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RESEARCH ARTICLE

Development of screening method for drought tolerance in cotton genotypes based on ABA, chlorophyll stability index and drought tolerant index

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SUMMARY

Drought stress adversely affects the growth, development and ultimately yield of cotton. The growth and productivity of cotton plants depend largely on their vulnerability to environmental stress. Water stress is commonly attributed to situations where the water loss exceeds sufficient absorption intensity causing a decrease in plant water content, turgor reduction and, consequenctly, a decrease in cellular expansion and alterations of various essential physiological and biochemical processes that can affect growth or productivity. The experiment was conducted by adopting Factorial Randomized Block Design with three replications. The treatments comprised of water stress imposed at vegetative, squaring and boll development stages of crop growth. Withholding water at any growth stage significantly increased the leaf ABA content. Among the different treatments, stress at squaring had a major impact over the ABA quantity enhancement. Chlorophyll stability index is a measure of integrity of membrane or heat stability of the pigments under stress conditions. The genotype KC 2 X MCU 13 showed tolerance to water stress as it accumulated relatively higher ABA.

Key Words : ABA, Chlorophyll stability index, Yield, Drought, Drought tolerant index.

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Water scarcity limits crop production and further expansion of agriculture in the world. Water stress is characterized by reduction of water content, turgor and total water potential leads to closure of stomata, decrease in cell enlargement and growth. Effective screening methods must evaluate plant performance at critical developmental stages, be complete rapidly, use small amounts of plant material, and screen large number of plants (Johanson, 1980). A screening method for drought tolerance in crop plants that fulfills all of these important requirements has eluded researches to date. Chlorophyll stability is a function of temperature, and it is found to correlate with drought tolerance. Chlorophyll stability index is a measure of integrity of membrane or heat stability of the pigments under stress conditions (Koleyoreas, 1958). The CSI is a single parameter used to measure frost (or) drought

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resistance of a plant. Sairam *et al*. (1996) reported that both drought stress and temperature stress decreased membrane stability, chlorophyll content and chlorophyll stability index in all wheat genotypes.

The high chlorophyll stability indices help the plants to withstand stress through better availability of chlorophyll. This leads to increased photosynthetic rate and more dry matter production (Madhanmohan *et al*., 2000). Stress may induce common responses such as enhancement of plant hormones. For instance, wounding can induce the production of increased ethylene, auxin, and abscisic acid (ABA). Since many kinds of stresses including water, salt, and cold temperatures, induce ABA synthesis, ABA may be considered a plant stress hormone. It regulates several important aspects of plant growth and development. Recent studies have demonstrated a pivotal role for ABA in modulation at the gene level of adaptative responses for plants in adverse environmental conditions. ABA is also involved in several other physiological processes such as stomatal closure, embryo morphogenesis, development of seeds, and synthesis of storage proteins and lipids, germination, leaf senescence, and defense against pathogens. Nevertheless, ABA acts as a mediator in controlling adaptative plant responses to environmental stresses.

The phytohormone abscisic acid (ABA) plays a regulatory role in many physiological processes in plants. Different stress conditions such as water, drought, cold, light and temperature results in increased amount of ABA. Recent studies have demonstrated a pivotal role for ABA in modulation at the gene level of adaptative responses for plants in adverse environmental conditions. ABA is also involved in several other physiological processes such as stomatal closure, embryo morphogenesis, development of seeds, and synthesis of storage proteins and lipids (Rock and Quatrano, 1995), leaf senescence (Zeevaart and Creelman, 1988) and defense against pathogens (Dunn *et al*., 1990). ABA is an essential mediator in triggering plant responses to adverse environmental stimuli and is known to occur in a number of crop plants which include rice, barley and cotton.

MATERIAL AND METHODS

For present investigation, twenty one genotypes including eight parents, four F_1 hybrids, five F_2's and four back crosses, four F_3 populations, one F_4 population along with parents were subjected for genetic diversity analysis using physiological features. Field trails were conducted at *Kharif* 2008-09 in the Department of Cotton, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore.

