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Physiological and biochemical evaluation for drought tolerance in wheat germplasm collected from arid western plains of India

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Wheat is an important cereal crop and securing an increase in wheat production is necessary to feed the growing population. Integration of drought tolerance into new varieties attracts researchers as it helps to develop climate resilient wheat. Here, we tried to identify new sources of drought tolerance in crops using the targeted germplasm collection approach and to establish the relationship between different morpho-physiological, biochemical traits and stress susceptibility index. A set of 18 wheat genotypes collected using focused identification germplasm strategy (FIGS) was evaluated for drought tolerance at seedling stage. Stress was imposed by keeping the pots at 40 % field capacity for one week followed by watering to allow recovery. Shoot length, shoot dry weight, relative water content, chlorophyll, membrane stability and seedling survival declined, and proline content increased in all the genotypes under stress. Root length and dry root weight also increased in IC333095 and IC112205 in response to water scarcity. Correlation study showed positive correlation between seedling survival and shoot length (0.51), relative water content (0.44) at $P<0.05$ and shoot dry weight (0.64) and root dry weight (0.70) at $P<0.01$. Based on different morphological and physiological parameters and drought susceptibility index, IC333095 (0.37), IC615005 (0.44), Dharwad Dry (0.44) and C306 (0.45) were considered drought tolerant genotypes. Further, it was concluded that shoot dry weight, root dry weight, shoot length and relative water content are the most reliable parameters for phenotyping against drought stress at an early stage.

Keywords: Abiotic stress, Drought susceptibility index, FIGS, Phenotyping, *Triticum aestivum*

Drought is the most devastating stress limiting crop production worldwide. In the developing world, wheat is produced under rain fed condition without supplementary irrigation[1]. It has been estimated that drought annually[2] hits half of the wheat-cultivated area. Wheat growth is particularly sensitive to drought during seedling establishment, booting and grain filling stages[3]. Autumn sown wheat faces water deficit at early growth stages. Low soil moisture during germination reduces germination percentage and delays the germination process affecting maturity and yield of plants[4]. Water scarcity at an early stage leads to non-vigorous plant stand and poor sink development, which affect yield adversely at maturity[5]. Depending upon the stress intensity, seedling stage drought may be more detrimental to yield in comparison to stress at later growth stages[6]. Therefore, it is necessary to ensure the proper seedling establishment and vigorous plant stand soon after germination.

Screening of crop germplasm is widely accepted strategy to identify trait specific lines. The Focused identification of germplasm strategy (FIGS) is a targeted germplasm collection method, which helps in improving the efficiency of identification of trait specific germplasm. The FIGS approach uses the environmental data to identify the most suitable collection site, so that germplasm collected from these sites possess the maximum probability for the presence of trait.

Phenotyping for drought tolerance at seedling stage is a common approach used in wheat, barley, triticale, maize, and rice[7-10]. It is rapid, cost effective and reliable for evaluation of plant's performance[10]. Reports are available on the correlation between drought tolerance at seedling stage and reproductive stage in wheat and cowpea establishing the importance of screening for drought tolerance at seedling stage[11,12]. Seedling survival, dry weight, root shoot ratio and root length, relative water content, seed reserve mobilization are the traits, which have used for screening of germplasm for drought tolerance[13-15].

Though a large number of studies have been conducted on drought tolerance in wheat, screening of wheat germplasm using the appropriate collection and evaluation strategy has not gained much attention.

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Keeping it in view, the present study was carried out to identify drought tolerant wheat genotypes by of morpho-physiological and biochemical responses under drought stress.

Materials and Methods

Germplasm collection

The FIGS was used to collect the wheat germplasm, and the sites were identified based on environmental factors (temperature, rainfall), altitude/geo-coordinates, soil type, cultivation practices used in the area by the farmers. The germplasm lines were collected from Pratapgarh, Banswara and Dungarpur in southern Rajasthan, which comes under the arid western plain agro-ecological region of Indian council of agricultural research. Wheat was the staple crop in the area, which was cultivated under rainfed conditions. Mean temperature ranged >15-18°C during anthesis and prevailed >35°C during grain filling period. Rainfall was in the range of 750-900 mm. All the wheat local cultivars collected were grown without any irrigation in the remote pockets of these districts and the natural rainfall during *rabi* was hardly 10 mm during crop growth period.Soil type was a red type with poor fertility.

