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In vitro screening for salinity stress and drought tolerance in rainout shelter among parental genotype seedlings of cotton (*Gossypium hirsutum* L.)

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Abstract
Among the abiotic stresses salinity and drought stresses are the major ones that cause severe losses to cotton yields. To cultivate the suitable ones that can tolerate those stresses is of great importance. Two parental cultivars CPD-813 and 8-1-2, which had been taken from Multi Environment Stress Resistance System were screened for salinity stress under in-vitro conditions and for drought, in a rainout shelter. Nine different traits of root and shoot were studied in case of drought screening at rainout shelter and seven traits for salinity stress. 8-1-2 genotype was found to be more tolerant to drought and salinity stress. Utilitarian genetic variability can be used in different crop improvement programs and for studying the molecular basis of complex trait drought tolerance.

Keywords: *In vitro* screening, salinity stress, drought stress, rainout shelter, cotton seedlings

Introduction
Cotton is a significant fibre yielding and vegetable oil crop widely grown all around the world. In India, it is grown in 122.38 lakh hectares with an overall production of 361 lakh bales having productivity of 501 kg/ha (Cotton Corporation of India 2018-2019). Drought and salinity stress are the two major abiotic stresses which are responsible for remarkable losses in cotton yield (Bohnert, 1995) [2], (Turner, 1997; Sinclair, 2005) [10, 9]. Due to salinity stress, the relative growth and the yield and quality are highly affected (Shannon, 1984) [8]. So, the biomass production of a plant is considered one of the most important criteria for selection under such salinity conditions (Kingsbury & Epstein, 1984; Jafri & Ahmad, 1994; Martin *et al*., 1994) [5, 4, 6]. In the case of drought, morpho-physiological traits during seedling growth study depict the response of the genotype to external environmental conditions and buffering capacity of the genotype. (Chaves and Rodrigues, 1987; Winter *et al*., 1988; Xu and *et al*., 1995; Pace *et al*., 1999) [3, 2, 14, 7]. It was observed that drought-stressed cotton seedlings had shown a significant increase in taproot length followed by a decrease in diameter (Pace *et al*., 1999) [7]. The current research was done to trace out the difference between the two genotypes for salinity and drought stress in the case of different traits.

Materials and Methods
Two parental genotypes namely CPD-813 and 8-1-2 taken from Multi Environmental Stress Resistance System (www.cicr.org.in), Cotton: Biotechnological Approaches (Usha Barwale Zehr). Both the genotypes were subjected to in-vitro screening for salinity stress and drought tolerance under rainout shelter conditions. The procedure followed to study the morphological parameters *in vitro* screening for salinity stress of the two parents i.e., CPD- 813 and 8-1-2 is as follows. MS Agar media was prepared with 1-litre distilled water to which 30 grams of sucrose was added. The concentrations of calcium chloride, magnesium sulphate, sodium bicarbonate, sodium chloride and agar to be added to 500 ml separately to maintain an EC -6 levels. The pH of the solution to which salts were added was stabilized at 5.6-5.8 (Ashraf, 2002) [1] Seeds were sown on this media in test tubes and allowed to germinate. The following observations were recorded 8 days after sowing on the growing plantlets. Root length (cm), Shoot length (cm) Root fresh weight (g) Root dry weight (g) Shoot fresh weight (g) Shoot dry weight (g) Total biomass dry weight (g).

Screening for drought
This experiment included the root and shoot studies on two parents, CPD- 813 and 8-1-2 in

pots (plastic pipes of 15 cm diameter and 4 feet length) where the two parents were tested in five replications each following the methodology described below.

