed yield of chickpea and subsequently subjected to PCA biplot analysis for extraction of key information. PCA had converted the DTIs into twelve factors which were as following; F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11 and F12. Separately F1 and F2 contributed 61.07% and 27.16% of total data variability and their cumulative contribution was 88.23%. Three factors (F1, F2 and F3) had more than 1 eigen value and only these factors were suitable for further data analysis (Table 4).

Correlation of chickpea seed yield (SY) with drought tolerance indices was analyzed. SY (T_1) had positive correlation with YI (T_1), YI (T_2), YI (T_3), STI (T_1&T_2), STI (T_1&T_3), STI (T_2&T_3), GMP (T_1&T_2), GMP (T_1&T_3), GMP (T_2&T_3), SSI (T_1&T_2), SSI (T_1&T_3), SSI (T_2&T_3), MP (T_1&T_2), MP (T_1&T_3), MP (T_2&T_3), SY (T_2) and SY (T_3) while had negative correlation with YSI(T_1&T_3), YSI (T_1&T_2), and YSI (T_2&T_3). SY (T_2) had strong positive correlation with YI (T_1), YI (T_2), YI(T_3), STI (T_1&T_2), STI (T_1&T_3), STI (T_2&T_3), MP (T_1&T_2), MP (T_1&T_3), MP (T_2&T_3), GMP (T_1&T_2), GMP (T_1&T_3), GMP (T_2&T_3) and SY (T_1) while it had negative correlation YSI (T_1&T_2), SSI (T_1&T_2) and YSI (T_1&T_3). SY (T_3) had strong positive correlation STI (T_2&T_3), STI (T_1&T_3), YI (T_3), GMP (T_2&T_3) and GMP (T_1&T_3) but it had negative correlation with SSI (T_1&T_2), SSI (T_2&T_3), SSI (T_1&T_3) and YSI (T_1&T_2) (Fig. 1).

Biplot based on DTIs categorized the chickpea genotypes into four distinct groups. Group-I comprised of D086-11, D078-11, K0041-11, CM1529/03, CH53/07, CH30/06, K0034-11, D072-11, D088-11, CH70/06, D090-11, CM510/06, K0065-11 and CM98/05 genotypes. Group-II comprised of CH36/06, CH81/06, CH16/06, K0063-11, D097-11, CH84/06, CH107/06, D098-11, and CH85/06 genotypes. Group-III consisted of 11K113, K0048-11, K0070-11, D089-11, TGDX201 and D094-11 genotypes. K055-11, K064-11, K051-11, CH46/07, CH48/07, K008-11, CM1592/08, K0032-11 and CM562/05 were present in Group-IV (Fig. 1).

