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Screening of *Hirsutum* Cotton Genotypes for Drought Tolerance under Different Osmotic Potential and Field Capacities

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Abstract

A Lab experiments were conducted during 2016 at Agriculture Research Station, Crop physiology division, Hebballi farm, UAS, Dharwad, to screen twenty one cotton genotypes under water stress condition for seed germination and seedling growth. Study consisted of 19 *Gossypium hirsutum* genotypes with three checks (LRA-5166 (National check), RAH-100 (Zonal check) and Sahana (Regional check)). Seed germination and seedling growth study was conducted under different osmotic potentials using PEG- 6000 (0.0 MPa), −0.140 MPa and −0.39 MPa and pot study with different moisture levels (100, 50 and 25% field capacity). Results indicated that as the PEG concentration and stress level increases, the root length, root weight, lateral root number, proline content increased and chlorophyll content decreased. Whereas, shoot biomass decreased resulting in increase in root to shoot ratio. The varieties viz., Sahana, BS-37, LRA-5166, CCH-12-3, GBHV- 177, BS-39, GBHV-182, ARBH-1352, which showed higher seedling indices like seedling vigor index, shoot vigor index, and root vigor index under higher osmotic potential and moisture stress condition and can be considered as drought tolerant varieties based on seed germination and seedling growth studies and the genotypes viz., RAH-100, RAH-806, GSHV-169, AHK-09-05, NDLH-1943, NDLH-1938 were considerd as a less tolerant genotypes due to decrease in the germination %, seedling indices, root to shoot ratio, morphological characters, proline content, chlorophyll content of cotton plants under different PEG concentration and field capacity.

Keywords: PEG-6000, cotton, chlorophyll, proline, stress indices

1. Introduction

Cotton (*Gossypium* spp.) “the silver fiber” is an important commercial crop of India, playing a significant role in Indian farming and industrial economy of country, by providing 65–70% of raw material for the textile industry of our country. Cotton is cultivated in 70 countries of the World with the total coverage of 33.1 mha, production of 116.6 mbales and a productivity of 76.6 kg lint ha^{-1}. India being the traditional home for cotton and cotton textiles, the cultivated area occupying about 11.8 mha producing 35.2 mbales with the productivity of 504 kg lint ha^{-1}. In Karnataka, it is grown in an area of 0.61 mha with a production of 0.2 m bales and productivity of 556 kg lint ha^{-1} (Anonymous, 2016). Though, India has the largest area under cotton, it ranks third in production due to low productivity. The major reasons for low yield in India are biotic, abiotic, and technological problems. One of the major abiotic stresses affecting plant productivity is water stress resulting through drought which limits crop growth and productivity (Anjum et al., 2011). Water availability and quality affect the growth and physiological processes of all plants as water is the primary component of actively growing plants ranging from 70–90% of plant fresh mass (Babu, 2015). Due to its predominant role in plant nutrient transport, chemical and enzymatic reactions, cell expansion and transpiration, water stresses result in anatomical and morphological alterations as well as changes in physiological and biochemical processes affecting functions of the plants. Plant water deficits depend both on the supply of water to the soil and the evaporative demand of the atmosphere. In general, plant water stress is defined as the condition where a plant’s water potential and turgor are decreased enough to inhibit normal plant function. The effect of water stress depends on the severity and duration of the stress, the growth stage at which stress is imposed and the genotype of the plant (Akram, 2011).

The PEG-6000 was first time used as an inducer and identifier to screen and select drought resistant tobacco cell lines. Chinese researchers used to do cotton drought evaluation and identification by repeated drought induction method. Water stress induced by PEG, leads to decrease in the germination index and the morphological development of organs from young cotton plants and also reported that water absorption, retention and biomass gain were affected by water stress (Babu et al., 2014).

Evaluation of the germination capacity of seeds, seedling vigour, morpho-physiological and biochemical traits under different field capacities in pot is one of the most common methods used for selection and breeding of the drought tolerant resources. Hence, the experiment was carried out to evaluate *hirsutum* cotton varieties under drought stress, using PEG-6000 and pot experiments with varying moisture stress to screen the varieties.

2. Materials and Methods

The laboratory and pot culture experiments were carried out during 2016 in Division of Crop Physiology, Agriculture Research Station, Dharwad, Karnataka, India with 19 cotton varieties viz., TSH-04/115, GBHV-182, GBHV-177, PH-1060, CCH-12-3, GSHV-169, TCH-1777, SCS-1213, SCS- 1062, AKH-09-5, NDLH-1943, CNH- 1110, ARBH-1352, NDLH-1938, RAH- 806, BS-37, BS-39, and GJHV- 516 and three checks viz., LRA-5166 (National check), RAH-100 (Zonal check) and Sahana (Local chek) to assess their performance for drought tolerance.

2.1. Seed germination and seedling vigour under osmotic stress using PEG-6000

Poly ethylene glycol-6000 (PEG-6000) solutions of different osmotic potentials of 0.0 MPa, -0.140 MPa and -0.39 MPa were used to conduct the experiment .The concentrations of PEG-6000 required to obtain these values, were determined, by using the equation given by Michel and Merrill (1973) and Babu et al.,(2014).

$$\Psi s=-(1.18\times10^{-2})C-(1.18\times10^{-4})C_2+(2.67\times10^{-4})CT+(8.39\times10^{-7})\ C_2T$$

Where, Ψs=osmotic potential (bar), C and C_2=concentration (g l^{-1} PEG-6000 in water) and T=temperature (°C).

The delinted seeds were initially disinfected with 0.1% $HgCl_2$ for 5 minutes. Six seeds were kept on top portion of the filter paper/glass plate at 5 cm spacing. The seeds were covered with a small strip of filter paper. Suitable holding material was used to avoid the fall of seeds in slanting position. Initially little quantity of respective prepared PEG solution was added on to the small strip of filter paper which helps in adsorption of seeds in to filter paper firmly. Glass plate was inserted in polythene cover. The plate along with the poly bags were transferred on the supporting wooden block in slanting position. 250 ml of corresponding concentrations (0%, 10% and 20%) of PEG-6000 osmotic solutions were added separately into the respective polythene bag. The PEG solution moved upward and reached to the seeds by capillary movement through filter paper. Seedlings were allowed to grow under room temperature. Fresh PEG solutions were added in regular intervals of three days to maintain the level of solution. Each treatment replicated twice to fit into design for statistical analysis.

