

EVALUATING THE POTENTIAL OF NORMAL WATERING AND POLYETHYLENE GLYCOL (PEG-6000) ON MORPHOLOGICAL TRAITS OF SPRING WHEAT SEEDLINGS OF ADVANCE LINES

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Background: Water stress is crucial factor limiting wheat seedling's, nutritional, growth parameters, and development, ultimately limited production. **Objectives:** The aim of present study was to assess the potential of water stress treatments such as under normal watering (T0), under polyethylene glycol (PEG-6000) at 0.5 MPa solution (T1), and under PEG 6000 at 0.75 MPa solution (T2) on quantitative wheat seedling traits such as shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), and root dry weight (RDW), of five winter wheat cultivars and their ten advance (F2) lines, under In situ condition, result indicated that the maximum shoot length (SL) of wheat line TJ-83 × Sarsabz was 19.53 cm under PEG-6000 at 0.5 MPa treatment. **Methods:** The present study was performed under control condition at Nuclear Institute of Agriculture (NIA), Tandojam Pakistan, along with a completely block design with split-plot along with three replication. **Results:** The highest root length (RL) of wheat line TJ-83 × Sarsabz was noted by 26.60 cm under normal watering. The maximum shoot fresh weight (SFW) of wheat cultivar, NIA-Sarang was noted up to 1.62 g under normal watering. The root fresh weight (RFW) of TD-1 cultivar was significantly increased by 1.38 g under normal water condition. The shoot dry weight (SDW) of wheat line TJ-83 × Kiran-95 was dramatically increased by 1.44 g under PEG-6000 at 0.5 MPa treatment. Similarly, shoot dry weight (SDW) of wheat line TJ-83 × Sarsabz was noted by 1.44 g under normal water condition. The root dry weight (RDW) of wheat line TJ-83 × Kiran-95 was significantly increased by 1.26 g under PEG-6000 at 0.5 MPa treatment. **Conclusion:** Overall results revealed that wheat cultivar NIA-Sarang, wheat advance line, TJ-83 × Kiran-95, and TJ-83 × Sarsabz showed drought tolerance, which could be utilized in future breeding scheme of developing drought tolerant wheat genotypes.

Keywords: PEG-6000; cultivar; advance lines; seedling; morpho-traits; *Triticum aestivum*.

INTRODUCTION

Global human population expected to exceed 9 billion by 2050, requiring at least 60% increase in wheat yield (Abah et al., 2023; Rodríguez et al., 2024; Hossain et al., 2025). Climate change is intensifying drought occurrences, leading to widespread water scarcity and diminished wheat (*Triticum aestivum* L.) yields internationally (Roush, 2023; Panhwar et al., 2024). At the same time, low-quality products can cause iron deficiency conditions (Kuzminska et al., 2018). There is also a strong dependence of yields on natural phenomena, such as uneven distribution of precipitation (Khan et al., 2023), and periodic droughts (Liu et al., 2024), which motivates scientists to look for ways to ensure yield stability. Wheat is the most important staple grain food crop, ranks 2nd amongst cereals (Giraldo et al., 2019). Seed germination and seedling emergence utilized in different tests and selection procedures to evaluate water stress tolerant genotypes (Ahmed et al., 2019). Due to linear correlation of germinating seeds and seedling stage have markedly varying processes (Ashraf & Foolad, 2005). Seeds imbibe and embryo development depended upon use activation of dormant enzyme system and use of reserved food in seed (Rosental et al., 2014). After development of first seed leaf, photosynthesis takes place in seedling (Evers et al., 2010). Seed germination and seedling development directly affected by the stress developed during growth (Khurana and Singh, 2001). Moisture stress during growth affected plant developmental stages (Bhattacharya & Bhattacharya, 2021). Resistance to drought is genotypic specific response, utilization of endosperm, percentage of germinated seeds, seedling

percentage and seedling recovery (Grzesiak et al., 2013). Screening for water stress tolerance, and estimation of response of genotype is tedious and impossible. Drought affected vegetation double during last 4 decades. About 26% of arable land of World has been degrading due to drought. About 12 million ha of World damaged every year by drought (Azadi et al., 2018; AbdelRahman, 2023). From 79.6 million ha of Pakistan, 62 million ha is under drought while about 27% of cultivated land faced water stress (Shahid & Venturi, 2023). In crop plant drought mechanisms are avoidance or escape. To repair drought effects various mechanisms have been made to overcome drought losses viz. micro irrigation, dry farming, mulch mechanisms, induced raining, underground watering and utilization of water stress tolerant varieties (Ramón Vallejo et al., 2012; Jovanovic et al., 2020). Development of drought tolerant genotypes is suitable strategy for drought areas (Blum, 2016). Various crop characters were affected due to water stress including tallness, area of leaves, green pigments, stomata function, osmotic adjustment, water retention etc (Panhwar et al., 2021). Drought damages could be study through plant characters during water stress (Chen et al., 2022). Limited intake of NPK by plants studied after drought via traits. Limited N uptake under water stress affected leaf water; chlorophyll and photosynthesis, which restricted plant development and yield (Sallam et al., 2019).