10 pots were taken and saturated with water. Five were considered as control and another five as treatment. Control pots were watered every three days consecutively but the treatment pots were imposed moisture stress for 7 days and 14 days and moisture level were compared with control. The intention was to get less than 50 per cent moisture level in the treatment pots as compared to control pots. It was found that the 15-day period was to be taken as a drought induction period. The two varieties were grown both in control and treatment pots and compared with each other for the characters shown below through the destruction method. Taproot length (cm), Shoot length (cm), root fresh weight (g), Shoot fresh weight (g), root dry weight (g), Shoot dry weight (g), Ratio of Root dry weight: Shoot dry weight (RW: SW), Number of lateral roots Chlorophyll Stability Index (CSI)

The per cent reduction or increase in the trait expression upon imposition of the stress on both genotypes in both the experiments was compared by the 'two-sample t-test between per cents for all the traits. Stat Pac. Inc. (2011), a statistical package was used for calculation. Significant per cent changes between the two genotypes under treatment give the differential response of the genotype *In vitro* study for salinity stress in two genotypes

Results and discussion

The following traits were studied viz., shoot fresh weight, shoot length, root fresh weight, root length, total dry weight (biomass), root dry weight and shoot dry weight after 8 days of inoculation of the germinated embryo in the test tubes of control and treatment. It was observed that for all the traits except root dry weight, the genotype 8-1-2 had higher values than CPD-813 under control. And, in the treatment, all the traits of 8-1-2 had higher per se values than CPD-813. Upon imposition of salinity stress, the per cent change in trait expression of both genotypes ranged from 24.28 per cent (8-1-2 for shoot length) to 82.20 per cent (CPD-813 for root length). However, CPD-813 consistently had a greater per cent change compared to 8-1-2 across all the traits except, shoot dry weight. The two-sample t-test between per cents was significant for total dry weight and shoot dry weight only (Table 1). Response to salinity stress can be observed in figure1. The results of the pot experiment are presented in Table 2 and the response of the genotypes in figures 2 and 3. At both stages of observation (except for chlorophyll stability index at 15 days) genotype 8-1-2 had higher per se values than CPD-813 across all traits. The same trend continued upon imposition of drought stress with 8-1-2 having higher per se values than CPD-813 for all traits except root to shoot dry weight ratio at 15 days. The two important morphological underground traits supposed to be indicators of drought tolerance i.e., taproot length and the number of lateral roots had greater values in 8-1-2 than CPD-813. The per cent reduction in trait expression across all the 9 traits observed at both stages (15 and 30 days) after drought imposition in both the genotypes was appreciable, ranging from 2.34 (shoot dry weight at 15 days in 8-1-2) to 63.11 (root fresh weight at 30 days in CPD-813). The genotype 8-1-2 showed a lesser reduction in trait expression compared to CPD-813 for 5 traits at both 15 and 30 days. However, at 15 days and 30 days, 8-1-2 showed a greater reduction in root dry weight and shoot dry weight than CPD-813, respectively. For the root to shoot dry weight ratio and chlorophyll stability index, 8-1-2 showed greater reduction than CPD-813 at both stages. The two-sample t-test between per cents done to see if any difference existed between the response of the two genotypes to drought stress revealed a significant difference between the two genotypes for all traits except shooting fresh weight. Other traits (except root dry weight at 15 days, root to shoot dry weight ratio at 30 days and chlorophyll stability index at 30 days) showed a significant difference between the two genotypes, 8-1-2 and CPD-813 to stress imposition.

Table 1: Differences in various traits between two genotypes for salinity stress response under *in vitro* conditions at ARS, Dharwad

Genotypes (G)	Shoot fresh weight (g)	Shoot length (cm)	Root fresh weight (cm)	Root length	Total dry weight (g)	Root dry weight (g)	Shoot dry weight (g)
Control (normal condition)							
8-1-2	0.1880	4.8500	0.0430	4.4800	0.036	0.0050	0.0310
CPD-813	0.1500	4.3500	0.0360	3.3500	0.026	0.0060	0.0200
Treatment (T) (after salinity stress)							
8-1-2	0.1420	2.8300	0.0350	2.5200	0.0210	0.0040	0.0170
CPD-813	0.0710	0.8400	0.0240	1.0000	0.010	0.0010	0.0090
Per cent reduction in trait expression after salinity stress imposition							
8-1-2	32.66	24.28	56.50	50.85	31.47	25.00	78.69
CPD-813	52.10	51.81	75.71	82.20	42.42	75.00	63.17
Two sample t-test between per cents							
t (Table)	0.62	0.89	0.64	1.05	0.35	1.58	0.54
t (cal)	0.55	0.39	0.54	0.32	0.72*	0.15	0.60*

Table 2: Differences in various traits between the two genotypes among control and treatment for root and shoot characters in the pot experiment at ARS Dharwad.