Evaluation for drought tolerance

A pot experiment was conducted with eighteen wheat accessions collected by using the focused identification of germplasm strategy from drought-hit areas of Rajasthan and Madhya Pradesh, India. Two drought tolerant varieties (C306 and Dharwar Dry) were used as a check. The experiment was conducted in a green house in randomized complete block design and was repeated twice. Plants were grown in plastic pots (15 × 15 cm) filled with 1 kg sand. At the time of sowing, five seeds were sown in each pot. After germination, they were thinned to one plant per pot. Plants were supplemented with the Hoagland solution alternatively and were watered to maintain 80% field capacity. Stress was implemented after two weeks of sowing by withholding the watering up to 40% field capacity. After one week, plants were watered again to allow recovery. All the physiological parameters were recorded on the youngest fully expanded leaves. Seedling survival was recorded after one week of watering the plants. The plants were harvested carefully and washed properly to remove sand. Root and shoot length was measured immediately, and samples were dried in hot air oven at 65°C for 48 h. Dry weight was recorded after complete drying of the samples.

Chlorophyll content was recorded with a self-calibrating chlorophyll meter (Optiscience, USA) on the fully expanded leaves and expressed as SPAD units. Data was taken with three replications each from control and drought treatment.

A mid leaf section was collected and weighed to record fresh weight (FW). Samples were hydrated in double distilled water for 4 h and weighed to record turgid weight (TW). Then the samples were dried at 65°C for 48 h and dry weight was observed (DW). The relative leaf water content was calculated based on the following formula[16].

$$RWC\ (\%) = (FW- DW) / (TW- DW) \times 100$$

To determine proline content, leaf tissue (0.01 g) was homogenized in 10 mL 3 % sulfosalicylic acid. The extract was centrifuged at 5000 g for 20 min. The supernatant (2 mL) was mixed with an equal amount of glacial acetic acid and ninhydrin solution. Samples were incubated in a boiling (100°C) water bath for one hr. After cooling, 5 mL toluene was added to the samples. Absorbance was recorded in toluene layer at 520 nm, and proline concentration in the samples was calculated using a standard curve[17].

To measure, membrane stability index (MSI), leaf tissue (0.5 g) was collected in glass vials containing 10 mL double distilled water. Samples were incubated in a water bath at 40°C for 30 min. After cooling to room temperature, electrical conductivity (C1) was recorded with a conductivity meter (Sanco, India). Again, the samples were kept in a boiling (100°C) water bath for 10 min, and electrical conductivity (C2) was measured at room temperature. Membrane stability index (MSI) was calculated as follows[18,19].

$$MSI = [1- (C1/C2)] \times 100$$

All the experimental data were analyzed using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA). Two-way analysis of variance (ANOVA) was used to analyze the effect of genotype and treatment combinations. Physiological and biochemical parameters were recorded with three replications. Differences at $P<0.05$ were considered statistically significant. Linear correlation analysis was performed to study the relationship between studied parameters.

Results

All the parameters changed significantly in the all the genotypes in response to stress and treatment, genotype and treatment and genotype interactions were

Table 1—Analysis of variance of morpho-physiological and biochemical traits traits for drought tolerance at seedling stage

Source of variance	df	Root length	Shoot length	Root dry wt.	Shoot dry wt.	Chlorophyll	RWC	Membrane Stability Index	Proline	SS
Genotype	19	11.84**	63.2**	0.022**	0.002**	0.695**	136.61**	70.54**	0.02**	5.80**
Treatment	1	0.85*	1130.37**	0.004**	0.058**	4.524**	5460.50*	40509.85**	1.04**	625.63**
Genotype* Treatment	19	1.95**	39.15**	0.025**	0.002**	0.255**	48.68*	75.62**	0.02**	5.80**
Error	80	0.69	2.27	0.00005	0.0001	0.0581	81.02	1.68	0.0004	0.425