Polyethylene glycol (PEG 6000) is a synthetic polymer, utilized to mimic drought or water-stressed conditions in soil, enabling researchers to study plant responses (Tahir et al., 2024). The multifaceted polymer, PEG-6000, has diverse applications across various fields, including biotechnology,

pharmaceuticals, agriculture, and scientific research (D'souza & Shegokar, 2016). Khan et al. (2013) assessed the impact of PEG-6000 at different application rates aiming to induce moisture stress at germination and seedling stage of wheat genotypes. Furthermore, Magar et al. (2019) assessed the potential of PEG on seedling of maize varieties under drought condition, as a result found that lines Arun-2, Rampur Composite and RL-105 are recommended for varietal wine betterment program for water stress conditions. Water deficit concentrated sugars, shock proteins, enzyme activation and ROS (Tefera et al., 2021). Reduced proteins under water deficit is due to hydrolysis, oxidation and inhibit protein synthesis (Ozturk et al., 2021). Due to water deficit, osmolytes (dehydrants) accumulated in water stress tolerant genotypes and protected cell structure from drought damages (Halder et al., 2022). Dehydrants are high hydrophilic and take part in drought tolerance through increasing water retention capacity, enhance chlorophyll, photosynthesis, ROS detoxification and increase presence of solutes (Priya et al., 2019). In water the maximum light energy not used by plants sufficiently under water deficit because of electron transport chain disturbed, ROS production and loss to thylakoid membranes (Zhu et al., 2021). Chlorophyll a fluorescence promoted structure and function of PSII to indicate water stress tolerant and susceptible varieties (Kalaji et al., 2018; Tcsimilli-Michael, 2020; Li & Kim, 2021). Plants have various responses to meet drought conditions and complete life process earlier of extreme drought (escape), conserve moisture by close of stomata and reduce lamina (avoidance), or osmotic adjustment (tolerance) (Pamungkas et al., 2022). Plants collect different organic/inorganic substance (sugars, polys, amino acids etc.) during drought and retain moisture level and turgid pressure to sustain photosynthesis (Zhang et al., 2022). Plant physical, physio-biochemical traits, genetic, genotypic studies and plant breeding method integration used to know mechanism of water stress tolerance of plants. Available germplasm examined for water deficit conditions and is suitable plan to study effects of drought (Tefera et al., 2021). Novelty point of view, there is a lack of knowledge on to assess the potential of normal watering,

PEG 6000 at 0.5 MPa, and PEG 6000 at 0.75 MPa solutions on seedling traits of winter wheat cultivars and their advance lines (F2) lines under field condition. The aim of present study was to examine the potential of wheat advance lines under PEG 6000 at 0.5 MPa and PEG 6000 at 0.75 MPa solutions on early wheat seedlings parameters viz., shoot length, root length, shoot fresh and dry weight, root fresh and dry weight of five winter wheat viz. TJ-83, Sarsabz, TD-1, NIA-Sarang, Kiran-95 and their ten F2 line seedling viz. TJ-83 × Sarsabz, TJ-83 × TD-1, TJ-83 × NIA-Sarang, TJ-83 × Kiran-95, Sarsabz × TD-1, Sarsabz × NIA-Sarang, Sarsabz × Kiran-95, TD-1 × NIA-Sarang, TD-1 × Kiran-95 and NIA-Sarang × Kiran-95. Therefore, this experiment conducted for study genotypic variability of seedling characters to draw relationship between seedling shoot and root traits for selecting water stress tolerant genotype in early growth stages. It was hypothesized that PEG 6000 can promote the seedling traits of wheat parents and their advance lines (F2) as compared to under normal watering.

MATERIALS AND METHODS

Study area

The present study was performed under control condition at Nuclear Institute of Agriculture (NIA), Tandojam Pakistan (25°25'16"N; 68°32'28"E). A completely block design with split-plot along with three replication. In the present work, five wheat (*Triticum aestivum* L.) cultivars such as TJ-83, Sarsabz, TD-1, NIA-Sarang and Kiran-95 and their ten F2 line seedling viz. TJ-83 × Sarsabz, TJ-83 × TD-1, TJ-83 × NIA-Sarang, TJ-83 × Kiran-95, Sarsabz × TD-1, Sarsabz × NIA-Sarang, Sarsabz × Kiran-95, TD-1 × NIA-Sarang, TD-1 × Kiran-95 and NIA-Sarang × Kiran-95 were obtained from Nuclear Institute of Agriculture Tandojam Pakistan. The impact of three water stress treatment including under normal watering (T0), PEG-6000 at 0.5 MPa (T1), and PEG-6000 at 0.75 MPa (T2) concentration was assessed on the seedling parameters such as shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight of five winter wheat parent cultivars and their ten crosses (F2). The flow diagram of methodology is indicated in (Figure 1).

