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Short Research Article

Evaluation of Drought Stress Tolerance Efficiency of Wheat (*Triticum aestivum* L.) Genotypes at Germination and Seedling Stages

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Abstract

Wheat (*Triticum aestivum* L.) is world-wide used as a staple food crop that significantly affected by abiotic stresses such as high-low temperatures and drought. Tolerance to water stress is a complicated parameter in which wheat performance influences by a number of characteristics. In the present investigation, a feasible unconventional strategy has been applied by employing PEG-6000 in order to *in vitro* screening of ten bread wheat cultivars for drought tolerance at germination and seedling stages during the year 2014-15. Water stress condition was induced by 15%, 20% and 25% of PEG-6000 containing nutrient solutions and maintained in a hydroponics culture treatment with three replications applying completely randomized design (CRD). After 10 days, seven stress-associated indices, PI, GSI, PHSI, RLSI, SLSI, DMSI and RWCSI from drought-stressed and non-stress wheat seedlings were estimated and demonstrated that all the indices efficiently illustrate highly drought tolerant and susceptible wheat genotypes. Statistical analysis including ANOVA, mean rank, standard deviation of ranks and rank sum (RS) revealed that germination percentage, shoot length and root length significantly declined due to water deficit and distinguished genotypes, SUJATA, MP1500 and HI1077 as highly drought tolerant genotypes and significantly superior in seedling growth tolerance under increased levels of water stress whereas, GW322, MP1531 and GW273 was found susceptible to drought stress. It was concluded that measuring stress by elicited indices varies with the stress severity and provides an effectual platform for rapid screening of tolerant genotypes against potent drought stress at seedling stage.

1. Introduction

Wheat (*Triticum aestivum* L.) is the first grain crop consumed as a staple food by more than 35% of the world population (Metwali et al., 2011) and generally grown under rainfed conditions in developing countries (Zala et al., 2014). Due to climate change, cultivation of wheat is significantly affected by abiotic stresses such as high-low temperatures and drought are the serious problems for agriculture reducing the crop productivity with losing yield up to 80% in arid and semi arid regions of the world (Fleury et al., 2010). Development stages such as germination, seedling or flowering are the most critical and sensitive for drought stress in the life cycle of plants (Ashraf and Mahmood, 1990). Therefore, plants develop different defence mechanisms (morphological, physiological and biochemical characteristics) which inhibit or eliminate the harmful effects of stresses (Marcińska et al., 2013).

Screening techniques have been accomplished under several breeding programmes for the development of drought tolerant varieties. Drought is a major constraint seriously influences wheat production and quality but development of tolerant cultivars is hampered by the lack of efficient assortment criteria (Mardeh et al., 2006). Many conventional and specific traits such as osmotic regulation, root-shoot length and root penetration have been suggested to select crop genotypes against drought stress (Fukai and Cooper, 1995). Baloch et al. (2012) also used some drought attributed traits such as seed germination, seedling vigour, root-shoot length, relative water content (RWC) to screen drought tolerant varieties/ genotypes. Plant breeders directly select tolerant genotypes according to grain yield (GY) in the target environments as the main criterion. To differentiate drought tolerant genotypes, Huang (2000) suggested a number of selection indices on the basis of a mathematical relationship between favorable and

stress conditions and has been proposed by estimating some indices to determine drought tolerance i.e. Stress tolerance level (TOL), stress tolerance index (STI), stress susceptibility index (SSI) and drought tolerance efficiency (DTE), which may be useful as an indicator to identify drought tolerant genotypes that perform well in stress environments (Kumar et al., 2014). Various plant physiological indices including RWC, MDA accumulation, free proline content etc., have also been widely used to evaluate tolerance genotypes under water stress conditions (Sultan et al., 2012).