Genotypes (G)	Root fresh weight (g)		Shoot fresh weight (g)		Taproot length (cm)		Shoot length (cm)		Number of lateral roots		Root dry weight (g)		Shoot dry weight (g)		Root: shoot dry weight ratio		Chlorophyll stability index	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
Control (normal watering condition)																		
8-1-2	2.45	12.40	17.85	21.11	26.74	40.58	38.40	45.40	70.62	78.58	1.58	1.60	5.12	7.82	0.31	0.20	55.93	45.17
CPD-813	1.58	8.54	13.26	17.56	18.56	31.86	32.40	38.10	55.76	68.36	0.81	0.86	3.65	5.22	0.24	0.16	59.23	32.42
Treatment (T) (after drought stress)																		
8-1-2	1.67	6.73	15.46	20.12	36.42	50.40	39.80	50.40	83.12	86.44	0.79	1.42	5.00	6.37	0.16	0.22	69.64	70.22
CPD-813	1.06	3.15	11.11	14.26	28.60	42.80	34.90	44.80	77.00	79.36	0.73	0.74	3.40	4.53	0.21	0.16	63.45	47.44
Per cent change in the two genotypes after imposition of drought																		
8-1-2	31.84	45.73	13.39	4.69	26.58	19.48	3.52	9.92	15.04	9.09	50.00	11.25	2.34	18.54	48.80	-8.95	19.69	35.67
CPD-813	32.91	63.11	16.21	18.79	35.10	25.56	7.16	14.96	27.58	13.86	9.88	13.95	6.85	13.22	8.87	-5.27	6.65	31.66
Two sample t-test between per cents																		
t (Table)	0.04	0.55	0.13	0.69	0.29	0.23	0.25	0.24	0.48	0.23	1.38	0.13	0.34	0.23	1.34	0.23	0.61	0.13
t (cal)	0.97*	0.59*	0.90	0.50	0.77*	0.82*	0.80*	0.81*	0.64*	0.81*	0.20	0.90*	0.74*	0.82*	0.20	0.83*	0.56	0.89*

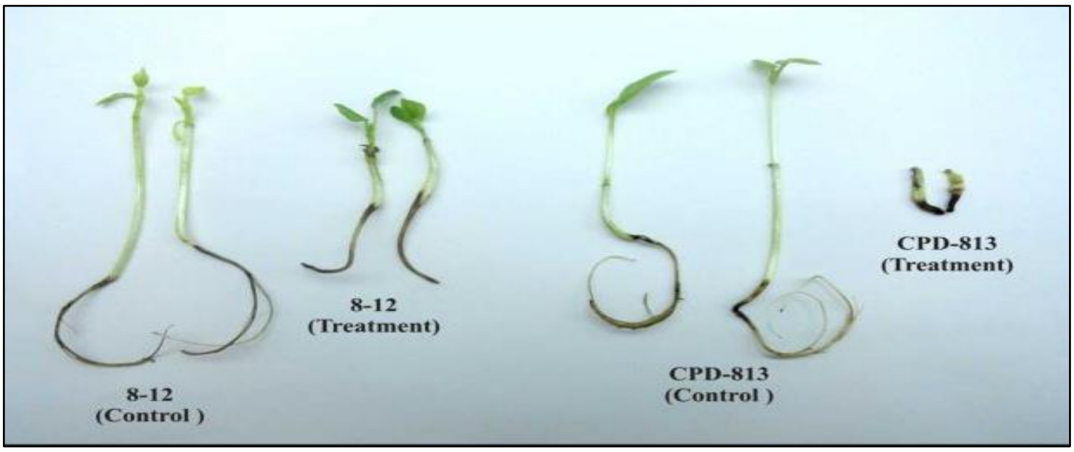

Fig 1: Salinity stress response (up)