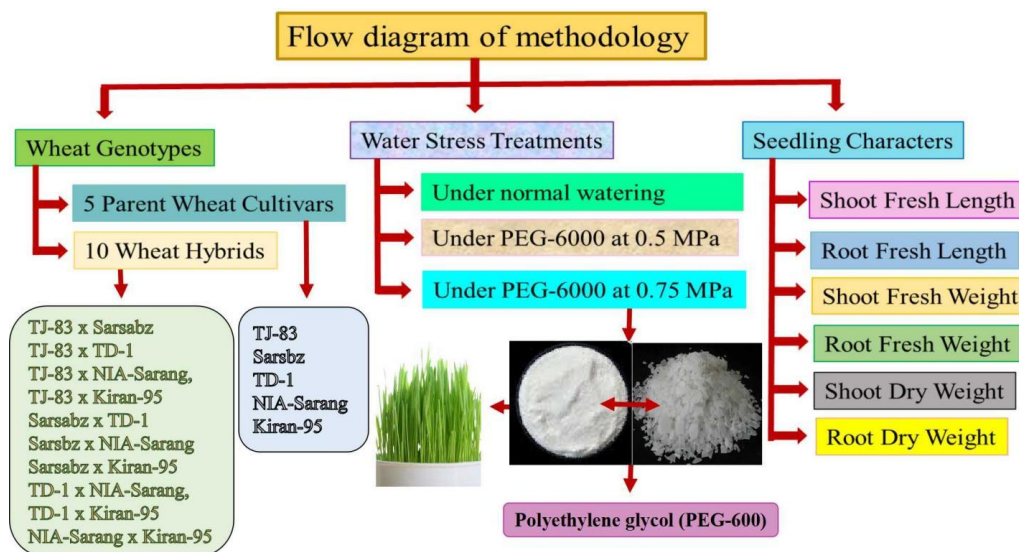


Figure 1. Flow diagram of research methodology

Experimental set-up

In this research work, the impact of three treatments such as normal watering (distilled water), polyethylene glycol (PEG-6000) at 0.5 MPa, and PEG-6000 at 0.75 MPa on the seeding parameters of 5 wheat cultivar and their 10 crosses (F2) lines under control condition. In addition, PEG-6000 in the form of crystalline was ground with the help of mortar and pestle into powder form was dissolved in distilled water by using Spanner-type thermocouple psychomotor according to (Brown & van Haveren, 1972), aiming to make PEG-6000 solution and applied as PEG-6000 at 0.5 MPa, and PEG-6000 at 0.75 MPa treatment in the studied bowls. The bowls were kept at 22 °C at darkness of growth cabinet. The seeds of studied wheat genotypes were disinfected by using sodium hypochlorite (1%). Approximately 10 healthy treated seeds of each wheat cultivar and their 10 crosses (F2) lines grown in each plastic bowl. This study, comprising 15 genotypes, 3 replication and treatments such as normal watering, PEG-6000 at 0.5 MPa, and PEG-6000 at 0.75 MPa, as a result total 135 bowls. The temperature was adjusted at 15 °C / 20 °C night/day, relative humidity 63 – 75% and radiant energy flux of 0.1 μE/cm²/s. Ten seeds of each genotype per replicate placed on bowl covers with appropriate holes first on control and water stress treatment solutions developed from DH₂O as under normal watering as control treatment (T0), PEG-6000 at 0.5 MPa (T1), and PEG-6000 at 0.75 MPa (T2). The oven dried plant material was kept at 19 °C for 48 h. No any chemical fertilizer was applied in this investigation.

Plant harvesting

All the seedling plants from each plastic bowls were harvested after 20 days of sowing for test the morphological parameters such as shoot length, root length, shoot fresh and dry weight, root fresh and dry weight. The root length, shoot length, fresh root and shoot fresh weight was noted after harvesting of wheat plants. After that, all the root and shoot biomass were kept in oven at < 65 °C for 2 – 3 days for drying process and after dried plant root and shoot dry weight was noted.

Statistical analysis

Analysis of data for mean squares conducted by the use of computer based software Statistix version 8.1 to determine genotype, treatments and interaction differences. All graphs made by using ORIGIN Pro.8.5 Version. Correlation matrix graphs were made by using STHDA tool.

RESULTS AND DISCUSSIONS

Evaluation of cultivars and their crosses/F2 lines for seedling parameters under normal watering

The highest shoot length (SL) was noted by 17.73 cm for line TJ-83 × NIA-Sarang, whereas the lowest shoot length (SL) was noted by 9.20 cm for NIA-Sarang × Kiran-95 as compared to other lines under normal watering (Figure 2a). The maximum root length (RL) was observed by 17.73 cm for TJ-83 × Sarsabz, however the minimum root length (RL) was received by 11.29 cm for NIA-Sarang × Kiran-95 line than other wheat lines under normal watering (Figure 2b). The maximum shoot fresh weight (SFW) of NIA-Sarang cultivar was noted up to 1.62 g, whereas, the minimum shoot fresh weight (SFW) of NIA-Sarang × Kiran-95 line was observed by 0.60 g as compared to other lines under normal watering (Figure 2c). It has been noted that the greatest root fresh weight (RFW) of TD-1 cultivar was observed by 1.38 g, however, the lowest root fresh weight (RFW) of Sarsarz × NIA-Sarang was noted by 0.44 g than other wheat lines under

normal watering (Figure 2d). The results indicated that the maximum shoot dry weight of TJ-83 × Sarsabz line was observed by 1.44 g, whereas, the minimum shoot dry weight (SDW) of TJ-83 wheat cultivar was observed by 0.18 g as compared with other wheat varieties under normal watering (Figure 2e). The great root dry weight of TJ-83 × Sarsabz and TJ-83 × Kiran 95 lines were observed by 0.82 g respectively, on the other hand the minimum root dry weight (RDW) of TJ-83 cultivar was noted by 0.08 g in comparison with other wheat varieties under normal watering (Figure 2f). The above results indicated that the maximum seedling traits of wheat genotype such TJ-83 × NIA-Sarang wheat line, TJ-83 × Sarsabz wheat line, NIA-Sarang and TD-1 cultivar, TJ-83 × Sarsabz line, TJ-83 × TD-1 and TJ-83 × Kiran-95 were increased significantly under normal watering treatment. Drought effects also stated by (Halder et al., 2022; Panhwar et al., 2021; Hussain et al., 2022). Moreover, (Chen et al., 2022; Panhwar et al., 2022; Ahmad et al., 2022) showed results of drought tolerance in wheat. In the earlier study, Akram (2011) revealed that consecutive stresses can caused severe reduction in the growth and yield components of wheat cultivars. Khan et al. (2010) reported that the shoot length, root length, fresh weight and dry weight of wheat varieties were reduced under the water stress condition.