Wheat breeding requires the investigation for traits related to drought tolerance is an important action. In field experiments, screening of different cultivars is the most reliable approach to assess their drought tolerance. However, winter wheat requires 2~3 years and a very large area if several cultivars are evaluated at the same period. Therefore, the various techniques for evaluating drought tolerance of wheat plants in laboratory have been developed and employed to obtain appropriate results for it. Certain stimulators such as polyethylene glycol (PEG), Mannitol used to induce water stress in plants (Emmerich and Hardegree, 1990). Among these chemicals, application of PEG-6000 is an unconventional approach and successfully used to screen drought tolerant varieties/genotypes at early stage of seedlings under laboratory condition (Lu and Neumann, 1998). Polyethylene glycol acts as a non-penetrating osmotic agent resulting increased solute potential and blockage of absorption of water by the root system. PEG-6000 in a hydroponic solution changes in the water potential of the plant with decreasing its growth and biomass production (Grzesiak et al., 2003). The present study was aimed to develop a rapid and straight forward screening technique for wheat genotypes against drought stress at early seedling stage using PEG-6000 as well as to establish associations among drought related seedling traits. The screened drought tolerant cultivars will then be used for molecular study and manipulating susceptible genotypes into drought conditions under wheat breeding programmes.

2. Materials and Methods

2.1. Plant material

Ten genotypes of bread wheat (Table 1). Were screened for drought tolerance in an experiment laid out in completely randomized design (CRD) with three replicates incubated at 25±2 °C during the year 2014-15. PEG-6000 as drought stimulator was used at three water stress levels, 15, 20 and 25% with control (distilled water) containing 1.1 g l^{-1} Murashig-Skoog media as hydroponic cultures for 10 days. To protect from microbial attacks, seeds were surface sterilized with 1% Sodium hypochlorite for two minutes followed by three washings with distilled water. Thirty seeds of each genotype were positioned on net at media surface in separate beakers.

Table 1: List of ten wheat genotypes with their pedigree

Sl. no.	Genotype	Pedigree
1.	GW273	CPAN2084/VW205
2.	GW322	PBW173/GW196
3.	HI1077	GALL/AUSTII61-151/CNDN066/3*KAL
4.	GW190	VEE/3/BB*S*/SKA/ARJUN
5.	DL803-3	K7537/HD2160MUT/HD2278/DL896-2
6.	MP3020	C306/CB spring (BW) 84
7.	HI1500	HW2002*2/STREMOALLI/PWC5
8.	HI1531	HI1182/C[AN1990]
9.	HW2004	C306*7/TR380-14#7/3AG14
10.	SUJATA	Selection from C306

Germinated seeds were counted at two days interval (2, 4, 6, 8, and 10 days) and seedling growth parameters viz., fresh weight, turgid weight, shoot length and root length were recorded after 10 days. Plant dry weight was recorded after drying at 70 °C to a constant weight. From these seedling growth parameters, the promptness index (PI), germination stress tolerance index (GSI), plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and dry matter stress tolerance index (DMSI) were calculated using following formulas.

i) $PI=nd_2(1.0)+nd_4(0.8)+nd_6(0.6)+nd_8(0.4)+nd_{10}(0.2)$, where, nd_2, nd_4, nd_6, nd_8 and nd_{10} are the number of seeds germinated in the 2, 4, 6, 8 and 10 days, respectively; ii) G.S.I.=[P.I of stressed seeds/P.I control seeds]×100; iii) PHSI=(Plant height of stressed plant/Plant height of control plants)×100; iv) RLSI=(Root length stressed plant/Root length of control plants)×100; v) SLSI=(Shoot length stressed plant/Shoot length of control plants×100; vi) DMSI=(Dry matter of stressed plant/Dry matter of control plants)×100; vii) RWCSI=(Relative water content of stressed plant/Relative water content of control plants)×100.

2.2. Statistical analysis

The experimental data were analyzed by mean variables and mean values taken from measurement of three replicates. Collected data was subjected to statistical analysis of variance (ANOVA) using Windostat 9.1 advance statistical software package. The combined ANOVA was carried out to estimate the main effects of the different sources of variation. Relationship among genotypes and stress indices was estimated based on seedling parameters and the ranking method to determine the drought tolerant and susceptible genotypes.