*Significant at 0.05 and ** significant at 0.01 probability level

Table 2—Changes in morpho-physiological parameters in wheat accessions under drought stress

Traits	Mean		Range		%Reduction/Increase
	Control	Drought	Control	Drought	
Shoot length (cm)	29.86	22.72	20.6-38.76	15.9- 27.2	(-) 20.56
Root length (cm)	12.04	10.87	9.50-14.17	7.17-12.53	(-) 9.71
Shoot dry weight (g)	0.11	0.06	0.06-0.15	0.03-0.10	(-) 45.45
Root dry weight (g)	0.08	0.07	0.05-0.11	0.03-0.14	(-) 12.5
Chlorophyll content index	1.79	1.40	1.23-3.07	1.13-2.43	(-) 21.78
Proline(µM g^{-1} fresh weight)	0.14	0.33	0.07-0.18	0.21-0.54	(+) 135.71
Relative water content (%)	83.51	64.64	80.27-95.61	51.28-74.25	(-) 22.59
Membrane stability index	98.00	`61.25	95.97-98.81	46.00-69.61	(-) 37.5
Seedling survival (%)	100	54.3	100	13.33-83.33	(-) 45.7
DSI seedling survival	-	1.00	-	0.37-1.93	-

significant (P<0.01) (Table 1). Percent reduction in chlorophyll content was 6-59.1 % compared to control condition (Table 2). Chlorophyll content index was the highest in drought tolerant variety Dharwad dry (2.4) followed by KP1870 (1.8) and IC252441 (1.7).

Shoot growth was affected adversely due to water scarcity. Genotype, treatment and their interactions were significant at 0.01 probability level (Table 1). We observed 3.6-39.4 % reduction in shoot length in different genotypes (Table 2). IC333095 registered the maximum shoot length (27.7 cm) followed by Dharwad dry (26.9 cm) and IC615005 (24.5 cm) under stress. KP1870 registered the lowest shoot length (15.9 cm) among the studied genotypes.

Different genotypes responded differently with respect to root length under water scarcity, and changes were significant with respect to genotype (P<0.01), treatment (P<0.05), genotype and treatment interaction (P<0.01) (Table 1, 2). Root length increased in Dharwad dry (3.6%), C306 (8.6 %), IC333095 (1.6%) and IC112205 (3.8 %) compared to control, while; it reduced 4.2-32.0% in rest of the genotypes. Highest root length was observed in Dharwad dry (12.5 cm), while, it was the lowest in KP1870 (7.2 cm).

All the genotypes showed a significant reduction in the dry matter under water limited environment (Tables 1 and 2). Percent reduction in shoot dry weight due to stress was 1.3-72.2% in different genotypes. IC333095 exhibited the maximum shoot dry weight (0.10 g) followed by Dharwad dry (0.08 g). Shoot growth was the most adversely affected in KP1870, where dry weight was 0.03 g under drought condition.

Genotype, treatment and their interaction led to significant variation in root dry weight (Table 1). It varied from 0.03-0.14 g among different genotypes under stress (Table 2). IC112205 and IC333095 registered higher root dry weight (0.14 g and 0.13 g, respectively) than drought tolerant varieties Dharwad dry (0.10 g) and C306 (0.09 g). Root dry weight was the lowest (0.03 g) in KP1870 and IC614999 in stress condition.

MSI reduced significantly in all the genotypes under the influence of water scarcity (Table 1). Percent reduction in MSI ranged from 95.97-98.81 under control and 28.5-53.3 under drought stress (Table 2). IC333095 had the maximum MSI (69.61) followed by Dharwad dry (68.46) and C306 (66.85). Genotype IC615006 (46.00) showed the maximum membrane injury followed by IC615007 (48.05) under the influence of stress.

RWC is an important physiological trait representing the water status of the tissue. We observed 10.2-37.7% reduction in RWC during stress (Table 2). Genotypic differences in RWC were significant at P<0.01, while treatment, genotype,and treatment interaction were significant at P< 0.05 (Table 1). RWC

was the lowest in KP1857 (51.28%) followed by IC615011 and IC615008 (53.3%). KP1876 maintained the higher RWC (74.25%) compared to Dharwad dry (73.44%) and C306 (71.34%).