The germination percentage, root length and shoot length parameters were recorded on 12th days of imposing the treatments.

Germination (%) –Number of seeds germinated were counted and expressed in percentage.

Seedling vigour - Shoot and root vigour indices were calculated by multiplying shoot length/ root length/ seedling length) with germination percentage as described by Yu et al. (1999).

Shoot vigour index=Shoot length×germination %

Root length index=root length×germination %

Seedling vigour index=(root length+shoot length)×germination %

The data collected from the experiment was subjected to statistical analysis as described by Gomez and Gomez (1984) and analysed variance by two factorial CRD (Completely Randomized Design).

2.2. Pot experiment

Pot experiment with 21 cotton genotypes and three water stress levels viz., control (100% field capacity), moderate stress (50% field capacity) and severe stress (25% field capacity), imposed after 21 days of sowing and maintained up to 45 days. The plants were extracted on 45th day from pots for recording morphological observation viz*.,* Shoot length (cm), root length (cm), number of secondary roots, shoot dry weight (g plant^{-1}), leaf dry weight (g plant^{-1}), total dry matter and distribution (g plant^{-1}), root to shoot ratio were recorded as per the standard procedure. The leaf proline content was estimated by the method of Bates et al*.,* (1973) and Chlorophyll content by using dimethyl sulfoxide (DMSO) as given by Shoaf and Loum (1976).

3. Results and Discussion

3.1. Experiment-I (PEG-6000)

3.1.1. Germination percentage (%)

Significant differences were observed for germination percentage between genotype, stress levels and interaction effects (Table 1) All the genotypes showed reduction in germination percentage with increase in stress levels. However genotypes showed significant variation in rate of reduction over control. Among the PEG concentrations, control recorded significantly higher germination % (87.6), which was significantly differed with 10% (76.4) and 20 % (19.0). Whereas the genotypes, Sahana recorded highest (86.7) germination percentage followed by BS-37(83.3), LRA-5166(80.0), GBHV-177(80.0), CCH-12-3(73.3) and BS-39 (73.3). The genotypes such as, RAH-806 recorded less germination % (20.0) followed by TSH-04/115(29.0), CNH-1110(39.0), NDLH-1943(43.3), NDLH-1938(50.0) and RAH-100(53.3). The genotype Sahana, BS-37, LRA-5166, ARBH-1357, BS-39 and CCH-12-3 are germinated well under all the PEG concentrations, hence these genotypes may be considered as an osmotic stress tolerant. Decreased seed germination is due to reduction in imbibitions of water by seeds which leads to a series of metabolic changes, including general reduction in hydrolysis and utilization of the seed reserve. Increase osmotic stress limit results mobilization of reserves in several species and damages cellular machinery. Higher concentration of PEG is the lethal water potential for germination of cotton seeds; hence the germination was ceased (Babu et al., 2014 and Sidari et al., 2008).

3.1.2. Indices

Various indices worked out using seed germination per cent and seedling growth are presented in Table 1. The seedling vigour index increased from control to 10%, but it decreased with the increase in PEG-6000 concentration of 20%. The PEG concentrations, genotypes and their interactions differed significantly with respect to seedling vigor index. 10% PEG concentration were recorded significantly (2311.8) higher seedling vigor index than control and 20% (1535.3 and 39.25, respectively). The genotype, Sahana was recorded significantly higher (2422.0) seedling vigor index which was followed by BS-37, LRA-5166, GBHV-177, CCH-12-3 and BS-39 (2205.7, 2107.3, 2036.7, 1824.4 and 1790.8, respectively). Whereas, the genotype RAH-806 (87.0) recorded significantly less seedling vigor index followed by TSH-04/115, CNH-1110, NDLH-1943, NDLH-1938, RAH-100, (227.6, 499.9, 601.5, 718.9 and 832.8, respectively). Similarly Zhang et al. (2007) studied 13 cotton samples with PEG-6000 stress for 12 hours. After 12 hours osmotic treatment, the survival rates showed that drought tolerance by considering the seedling vigour index.

Among the PEG concentration, significantly higher shoot vigor index was recorded in control (638.7) than 10% and 20% (299.9 and 3.31, respectively). The genotype, Sahana was recorded (601.3) significantly higher seedling vigor index which was followed by BS-37, LRA-5166, GBHV-177, CCH-12-3 and BS-39 (544.2, 505.7, 477.0, 445.3 and 422.8 respectively). Whereas, the genotype RAH-806 (34.0) recorded significantly less seedling vigor index followed by TSH-04/115, CNH-1110, NDLH-1943, NDLH-1938, RAH-100, (59.2, 105.0, 155.3, 187.0 and 216.3, respectively).

Among the PEG concentration, 10% were recorded significantly higher root vigor index (2011.8) than control and 20% (896.5 and 36.0, respectively). The genotype, Sahana was recorded (1820.7) significantly higher root vigor index which was followed by BS-37, LRA-5166, GBHV-177, CCH-12-3 and BS-39 (1660.7, 1601.7, 1559.7, 1379.0 and 1368.0, respectively). Whereas, the genotype RAH-806 (53.0) recorded significantly less root vigor index followed by TSH-04/115, CNH-1110, NDLH-1943, NDLH-1938 and RAH-100, (168.5, 395.0, 446.2, 531.9 and 616.4, respectively).