Evaluation of cultivars and their crosses (F2 lines) for seedling parameters under PEG 6000 at 0.5 MPa

The results indicated that the maximum shoot length (SL) was found up to 19.53 cm for TJ-83 × Sarsabz F2 wheat line, while the minimum shoot length (SL) was observed by 10.09 cm for Sarbabsz wheat cultivar as compared with other wheat varieties under PEG 6000 at 0.5 MPa condition (Figure 3a). The maximum root length (RL) was observed by 19.10 cm for TJ-83 × TD-1 wheat F2 line, however, the minimum root length was received by 13.49 cm for Sarsabz wheat cultivar than other wheat varieties under PEG 6000 at 0.5 MPa condition (Figure 3b). The maximum shoot fresh weight (SFW) of TJ-83 × Sarsabz wheat F2 line noted up to 1.17 g, whereas, the minimum shoot fresh weight (SFW) of Kiran-95 wheat cultivar was observed by 0.52 g as compared with other wheat varieties under PEG 6000 at 0.5 MPa condition (Figure 3c). It was noted that the greatest root fresh weight (RFW) of NIA-Sarang wheat cultivar was observed by 0.41 g, however, the lowest root fresh weight (RFW) of wheat cultivar Kiran-95 was found up to 0.21 g than other wheat varieties under PEG 6000 at 0.5 MPa condition (Figure 3d). The results indicated that the maximum shoot dry weight (SDW) was observed 1.44 g by TJ-83 × Kiran-95 wheat F2 line, while the lowest shoot dry weight (SDW) of Kiran-95 cultivar was found up to 0.12 g rather than other wheat varieties under PEG 6000 at 0.5 MPa condition (Figure 3e). The maximum root dry weight (RDW) of TJ-83 × Kiran-95 F2 wheat line was found up to 1.26 g, however, the minimal root dry weight (RDW) of TD-1 cultivar was recorded by 0.07 g as compared to other wheat varieties under PEG 6000 at 0.5 MPa condition (Figure 3f). The above results indicated that the maximum seedling traits of wheat F2 lines such as TJ-83 × Sarsabz, TJ-83 × TD-1, TJ-83 × Sarsabz, NIA-Sarang cultivar, TJ-83 × Kiran-95 F2 wheat line, and TJ-83 × Kiran-95 F2 wheat line were observed under PEG 6000 at 0.5 MPa. Also, Khan et al. (2013) revealed that maximum root length was observed for Lasani-2008, shoot length for Chenab-70, dry root weight for LLR-14, dry shoot weight, fresh root weight, and fresh shoot weight for Seri-82 wheat variety, while the minimum fresh shoot weight was observed for LLR-21 wheat variety with application of PEG-6000.

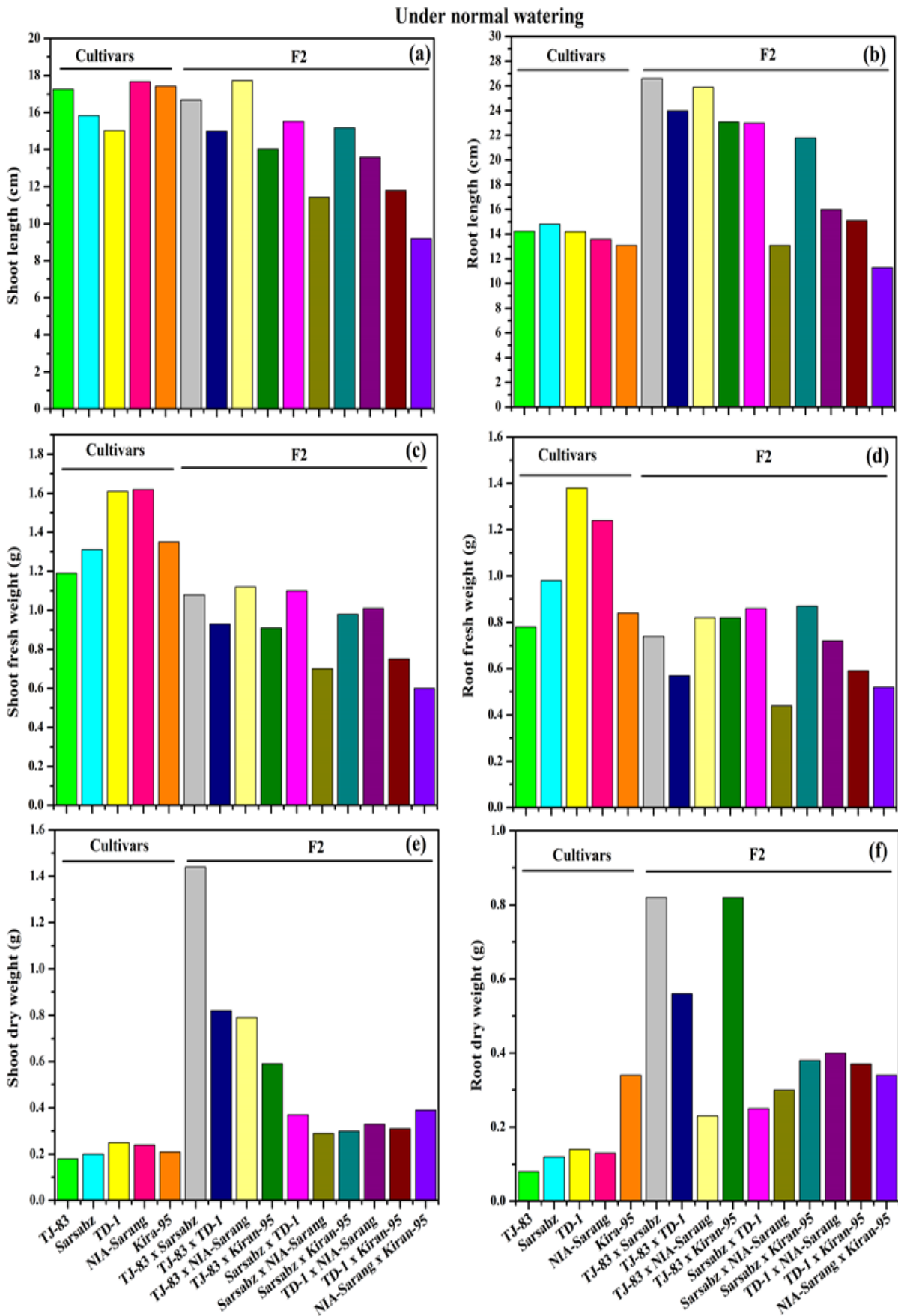


Figure 2. Impact of normal watering: a – on shoot length (SL); b – root length (RL); c – shoot fresh weight (SFW); d – root fresh weight (RFW); e – shoot dry weight (SDW); f – root dry weight (RDW)