Proline increased significantly in all the genotypes in response to stress condition (Tables 1 and 2). The increase was up to 7-fold depending upon the genotype. Proline content was the highest in genotype IC615005 (0.54 µM g^{-1} fresh weight) followed by C306 (0.49 µM g^{-1} fresh weight) and KP1868 (0.44 µM g^{-1} fresh weight). KP1870 exhibited the lowest proline (0.21 µM g^{-1} fresh weight) in response to drought.

Seedling survival showed the ability of a genotype to recover from the stress conditions. Genotype, environment and their interactions were highly significant (Table 1). Percent seedling survival varied from 13.3 to 83.3 among different genotypes (Table 2). Recovery was the maximum in IC333095 (83.3%) followed by C306, Dharwad dry and IC615005 (76.7%). When DSI was calculated based on seedling survival under stress, it ranged from 0.37 to 1.93 (Table 2). Genotypes with DSI <0.5 were selected as drought tolerant genotypes namely IC333095 (0.37), IC615005 (0.44), Dharwad dry (0.44) and C306 (0.45). Genotypes with moderate drought tolerance (1 < DSI > 0.5) were IC615003 (0.67), IC112205 (0.67), KP1876 (0.74), KP1868 (0.81), IC615011 (0.81), IC615006 (0.96) and IC615009 (0.96). Genotypes with DSI >1 were adjudged as drought susceptible genotypes. KP1870 showed the highest DSI (1.93) followed by IC614999 (1.70).

We studied correlation among the studied traits to find out the feasibility of parameters, which can be relied on for phenotyping (Table 3). Under control conditions, shoot dry weight was positively correlated withthe shoot length (r = 0.69), and root length showed a positive correlation with MSI (r = 0.68). Under stress conditions, more relationships between the parameters became evident. Root dry weight was positively correlated to shoot dry weight (r = 0.67). Seedling survival had moderate positively correlation with shoot length (r = 0.51) and RWC (r = 0.44) at $P<0.05$. Strong positive correlation was observed between seedling survival and shoot dry weight (r = 0.64) and root dry weight (0.70) at $P<0.01$.

Discussion

In arid and semi-arid areas, water scarcity is the major limitation to crop productivity. Drought response is the outcome of different morphological, physiological and biochemical alterations triggered at the molecular level. Reduced leaf water potential and turgor inhibit the growth during water scarce conditions[20]. In the present study, drought affected seedling growth culminating into mortality in genotype dependent manner. Consistent with the results reported in the earlier studies, drought induced reduction in shoot length, root length, shoot dry weight, root dry weight, chlorophyll, RWC, MSI, seedling survival, and increase in proline[4,9,12, 21-23]. The amplitude of change varied among different lines and the traits were affected by treatment, genotype and the interaction between treatment and genotype (Tables 1 and 2). Correlation analysis between morpho-physiological traits, seedling survival and DSI indicated that shoot dry weight, root dry weight, shoot length and RWC are correlated to seedling survival (Table 3).

Table 3—Correlation coefficient of different morphological and physiological parameters and drought susceptibility index under stress and non-stress condition

	Chlorophyll	Shoot length	Root length	Shoot dry wt.	Root dry wt.	Proline	Membrane Stability Index	Relative water content
Chlorophyll	1	0.35	0.03	0.39	0.02	0.21	0.05	0.21
Shoot length	0.04	1	0.29	0.69**	0.06	0.30	0.25	0.25
Root length	0.02	0.19	1	0.38	0.42	0.02	0.68**	0.23
Shoot dry wt.	0.23	0.43	0.03	1	0.21	0.11	0.36	0.01
Root dry wt.	0.21	0.40	0.22	0.67**	1	0.10	0.13	0.05
Proline	0.11	0.43	0.15	0.17	0.35	1	0.34	0.14
Membrane stability index	0.28	0.06	0.21	0.39	0.06	0.12	1	0.10
Relative water content	0.10	0.39	0.27	0.42	0.33	0.29	0.17	1
Seedling survival	0.10	0.51*	0.02	0.64**	0.70**	0.32	0.26	0.44*
Drought susceptibility index	0.12	-0.51*	0.02	-0.63*	-0.69**	-0.34	0.24	-0.45*