At 12th day under different PEG concentrations, the shoot length (Table 2a) was recorded significantly highest in control (7.03 cm) followed by 10% (3.31 cm) and less shoot length was recorded in 20% (0.07 cm). The genotype Sahana recorded significantly higher shoot length (6.10 cm) which was followed by BS-37, LRA-5166, GBHV-177, CCH-12-3, BS-39 and ARBH-1352 (5.48, 5.11, 4.80, 4.65, 4.38 and 4.00 cm, respectively). Whereas, the genotype RAH-806 (0.57 cm) and TSH-04/115 (0.88 cm) followed by CNH-1110, NDLH-1943, NDLH-1938, GSHV-169 and RAH-100 were recorded (1.57, 2.02, 2.45, 2.50 and 2.77 cm, respectively) significantly lower shoot length.

The root length differed significantly with respect to genotypes at 12th day presented in Table 2a. Genotypes, Sahana, BS-37, LRA-5166, GBHV-177, CCH-12-3, BS-39, ARBH-1352 and PH-1060 recorded significantly higher root length (18.69, 17.11, 16.49, 16.03, 15.28, 15.15, 13.73 and 13.31 cm, respectively) than genotypes RAH-806, TSH-04/115, CNH-1110, NDLH-1943, NDLH-1938, RAH-100 and AKH-09-5 where significantly lowest root length (5.34, 5.72, 7.10, 7.40, 7.93, 8.40 and 9.45 cm, respectively) were recorded. The root to shoot ratio was also differed significantly with respect to different concentrations of PEG-6000, genotypes and their interactions. Among the PEG-6000 concentrations root to shoot ratio was found maximum in 10% (6.37) which was followed by 20% and control (1.33 and 2.05, respectively) among the genotypes NDLH-1943and BS-37 recorded (8.58 and 7.36, respectively) significantly higher root to shoot ratio followed by, GBHV-177, LRA-5166 and Sahana (7.26, 5.12 and 4.14, respectively).

3.2. Pot experiment

Shoot length of the cotton varieties under different field capacities and their mean values were calculated and presented in Table 2b. Plant height could be considered an easy parameter to evaluate and compare different crop varieties for drought tolerance. Shoot length differed significantly with respect to moisture levels, genotypes and their interactions. Among the moisture levels, control recorded significantly higher shoot length (25.26 cm) which was followed by 50% and 25% of water supply (20.18 and 16.79 cm, respectively). There was a significant reduction in shoot length of all the varieties under drought stress. Among the genotypes BS-37 recorded (24.68 cm) which were on par with LRA-5166 and Sahana (24.26 and 23.86 cm, respectively) and which were followed by GBHV-177, CCH-12-3 and BS-39 (22.52, 22.13 and 21.83 cm, respectively). The genotype RAH-806 recorded significantly lower shoot length (17.64 cm) was on par with the TSH-04/115 and GSHV-169 (17.77 and 17.64 cm, respectively) which was followed by CNH-1110 and RAH-100 (18.32 and 19.60 cm, respectively). This might be due to under moisture stress condition the plant increases the root length, root volume, root weight and lateral roots to absorb water form deeper surfaces, this caused decrease in shoot biomass. The decreased shoot length and leaves helps in reducing transpiration water loss from shoot surfaces. Shoots elongation significantly decreased by concentration of 2-8 MPa whereas no hypocotyl elongation was observed at concentration of 10 and 12 MPa and shoot elongation completely inhibited, it was in conformity with result observed by Tonin et al. (2000).

The water regimes, genotypes and their interactions differed significantly with respect to root length presented in Table 2b. In general water stress results in thinning of the roots, because of reduced the air space between cells of xylem vessels and

Table 1: Effect of PEG concentration on germination percentage, per cent reduction C_2 and C_3 over C_1 over and seedling vigour indices of *Hirsutum* cotton varieties at 12th day

Genotypes	Germination (%)						Seedling vigour index				Shoot vigour index				Root vigour index			
	C_1	C_2	RO	C_3	RO	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
TSH-04/115	67.0	20.0	70.15*	0.0	100.00*	29.0	408.7	274.2	0.0	227.6	177.6	0.0	0.0	59.2	231.2	274.2	0.0	168.5
GBHV-182	93.0	90.0	3.23	20.0	78.49	67.7	1562.4	2801.7	17.0	1460.4	683.6	391.5	0.0	358.4	878.9	2410.2	17.0	1102.0
GBHV-177	100.0	100.0	0.00	40.0	60.00	80.0	2200.0	3818.0	92.0	2036.7	875.0	550.0	6.0	477.0	1325.0	3268.0	86.0	1559.7
PH-1060	93.0	90.0	3.23	20.0	78.49	67.7	1646.1	2969.1	23.0	1546.1	692.9	400.5	0.0	364.5	953.3	2568.6	23.0	1181.6
CCH-12-3	100.0	90.0	10.00	30.0	70.00	73.3	2180.0	3230.1	63.0	1824.4	865.0	468.0	3.0	445.3	1315.0	2762.1	60.0	1379.0
GSHV-169	93.0	85.0	100.00	10.0	100.00	62.7	1357.8	2369.0	0.0	1242.3	558.0	127.5	0.0	228.5	799.8	2241.5	0.0	1013.8
TCH-1777	87.0	80.0	8.05	10.0	88.51	59.0	1413.8	2024.8	2.8	1147.1	626.4	288.0	0.0	304.8	787.4	1736.8	2.8	842.3
SCS-1213	87.0	80.0	8.05	10.0	88.51	62.3	1422.5	2520.9	5.0	1316.1	626.4	355.5	0.0	327.3	796.1	2165.4	5.0	988.8
SCS-1062	87.0	80.0	8.05	10.0	88.51	59.0	1405.1	1944.8	3.5	1117.8	622.1	252.0	0.0	291.4	783.0	1692.8	3.5	826.4
AKH-09-5	80.0	80.0	0.00	10.0	87.50	56.7	1240.0	1787.2	0.0	1009.1	548.0	212.0	0.0	253.3	692.0	1575.2	0.0	755.7
NDLH-1943	80.0	50.0	37.50	0.0	100.00	43.3	1044.0	760.5	0.0	601.5	436.0	30.0	0.0	155.3	608.0	730.5	0.0	446.2
CNH-1110	67.0	50.0	25.37	0.0	100.00	39.0	787.3	712.5	0.0	499.9	314.9	0.0	0.0	105.0	472.4	712.5	0.0	395.0
ARBH-1352	93.0	90.0	3.23	20.0	78.49	67.7	1729.8	2999.7	25.0	1584.8	697.5	405.0	0.0	367.5	1032.3	2594.7	25.0	1217.3
NDLH-1938	80.0	60.0	25.00	10.0	87.50	50.0	1156.0	1000.8	0.0	718.9	480.0	81.0	0.0	187.0	676.0	919.8	0.0	531.9
RAH-806	60.0	0.0	100.00	0.0	100.00	20.0	261.0	0.0	0.0	87.0	102.0	0.0	0.0	34.0	159.0	0.0	0.0	53.0
BS-37	100.0	100.0	0.00	50.0	50.00	83.3	2420.0	4037.0	160.0	2205.7	1025.0	600.0	10.0	545.0	1395.0	3437.0	150.0	1660.7
BS-39	100.0	90.0	10.00	30.0	70.00	73.3	2155.0	3159.0	58.5	1790.8	850.0	418.5	0.0	422.8	1305.0	2740.5	58.5	1368.0
GJHV-516	93.0	90.0	3.23	20.0	78.49	67.7	1534.5	2733.3	13.0	1426.9	674.3	360.0	0.0	344.8	860.3	2373.3	13.0	1082.2
Sahana	100.0	100.0	0.00	60.0	40.00	86.7	2810.0	4198.0	258.0	2422.0	1090.0	675.0	39.0	601.3	1720.0	3523.0	219.0	1820.7
LRA-5166(NC)	100.0	100.0	0.00	40.0	60.00	80.0	2275.0	3941.0	105.6	2107.2	925.0	580.0	11.6	505.5	1350.0	3361.0	94.0	1601.7
R A H - 1 0 0 (LC)	80.0	70.0	12.50	10.0	87.50	53.3	1232.0	1266.3	0.0	832.8	544.0	105.0	0.0	216.3	688.0	1161.3	0.0	616.4
Mean	87.6	76.4		19.0			1535.28	2311.80	39.35		638.74	299.98	3.31		896.54	2011.83	36.04	1535.28
	C	G		C x G			C	G	C x G		C	G	CxG		C	G	CxG	
SEm+	1.24	0.47		2.15			0.38	0.14	0.66		0.20	0.08	0.35		0.37	0.14	0.63	
CD (p=0.05)	4.67	1.76		8.08			1.44	0.54	2.49		0.77	0.29	1.33		1.38	0.52	2.38	

