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Evaluation of Seedling Survivability and Growth Response as Selection Criteria for Breeding Drought Tolerance in Wheat

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Detection of genotypic variation in response to water stress at seedling stage could help in escalating selection intensity in breeding drought tolerant varities. Nine genotypes were tested for seedling survivability under drought stress. Four genotypes, i.e. 'Sarsabz', 'Sitta', 'Fareed' and 'FD-83', showed complete survival on resumption of irrigation after drought stress. These genotypes were late dying as they withered slowly under drought. Percent wilting and percent survival on resumption of irrigation were negatively correlated. Six genotypes were selected on the basis of seedling survivability (late and early dying) and evaluated for seedling growth response under drought. Root length and dry weight increased significantly under stress in 'Sitta', 'FD-83' and 'Fareed'. Drought stress also increased the root-to-shoot length ratio in 'FD-83' and 'Fareed'. However, seedling fresh and dry weight significantly reduced in 'Nesser' and 'Inqalab-91' under stress. In 'FD-83', seedling fresh and dry weight increased over control under stress. Results indicated that seedling survivability, root-to-shoot length ratio, root length and dry weight were most important traits for screening drought tolerance at seedling stage. On the basis of these indices, 'Sitta', 'Fareed' and 'FD-83' were classified as drought tolerant, 'Sarsabz' and 'Nesser' as moderately tolerant and 'Inqalab-91' as sensitive genotypes. Collectively, results suggested that selection by combining seedling survivability, growth response, RWC and leaf water potential can be efficiently used for rapid evaluation of drought tolerance in wheat breeding.

Keywords: biomass, root length, root-to-shoot ratio, *Triticum aestivum* L., water deficit

Introduction

Drought stress is one of the most important abiotic stresses, generally accompanied by heat stress in dry season (Dash and Mohanty 2001; Siddiqui et al. 2008). Drought is a worldwide problem, seriously influencing crop productivity (HongBo et al. 2006; Akhter et al. 2008). Gap in world food production and demand is mainly because of abiotic stresses such as drought, high temperature, frost etc. with drought being a major constraint. Breeding programmes of bread wheat seeking increased yield have usually attempted to improve drought tolerance of plants. Crop varieties with drought and thermo tolerance are need of the day as water is becoming scarce with every passing day. Before

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successful genetic manipulation it is important to characterize the physiological parameters of known drought tolerant or sensitive cultivars (Bray 1997).

To achieve this goal and speed up the breeding programmes, screening techniques for drought tolerance should be rapid, cost effective, reliable and capable of evaluating plant performance at seedling stage. Field screening for drought and thermo tolerance is difficult due to uncertain environmental changes (rainfall, temperature fluctuation, etc.).

Several methods based on physiological and agronomical traits as selection criteria, such as leaf relative water content (Sinclair and Ludlow 1985; Schonfeld et al. 1988), water use efficiency, early seedling vigour (Nagarajan and Rane 2000; Dhanda et al. 2004), shoot and root length, root-to-shoot length ratio (Dhanda et al. 2004) and seed reserve mobilization (Soltani et al. 2006) have been suggested to screen the germplasm for drought tolerance. Furthermore, seedling survivability, a simple method of screening germplasm lines which accurately discriminates between drought tolerance and susceptibility under artificial moisture stress conditions (Singh et al. 1999), has been used as a selection criterion in cowpea (Singh et al. 1999) and wheat (Tomar and Kumar 2004). In cowpea a close correspondence between drought tolerance in the seedling and reproductive stage has also been exhibited (Singh et al. 1999).

In present work, combination of seedling survivability and seedling growth response were tested as screening criterion for drought tolerance in wheat. Objective was to find out the indices that can be useful to differentiate between drought tolerance and susceptibility in wheat at seedling stage.

Materials and Methods

In first experiment nine wheat (*Triticum aestivum* L.) genotypes were screened for water stress tolerance using seedling survivability as criterion. These genotypes along with their origin are given in Table 1. Seeds were sown in soil (sandy loam: Sand 70%, clay 10% and silt 20%) filled plastic boxes with 2.5 cm row distance, a seed to seed distance of 3 cm and at a uniform depth of 1.5 cm. All boxes were given equal amount of water 16 h before sowing and there after no irrigation was provided. Data were recorded for percent wilting, due to drought, in different genotypes at 11^{th}, 12^{th} and 13^{th} day after seed sowing. On next day (14^{th}), boxes were irrigated to study the recovery response (survival) of genotypes. Data for percent survival were recorded 24 and 48 hours after irrigation. The earliest dying genotypes with low survivability and latest dying genotypes with high survivability were selected for second experiment in which seedling growth response was investigated.