Treatments :

T_1 - Control

T_2 - Stress at vegetative

T_3 - Stress at squaring

T_4 - Stress at boll development

Genotypes : (Table A)

Measurement of ABA levels:

The pattern of ABA accumulation during drought and degradation upon rewatering in the peduncle tissues was studied by means of quantifying the ABA content by HPLC (Krochko *et al*., 1998). Peduncle tissues were collected from control, drought stressed and rewatered plants and stored at -80°C after freeze drying. The tissues were ground using liquid nitrogen and dissolved in 80 per cent methanol. The samples were incubated at 4°C for 12 hrs. Then the extract was centrifuged at 3000 rpm for 5 minutes and the methanol in the supernatant was evaporated in a rotary flash evaporator. To this extract equal volume of phosphate buffer (pH 8.0) was added and the pH was adjusted between 8 and 9 using 0.1 N KOH. Then equal volume of ethyl acetate was

Table A : Varietal details :

Parents	F1 Hybrids	F2's	Back crosses
JKC 770	AS1x Suvin	KC2×MCU13 AS3×JKC 770	(AS2×MCU13)×MCU13
AS1	KC2×MCU 13	AS2×MCU 13	(AS2×MCU13)×AS2
AS2	AS2×MCU13	KC2×JKC 770	(KC2×MCU 13)×MCU 13
KC2	KC2×JKC 770	AS1×Suvin	(KC2×MCU13)×KC2
KC3			
MCU 13			
Suvin			
Surabhi			

added and centrifuged at 3000 rpm for 5 minutes. The ethyl acetate fraction containing chlorophyll was discarded. Then the pH of the extract was adjusted between 2 and 3 using 0.2 N HCl and it was extracted with ethyl acetate twice. The ethyl acetate fractions were pooled and evaporated in a rotary evaporator. The residue was dissolved in 4 ml methanol and used for HPLC quantification of ABA using a reverse-phase column by isocratic elution with a 75:25 (v/v) mixture of aqueous 1 per cent acetic acid and acetonitrile and UV detection at 262 nm. The ABA fraction was identified by retention time and comparison with known ABA standards. The peak areas were measured and the ABA concentration was quantified using the standard curve obtained using ABA.

Hormones were determined in three independent samples for each treatment or time point according to method (Silvia Forcat *et al.,* 2008).

Chlorophyll stability index (CSI) :

Chlorophyll stability index was estimated by the method described by Murthy and Majumdhar (1962) :

$$\textbf{Chlorophyll stability index (\%)} = \frac{\textbf{Total chlorophyll content (Treated)}}{\textbf{Total chlorophyll content (Control)}} \times \textbf{100}$$

Drought tolerant index (DTI) :

An attempt was made to develop a drought tolerant index based on two physiological parameters such as chlorophyll stability index and relative water content. In a similar fashion yield data's were also computed for arriving at a drought tolerance index. The following parameters were used, Zangi (1998) and Jafary (2002).

- The yield recorded in normal stress free environment (Yn)
- The yield recorded under drought (Yd)
- The yield recorded in all genotypes under normal environment (Yn*)

The drought tolerant index is defined by the above data for arriving at the following indices;

$$\textbf{Drought tolerance index (DTI)} = \frac{\textbf{(Yd x Yn)}}{\textbf{Yn *}}$$