3. Results and Discussion

3.1. Analysis of variance and mean comparison

All wheat genotypes exhibited a significant reduction in

germination percentage and plant growth parameters. The results of combined ANOVA indicated significant differences among genotypes and PEG concentrations for all traits (Table 2). Interaction between genotype×PEG concentration was found to be significantly correlated with GSI, PHSI, SLSI, DMSI, RLSI and RWCSI.

3.2. Plant growth parameters at germination and seedling stages

Germination percentage was recorded when the both plumule and radicle had emerged to 5 mm and white coleoptile was detectable after breaking the seed-coat. Earliest stage of germination was considered as Zadoks decimal growth scale for wheat after usual visual inspection (White and Edwards, 2008). Seed germination observed to be decrease as osmotic potential further fall towards the negative side. Application of 25% PEG solution severely affected seedling growth and causes cell mortality due to dehydration. Seed germination was lower under 20% PEG as compared to 15%. Whereas, per cent germination was significantly found to be lower in the genotypes GW273, GW322, GW190, DL803-3, MP1500, MP1531 and HW2004. Similarly, Guo et al. (2013) observed a decreased trend with increased PEG-6000 concentration and showed the reductions with greater above 15% concentration. Zala et al. (2014) reported that wheat genotypes including HW 2004 significantly showed higher percent germination in the reflecting the drought tolerance characteristics at low PEG concentration (5 and 10%). After 10 days of germination, GSI values found to be ranged from 69.28% to 86.54% for the 15% PEG compared to 55.61 to 74.72% at 20% PEG (Table 3). This indicates more prominent differences among genotypes at the lower osmoticum (Figure 1). At 15% PEG, the highest GSI value was recorded for wheat genotype HI1077 (86.54%) while the lowest (69.28%) for GW190. At 20% PEG, the highest GSI (74.72%) was recorded in Sujata, whereas lowest GSI (55.61%) in GW322 (Figure 1). Hegarty (1977) observed that water stress at seedling stage can result in delayed and reduced germination or may prevent germination completely.

Table 2: Analysis of variances for some growth parameters of wheat genotypes exposed to different PEG-6000 concentrations at germination and seedling growth stages

S.O.V.	df	Mean square					
		GSI	PHSI	RLSI	SLSI	DMSI	RWCSI
Genotypes (G)	9	42.286***	133.128***	158.901***	292.750***	1319.214***	5643.917***
PEG concentration (C)	1	1142.618***	3336.547***	12001.080***	5823.147***	2892.333***	16425.810***
G×C	9	27.691***	93.087***	582.824***	98.929***	168.333***	2206.315***
Error	36	1.226	1.568	2.051	1.631	1.525	1.549
CV%		2.923	3.079	2.626	4.605	5.034	2.340