Second experiment was conducted using six genotypes selected on the basis of their performance in seedling survivability experiment. Genotypes included in experiment were 'Nesser', 'Inqalab-91', 'Sitta', 'Sarsabz', 'FD-83' and 'Fareed'. Seeds were germinated in plastic pots filled with equal quantity of soil and irrigated at soil water holding capacity. In control pots, water was maintained at soil water holding capacity. While after sowing, no irrigation was provided to pots in which drought stress was induced. Data for seedling growth response studies were recorded when water in stressed pots was at about 10% of soil water holding capacity.

Table 1. Different wheat genotypes used in the study along with their origin

Sr. No.	Genotypes	Origin of genotypes
1	Pak-81	Punjab, Pakistan
2	Sarsabz	Sindh, Pakistan
3	Nesser	CIMMYT
4	Sitta	CIMMYT
5	Inqalab-91	Punjab, Pakistan
6	FD-83	Punjab, Pakistan
7	Fareed	Punjab, Pakistan
8	Shafaq	Punjab, Pakistan
9	Sehar	Punjab, Pakistan

Fresh weight of seedlings was recorded immediately after harvesting to avoid any evaporation. For dry weight estimations, pre-weighed seedlings were kept at 90 ºC till dry. Seedling, root and shoot dry weight was measured after complete drying when there was no further decrease in weight. Root and shoot length was measured by spreading them on a scale calibrated in cm.

Statistical analysis of data

All experiments were conducted in triplicates and descriptive statistics were applied to analyze and organize the resulting data. The F-test was performed to calculate differences in variance among samples. The significance of differences between means (for stressed and control) for different parameters was measured using Student's *t*-test (two-tailed), at 0.01 and 0.05 significance level. All the statistical calculations were performed using computer software Microsoft Excel 2002.

Results

Seedling survivability as selection criteria for drought

In seedling survivability experiment, the stress effect (wilting) started from the 11^{th} day after sowing. Withering of leaf progressed from tip to bottom starting with yellow tips, turning brown and consequently completely drying. This process advanced from bottom leaf to top leaf. Genotypic differences became more prominent with increasing days of water stress.

Percent wilting in different genotypes generally increased with increasing duration of water stress however, magnitude was variable (Fig. 1). At 11^{th} day, percent wilting ranged from 31% in 'Sarsabz' to 67% in 'Pak-81'. On the basis of percent wilting, genotypes clustered into three groups with low, medium and high wilting at this day. Four genotypes, i.e. 'Sitta', 'Sarsabz', 'FD-83' and 'Fareed', showed low wilting percentage. Two genotypes, 'Inqalab-91' and 'Sehar' showed medium wilting percentage while in remaining three genotypes ('Shafaq', 'Nesser' and 'Pak-81') it was relatively high.

At 12^{th} day, genotypic differences in terms of percent wilting became clearer as all genotypes clustered into two distinct groups. Four genotypes 'Sitta', 'Sarsabz', 'FD-83' and

'Fareed', showed low wilting percentage ranging from 50% to 57%. Wilting percentage was high in the remaining five genotypes and even up to 100% in 'Shafaq'.

At 13th day minimum percent wilting (60%) was observed in 'Fareed' while it was relatively higher in 'FD-83' (68%) and 'Sitta' (65%). These three genotypes were late dying under water stress as compared to the other tested varieties. 'Sarsabz' exhibited comparatively higher wilting (83.3%) than the first group while remaining five genotypes showed complete wilting at this day and were classified as early dying genotypes.