RESULTS AND DISCUSSION

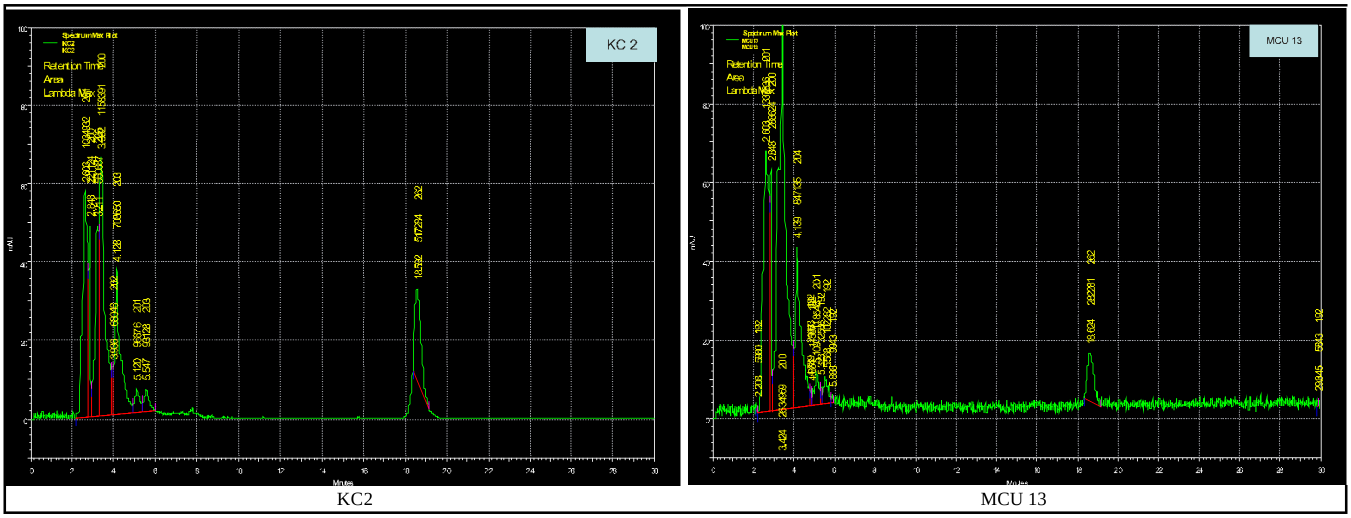
Cotton has a C_3 carbon metabolism, however, its photo- synthetic potential is relativily high (Ephrath *et al.*, 1990; Faver and Gerik,1996). Reduction of photosynthetic rate in cotton under water- limited environment is documented (Ephrath *et al.*, 1993; Pettigrew, 2004). Growth and yield of a crop plant is drastically affected directly or indirectly by altering metabolism, growth and development (Garg *et al*., 2002). However, reports on drought tolerance of cotton crop are limited. The phytohormone abscisic acid (ABA) plays a regulatory role in many physiological processes in plants. Withholding water at any growth stage significantly increased the leaf ABA content. Among the different treatments, stress at squaring had a major impact over the ABA quantity enhancement. Stress may induce common responses such as enhancement of plant hormones. Different stress conditions such as water, drought, cold, light, and temperature result in increased amounts of ABA. Genes encoding late embryogenesis abundant (LEA) proteins were consistently represented in differential screens for transcripts with increased levels during drought. These proteins were first described in research on genes abundantly expressed during the final desiccation stage of seed development. Circumstantial evidence for their involvement in dehydration tolerance is strong. The genes are similar to many of those expressed in vegetative tissues of drought-stressed plants (Rock and Quatrano, 1995 and Ingram and Bartels, 1996). ABA can also induce *lea* genes in seeds and vegetative tissue. A number of genes have been described that respond to drought and low temperature stress at the transcription level. The functions of some gene products have been predicted from sequence homology with known proteins and are thought to play a role in protecting cells from water deficits and low temperatures (Shinozaki *et al*., 1996 and Thomashaw, 1994). ABA is an essential mediator in triggering plant responses to adverse environmental stimuli. This is known to occur in a number of crop plants which include rice, barley, soybean, tomato and cotton. Leaf ABA content in wild plants increased with water stress. Upon rehydration, the ABA level

Table 1 : Effect of drought on ABA quantification (µg g^{-1}) at boll development different stage of cotton

Genotypes	ABA (µg g^{-1})
KC 2	18.592
MCU 13	18.624
Surabhi	18.464
KC2 × MCU 13	18.539

Table 2 : Effect of drought on chlorophyll stability index (CSI %) at different stages of cotton in F_1, F_2, back crosses along with parents