C_1: 0.0 MPa (0 bar); C_2: -0.140 MPa (-1 bar); RO: % Red. Over C_1; C_3:-0.39 MPa (-3.9 bar); *: per cent reduction

Table 2a: Effect of moisture stress on shoot length, root length and root and root to shoot ratio of *Hirsutum* cotton genotypes at 12th day in PEG-6000

Treatments	Shoot length (cm)				Root length (cm)				Root to shoot ratio			
	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
TSH-04/115	2.65	0.00	0.00	0.88	3.45	13.71	0.00	5.72	1.30	0.00	0.00	0.43
GBHV-182	7.35	4.35	0.00	3.90	9.45	26.78	0.85	12.36	1.29	6.16	0.00	2.48
GBHV-177	8.75	5.50	0.15	4.80	13.25	32.68	2.15	16.03	1.51	5.94	14.33	7.26
PH-1060	7.45	4.45	0.00	3.97	10.25	28.54	1.15	13.31	1.38	6.41	0.00	2.60
CCH-12-3	8.65	5.20	0.10	4.65	13.15	30.69	2.00	15.28	1.52	5.90	0.00	2.47
GSHV-169	6.00	1.50	0.00	2.50	8.60	26.37	0.00	11.66	0.00	0.00	0.00	0.00
TCH-1777	7.20	3.60	0.00	3.60	9.05	21.71	0.28	10.35	1.26	6.03	0.00	2.43
SCS-1213	7.20	3.95	0.00	3.72	9.15	24.06	0.50	11.24	1.27	6.09	0.00	2.45
SCS-1062	7.15	3.15	0.00	3.43	9.00	21.16	0.35	10.17	1.26	6.72	0.00	2.66
AKH-09-5	6.85	2.65	0.00	3.17	8.65	19.69	0.00	9.45	1.26	7.43	0.00	2.90
NDLH-1943	5.45	0.60	0.00	2.02	7.60	14.61	0.00	7.40	1.39	24.35	0.00	8.58
CNH-1110	4.70	0.00	0.00	1.57	7.05	14.25	0.00	7.10	1.50	0.00	0.00	0.50
ARBH-1352	7.50	4.50	0.00	4.00	11.10	28.83	1.25	13.73	1.48	6.41	0.00	2.63
NDLH-1938	6.00	1.35	0.00	2.45	8.45	15.33	0.00	7.93	1.41	11.36	0.00	4.25
RAH-806	1.70	0.00	0.00	0.57	2.65	13.38	0.00	5.34	1.56	0.00	0.00	0.52
BS-37	10.25	6.00	0.20	5.48	13.95	34.37	3.00	17.11	1.36	5.73	15.00	7.36
BS-39	8.50	4.65	0.00	4.38	13.05	30.45	1.95	15.15	1.54	6.55	0.00	2.69
GJHV-516	7.25	4.00	0.00	3.75	9.25	26.37	0.65	12.09	1.28	6.59	0.00	2.62
Sahana	10.90	6.75	0.65	6.13	17.20	35.23	3.65	18.69	1.58	5.22	5.62	4.14
LRA-5166(NC)	9.25	5.80	0.29	5.11	13.50	33.61	2.35	16.49	1.46	5.79	8.10	5.12
RAH-100 (LC)	6.80	1.50	0.00	2.77	8.60	16.59	0.00	8.40	1.26	11.06	0.00	4.11
Mean	7.03	3.31	0.07		9.83	24.21	0.96		1.33	6.37	2.05	
	C	G	CxG		C	G	CxG		C	G	CxG	
SEm±	0.023	0.009	0.040		0.039	0.015	0.067		0.027	0.010	0.048	
CD (p=0.05)	0.086	0.033	0.150		0.146	0.055	0.253		0.103	0.039	0.179	