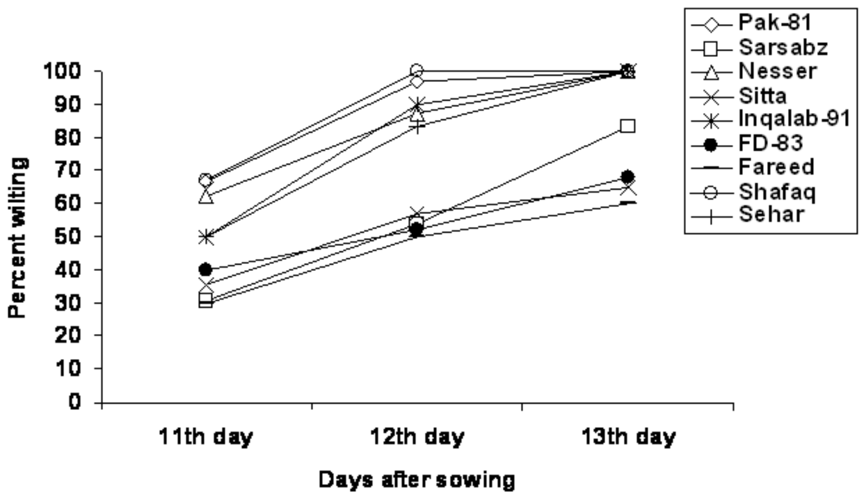

Figure 1. Percent wilting in different wheat genotypes subjected to water stress

On resumption of irrigation, recovery response in terms of percent survival was also recorded (Fig. 2). Three genotypes, i.e. 'Sarsabz', 'Sitta' and 'FD-83' completely recovered within 24 h after resumption of irrigation. However percent survival was low in other genotypes with minimum value in 'Inqalab-91' and highest in 'Fareed'.

During next 24 h 'Fareed' also completely recovered and at 48 h after resumption of irrigation, genotypes could be distinguished in two groups. First group showed complete recovery (100% survival) and includes 'Sarsabz', 'Sitta', 'Fareed' and 'FD-83' whereas second group was with low survival rate ranging from 22% ('Inqalab-91') to 59% ('Pak-81'). No change in seedling survival was observed beyond 48 h of resumed irrigation and a significant negative correlation was observed in percent wilting and percent survival (Fig. 3). Results indicated that genotypes which withered earlier also show poor recovery on resumption of irrigation and vice versa.

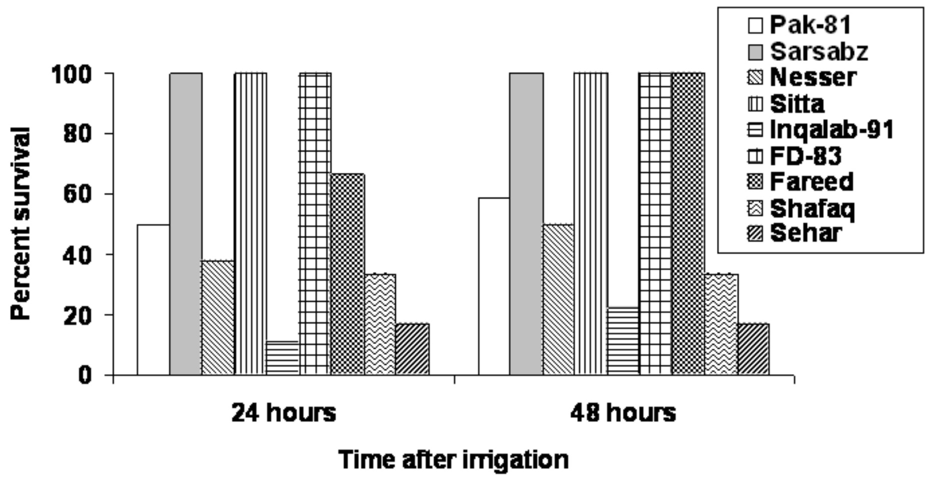

Figure 2. Percent survival (recovery) in different wheat genotypes after resumption of irrigation