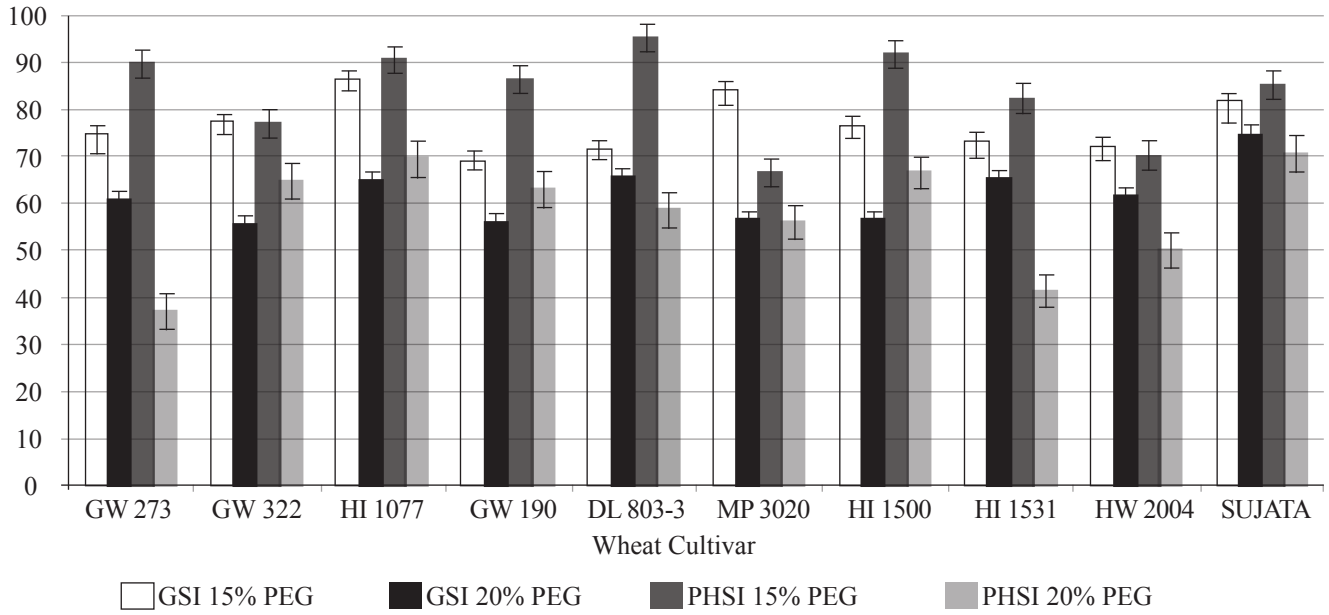

Figure 1: Mean of germination stress index (GSI) and plant height stress index (PHSI) of ten wheat cultivars in response to 15% and 20% PEG concentrations

Table 3: Mean showing standard deviation for growth characteristics of wheat genotypes under water stressed condition at germination and seedling growth stages