C_1: 0.0 MPa (0 bar); C_2: - 0.140 MPa (-1 bar); C_3: -0.39 MPa (-3.9 bar)

increases the tap root length. Among the water regimes, 100% field capacity recorded significantly higher root length (20.57 cm), which was followed by (18.01 cm) 50% field capacity and the lower root length was observed under 25% field capacity (16.57 cm). Among the genotypes BS-37 recorded (24.57 cm) higher root length which was followed by LRA-5166, Sahana, GBHV-177, CCH-12-3 and BS-39 (23.65, 22.68, 21.27, 20.87 and 20.16 cm, respectively) and the genotype RAH-806 recorded significantly lower root length (13.95 cm) was on par with the TSH-04/115, GSHV-169, CNH-1110 and RAH-100 (14.39, 13.07, 15.09 and 16.75 cm). This was due to the root traits play a major role in water stress tolerance under terminal water stress environments. In terms of root architecture, both more prolific root systems extracting more of the water in upper soil layers and longer root systems extracting soil moisture from deeper soil layers are important for maintaining yield under terminal water stress. Maruti and Katageri, (2015) reported that the genotypes such as IC 359963 (2.25), RDT 17 (5.1%) and CPD 446 (4.1%) recorded significantly thinner roots than Sahana (4.9%) in both normal and water stress condition, also significantly increases their primary root length.

Data on number of secondary roots is presented in the Table 2b were differed significantly with respect to moisture levels, genotypes and their interactions. The genotypes Sahana recorded significantly higher secondary roots (44.17) which were followed by BS-37, LRA-5166, GBHV-177 and CCH-12-3 (41.33, 41.08, 37.83 and 36.83, respectively). The genotype RAH-806 recorded significantly lower secondary root (16.00) was followed by TSH- 04/115, RAH-100 and GSHV-169 (19.00, 20.33 and 23.83, respectively). Among the different water

Table 2b: Effect of moisture stress on shoot length, root length, number of secondary root and root to shoot ratio of *Hirsutum* cotton genotypes grown in pot condition at 45 DAS

Treatments	Stem length (cm)				Root length (cm)				Number of secondary roots				Root to shoot ratio (Length basis)			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
TSH-04/115	20.95	18.20	14.16	17.77	16.21	14.70	12.25	14.39	17.50	23.50	16.00	19.00	0.773	0.808	0.865	0.815
GBHV-182	25.68	21.33	17.60	21.54	21.07	18.62	17.89	19.19	34.00	36.75	26.00	32.25	0.821	0.873	1.016	0.903
GBHV-177	28.16	20.63	18.79	22.52	23.70	20.41	19.69	21.27	38.50	41.50	33.50	37.83	0.842	0.990	1.048	0.960
PH-1060	25.67	21.45	17.24	21.45	21.23	18.73	17.53	19.16	25.00	36.00	26.50	29.17	0.827	0.873	1.016	0.906
CCH-12-3	27.75	20.63	18.00	22.13	23.33	20.41	18.88	20.87	37.50	41.00	32.00	36.83	0.841	0.989	1.049	0.960
GSHV-169	20.63	18.28	14.00	17.64	14.66	13.52	11.03	13.07	18.50	35.50	17.50	23.83	0.711	0.740	0.788	0.746
TCH-1777	23.97	19.06	17.50	20.18	19.60	16.42	17.15	17.72	31.50	40.50	16.00	29.33	0.818	0.861	0.980	0.886
SCS-1213	24.26	21.16	18.04	21.16	19.85	18.28	17.74	18.62	31.50	40.00	30.00	33.83	0.818	0.864	0.983	0.888
SCS-1062	23.43	19.27	17.00	19.90	19.11	16.17	16.66	17.31	30.50	40.00	26.00	32.17	0.816	0.839	0.980	0.878
AKH-09-5	23.32	19.10	16.13	19.52	18.87	15.93	15.68	16.82	30.00	40.00	25.00	31.67	0.809	0.834	0.972	0.872
NDLH-1943	24.10	18.53	15.00	19.21	18.83	15.19	13.97	16.00	22.50	31.50	25.00	26.33	0.781	0.820	0.931	0.844
CNH-1110	22.73	18.42	13.80	18.32	17.60	14.95	12.74	15.09	29.00	39.50	29.50	32.67	0.774	0.811	0.923	0.836
ARBH-1352	26.26	21.62	17.47	21.78	21.76	19.35	18.01	19.71	29.00	30.50	16.50	25.33	0.828	0.895	1.031	0.918
NDLH-1938	24.02	18.68	15.12	19.27	19.08	15.44	14.21	16.24	22.00	26.50	16.00	21.50	0.794	0.826	0.940	0.853
RAH-806	20.70	18.11	14.11	17.64	15.52	14.31	12.01	13.95	15.00	21.00	12.00	16.00	0.750	0.790	0.851	0.797
BS-37	32.82	22.34	18.90	24.68	28.09	24.73	20.89	24.57	39.50	48.00	36.50	41.33	0.856	1.107	1.105	1.023
BS-39	26.87	21.17	17.43	21.83	22.54	19.93	18.01	20.16	35.00	43.00	30.50	36.17	0.839	0.941	1.033	0.938
GJHV-516	24.85	21.11	17.96	21.31	20.34	18.38	17.89	18.87	28.50	38.50	29.50	32.17	0.818	0.871	0.996	0.895
Sahana	29.61	23.15	18.83	23.86	25.17	23.10	19.78	22.68	46.00	51.00	35.50	44.17	0.850	0.998	1.051	0.966
LRA-5166(NC)	31.22	22.63	18.92	24.26	26.54	24.01	20.41	23.65	41.00	47.75	34.50	41.08	0.850	1.061	1.079	0.997
RAH-100 (LC)	23.38	18.86	16.56	19.60	18.87	15.71	15.68	16.75	24.00	22.00	15.00	20.33	0.807	0.833	0.947	0.862
Mean	25.26	20.18	16.79		20.57	18.01	16.57		29.81	36.86	25.19		0.811	0.887	0.980	
	F	G	FxG		F	G	FxG		F	G	FxG		F	G	FxG	
SEm±	0.45	0.17	0.78		0.307	0.116	0.531		0.58	0.22	1.00		0.016	0.006	0.028	
CD (*p*=0.05)	1.70	0.64	2.94		1.154	0.436	1.998		2.18	0.82	3.78		0.061	0.023	0.106	