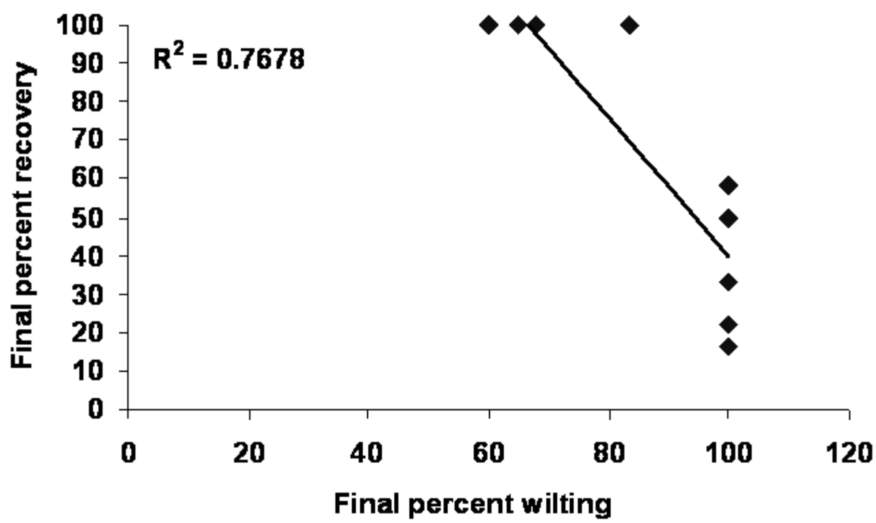

Figure 3. Correlation between final percent recovery (survival) and final percent wilting in wheat genotypes

It can be summarized that on the basis of low wilting and high seedling survival; four wheat genotypes, i.e. 'Sarsabz', 'Sitta', 'Fareed' and 'FD-83' performed better under water stress. Other five genotypes were less efficient under applied water stress.

Seedling growth response to drought

In growth response studies, substantial variation in response to water stress at the seedling stage was observed among tested wheat genotypes. Fresh weight of seedling was significantly decreased in 'Nesser' ($p < 0.01$) and 'Inqalab-91' ($p < 0.05$) under water stress (Fig. 4a). Applied stress reduced the seedling fresh weight up to 29% in 'Nesser' and 38.6% in 'Inqalab-91'.

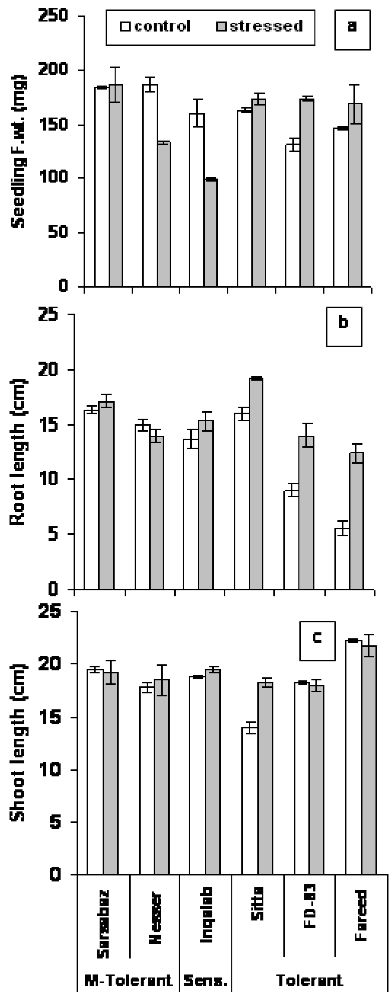

Figure 4. Effect of water stress on seedling fresh weight (a), root (b) and shoot (c) length in different wheat genotypes. The values are means of three replicates ± standard error (SE)

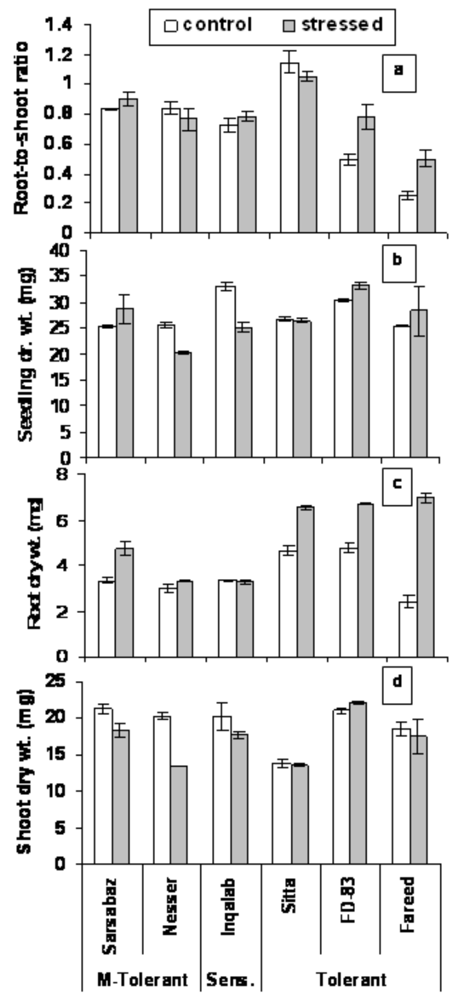

Figure 5. Effect of water stress on root to shoot length ratio (a), seedling (b), root (c) and shoot (d) dry weight in different wheat genotypes. The values are means of three replicates ± standard error (SE)

While in case of ‘FD-83’, seedling fresh weight increased significantly under water stress. Increase over control was 24.7% in this genotype. Seedling fresh weight remained unaffected under water stress in ‘Sitta’ and ‘Fareed’.