Cultivar	GSI		PHSI		RLSI		SLSI		DMSI		RWCSI	
	15% PEG	20% PEG	15% PEG	20% PEG	15% PEG	20% PEG	15% PEG	20% PEG	15% PEG	20% PEG	15% PEG	20 % PEG
GW273	75.12^{pq}±2.2	60.97^{cj}±2.5	89.86^{b}±2.6	37.20^{fj}±1.8	135.91^{h}±2.6	45.57^{ef}±2.2	70.17^{cj}±2.1	14.36^{kl}±1.0	18.71^{kl}±2.0	10.05^{gk}±2.0	184.10^{b}±1.6	43.805^{fg}±2.5
GW322	77.47^{lc}±2.1	55.61^{dj}±2.7	77.20^{ef}±1.1	64.95^{b}±3.9	130.55^{cj}±2.5	75.10^{dg}±3.2	39.82^{kl}±2.3	18.26^{ck}±1.4	17.95^{kl}±3.1	5.35^{hl}±1.5	335.96^{a}±3.3	126.71^{a}±0.5
HI1077	86.54^{fg}±0.9	65.09^{bh}±1.6	90.82^{b}±2.0	69.65^{fg}±1.2	105.86^{gj}±3.3	62.60^{ef}±3.2	62.05^{dj}±0.8	45.06^{b}±2.0	89.34^{fg}±0.9	71.89^{gh}±2.4	67.92^{lh}±2.1	58.58^{ef}±2.7
GW190	69.28^{fj}±1.4	56.14^{ej}±1.9	86.51^{cg}±0.9	63.21^{hj}±1.9	143.80^{ab}±2.5	49.63^{fj}±2.1	54.47^{ej}±1.1	34.21^{cj}±1.3	43.24^{ek}±1.3	15.21^{fj}±2.1	73.92^{gg}±0.9	58.71^{ej}±1.9
DL803-3	71.75^{gh}±2.2	65.62^{bh}±1.6	95.43^{ab}±1.8	58.61^{cj}±3.5	123.82^{dj}±1.5	77.59^{cg}±2.6	71.07^{cj}±2	27.66^{dj}±3.5	19.26^{kl}±1.0	12.92^{fg}±1.1	95.50^{fj}±2.3	58.80^{ej}±1.3
MP3020	84.47^{ab}±2.1	56.32^{ej}±1.8	66.82^{gg}±0.9	56.06^{cj}±3.2	97.26^{gj}±2.5	82.41^{bj}±2.5	56.00^{gg}±3.3	26.51^{dj}±0.5	44.94^{ek}±1.7	20.75^{ek}±2.8	134.48^{bc}±3.1	92.77^{bj}±1.8
MP1500	76.82^{cg}±0.8	56.52^{ej}±2.5	92.07^{bj}±2.4	66.67^{gh}±2	137.20^{bj}±1.1	76.61^{cj}±1.4	77.69^{bj}±1.9	53.06^{gk}±2.4	67.32^{dj}±2	59.57^{ej}±3	66.34^{lh}±1.7	55.56^{ej}±2.1
MP1531	73.50^{de}±2.9	65.23^{bh}±3.2	82.61^{dj}±2.3	41.40^{ej}±0.9	118.04^{ej}±3.4	43.54^{gg}±2.0	54.02^{gg}±2.7	19.82^{ek}±1.8	84.80^{bj}±1.9	23.70^{dg}±0.6	61.43^{lj}±3.0	29.20^{fg}±1.2
HW2004	72.28^{def}±1.6	61.81^{bc}±1.9	70.33^{fg}±1.8	50.15^{gh}±1.1	97.80^{gg}±2.1	71.51^{dj}±2.2	67.66^{cj}±3.5	12.66^{kl}±0.8	74.24^{cg}±2.5	24.69^{dg}±3.7	110.71^{dj}±2.2	44.30^{gg}±3.4
SUJATA	81.97^{bc}±1.6	74.72^{ag}±1.9	85.30^{cd}±2.1	70.72^{dg}±2.6	78.39^{hj}±2.6	94.15^{bj}±1.7	86.89^{ag}±2.1	46.99^{bh}±2.2	88.83^{bg}±2.2	63.99^{bg}±2.0	105.14^{cg}±2.6	93.91^{bj}±2.0
Mean	76.92	61.80	83.69	57.86	116.86	67.87	63.99	29.86	54.86	30.81	123.55	66.24

During present investigation, all the genotypes displayed reducing trend in seedling germination at 15% followed by 20% PEG as compared to control. Germination percentage and seedling growth have been reported to decrease at low moisture levels (Ashraf and Abu-Shakra, 1978). High values of the GSI indicated a high rate of germination which was inversely related to moisture stress. Increased concentration of drought inducer chemical significantly inhibits germination and growth of oxidative stressed plants due to suppression of cell expansion and cell growth in response to low turgor pressure (Nabil et al., 1995; Ogbonnaya, 2003).

The results based on plant height, root length, shoot length revealed that wheat genotypes varied from each other under all environments. PHSI data revealed significant differences among genotypes, PEG concentrations and genotypes×PEG concentration interactions. The highest PHSI value over the treatment was 95.43% for DL803-3 and the lowest (37.20%) for GW273. Overall experiment, PHSI decreased at both 15% and 20% PEG concentrations in all the genotypes (Figure 1). Ozturk (1999) has also reported that plant height decreased under the water stress condition. Lower water supply negatively affects all growth characters represented as plant height as compared with normal water supply (Hammad and Ali, 2014) due to restrict internode elongation and leaf expansion through inhibiting cell expansion. Furthermore, drought stress may reduce plant height due to dehydration of protoplasm, decrease in relative turgidity associated with turgor loss and cell division (Delfine et al., 2002; Hussain et al., 2008). Root is the first part of plant to be induced by drought stress (Shimazaki et al., 2005). Under drought stress condition, roots continue to grow to find water, but the airy parts of plant are restricted to expand (Nezhadahmadi et al., 2013). This different growth response of shoots and roots to drought is an adaptation to arid conditions (Sharp and Davis, 1989; Spollen et al., 1993). In the present study, wheat cultivars also differed significantly for increased shoot and root length under PEG induced water stress conditions. Root and shoot length increased with 15% PEG as compared to control however, further increase in PEG (20%) drastically reduced root-shoot length lower than the control and 15% PEG concentration. While comparing the growth performance of wheat genotypes under drought it was observed that increased PEG concentration decline the values of RLSI. Cultivar GW190 (143.80%) and Sujata (94.15%) showed maximum RLSI with both the PEG treatments (Figure 2). The SLSI values also reduced when genotypes were exposed to 20% PEG concentration (Figure 2). As observed in the present investigation, Younis et al. (2000); Bibi et al. (2010) also revealed that water stress suppresses shoot growth and increases the root length as compared to the plants under controlled conditions. In wheat, the root growth was not