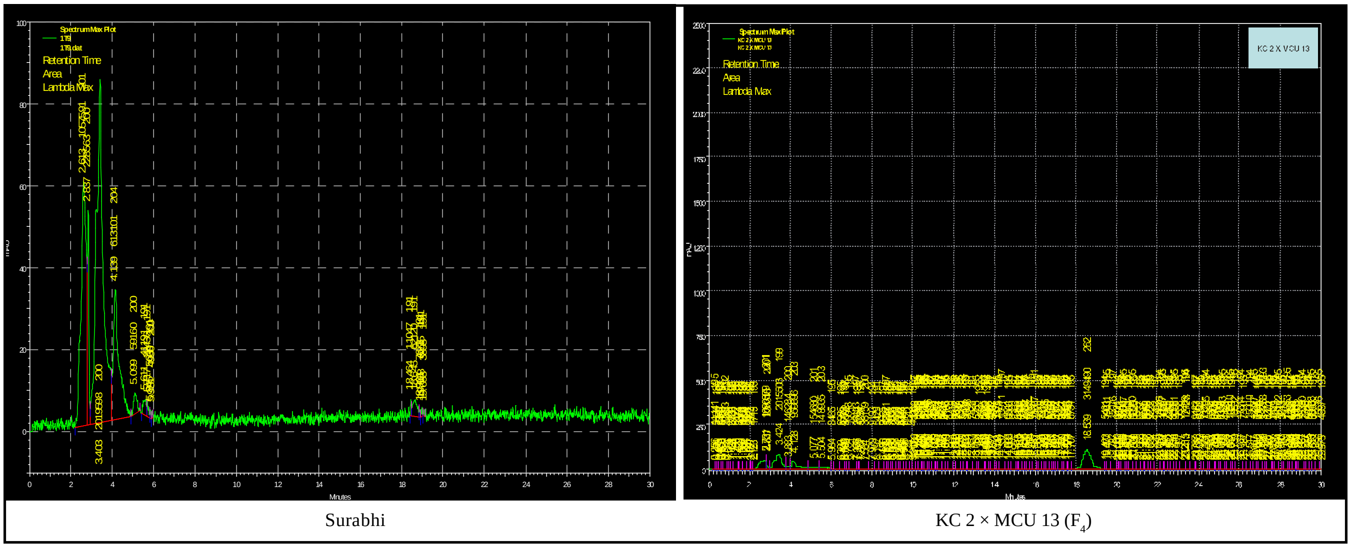
Stages Genotypes	Vegetative					Squaring					Boll development				
Parents	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
MCU 13	69.5	58.27	63.32	59.2	62.55	68.22	69.63	62.29	71.39	64.92	78.39	69.38	70.82	68.29	66.86
AS 2	74.33	59.39	68.13	71.2	68.26	69.29	72.65	64.92	70.48	68.74	78.39	74.38	76.29	72.27	70.62
JKC 770	68.07	63.29	63.17	69.38	66.73	65.29	66..26	60.39	65.64	64.99	73.28	70.73	69.29	69.28	66.73
KC 2	74.48	70.21	71.58	71.28	71.89	76.31	78.28	71.45	74.29	73.31	83.29	79.83	78.39	78.53	75.22
AS 1	64.26	62.38	68.06	64.47	66.04	65.17	66.28	55.39	70.26	65.26	69.12	69.28	65.29	69.26	66.11
Surabhi	56.59	52.36	53.69	61.87	56.13	61.19	63.27	58.38	62.25	58.41	73.12	70.28	63.27	68.27	61.36
KC 3	66.56	61.98	70.36	62.56	65.37	65.39	66.29	57.27	68.39	64.91	70.72	69.97	65.39	67.28	65.89
Suvin	66.29	61.23	70.09	69.85	66.87	68.29	69.28	56.92	70.20	66.56	75.18	73.25	67.92	72.28	68.16
F_1 Hybrids															
AS1 × Suvin	58.23	54.21	54.27	56.18	55.72	69.39	69.19	60.64	63.30	67.30	74.33	67.88	72.34	65.34	63.31
KC 2 × MCU 13	63.32	54.87	59.28	60.27	59.44	77.39	68.92	64.67	67.29	71.22	80.21	75.33	78.23	76.65	68.36
AS 2 × MCU 13	60.72	57.28	59.72	58.12	58.96	67.87	65.28	61.98	61.59	68.28	76.43	69.75	74.34	68.34	64.90
KC 2 × JKC 770	63.91	58.28	61.28	65.18	62.16	76.28	73.39	67.68	70.45	78.20	86.54	80.54	83.24	78.88	71.86
F_2'S															
KC 2 × MCU 13	73.43	67.12	76.56	70.38	71.87	80.81	76.88	72.19	73.34	73.62	81.1	74.38	75.46	75.45	74.47
AS 3 × JKC 770	74.55	68.73	70.56	78.33	73.79	78.19	72.18	74.39	83.45	75.24	82.19	73.20	84.35	70.67	75.92
AS 2 × MCU 13	64.24	58.34	65.56	66.28	63.61	73.56	65.18	72.18	77.56	67.39	72.91	70.87	74.35	65.58	68.40
KC 2 × JKC 770	76.88	74.35	77.76	75.55	76.14	85.45	82.18	73.29	84.89	78.50	88.32	80.92	85.19	82.64	80.15
AS 1 × Suvin	62.45	56.34	66.56	60.65	61.50	71.23	65.27	70.1	80.65	66.08	82.51	79.30	76.29	78.39	69.81
Back crosses															
(AS2×MCU13) × MCU13	62.30	52.19	55.49	57.30	58.32	68.56	69.20	64.24	67.47	62.34	78.2	74.55	73.62	72.20	65.86
(KC2×MCU13) × KC2	66.78	63.83	65.29	67.45	65.84	74.39	73.49	63.54	71.28	67.99	82.76	78.92	76.90	75.20	70.98
(AS2×MCU13) × AS2	66.72	64.20	64.72	69.37	66.25	76.55	75.56	66.89	73.92	69.35	81.43	78.30	77.83	74.38	71.82
(KC2×MCU13) × MCU13	64.93	62.87	60.71	63.29	62.95	71.3	70.85	62.15	68.90	65.33	78.56	75.55	73.33	70.47	67.94
Mean	66.60	61.03	65.06	65.63	64.78	71.91	70.66	64.81	71.29	68.47	78.43	74.12	74.39	72.36	69.27
	T	G	T×G			T	G	T×G			T	G	T×G		
S.E. ±	1.982	2.250	4.500			0.930	2.132	4.265			1.015	2.326	4.652		
C.D. (P=0.05)	1.938	4.421	8.871			1.837	4.211	8.422			2.004	4.652	9.186		