Root length significantly increased under water stress in three genotypes i.e. ‘Sitta’ ($p < 0.05$), ‘FD-83’ ($p < 0.05$) and ‘Fareed’ ($p < 0.01$) (Fig. 4b). Increase in root length due to water stress was 55% in ‘FD-83’ and 22% in ‘Sitta’ while it was above two folds in ‘Fareed’. In other three genotypes, root length was not influenced by applied water stress.

Shoot length was significantly increased ($p < 0.01$) only in ‘Sitta’. A 30% increase in shoot length over control was observed in this genotype. Other genotypes showed no response for shoot length trait under water stress (Fig. 4c).

Root-to-shoot ratio which is considered to be an important trait under water stress significantly increased in ‘FD-83’ and ‘Fareed’ (Fig. 5a). In case of ‘FD-83’, increase in root-to-shoot ratio due to water stress was 58%. Root-to-shoot ratio was two fold in ‘Fareed’ under water stress compared to control.

Water stress reduced the seedling dry weight in ‘Nesser’ and ‘Inqalab-91’ (Fig. 5b). Reduction in biomass accumulation was highest (24%) in ‘Inqalab-91’. However in ‘FD-83’, seedling dry weight increased ($p < 0.05$) over control under stressed condition.

Biomass accumulation in roots significantly increased under water stress in three genotypes i.e. ‘Sitta’, ‘Fareed’ and ‘FD-83’ (Fig. 5c). Increase in root dry weight under water stress was 29% in ‘Sitta’ and ‘FD-83’ and 65% in ‘Fareed’. Shoot dry weight reduced ($p < 0.01$) only in ‘Nesser’ and it was 34% less as compared with control (Fig. 5d).

Leaf water potential of wheat genotypes reduced (more –ve) significantly under water stress and also varied among genotypes (Fig. 6a). In case of wheat genotype ‘Fareed’ least (34%) stress induced reduction in water potential was observed. This was followed by ‘FD-83’ (83%) and ‘Sitta’ (84%). Reduction in leaf water potential was comparatively higher in other genotypes i.e. ‘Inqalab-91’ (145%), ‘Sarsabz’ (114%) and ‘Nesser’ (115%).

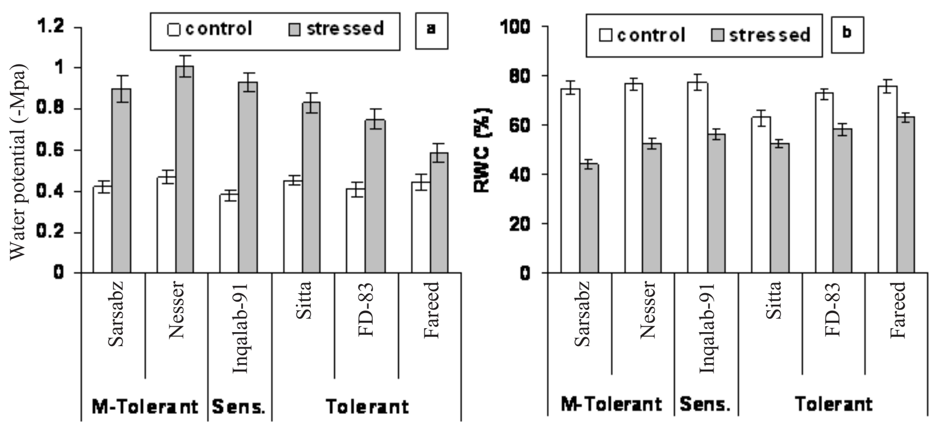

Figure 6. Effect of water stress on leaf water potential (a) and relative water content (b) in different wheat genotypes. The values are means of three replicates ± standard error (SE)

Relative water content (RWC) also decreased in all genotypes under water stress as compared to controls (Fig. 6b). However magnitude of decrease varied in genotypes. Stress induced decline in RWC was less in relatively tolerant genotypes, i.e. 'Fareed' (16.4%), 'Sitta' (16.6%) and 'FD-83' (19.8%) as compared to other three genotypes, i.e. 'Sarsabz' (41%), 'Inqalab-91' (27.4%) and 'Nesser' (31.7%).