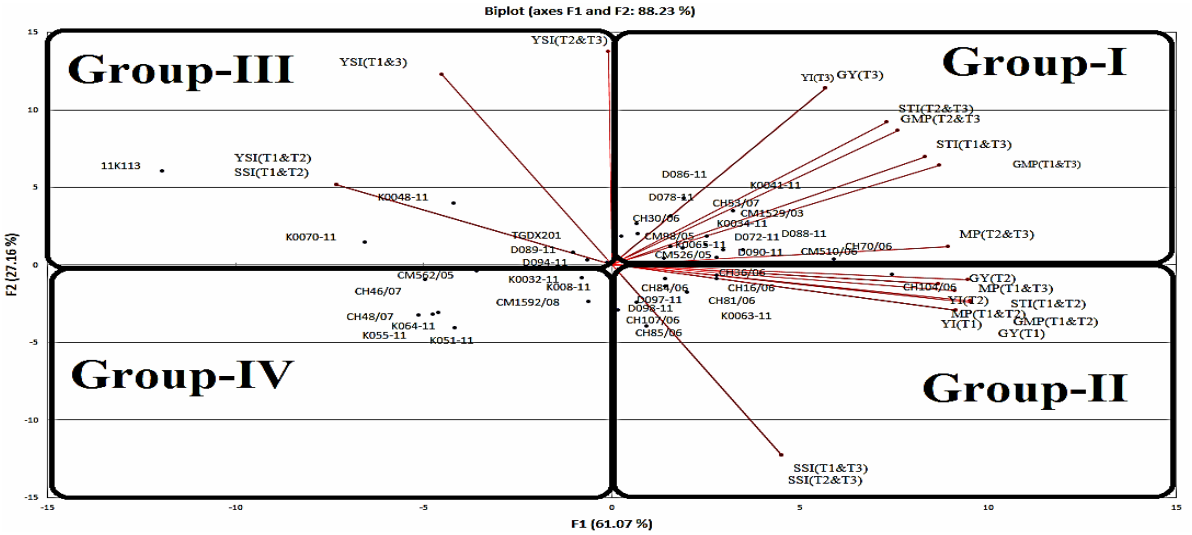

Fig. 1. PCA based biplot of 40 chickpea genotypes for drought tolerance indices

SSR marker assisted selection: QTL mapping was worked out by numerous researchers on chickpea for drought tolerance. Loci conferring drought tolerance were mapped, confirmed and validated in germplasm (Hüttel *et al*., 1999; Winter *et al*., 2000; Tar'an *et al*., 2007; Rehman, 2009; Varshney *et al*., 2009; Rehman *et al*., 2011). Marker assisted selection for drought tolerance in chickpea was carried out by using twenty eight well documented and QTL mapped SSR markers. Size for their molecular weight and association of alleles with tolerance or susceptibility is also previously established. All of these SSR primers were previously reported in literature. Among these SSR primers, TA72, ICCeM006, ICCeM005, TA194, TA27, TA21, TA28 and CaSTMS-11 were not amplified. H5AO8, ICCEM0249, GA24, TAA170, TA37, TR56, TS29, ICCeM0055, ICCeM0040, ICCeM0058 and TR24 were monomorphic. Only nine SSR markers were found to be polymorphic which were following; TA8, TA14, TA25, TA125, TA80, H3DO5, TR19, TA110 and ICCeM0035 (Table 5). Gel picture with bands of forty chickpea accessions based on H3DO5 primer was presented in Fig. 2.

TA110, H3DO5, TA8 and ICCeM0035 SSR primers have discriminated 24, 25, 26 and 28 genotypes respectively as drought tolerant. TA125 and TA25 primers have discriminated 22 out of 40 chickpea genotypes as susceptible and 18 genotypes as drought tolerant. TA14 and TR19 have assorted 20 out of 40 chickpea genotypes as drought tolerant.

CH30/06 and CH36/06 were declared by eight SSR markers to be drought tolerant whereas, CM562/05 and CM1592/08 were categorized by seven SSR primers as to be drought tolerant. D086-11, CH70/06, D089-11, CH16/06, D097-11, CH81/06, K0070-11 and 11K113 were rated by six SSR primers to be drought tolerant. D088-11, D094-11, K0048-11 and K008-11 genotypes were categorized to be drought tolerant by only three SSR primers.

DTIs categorized the chickpea genotypes into four distinct group based on their relative performance under different water treatments. Group wise results of SSR primers were described as following;

Group-I: This was comprised of 15 chickpea genotypes, H3DO5 and TA8 marker described 11 chickpea genotypes as a tolerant and results of these markers were strongly correlated with DTIs. In this group, CH30/06 was rated by 8 primers as drought tolerant whereas, DO86-11, CH70/06 and CM526/05 were rated by 6 primers as drought tolerant. All other genotypes in this group not had strong correlation with studied SSR primers based on drought tolerance indices as rated only by fewer primers as drought tolerant (Table 5).

Group-II: DTIs categorized, 10 genotypes in this group through PCA based biplot analysis. TR19 and ICCM0035 primers differentiated 8 genotypes as drought tolerant so, results of these SSR primers had strong parallelism with results of DTIs. Seven chickpea genotypes were found to be tolerant with the help of TA80 primer in 2nd group so, results of this primer were also correlated with DTIs (Table 5). CH36/06 was rated by 8 SSR primers as drought tolerant while CH81/06, CH16/06 and DO97-11 were rated by six SSR primers as drought tolerant.

Group-III: DTIs categorized six chickpea genotypes in this group. D089-11, K0048-11, TGDX201, K0070-11, D094-11, and 11K113 were included in this group. ICCM0035 primer discriminated 5 genotypes to be drought tolerant (Table 5). TA110, TA8 and TA25 primer distinguished, 4 genotypes as drought tolerant and 2 genotypes as susceptible. K0070-11, D089-11 and 11K113 were rated as drought tolerant by six SSR primers.

Group-IV: DTIs summed up nine chickpea genotypes in this group. TA25 had distinguished six genotypes as drought sensitive so, its results were associated with DTIs. TA125, TR19 and TA14 primer, rated five genotypes as drought sensitive and four genotypes as tolerant (Table 5). K008-11 was rated by six SSR primers as susceptible whereas, contradictory results were also present which showed that CM562/05 and CM1592/08 were rated by seven SSR primers as drought tolerant despite of their presence in group-IV.