markedly decreased under drought (Rao et al., 1993), whereas, moderate and high drought conditions reduces the growth rate of wheat roots (Noctor and Foyer, 1998). Wheat seedlings showed drastically decreased root and shoot length under PEG induced stress (Jajarmi et al., 2009). Decreasing trend for shoot length and root length also reported with increasing concentrations of poly ethylene glycol (Khan et al., 2013) and osmotic stress in wheat (Dhanda et al., 2004).

The DMSI values declined from 71.89 to 5.35% when exposed to 20% PEG as compared to 15% PEG (89.34 to 17.95%) (Figure 3). Reduced biomass under drought stress have been observed in several plant species including soybean (Specht et al., 2001), sunflower (Vanaja et al., 2011; Saensee et al., 2012), maize (Vanaja et al., 2011) and wheat (Wahid and Rasul, 2005). This parameter also used for measuring genotypic yield potential among wheat genotypes under drought stress conditions (Brukner and Frohberg, 1987; Clarke et al., 1992). A most common detrimental effect of water stress level is the decline in fresh biomass and dry matter production in wheat (Peschke et al., 1997) due to reducing photosynthates production under water deficit conditions (Tezara et al., 1999). Relative water content (RWC) is the suitable index of plant water status in terms of the physiological consequence of cellular water deficit induced with PEG. Exposure of plants to drought led to noticeable decreases in relative water content. Some genotypes of wheat, e.g. Genotype GW322 maintained relative water content extremely well at both the drought stress levels (Figure 3). Siddique et al. (2004) have also reported the decline of relative water content in wheat under drought stress conditions. Consistently diminishing of RWC in response to PEG-induced water stress has been reported in wheat (Bajji et al., 2001). Physiological parameters such as relative water content (RWC) is very responsive to drought stress and have been shown to correlate well with drought tolerance (Jamaux et al., 1997; Altinkut et al., 2001). Many studies show a reduction in leaves RWC, when plants subjected to drought (Talamè et al., 2006; Aprile et al., 2013). Dedio (1975), Clarke and McCaig (1982), Ashraf et al. (l994) investigated that wheat varieties had