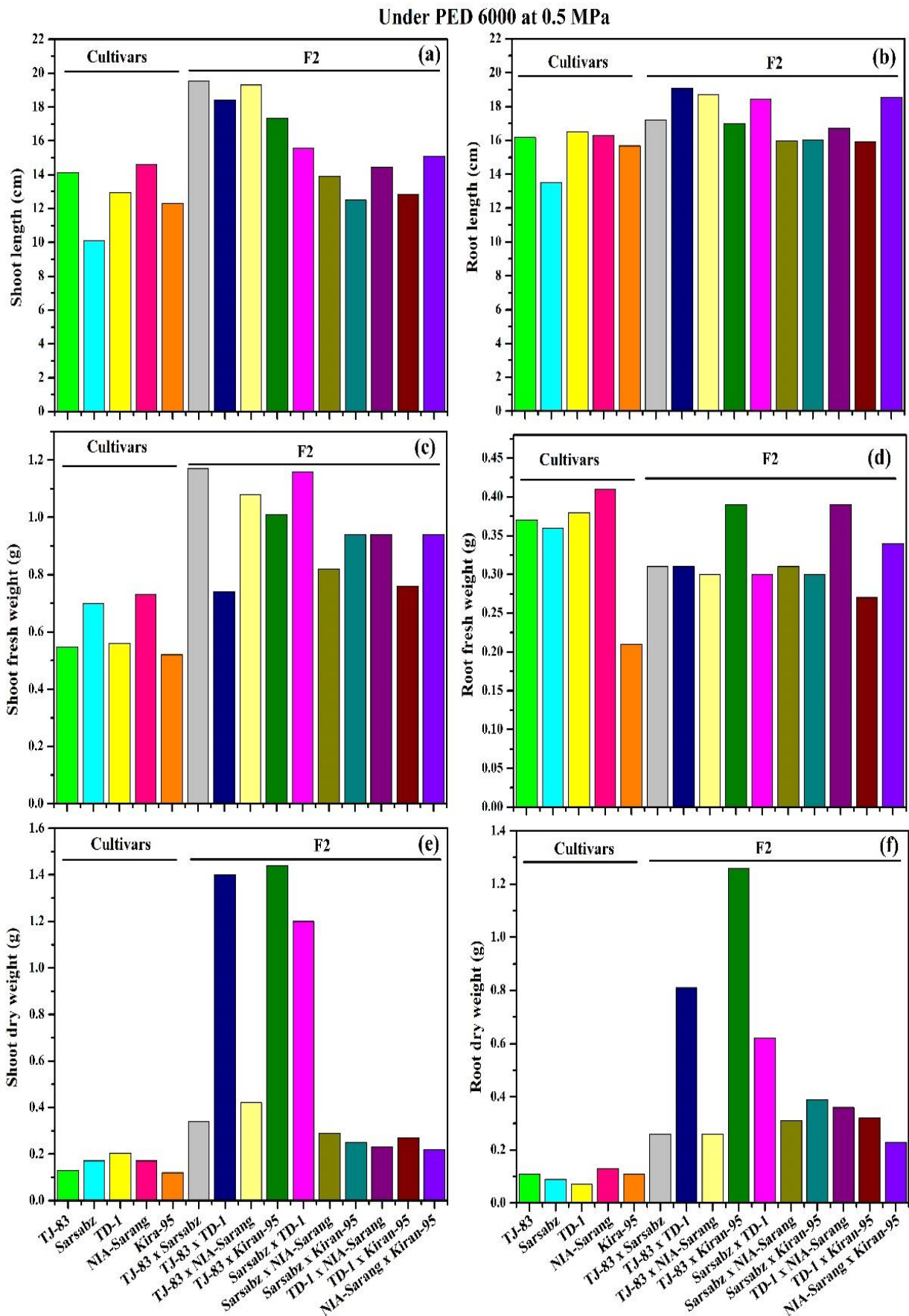


Figure 3. Impact of PEG-6000 at 0.5 M Pa: a – on shoot length (SL); b – root length (RL); c – shoot fresh weight (SFW); d – root fresh weight (RFW); e – shoot dry weight (SDW); f – root dry weight (RDW)

Evaluation of cultivars and their crosses (F2 lines) for seedling parameters under PEG- 6000 at 0.75 MPa