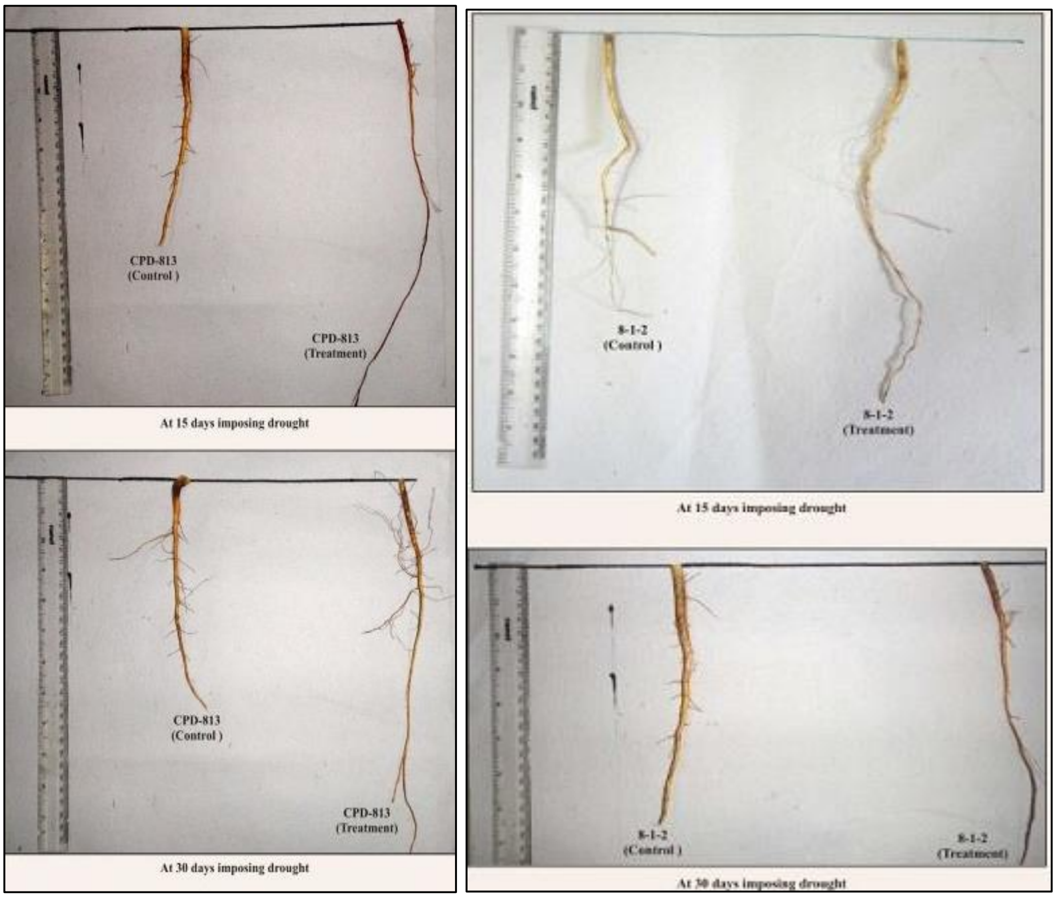

Fig 2, 3: Response of taproot to imposed drought (down)

Conclusion

Considering the diverse nature of CPD-813 and 8-1-2 established in the field, pot experiments and laboratory *in vitro* conditions and also the differential stress response between the two genotypes, they can be used in further studies to understand the molecular basis of diversity. The hybrid CPD-813 x 8-1-2 had the highest root to shoot ratio for which it was selected and its F3 lines have now given high seed cotton yield, certainly a notable achievement of the present study. Parental genotypes 8-1-2 and CPD-813 can be subjected to molecular breeding methods to find out the genetic basis of drought and salinity stress response.

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