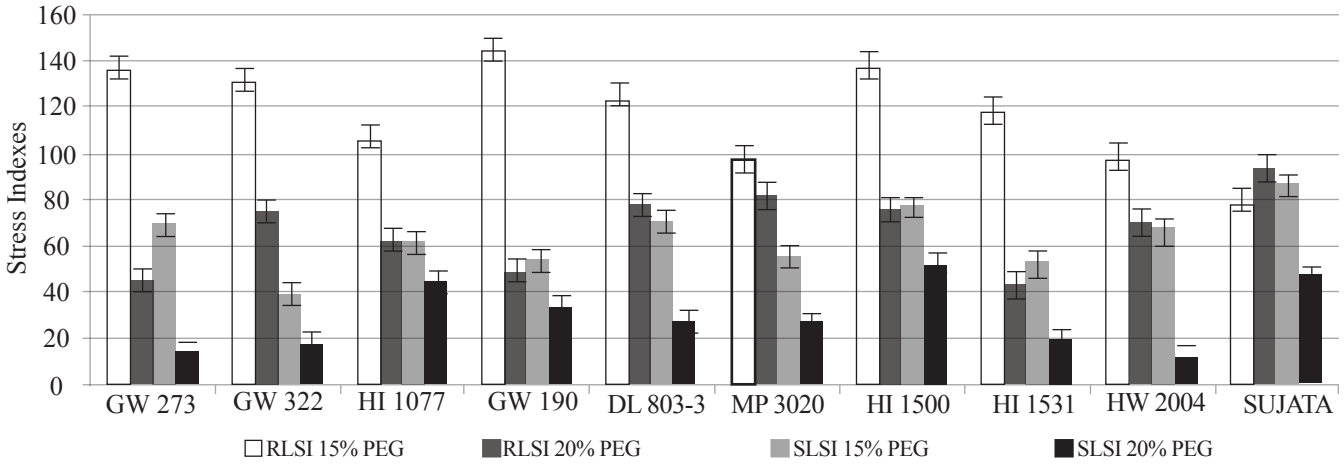

Figure 2: Mean of root length stress index (RLSI) and shoot length stress index (SLSI) of ten wheat cultivars in response to 15% and 20% PEG concentrations

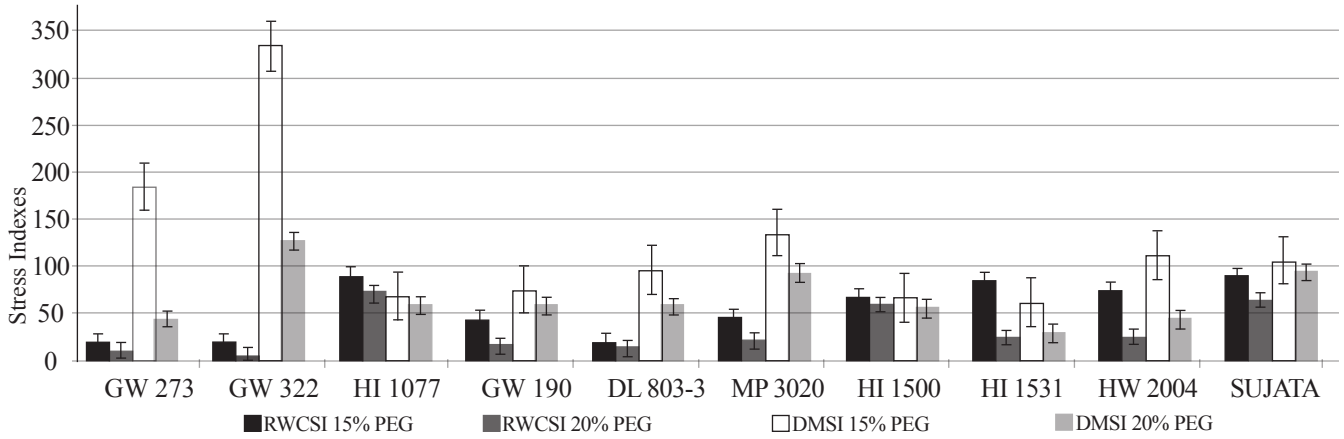

Figure 3: Mean of dry matter stress index (DMSI) and relative water content stress index (RWCSI) of ten wheat cultivars in response to 15% and 20% PEG concentrations

Table 4: Ranks (R), ranks mean ($\bar{R}$), standard deviation of ranks (SDR) and rank sum (RS) on growth characteristics of seedling indicators of drought tolerance