F: Field capacity; G: Genotypes; F_1: 100 % Field capacity; F_2: 50% Field capacity; F_3:25% Field capacity regimes 50% field capacity recorded significantly higher number of secondary roots (36.86), which was followed by control (29.81) where as 25% field capacity recorded significantly (25.19) lower number of secondary roots. It was observed from the experiment that slight increase in stress condition increases secondary root numbers but severs stress condition decrease secondary root number and similar results are reported by Maruti and Katageri (2015).

Moisture levels, genotypes and their interactions differed significantly with respect to root to shoot ratio (Table 2b). The genotypes BS-37 recorded significantly higher (1.023) root to shoot ratio which was on par with LRA-5166, Sahana, GBHV-177, CCH-12-3 and BS-39(0.997, 0.966, 0.960, 0.960 and 0.938, respectively). The genotype GSHV-169 recorded significantly lower root to shoot ratio (0.746) was on par with the RAH-806 and TSH- 04/115 (0.797 and 0.815, respectively) and followed by CNH-1110, NDLH-1938 and RAH-100 (0.836, 0.853 and 0.862, respectively). Among the moisture levels 25% field capacity recorded significantly higher root to shoot ratio (0.980), which was followed by 50% field capacity (0.887) where as 100% field capacity (0.811) recorded significantly lower root to shoot ratio. Mc Michael and Quisenberry (1991) in cotton and Ogbonnaya et al. (2003) in cowpea reported that the water stress tolerant genotypes had higher root to shoot ratio than susceptible ones.

The results obtained on total dry matter accumulation and its distribution on leaf, stem and root dry weight during the growth as influenced by different field capacities, genotypes and their interactions are presented in Table 3. With respect to stem dry weight, different water regimes, genotypes and their interactions differed significantly. Among the field capacities, 100% recorded significantly higher stem dry weight

Table 3: Effect of moisture stress on stem dry weight, root dry weight and rot to shoot ratio of *Hirsutum* cotton genotypes grown in pot condition at 45 DAS

Treatments	Stem dry weight (g plant^{-1})				Root dry weight (g plant^{-1})				Root to shoot ratio (dry weight basis)			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
TSH-04/115	0.69	0.60	0.47	0.59	0.295	0.267	0.223	0.262	0.43	0.45	0.48	0.45
GBHV-182	1.04	0.86	0.71	0.87	0.546	0.482	0.463	0.497	0.53	0.56	0.65	0.58
GBHV-177	1.09	0.80	0.73	0.87	0.579	0.499	0.481	0.520	0.53	0.62	0.66	0.60
PH-1060	1.03	0.86	0.69	0.86	0.566	0.499	0.467	0.511	0.55	0.58	0.68	0.60
CCH-12-3	1.10	0.82	0.72	0.88	0.591	0.517	0.478	0.528	0.54	0.63	0.67	0.61
GSHV-169	0.56	0.49	0.38	0.48	0.246	0.227	0.185	0.219	0.44	0.46	0.49	0.46
TCH-1777	0.94	0.75	0.69	0.79	0.464	0.389	0.406	0.420	0.49	0.52	0.59	0.53
SCS-1213	0.96	0.84	0.72	0.84	0.478	0.440	0.427	0.449	0.50	0.53	0.60	0.54
SCS-1062	0.92	0.76	0.67	0.78	0.434	0.367	0.378	0.393	0.47	0.49	0.57	0.51
AKH-09-5	0.92	0.75	0.64	0.77	0.429	0.363	0.357	0.383	0.47	0.48	0.56	0.50
NDLH-1943	0.88	0.68	0.55	0.70	0.395	0.318	0.293	0.335	0.45	0.47	0.54	0.49
CNH-1110	0.76	0.62	0.46	0.62	0.332	0.282	0.240	0.285	0.43	0.46	0.52	0.47
ARBH-1352	1.05	0.86	0.70	0.87	0.569	0.506	0.471	0.516	0.54	0.59	0.67	0.60
NDLH-1938	0.93	0.72	0.59	0.75	0.410	0.331	0.305	0.349	0.44	0.46	0.52	0.47
RAH-806	0.60	0.53	0.41	0.51	0.254	0.234	0.196	0.228	0.42	0.44	0.48	0.45
BS-37	1.25	0.85	0.72	0.94	0.680	0.599	0.506	0.595	0.54	0.70	0.70	0.65
BS-39	1.07	0.85	0.70	0.87	0.580	0.512	0.463	0.518	0.54	0.61	0.66	0.60
GJHV-516	0.97	0.83	0.70	0.83	0.516	0.467	0.454	0.479	0.53	0.56	0.65	0.58
Sahana	1.13	0.88	0.72	0.91	0.626	0.574	0.492	0.564	0.55	0.65	0.69	0.63
LRA-5166(NC)	1.19	0.86	0.72	0.92	0.642	0.581	0.494	0.573	0.54	0.67	0.69	0.63
RAH-100 (LC)	0.91	0.74	0.65	0.77	0.410	0.341	0.341	0.364	0.45	0.46	0.53	0.48
Mean	0.95	0.76	0.63		0.478	0.419	0.387		0.494	0.542	0.599	
	F	G	F×G		F	G	F×G		F	G	F×G	
SEm±	0.45	0.17	0.78		0.008	0.003	0.014		0.010	0.004	0.017	
CD (*p*=0.05)	1.70	0.64	2.94		0.030	0.012	0.053		0.037	0.014	0.065	

F: Field capacity; G: Genotypes; F_1: 100 % Field capacity; F_2: 50% Field capacity; F_3: 25% Field capacity

(0.950 g) which was followed by 50% and 25% of water supply (0.760 and 0.630 g respectively). Among the genotypes BS-37 recorded (0.940 g) which was on par with LRA-5166 and Sahana (0.924 and 0.910 g, respectively) and which was followed by CCH-12-3, GBHV-177, BS-39 and GBHV-182 (0.880, 0.875, 0.873 and 0.868 g respectively). The genotype GSHV-169 recorded significantly lower stem dry weight (0.477 g) which was followed by RAH-806 and TSH-04/115 (0.514 and 0.586 g, respectively).