The results indicated that the maximum shoot length (SL) was found up to 17.13 cm for TD-1 × NIA-Sarang F2 wheat line, while the minimum shoot length (SL) was observed by 5.24 cm for Kiran-95 wheat cultivar as compared with other wheat varieties under PEG-6000 at 0.75 MPa condition (Figure 4a). The maximum root length (RL) was observed by 18.13 cm for TD-1 × NIA-Sarang F2 wheat line, however the minimum root length (RL) was received by 9.48 cm for Kiran-95 cultivar than other wheat varieties under PEG-6000 at 0.75 MPa condition (Figure 4b). The highest shoot fresh weight (SFW) was observed by 0.99 g in TD-1 × NIA-Sarang F2 wheat line, however, the lowest shoot fresh weight (SFW) of Kiran-95 wheat cultivar was recorded up to 0.16 g than other varieties under PEG-6000 at 0.75 MPa condition (Figure 4c). The results highlighted that the maximum root fresh weight (RFW) of Sarsabz × Kiran-95 F2 wheat line was found up to 0.40 g, while the minimum root fresh weight (RFW) of wheat cultivar Kiran-95 was recorded up to 0.13 g in comparison with other wheat varieties under PEG-6000 at 0.75 MPa condition (Figure 4d). The results revealed that the highest shoot dry weight (SDW) of TJ-83 × TD-1 F2 wheat line was noted by 0.85 g, while the minimum shoot dry weight (SDW) of TJ-83, TD-1 and Kiran-95 wheat cultivars was found up to 0.08 g respectively, than other wheat varieties under PEG-6000 at 0.75 MPa condition (Figure 4e). The highest root dry weight (RDW) of TJ-83 × TD-1 F2 wheat line was received by 0.75 g, while the lowest root dry weight (RDW) of Kiran-95 cultivar was noted up to 0.05 g in comparison with other wheat varieties under PEG 6000 at 0.75 MPa condition (Figure 4f). The above results revealed that the maximum seedling traits were of wheat lines viz., TD-1 × NIA-Sarang, Sarsabz × Kiran-95, TJ-83 × TD-1 and TJ-83 × TD-1 under PEG 6000 at 0.75 MPa. Boutraa et al. (2010) observed the reduction in growth traits of Hab-Ahmar wheat followed by Sindy-2 then Sindy-1 varieties under severe water stress condition. Chachar et al. (2016) examined that the maximum shoot length, root length, shoot and root fresh and dry weight observed in Khirman wheat cultivar with application of PEG-6000 under control condition.

Evaluation of reduction in shoots and root length of wheat varieties under water stress condition

The data in (Table 1) revealed that the maximum reduction (RD1) in the shoot length of Kiran-95 wheat cultivar was observed by (-12.16) with application of PEG-6000 at 0.5 MPa as compared with under normal watering (T0). The highest reduction (RD2) in the shoot length (SL) of TJ-83 was noted (-6.433) with application of under PEG-6000 at 0.75 MPa in comparison with under normal watering (T0). The greatest reduction (RD1) in the root length (RL) of TJ-83 × NIA-Sarang and Sarsabz × TD-1 F2 wheat line was observed by (-10.83), respectively under PEG-6000 at 0.5 MPa application as compared with under normal watering (T0). The results indicated that the highest reduction (RD2) in the root length of TJ-83 × NIA-Sarang F2 wheat line was noted by (-7.20) respectively under PEG-6000 at 0.75 MPa application as compared to under normal watering (T0).

Evaluation of reduction in shoot and root fresh weight of F2 lines under PEG-6000 stress condition

The data in (Table 2) depicted that maximum reduction (RD1) in the shoot fresh weight of TJ-83 × Kiran-95 F2 wheat line was

observed by (-0.114) under PEG-6000 at 0.5 MPa application as compared with under normal watering (T0) treatment. Furthermore, the highest reduction (RD2) in the shoot fresh weight of TD-1 cultivar was (-1.29) under PEG-6000 at 0.75 MPa as compared to under normal watering (T0) treatment. The results highlighted that the maximum reduction (RD1 and RD2) in the root fresh weight of TD-1 cultivar was noted by (-1.0) under PEG-6000 at 0.5 MPa treatment, and (1.006) under PEG-6000 at 0.75 MPa treatment as compared with under normal watering (T0) treatment.

Evaluation of reduction in shoot and root dry weight of wheat genotypes under water stress condition

As shown in (Table 3) the data revealed that the highest reduction (RD1) in the shoot dry weight of Sarsabz × NIA-Sarang F2 wheat line was recorded up to (-1.97) under PEG-6000 at 0.5 MPa application in comparison with under normal watering (T0) treatment. The greatest reduction (RD2) in the shoot dry weight of TJ-83 × Sarsabz F2 wheat line was noted by (-1.213) under PEG-6000 at 0.75 MPa as compared to under normal watering (T0) treatment. It has been observed that the maximum reduction (RD1) in the root dry weight of TJ-83 × Kiran-95 F2 wheat line was up to (-1.196) under PEG-6000 at 0.5 MPa application as compared to under normal watering (T0) treatment. The highest reduction (RD2) in the root dry weight of TJ-83 cultivar was observed by (-0.373) under PEG-6000 at 0.75 MPa application as compared with under normal watering (T0) treatment. The reduction in seedling growth may cause by restrictions in cell divisions and enlargement as a result, reduced water absorption by plant (Basal et al., 2020; Koskosidis et al., 2020). In the earlier study, Chachar et al. (2016) stated that the highest reduction in wheat varieties was observed at higher osmotic stress evoked by PEG-6000 (-1.0 MPa).

Mean squares of seedling traits under water stress condition

Table 4 indicated that the studied replications had shown the non-significant ($P > 0.01\%$) relationship with seedling traits such as shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW) of cultivars and their F2 lines under normal watering, PEG-6000 at 0.5 MPa, and PEG-60000 at 0.75 MPa treatments. The highly significant relation found among treatment and shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW). The replication × treatment relationship was highly positive with shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW). The genotypes relationship was highly significant with shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW). The treatment × genotype relation was highly significant with shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW). The replication × treatment × genotypes relation was highly significant with shoot length and root length of wheat varieties under water stress condition. Our results are in-line with, Ghaffar et al. (2023), who reported highly significant differences ($> 0.01\%$) for shoot and root fresh weight, root and shoot dry weight. Panhwar et al. (2022) indicated highly significant differences among wheat genotypes for morphological traits.