KC2

MCU 13

Fig. 1: Effect of drought on ABA accumulation in KC 2 and MCU 13



Surabhi

KC 2 × MCU 13 (F_4)

Fig. 2 : Effect of drought on ABA accumulation in Surabhi and KC 2 × MCU 13 (F_4)

Table 3 : Effect of drought on yield components of cotton in F_1, F_2, back crosses along with parents

Stages Genotypes	No of flowers per plant					Seed cotton yield (g pl^{-1})				
Parents	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
MCU 13	52.8	49.5	46.9	48.6	49.5	108.23	94.59	85.45	88.27	96.09
AS 2	45.7	44.4	41.2	42.5	43.5	126.43	112.34	94.82	107.29	110.22
JKC 770	38.3	26.1	22.6	24.1	27.8	103.68	95.48	77.29	86.3	90.69
KC 2	73.2	62.1	51.5	56.2	60.3	133.16	123.16	108.17	128.32	120.28
AS 1	57.6	51.3	46.6	49.4	51.2	118.63	110.35	93.76	99.59	105.58
Surabhi	44.2	42.6	41.3	43.4	42.6	93.6	86.9	62.9	78.34	80.44
KC 3	48.9	46.5	43.2	44.8	45.9	102	96.4	89.76	95.2	95.5
Suvin	59.6	55.5	52.5	54.2	54.8	102	96.4	89.76	95.2	95.5
F_1 Hybrids										
AS1 × Suvin	37.5	35.5	33.6	35	35.40	99.96	83.27	73.1	70.38	81.68
KC 2 × MCU 13	63.3	52.7	45.7	48.9	52.65	144.824	88.39	95.33	120.29	112.21
AS 2 × MCU 13	26.8	26	22.7	25	25.13	68.432	64.29	57.33	61.21	62.82
KC 2 × JKC 770	42.8	41.7	40.7	41.5	41.68	92.778	79.39	68.34	72.29	78.2
F_2'S										
KC 2 × MCU 13	55.3	49.6	46.1	48.2	49.8	157.973	121.23	116.38	120.38	128.99
AS 3 × JKC 770	33.8	32.3	28.9	31.2	31.55	58.045	42.66	35.34	49.72	46.44
AS 2 × MCU 13	34.5	33.1	29.3	31.2	32.03	89.11	60.72	54.22	63.54	68.01
KC 2 × JKC 770	36.1	35.2	31.7	32.9	33.98	69.09	55.28	45.23	54.46	56.25
AS 1 × Suvin	54.7	54.2	49.5	52.6	52.75	140.008	99.82	103.29	122.26	116.28
Back crosses										
(AS2×MCU13) × MCU13	37.1	35.2	33.2	34.1	34.90	71.968	66.23	65.29	66.39	67.47
(KC2×MCU13) × KC2	24.4	23	21.2	21.6	22.55	81.263	60.38	62.28	57.19	65.28
(AS2×MCU13) × AS2	39.6	37.4	36.2	26.9	35.03	65.8	58.92	42.1	55.1	55.48
(KC2×MCU13) × MCU13	57.9	56.4	53.2	53.8	55.33	108	92.88	78.36	85.12	92.59
Mean	45.91	42.4	38.94	40.29	41.83	101.67	85.19	76.12	84.61	86.95
	T	G	T×G			T	G	T×G		
S.E. ±	1.584	0.691	3.198			3.632	1.585	7.264		
C.D. (P=0.05)	3.127	1.365	6.255			7.171	3.129	14.342		