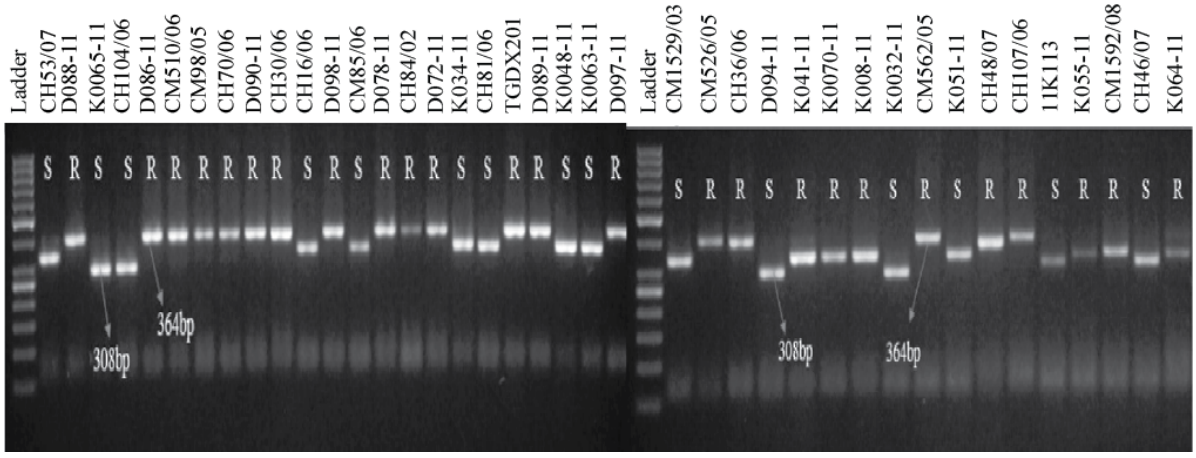

Fig. 2. DNA banding profiles of 40 chickpea genotypes using "H3DO5" primer differentiating Resistant (R) and susceptible (S) genotypes for drought stress. Allele of 308bp size is associated with drought susceptibility.

Table 5. Genotypic performance based on drought tolerance indices (DTIs) and SSR markers.

S.#	Genotype	DTI group	TA125	H3DO5	TA110	TA25	TA14	TA80	TR19	TA8	ICCM0035
1.	CH53/07	I	R	S	S	R	R	S	R	S	S
2.	D088-11	I	S	R	S	S	R	S	S	R	S
3.	K0065-11	I	R	S	R	R	S	R	S	S	S
4.	D086-11	I	R	R	R	S	R	S	S	R	R
5.	CM510/06	I	S	R	R	S	R	S	S	R	R
6.	CM98/05	I	S	R	S	S	S	R	R	R	R
7.	CH70/06	I	S	R	R	R	S	R	S	R	R
8.	D090-11	I	R	R	R	S	S	S	S	R	R
9.	CH30/06	I	R	R	S	R	R	R	R	R	R
10.	D078-11	I	S	R	S	R	S	S	R	R	R
11.	K034-11	I	R	S	R	S	R	S	R	S	S
12.	D072-11	I	S	R	R	S	R	S	S	R	R
13.	K041-11	I	R	R	S	R	S	R	S	S	S
14.	CM1529/03	I	R	S	S	S	R	S	R	R	S
15.	CM526/05	I	S	R	S	R	R	R	S	R	R
16.	CH104/06	II	S	S	S	S	R	R	R	S	R
17.	CH16/06	II	R	S	S	R	R	R	R	S	R
18.	CM85/06	II	S	S	R	S	R	R	R	S	R
19.	CH84/02	II	S	R	R	S	S	S	R	R	R
20.	CH81/06	II	R	S	R	S	S	R	R	R	R
21.	D098-11	II	S	R	R	S	S	S	S	R	R
22.	D097-11	II	S	R	R	R	S	R	R	R	S
23.	K0063-11	II	R	S	S	R	S	R	R	S	S
24.	CH36/06	II	R	R	S	R	R	R	R	R	R
25.	CH107/06	II	S	R	R	S	R	S	S	R	R
26.	D089-11	III	S	R	S	R	S	R	R	R	R
27.	TGDX201	III	S	R	R	S	R	S	S	R	R
28.	K0048-11	III	S	S	R	R	S	R	S	S	S
29.	D094-11	III	S	S	R	S	S	S	S	R	R
30.	K0070-11	III	R	R	R	R	S	R	S	S	R
31.	11K113	III	R	S	S	R	R	S	R	R	R
32.	K008-11	1V	R	R	S	S	S	R	S	S	S
33.	K0032-11	1V	R	S	R	S	S	R	S	R	R
34.	CM562/05	1V	S	R	R	S	R	R	R	R	R
35.	K051-11	1V	S	S	R	R	S	S	R	S	R
36.	CH48/07	1V	S	R	R	S	R	R	S	R	S
37.	K055-11	1V	S	R	S	R	S	S	R	S	R
38.	CM1592/08	1V	R	R	R	S	R	R	S	R	R
39.	CH46/07	1V	R	S	R	S	R	S	R	R	S
40.	K064-11	1V	S	R	R	R	S	S	S	S	R