Above Diagonal-Correlation coefficient among traits in control condition; below diagonal- Correlation coefficient among traits under drought stress; * and ** denotes significance at $P<0.05$ and $P<0.01$, respectively

Drought tolerant wheat variety registered the highest germination percentage, shoot length and shoot weight and root weight under polyethylene glycol induced stress conditions. Growth depends upon the maintenance of turgor pressure inside the cell. During water deficit, when the plant is not able to maintain its water potential, growth is inhibited. Therefore different morphological parameters related to growth showed significant reduction in response to stress. Different workers in wheat and other crops recorded similar observations under drought. It was observed that root dry weight and shoot dry weight was correlated to drought tolerance[4,21], while, also proposed shoot length as potential selection trait for drought tolerance in wheat[24]. We also observed similar relationship in the present study. The role of root growth in response to drought is well documented in wheat, and the strong negative correlation between root length and DSI reflected the potential of root traits in drought tolerance [24,25]. We also observed a strong negative correlation between seedling survival and root length (P<0.01). Water use efficiency, biological yield, shoot dry weight, root shoot ratio and wilting percentage also served as potential traits for selection of drought tolerant genotypes in wheat[26]. A significant correlation between DSI and different growth related traits showed that genotypes with the maximum growth potential might sustain stress more efficiently as reported earlier in wheat. The relation between plant height and seedling traits is well documented in different studies [12,27]. Tall wheat varieties were reported to be more tolerant to post anthesis drought stress because of their capability to partition more stem reserves towards grain filling[28]. The current findings agree with the observations made by above authors regarding the role of growth traits in drought tolerance.

Maintaining cell water status is crucial to growth and development. RWC was proposed to be closely linked to drought tolerance[9,10]. Though we reported a consistent reduction in RWC in all the studied lines, in Dharwar Dry, C 306 and KP 1876 was correlated withseedling survival under stress. We also reported a significant negative correlation between DSI and RWC could be elucidated. These results are in agreement with theearlier study where maintenance of tissue water status under stress condition led to drought tolerance in maize and triticale genotypes[9].

Drought stress leads to chlorophyll degradation and is one of the most frequently used parameters. Chlorophyll content reduced in all the genotypes in response to stress but was not correlated to drought recovery. Similarly, the osmotic adjustment is an important mechanism and accumulation of proline, and other incompatible solutes havebeen used as an indicator of drought tolerance. Proline increased multiple folds in different genotypes under water-limited environment, but its relation to drought recovery was non-significant. Therefore, it was concluded that it was a common strategy used by all the genotypes to combat water scarce conditions.

Membrane stability was proposed as an indicator of drought and heat tolerance at seedling and anthesis stage in wheat[23]. The trait was positively correlated with the thousand-grain weight under drought as well as heat stress. We observed that IC333095, the drought tolerant genotype showed the maximum membrane stability followed by Dharwar Dry and C306. However, we did not observe any significant correlation between seedling survival and membrane stability.

Drought tolerance is a complex trait and selection for drought tolerance is hampered due to difficulty in identification of potential traits for phenotyping under water-limited environment[3]. Incorporation of drought tolerance is the immediate requirement to develop climate resilience in our food crops[29].

In the present study, drought adaptive capabilities were assessed based on seedling growth and survival in response to stress. Though positive correlations have been established between drought tolerance at seedling stage and reproductive stage, it may not necessarily replicate field drought adaptability. Phenotyping at seedling stage with different morpho-physiological traits indicates the potential of wheat genotypes for drought tolerance.

Conclusion

In the present study, two accessions namely IC333095 and IC615005 were selected as drought tolerant based on morpho-physiological parameters and drought susceptibility index. Root dry weight, shoot dry weight, root length and relative water content are suitable traits, which can be used for screening of wheat lines for drought tolerance at early growth stage.

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