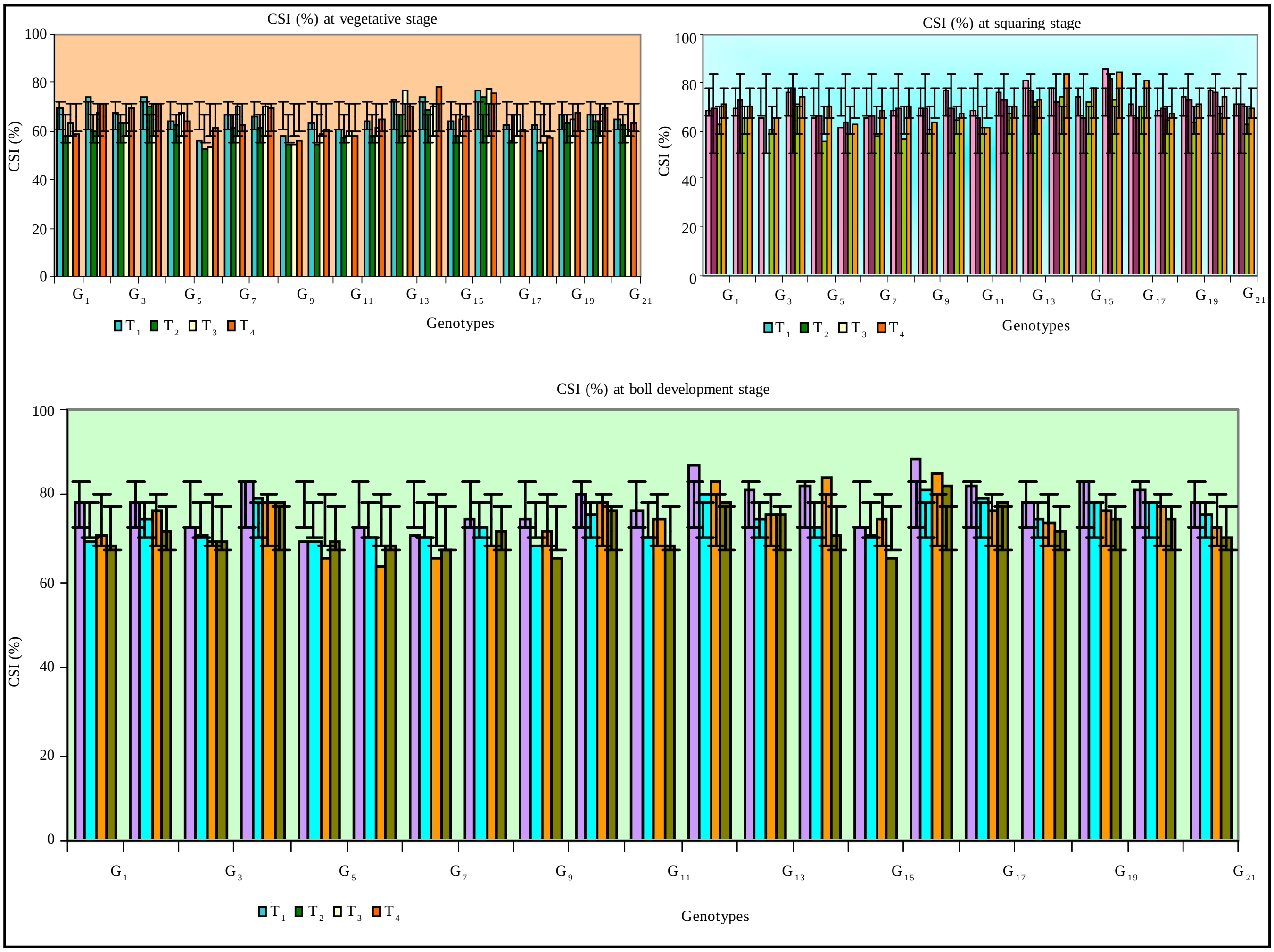


Fig. 3 : **Effect of drought on chlorophyll stability index (CSI %) at different stages of cotton in F_1, F_2, back crosses along with parents**

ceased to increase and returned to pre-stressed levels. Substantial evidence suggests that increased ABA levels limit water loss by reducing stomatal aperture (Table 1 and Fig.1 and 2).