Discussion

Terminal drought stress had reduced the seed yield in chickpea whereas, three water treatments had different magnitude of effects. Irrigation treatments had promoted the vegetative growth and produced lower yield than rainfed treatment which was shown by higher seed yield of chickpea genotypes under rainfed treatment. It was reported that availability of higher moisture contents through irrigation cause the lodging of crop and reduced harvest index in sub-tropical regions of India (Saxena, 1984). Current study was conducted at NIAB, Faisalabad situated in sub-tropical regions of Pakistan. As it is mentioned above that Faisalabad is located at Longitude 73°-74° East and latitude 30°-31.5° North, with elevation of 184 metres. So, irrigation to chickpea genotypes in this region at flowering stage was responsible for reduction of yield. Kanouni (2001) and Bakhsh *et al*. (2007) also concluded that application of irrigation at flowering stage increased the vegetative growth and delayed flower setting subsequently reduced yield. Patil, (2013) described an alternative option for application of irrigations at branching and pod formation could be used for higher seed yield.

PCA biplot was used for genotypic sortation based on DTIs. Among different factors, F-1 and F-2 contributed 88% cumulative variation and showed that biplot on the basis this data was most appropriate analysis to be used. SY (T_1), SY (T_2), MP (T_1&T_2), MP (T_2&T_3), MP (T_1&T_3), GMP (T_1&T_2), YI (T_1), YI (T_2) and STI (T_1&T_2) had positive correlation. MP had positive correlation with SY (T_1) and SY (T_2). Stronger correlation of GMP and STI with yield indicates that increase in yield was directly linked with drought tolerance and while lower values reflect the susceptible response. SY (T_1), SY (T_3), STI (T_1&T_3), STI (T_2&T_3), GMP (T_1&T_3) and GMP (T_2&T_3) were positively correlated with each other. It is reported that MP had positive correlation with Yp (yield under normal condition) and Ys (yield under stress condition). Ys had positive correlation with GMP and STI that was in accordance with current findings. Ys has negative correlation with SSI (Choukan *et al.,* 2006). SSI positively correlated with seed yield under normal conditions and negative correlation with seed yield under stress conditions (Narayan & Misra, 1989). Blum (1988) described that genotypes producing higher yield under normal and stress conditions were known to be drought tolerant.

DTIs distinguished the all studied chickpea genotypes into 4 distinct groups based on their performance. Distinctions of these groups and belonging genotypes are given as following;

- Group-I comprised of genotypes with better performance and higher yield both under normal and water deficit conditions (K0041-11, D086-11, CH53/07, D078-11, K0034-11, CM1529/03, CH30/06, D088-11, CH70/06, D072-11, D090-11, K0065-11, CM510/06, and CM98/05). These fourteen genotypes were found to be drought tolerant and producing higher economical yield so, preferably selected to grow on marginal lands.

- Group-II consisted of genotypes with higher yield only under normal condition and not under stress conditions (CH36/06, CH81/06, CH16/06, CH84/06, K0063-11, D098-11, D097-11, CH107/06 and CH85/06). Performance of these genotypes was highly reduced by drought stress and their yield potential was also lower than genotypes of group-I so, these were preferred to grow only under stress free environment.

- Group-III comprised of chickpea genotypes with relatively better yield only under stress conditions (11K113, K0070-11, K0048-11, D089-11, TGDX201 and D094-11). Their relative performance under stressed conditions was better than normal conditions but their yield potential was lower than Group-I & II.

- Group-IV encompassed genotypes lower yield both under normal conditions and stress conditions (K051-11, K055-11, CH48/07, K064-11, CM1592/08, CH46/07, K0032-11, K008-11 and CM562/05). These genotypes were not to be used for cultivation under drought stressed conditions as their yield potential was very lower and performance was also very poor. However, can be used as parent in certain hybridization program.