Under PED 6000 at 0.75 MPa

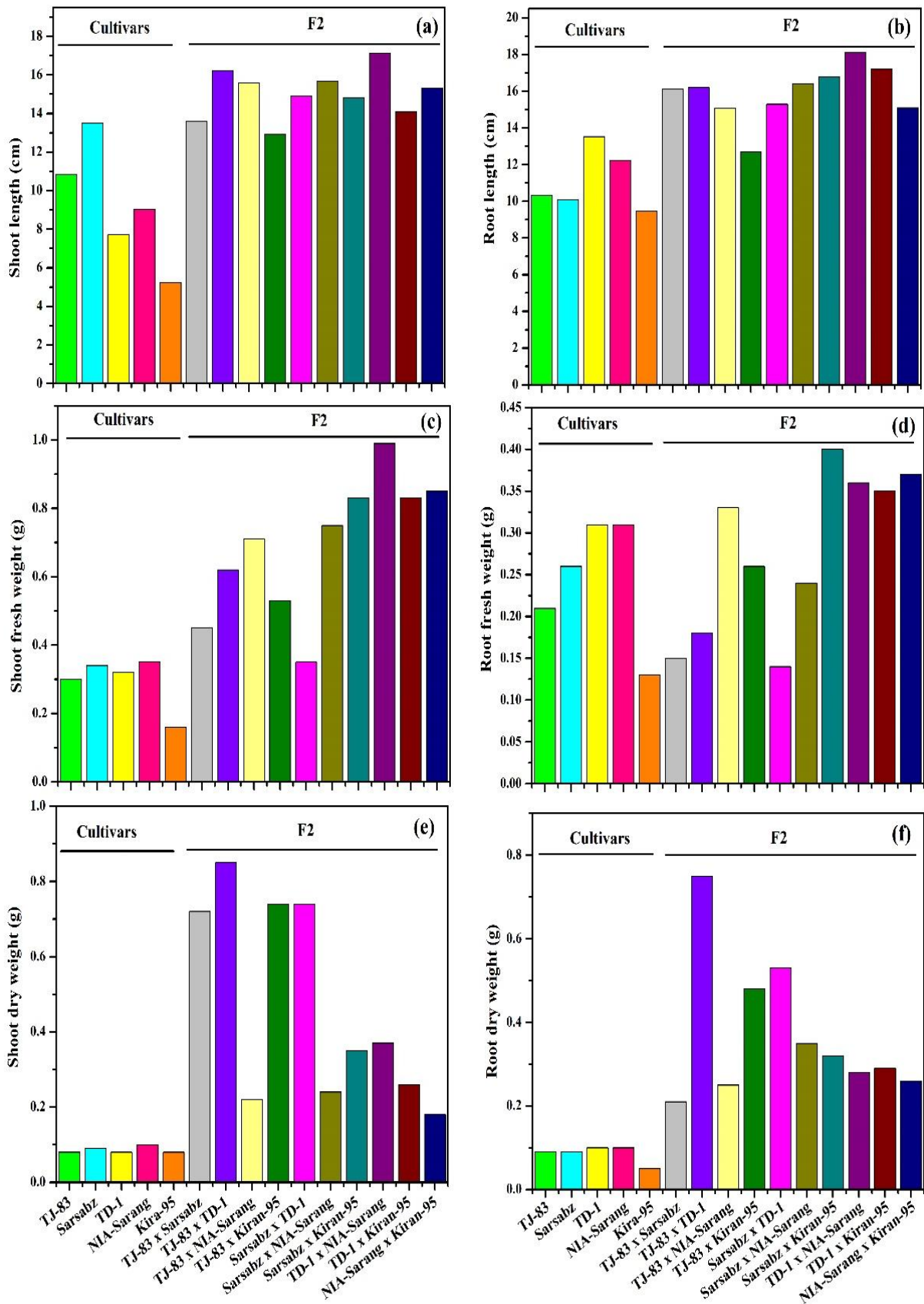


Figure 4. Impact under PEG- 6000 at 0.75 MPa: a – on shoot length (SL); b – root length (RL); c – shoot fresh weight (SFW); d – root fresh weight (RFW); e – shoot dry weight (SDW); f – root dry weight (RDW)

Table 1. Estimation of reduction in shoot and root length of wheat cultivars and F2 lines under normal watering, PEG-6000 at 0.5 MPa, and 0.75 MPa treatments