Discussion

Present findings provide evidence for usability of seedling survivability as selection criteria for drought tolerance which is in accordance with reported high efficacy of seedling survivability as screening method for selecting drought-tolerant cowpea (Singh et al. 1999) and wheat (Tomar and Kumar 2004) varieties. Genotypes which withered earlier also show poor seedling survivability on resumption of irrigation. Based on high seedling survival, four wheat genotypes, i.e. 'Sarsabz', 'Sitta', 'Fareed' and 'FD-83' showed better drought tolerance, which was further confirmed by seedling growth response studies. In connection, leaf death score of seedlings has also been used in rice breeding programmes as a selection index for drought resistance (Mitchell et al. 1998). It can be concluded that seedling survivability can be effectively used for screening drought tolerance in wheat at seedling stage.

Decreased seedling growth has been reported under drought in wheat (Soltani et al. 2006) and triticale (Kaydan and Yagmur 2008). In our studies fresh weight of seedling also decreased up to 38.6% in 'Inqalab-91', indicating decreased seedling growth under drought stress.

Root length is an important trait against drought stress in plant varieties; in general, varieties with higher root growth have ability for resistance to drought (Leishman and Westoby 1994). This was further strengthened by our findings as 22% to 105% increase in root length was observed in three genotypes showing drought tolerance. Stimulated root growth of wheat strains under drought stress has been reported (Ren et al. 2000). The increased root length under decreased osmotic potential (–0.45 MPa of PEG) has been reported in triticale (Kaydan and Yagmur 2008). It has been proposed that progress in breeding wheat for drought tolerance can be expected from selection for incresed root length (Dhanda et al. 2004).

Due to high heritability and genetic advance great benefit from selection for drought tolerance can be expected for incresed root-to-shoot length ratio (Dhanda et al. 2004). Root-to-shoot length ratio increased to 58% in 'FD-83' and was about twofold in 'Fareed' under stressed condition. Similar to present findings, root-to-shoot length ratio increased with increasing water stress and NaCl salinity, with more pronounced effect under water stress, in triticale (Kaydan and Yagmur 2008). Root-to-shoot length ratio also increased with increasing PEG and NaCl concentrations in durum wheat (Bajji et al. 2000) and by osmotic stress in bread wheat (Dhanda et al. 2004). Drought stress induced increase in root to shoot length ratio has also been reported in cowpea (Matsui and Singh 2003).

Drought tolerance has been reported to be associated with the increase in root dry matter in cowpea (Matsui and Singh 2003). Biomass accumulation in wheat roots also increased (ranging up to 65% in 'Fareed') under water stress in three genotypes, i.e. 'Sitta', 'Fareed' and 'FD-83'. Increased root dry weight was interrelated to drought tolerance in these genotypes. The superior root and shoot mass following drought stress have been proposed as reliable drought selection criteria for different plant species, including wheat (McMichael and Quisenberry 1991; Yang et al. 1991; Basal et al. 2005). Conclusively, if selection based on seedling survivability could be combined with extensive root system, the result could be superior adaptation to dry land environments.

Soil moisture deficit as a major adverse factor, in arid and semi-arid zones, can lower leaf water potential, leading to reduced turgor and ultimately lower crop productivity (Ezzat-Ollah et al. 2007). Drought induce reduction in the leaf water potential and relative water content has been reported in many crops including wheat (Selote et al. 2004). Similarly, a decline in both these parameters under water deficit stress in wheat seedlings was also observed in present study. Moreover, a higher reduction in these water relation parameters was observed in relatively less tolerant wheat genotypes used in present study. Similar higher reduction in leaf water potential and RWC in drought susceptible wheat genotypes as compared to tolerant ones has been reported previously (Subrahmanyam et al. 2006). These simple physiological traits thus deserve attention while screening wheat for drought tolerance.

In conclusion, this study proves that seedling survivability, enhanced root growth (increased root length, dry weight and root-to-shoot length ratio), RWC and leaf water potential are useful indices for rapid evaluation of drought response in wheat breeding. On the basis of these indices, three genotypes 'Sitta', 'Fareed' and 'FD-83' showed tolerant behavior towards water stress. Two genotypes 'Sarsabz' and 'Nesser' were moderately tolerant and 'Inqalab-91' was designated as water stress sensitive.

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