Precise genotypic selection for drought tolerance could be done with genomic assisted selection tools viz, marker assisted selection (MAS; Varshney *et al*., 2005). Variety development was accomplished with marker assisted breeding in cereals and soybean (Varshney *et al*., 2006; Varshney *et al*., 2010). Marker assisted breeding (MAB) had extensive potential for improvement in drought tolerance of chickpea through gene pyramiding, indirect selection and accumulation of multiple stress tolerance related genes (Gaur *et al*., 2012). Genomic based SSR primers are highly polymorphic than EST-SSR primers (Varshney *et al*., 2005) so, we used genomic based SSR primers. Among these SSR primers few members of ICCeM series were used, among them ICCeM0006 and ICCeM0005 primers were not amplified, ICCeM0040, ICCeM00055 and ICCeM00058 primers found to be monomorphic whereas only ICCeM0035 primer was polymorphic. Varshney *et al*. (2009) reported that ICCeM primer series have moderate genotype discrimination power.

Bharadwaj *et al.* (2011) used TA 80, TA 125 and TA110 primers for evaluation of chickpea genotypes against ascochyta blight whereas, in current study these were used for assessment of drought tolerance in chickpea genotypes. These three SSR primers were found to be highly polymorphic for drought related response. Choudhary *et al*. (2012) reported that TA125 and TA110 primers were monomorphic but these were polymorphic in current study and these differences were attributed to the differences in genetic background of germplasm. Choudhary *et al*. (2012) reported in accordance with current results the polymorphic pattern for TA8 and TA80 SSR primers.

We studied the correlation of SSR primers with drought tolerance indices. Forty chickpea genotypes were characterized into four different groups on the basis of drought tolerance indices. Group-I was known as genotypes which have better performance and higher

yield under normal and deficit water provisions. H_3DO_5, TA14 and TA8 primers were found to be highly correlated with the results of drought tolerance indices for Group-I. H_3DO_5 and TA8 were found to be present on chromosomal linkage group1 (LG1) while TA14 was present on linkage group3 (Rehman, 2009). Presence of H_3DO_5 and TA8 on LG1 showed that these were linked and could be explored for further introgression of this region in germplasm which could be helpful for improvement of drought tolerance. TA8 was previously found to be linked with harvest index, drought tolerance score and canopy temperature differential (Rehman *et al*., 2011). We found this to be linked with drought tolerance and higher yield of chickpea. TA14 was found to be linked with canopy temperature differential (Rehman *et al*., 2011) whereas, in current study we found it to be linked with drought tolerance and higher yield.

Second group has ten genotypes. TA80, TR19 and ICCeM0035 declared 7-8 genotypes as tolerant. TA80 was found to be linked with canopy temperature differential and we found it to be linked with higher yield under normal conditions (Rehman *et al*., 2011). Third group comprised of six genotypes whereas, TA110, TA25, ICCeM0035 and TA8 rated four out of six genotypes as tolerant. This showed that their linked genes were upregulated under stressed condition and downregulated under normal water availability. TA110 was present on LG2 which was considered as hot spot region for biotic and abiotic stress tolerance (Rehman, 2009). TA25 was found to be present on LG3 and have strong linkage with botrytis grey mould disease (BGM) resistance (Anuradha *et al*., 2011) whereas, we found it to be linked with higher stressed yield relative to normal conditions. Higher stressed yield showed that genes flanked by these primers were overexpressed or unregulated only under water stressed condition.

Genotype 11K113 was found to be early flowering with very poor seed yield under irrigated, rainfed and tunnel conditions but six markers rated this genotype as tolerant against drought stress. Piepho, (2000) described that such conflicting results showed that either genotypes have tolerant gene but their yield potential is very low or epistatic effects might be prevailing there. K008-11 was rated by six SSR primers as drought susceptible in Group-IV. CM562/05 and CM1592/08 were showing the conflicting results. As seven polymorphic markers rated them as drought tolerant but under field conditions these showed very low yield and drought susceptibility.

It was finally concluded from the experiment that significant differences were present among 40 chickpea genotypes for seed yield under different water treatments. DTIs categorized the genotypes into four distinct groups. Fifteen genotypes of Group-I were considered as drought tolerant because of high yield potential and better performance under normal and stressed conditions. H3DO5 and TA8 primers were found to have strong correlation with results of Group-I. DO86-11, CH70/06 and CM526/05 were rated by six SSR primers as drought tolerant in Group-I so, these genotypes were preferably selected as drought tolerant in Group-I.

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