The chlorophyll stability index is an indicative of the maintenance of photosynthetic pigments under drought situation. KC 2 × MCU 13 in both F_1 and F_2 generation have recorded higher values (68.36 and 74.47) at boll development stage than the rest of the combinations and parents irrespective of treatments, indicating that this combination posses drought tolerance characteristics (Table 2 and Fig. 3). Stress given at vegetative and squaring stages invariably show reduction in CSI. Among the genotypes the KC 2 and AS 2 maintained a good mean values (71.89, 73.31 and 75.22; 68.26, 68.74 and 70.62 at vegetative, squaring and boll development stages, respectively) which indicates that these two genotypes are able to withstand drought condition. On the other hand, the genotype MCU 13 and KC 3 was found to rank the medium indicating the moderately tolerant nature as for as the moisture stress is concerned. The CSI was found to be high in the case of (KC 2 × MCU 13) × KC 2 (82.76), when compared to others.

The susceptible genotype Surabhi, showed the lowest mean value of 56.13, 58.41 and 61.36 at vegetative, squaring and boll development stages, respectively. In general, the tolerant genotype, namely KC 2, AS 2, KC 2 × MCU 13 (F_1 and F_2) and KC 2 × JKC 770 have shown least reduction in CSI when compared to Surabi and AS 1. The high cholorophyll stability indices help the plants to with stand stress through better availability of cholorophyll. This leads to increase photosynthetic rate and more dry matter production (Madhanmohan *et al.*, 2000). The seed cotton yield

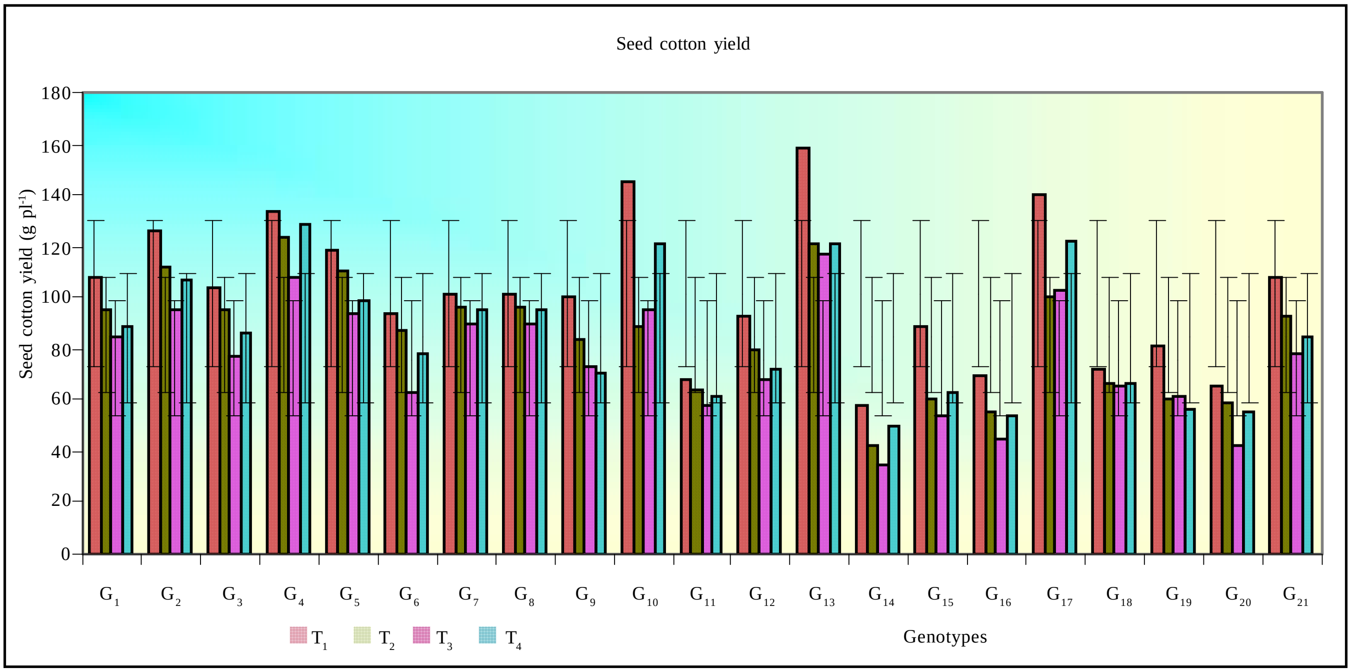


Fig. 4 : Effect of drought on yield components of cotton in F_1, F_2, back crosses along with parents

Table 4 : Drought tolerance index in chlorophyll stability index at squaring stage and seed cotton yield