Root dry weight reflects the amount of photosynthates diverted towards the roots. It is more important than fresh weight to identify the water stress tolerance, because the fresh root weight involves varying amount of water hold in the root biomass. According to Rezaeieh and Eivazi (2012), root dry weight was the best indicator and easiest typical trait to determine the water stress tolerance of maize. Pace et al. (1999); Dewi (2009) reported, that the water stress tolerant cultivars are maintained higher root dry weight and also record the higher number of secondary roots and tap root length in water stress condition. The result with respect to root dry weight is presented in Table 3. Where root dry weight differed significantly with respect to water regimes, genotypes and their interactions. The 100% field capacity recorded significantly higher root dry weight (0.478 g), which was followed by 50% field capacity (0.419 g) and the lower root dry weight was observed under 25% field capacity (0.387 g). Among the genotypes BS-37 recorded (0.595 g) higher root dry weight which was followed by LRA-5166, Sahana, GBHV-177, CCH-12-3 and BS-39 (0.573, 0.564, 0.520, 0.528 and 0.518 g, respectively) and the genotype GSHV-169 recorded significantly lower shoot length (0.219 cm) was on par with RAH-806 (0.228 cm) which was followed by TSH-04/115, CNH-1110 and RAH-100 (0.262, 0.285 and 0.364 cm, respectively). Increase in root dry weight may be due to higher primary root length, higher secondary root number and thicker roots and higher primary root length under water stress condition. It indicates that such genotypes have greater flexibility to adjust with the changing moisture level during crop growth stage but higher decreased root dry weight under drought condition as compared to the normal condition was due to decreasing primary root length, secondary root number and higher thinning of roots (Maruti and Katageri, 2015).

Significant differences were observed for root to shoot ratio between genotype, water regimes and interaction effects (Table 3). The genotypes BS-37 recorded significantly higher (0.65) root to shoot ratio which was on par with LRA-5166 and Sahana (0.63 and 0.63, respectively) and followed by GBHV-177, CCH-12-3 and BS-39 (0.60, 0.61 and 0.58, respectively). Whereas, the genotype RAH-806 recorded significantly lower root to shoot ratio (0.45) which was on par with the TSH-04/115 and GSHV-169 (0.45 and 0.46, respectively) followed by CNH-1110 and RAH-100 (0.47 and 0.48, respectively). Among the different water regimes 25% field capacity recorded significantly higher root to shoot ratio (0.60), which was followed by 50% field capacity (0.54) where as 100% field capacity (0.49) recorded significantly lower root to shoot ratio.

The results obtained on leaf dry weight and total dry weight differed significantly with respect to water regimes, genotypes and their interaction are presented in Table 4. Present investigation revealed that the reduction in stem dry weight, leaf dry weight and total biomass as increased in the stress levels from 100 to 25% field capacity. The 100% field capacity recorded higher leaf dry weight and total dry weight (1.037 and 1.989 g, respectively) followed by 50% field capacity (0.829 and 1.589 g, respectively). Significantly lower leaf dry weight and total dry weight (0.700 and 1.334 g, respectively) was recorded under 25% field capacity. The genotypes BS-37 recorded significantly higher leaf dry weight and total dry weight (1.077 and 2.017 g, respectively) which was on par with LRA-5166 (1.024 and 1.948 g, respectively) and Sahana (1.011 and 1.921 g, respectively) and followed by GBHV-177 (0.962 and 1.836 g, respectively), CCH-12-3 (0.965 and 1.528g, respectively) and BS-39 (0.952 and 1.825 g, respectively). The genotype RAH-806 recorded significantly lower leaf dry weight and total dry weight (0.595 and 1.109 g, respectively) was followed by TSH- 04/115 (0.651 and 1.237 g, respectively), GSHV-169 (0.544 and 1.021 g), CNH-1110 (0.670 and 1.286 g, respectively) and RAH-100 (0.846 and 1.613 g, respectively). This study showed that the rapid decrease in plant biomass from 100% to 25% field capacity i.e., both in stress condition and among the genotypes, is mainly because of reduction in photosynthetic activity and other metabolic reaction because of drought condition (Rezaeieh and Eivazi, 2012). That mean changes occured due to stress condition was adoptive mechanism which uses most of its energy to accumulation of osmoregulants, activation of most of oxidative enzymes and also accumulation and translocation of assimilates to stem and root.

Significant differences were observed for proline and total chlorophyll content between genotype, water regimes and interaction effects (Table 4). Proline is a major osmoregulent, it is produced in larger amount under stress as compared to the normal conditions (Unyayar et al., 2004). Drought stress condition increases the proline content in the leaves. The proline was found higher in 25% field capacity (71.80 mg g^{-1} fr. Wt.) was followed by 50% field capacity (63.61 mg g^{-1} fr. Wt.) and lower proline content (46.61 mg g^{-1} fr. Wt.) was observed under 100% field capacity. Among the genotypes CCH-12-3 (70.46 mg g^{-1} fr. Wt.) and LRA-5166 recorded (69.93 mg g^{-1} fr. Wt.) significantly maximum proline content which was on par with the Sahana and (68.37 mg g^{-1} fr. Wt.) and followed by GBHV-182, GBHV-177, BS-37 and BS-39. Whereas lower proline content was recorded by GSHV-169 (49.29 mg g^{-1} fr. Wt.) followed by RAH-806, TSH-04/115 and RAH-100 (51.51, 54.24 and 56.96 mg g^{-1} fr. Wt., respectively). By measuring the chlorophyll content of a plant tissue, a reliable estimate of photosynthetic rate in green tissue of plants can be estimated. The genotype CCH-12-3 and Sahana recorded significantly