Cultivar	GSI				PHSI				RLSI				SLSI				DMSI				RWCSI				$\bar{R}$	Rank	SDR	RS	Final
	15% PEG	R	20% PEG	R	15% PEG	R	20% PEG	R	15% PEG	R	20% PEG	R	15% PEG	R	20% PEG	R	15% PEG	R	20% PEG	R	15% PEG	R	20% PEG	R		mean			Rank
GW273	75.12	6	60.97	6	89.86	4	37.2	10	135.91	3	45.57	9	70.17	4	14.36	9	18.71	9	10.05	9	184.1	2	43.81	9	80	6.7	2.84	9.51	8
GW322	77.47	4	55.61	10	77.2	8	64.95	4	130.55	4	75.1	5	39.82	10	18.26	8	17.95	10	5.35	10	335.96	1	126.71	1	75	6.3	3.49	9.74	10
HI1077	86.54	1	65.09	4	90.82	3	69.65	2	105.86	7	62.6	7	62.05	6	45.06	3	89.34	1	71.89	1	67.92	8	58.58	6	49	4.1	2.61	6.69	3
GW190	69.28	10	56.14	9	86.51	5	63.21	5	143.8	1	49.63	8	54.47	8	34.21	4	43.24	7	15.21	7	73.92	7	58.71	5	76	6.3	2.46	8.80	7
DL803-3	71.75	9	65.62	2	95.43	1	58.61	6	123.82	5	77.59	3	71.07	3	27.66	5	19.26	8	12.92	8	95.5	6	58.8	4	60	5.0	2.52	7.52	4
MP3020	84.47	2	56.32	8	66.82	10	56.06	7	97.26	9	82.41	2	56.00	7	26.51	6	44.94	6	20.75	6	134.48	3	92.77	3	69	5.8	2.70	8.45	5
MP1500	76.82	5	56.52	7	92.07	2	66.67	3	137.2	2	76.61	4	77.69	2	53.06	1	67.32	5	59.57	3	66.34	9	55.56	7	50	4.2	2.48	6.65	2
MP1531	73.5	7	65.23	3	82.61	7	41.4	9	118.04	6	43.54	10	54.02	9	19.82	7	84.8	3	23.7	5	61.43	10	29.2	10	86	7.2	2.55	9.72	9
HW2004	72.28	8	61.81	5	70.33	9	50.15	8	97.8	8	71.51	6	67.66	5	12.66	10	74.24	4	24.69	4	110.71	4	44.3	8	79	6.6	2.15	8.73	6
SU-JATA	81.97	3	74.72	1	85.3	6	70.72	1	78.39	10	94.15	1	86.89	1	46.99	2	88.83	2	63.99	2	105.14	5	93.91	2	36	3.0	2.73	5.73	1

higher RWC under drought stress conditions indicating more drought resistant. Present study on wheat varieties confirmed the above findings.

3.3. Determination of wheat genotypes for drought tolerance based on ranking method

For evaluation of wheat genotypes for drought tolerance, ranking method was used to determine overall judgment. Based on mean rank and standard deviation of rank in terms of morpho-physiological stress indices, the most responsive drought tolerant genotypes were identified. Cultivar Sujata (RS=5.73), followed by MP1500 (RS=6.65) and HI1077 (RS=6.69) were the highly drought tolerant genotypes. Genotypes GW322 (RS=9.74), MP1531 (RS=9.72) and GW273 (RS=9.51) were the most sensitive to drought (Table 4). The morpho-physiological response of plants to water deficit-induced changes at germination and seedling stages has also been reported by Saensee et al. (2012). Mohammadi et al. (2011) used same methods for screening quantitative indicators of drought tolerance in wheat. The present study showed clear difference in the contribution of morpho-physiological defense system in the drought tolerance of wheat genotypes when subjected to drought stress. The exposure of water deficit led to differential GSI, PHSI, DMSI, RLSI and RWCSI response in all the genotypes.

4. Conclusion

All the germination and seedling growth traits under the study can be used as a selectable trait to discrimination between tolerance and susceptible genotypes under drought stress in breeding programmes. Stress by elicited indices provides an effectual platform for rapid screening of tolerant genotypes against potent drought stress and that priming from harsh environments is a promising, novel way to improve plant water use efficiency. These new advancements importantly contribute towards solving food security issues in changing climates.

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