Parents	DTI at vegetative stress		DTI at squaring stress		DTI at boll development stress		Mean	
	CSI	Seed cotton yield	CSI	Seed cotton yield	CSI	Seed cotton yield	CSI	Seed cotton yield
MCU 13	0.92	0.99	0.82	0.89	0.94	0.92	0.89	0.94
AS 2	0.97	1.37	0.87	1.16	0.94	1.31	0.93	1.28
JKC 770	0.84	0.96	0.76	0.78	0.83	0.87	0.81	0.87
KC 2	1.16	1.59	1.05	1.39	1.10	1.65	1.10	1.54
AS 1	0.84	1.27	0.70	1.08	0.89	1.14	0.81	1.16
Surabhi	0.75	0.79	0.69	0.57	0.74	0.71	0.73	0.69
KC 3	0.84	0.95	0.72	0.89	0.86	0.94	0.81	0.93
Suvin	0.91	0.95	0.75	0.89	0.93	0.94	0.86	0.93
F_1 Hybrids								
AS 1 × Suvin	0.93	0.81	0.81	0.71	0.85	0.68	0.86	0.73
KC 2 × MCU 13	1.03	1.24	0.97	1.34	1.01	1.69	1.00	1.42
AS 2 × MCU 13	0.86	0.43	0.81	0.38	0.81	0.41	0.83	0.40
KC 2 × JKC 770	1.08	0.71	1.00	0.61	1.04	0.65	1.04	0.66
F_2'S								
KC 2 × MCU 13	1.20	1.85	1.13	1.78	1.15	1.84	1.16	1.82
AS 3 × JKC 770	1.09	0.24	1.12	0.20	1.26	0.28	1.16	0.24
AS 2 × MCU 13	0.93	0.52	1.03	0.47	1.10	0.55	1.02	0.51
KC 2 × JKC 770	1.36	0.37	1.21	0.30	1.40	0.36	1.32	0.35
AS 1 × Suvin	0.90	1.35	0.97	1.40	1.11	1.66	0.99	1.47
Back crosses								
(AS2 × MCU13) × MCU 13	0.92	0.46	0.85	0.45	0.89	0.46	0.89	0.46
(KC2 × MCU13) × KC 2	1.06	0.47	0.91	0.49	1.03	0.45	1.00	0.47
(AS2 × MCU13) × AS 2	1.12	0.38	0.99	0.27	1.09	0.35	1.07	0.33
(KC2 × MCU13) × MCU 13	0.98	0.97	0.86	0.82	0.95	0.89	0.93	0.89
Mean	0.92	0.84	0.82	0.75	0.94	0.83	0.89	0.81

recorded as 128.99 in KC 2 × MCU 13 (F_2) irrespective of treatments (Table 3 and Fig. 3). Significant differences were also observed between the genotypes, treatments and their interactions. The genotypes KC 2 and AS 2 have the highest value of seed cotton yield (120.28 and 110.22) than other genotypes at all stages irrespective of the treatmental effects. Among the backcrosses, (KC 2 × MCU 13) × MCU 13 the tolerant genotype exhibited the highest seed cotton yield of 92.59 (Table 3). Maximum number of flowers and bolls produced were found to be highest in KC 2, AS 2 and MCU 13 followed by Suvin, AS 1 and KC 3 in parental genotypes. According to Anderson (1972), water deficit at flowering and pollination (60-70 DAS) even for a short period resulted in an irreversible damage to plant and lowered the yield.

Drought is a multifaceted parameter influenced both by the genotypes as well as the environment. Drought tolerant mechanism is by and larged much complicated and deserves to be quantified. In this direction an attempt was made to categorized drought tolerance in the cotton genotypes studied. From the perusal of the data (Table 4) it is seen that the genotype KC 2 has the highest mean value of drought tolerant index in chlorophyll stability index (39.62) followed by AS 2 (33.41). KC 2 × MCU 13 (36.03 in F_1; 41.66 in F_2). The cross KC 2 × JKC 770 recorded the highest value of drought tolerant index in CSI. Here KC 2 × JKC 770 recorded the lowest mean value of drought tolerant index in seed cotton yield. Among the genotypes Surabhi which was susceptible for water stress recorded the lowest mean value (26.08 and 35) for drought tolerant index in CSI and seed cotton yield irrespective of the treatments. Among the several genotypes studied the cross combination KC 2 × MCU 13 has shown good performance even under drought situation and in fact this genotype has proved to be the best. Again a drought tolerant index was developed for further use by the plant breeders and crop physiologist for further cotton research and development.

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