Table 4: Effect of moisture stress on leaf dry weight, total dry weight and biochemical parameter of Hirsutum cotton genotypes grown in pot condition at 45 DAS

Treatments	Leaf dry weight (g plant^{-1})				Total dry weight (g plant^{-1})				Proline (mg g fr. Wt.$^{-1}$)				Total Chl. (mg g fr. Wt.$^{-1}$)			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
TSH-04/115	0.813	0.653	0.487	0.651	1.504	1.254	0.954	1.237	40.46	55.20	67.07	54.24	3.252	1.991	1.806	2.350
GBHV-182	1.080	0.905	0.783	0.923	2.115	1.765	1.493	1.791	60.56	66.19	71.24	66.00	5.049	2.588	2.438	3.358
GBHV-177	1.190	0.872	0.823	0.962	2.284	1.673	1.552	1.836	50.84	68.33	76.62	65.26	3.682	3.761	2.975	3.473
PH-1060	1.090	0.913	0.733	0.912	2.116	1.770	1.423	1.770	52.75	66.29	71.32	63.45	3.781	2.705	2.467	2.985
CCH-12-3	1.180	0.903	0.812	0.965	2.284	1.723	1.528	1.845	67.32	68.15	75.91	70.46	6.561	3.550	2.778	4.296
GSHV-169	0.675	0.541	0.417	0.544	1.233	1.035	0.795	1.021	33.79	52.17	61.91	49.29	4.867	1.902	1.744	2.838
TCH-1777	1.045	0.820	0.753	0.872	1.988	1.570	1.442	1.667	40.73	63.46	70.25	58.15	3.110	2.453	2.297	2.620
SCS-1213	1.058	0.884	0.765	0.902	2.020	1.723	1.480	1.741	52.29	64.30	70.28	62.29	3.705	2.458	2.353	2.839
SCS-1062	1.015	0.890	0.736	0.880	1.935	1.646	1.404	1.662	41.47	63.02	69.69	58.06	4.002	2.309	2.097	2.803
AKH-09-5	0.980	0.849	0.678	0.836	1.901	1.603	1.315	1.606	34.96	62.30	69.56	55.60	4.161	2.275	2.035	2.824
NDLH-1943	0.925	0.718	0.577	0.740	1.803	1.393	1.124	1.440	40.99	60.84	68.15	56.66	3.130	2.055	1.874	2.353
CNH-1110	0.835	0.683	0.493	0.670	1.599	1.302	0.956	1.286	36.12	58.79	67.36	54.09	3.982	2.019	1.853	2.618
ARBH-1352	1.110	0.954	0.749	0.938	2.161	1.819	1.448	1.809	46.58	66.39	73.02	62.00	3.587	2.729	2.493	2.936
NDLH-1938	0.950	0.775	0.598	0.774	1.880	1.498	1.183	1.520	40.80	61.08	68.45	56.78	3.128	2.183	1.900	2.404
RAH-806	0.775	0.564	0.447	0.595	1.378	1.091	0.858	1.109	33.95	54.95	65.65	51.51	3.597	1.953	1.805	2.452
BS-37	1.340	0.984	0.908	1.077	2.590	1.835	1.627	2.017	43.12	70.11	83.76	65.66	3.380	4.467	3.894	3.914
BS-39	1.175	0.938	0.742	0.952	2.250	1.784	1.440	1.825	50.48	67.20	75.67	64.45	3.631	3.546	2.503	3.227
GJHV-516	1.060	0.845	0.766	0.890	2.034	1.672	1.470	1.725	54.03	65.99	70.52	63.52	3.937	2.535	2.400	2.957
Sahana	1.245	0.920	0.868	1.011	2.374	1.803	1.586	1.921	57.00	69.18	78.92	68.37	4.056	4.640	3.741	4.146
LRA-5166(NC)	1.265	0.915	0.891	1.024	2.454	1.777	1.612	1.948	56.41	69.68	83.69	69.93	4.070	4.181	2.983	3.745
RAH-100 (LC)	0.963	0.892	0.682	0.846	1.878	1.630	1.330	1.613	39.96	62.28	68.65	56.96	2.092	2.186	1.914	2.064
Mean	1.037	0.829	0.700		1.989	1.589	1.334		46.41	63.61	71.80		3.846	2.785	2.398	
	F	G	F×G		F	G	F×G		F	G	F×G		F	G	F×G	
SEm±	0.017	0.006	0.029		0.032	0.012	0.055		1.17	0.44	2.02		0.088	0.033	0.152	
CD (*p*=0.05)	0.063	0.024	0.110		0.120	0.045	0.208		4.39	1.66	7.61		0.330	0.125	0.571	

F: Field capacity; G: Genotypes; F_1: 100 % Field capacity; F_2: 50% Field capacity; F_3:25% Field capacity higher total chlorophyll content (4.296 and 4.146 mg g^{-1} fr. Wt.) which was followed by BS-39 and LRA-5166 (3.914 and 3.745 mg g^{-1} fr. Wt., respectively) and lower total chlorophyll content was observed in RAH-100 (2.064 mg g^{-1} fr. Wt.) followed by TSH-04/115 and NDLH-1943 (2.350 and 2.353 mg g fr. Wt. $^{-1}$, respectively).

4. Conclusion

The germination percentage, shoot length, root length, root to shoot ratio, proline content, total chlorophyll content and seedling indices were recorded. All these observations summarized that, genotypes viz*.,* Sahana, BS-37, LRA-5166, CCH-12-3 GBHV- 177, BS-39, GBHV-182, ARBH-1352 were found to be drought tolerant at higher osmotic potential of -0.39 MPa and -0.14 MPa and at lower field capacities of 25 and 50%.

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