| Genotypes | Shoot length, cm | | | | | Root length, cm | | | | |
|-----------------------|------------------|---------|----------|---------------|---------------|-----------------|----------|---------|---------------|--------------|
| | T0 | 0.5 MPa | 0.75 MPa | RD1 | RD2 | T0 | 0.75 MPa | 0.5 MPa | RD1 | RD2 |
| TJ-83 | 17.273 | 10.84 | 14.097 | -3.176 | -6.433 | 16.217 | 10.32 | 14.173 | -5.90 | -2.044 |
| Sarsabz | 15.84 | 12.49 | 14.09 | -3.35 | -1.75 | 14.82 | 10.09 | 13.493 | -4.73 | -1.33 |
| TD-1 | 15.033 | 7.71 | 12.907 | -7.323 | -2.126 | 16.20 | 13.51 | 14.503 | -2.69 | -1.70 |
| NIA-Sarang | 17.647 | 9.043 | 14.577 | -8.604 | -3.07 | 16.603 | 12.22 | 13.303 | -4.38 | -3.297 |
| Kiran-95 | 17.397 | 5.24 | 12.31 | -12.16 | -5.087 | 15.103 | 9.48 | 13.68 | -5.62 | -1.42 |
| TJ-83 × Sarsabz | 19.667 | 13.57 | 16.533 | -6.097 | -3.134 | 17.66 | 14.13 | 16.167 | -3.53 | -1.49 |
| TJ-83 × TD-1 | 18.967 | 14.20 | 16.40 | -4.77 | -2.57 | 24.00 | 16.20 | 19.10 | -7.80 | -4.90 |
| TJ-83 × NIA-Sarang | 19.733 | 15.57 | 17.30 | -4.163 | -2.433 | 25.90 | 15.07 | 18.70 | -10.83 | -7.20 |
| TJ-83 × Kiran-95 | 17.033 | 12.93 | 14.333 | -4.103 | -2.70 | 23.067 | 12.70 | 17.00 | -10.37 | -6.07 |
| Sarsabz × TD-1 | 16.533 | 14.9 | 15.567 | -1.633 | -0.97 | 23.00 | 15.27 | 18.433 | -10.83 | -4.57 |
| Sarsabz × NIA-Sarang | 15.433 | 11.67 | 13.90 | -3.763 | -1.533 | 16.10 | 13.37 | 15.967 | -2.73 | -0.13 |
| Sarsabz × Kiran-95 | 15.167 | 12.83 | 14.467 | -2.337 | -0.70 | 21.767 | 15.77 | 18.033 | -5.997 | -3.73 |
| TD-1 × NIA-Sarang | 17.567 | 13.13 | 14.433 | -4.437 | -3.134 | 18.13 | 14.17 | 16.70 | -3.96 | -1.43 |
| TD-1 × Kiran-95 | 14.767 | 11.10 | 12.833 | -3.667 | -1.934 | 17.231 | 14.20 | 15.933 | -3.031 | -1.298 |
| NIA-Sarang × Kiran-95 | 16.20 | 10.83 | 15.067 | -5.37 | -1.133 | 18.267 | 11.10 | 15.533 | -7.167 | -2.734 |

Table 2. Estimation of reduction in shoot and root fresh weight of wheat cultivars and F2 seedling under normal water, PEG-6000 at 0.5 MPa and 0.75 MPa treatments

| Genotypes | Shoot fresh weight, g | | | | | Root fresh weight, g | | | | |
|-----------------------|-----------------------|---------|----------|---------------|--------------|----------------------|----------|---------|-------------|---------------|
| | T0 | 0.5 MPa | 0.75 MPa | RD1 | RD2 | T0 | 0.75 MPa | 0.5 MPa | RD1 | RD2 |
| TJ-83 | 1.18 | 0.546 | 0.301 | -0.634 | -0.88 | 0.766 | 0.338 | 0.337 | -0.428 | -0.429 |
| Sarsabz | 1.313 | 0.67 | 0.338 | -0.643 | -0.98 | 0.98 | 0.360 | 0.360 | -0.62 | -0.62 |
| TD-1 | 1.61 | 0.561 | 0.320 | -1.05 | -1.29 | 1.38 | 0.384 | 0.374 | -1.0 | -1.006 |
| NIA-Sarang | 1.615 | 0.733 | 0.346 | -0.89 | -1.27 | 1.24 | 0.411 | 0.30 | -0.83 | -0.94 |
| Kiran-95 | 1.35 | 0.49 | 0.161 | -0.86 | -1.19 | 0.835 | 0.208 | 0.11 | -0.63 | -0.73 |
| TJ-83 × Sarsabz | 1.07 | 1.17 | 0.451 | -0.10 | -0.62 | 0.742 | 0.314 | 0.114 | -0.428 | -0.628 |
| TJ-83 × TD-1 | 0.92 | 0.73 | 0.615 | -0.19 | -0.30 | 0.558 | 0.278 | 0.17 | -0.28 | -0.39 |
| TJ-83 × NIA-Sarang | 1.12 | 1.084 | 0.714 | -0.02 | -0.406 | 0.816 | 0.297 | 0.147 | -0.519 | -0.67 |
| TJ-83 × Kiran-95 | 1.014 | 0.90 | 0.517 | -0.114 | -0.497 | 0.916 | 0.376 | 0.179 | -0.54 | -0.736 |
| Sarsabz × TD-1 | 1.146 | 1.102 | 0.35 | -0.44 | -0.706 | 0.863 | 0.289 | 0.154 | -0.573 | -0.709 |
| Sarsabz × NIA-Sarang | 0.789 | 0.745 | 0.70 | -0.044 | -0.089 | 0.443 | 0.305 | 0.19 | -0.133 | -0.253 |
| Sarsabz × Kiran-95 | 0.98 | 0.929 | 0.828 | -0.05 | -0.15 | 0.871 | 0.300 | 0.17 | -0.571 | -0.701 |
| TD-1 × NIA-Sarang | 1.01 | 0.919 | 0.98 | -0.091 | -0.03 | 0.695 | 0.393 | 0.193 | -0.307 | -0.51 |
| TD-1 × Kiran-95 | 0.83 | 0.7642 | 0.734 | -0.066 | -0.096 | 0.588 | 0.24 | 0.123 | -0.35 | -0.467 |
| NIA-Sarang × Kiran-95 | 0.946 | 0.8403 | 0.60 | -0.106 | -0.346 | 0.524 | 0.327 | 0.26 | -0.194 | -0.264 |

Table 3. Estimation of reduction in shoot and root dry weight of wheat cultivars and F2 seedling under normal water, PEG-6000 at 0.5 MPa and 0.75 MPa treatments

| Genotypes | Shoot fresh weight, g | | | | | Root fresh weight, g | | | | |
|-----------------------|-----------------------|----------|---------|--------------|---------------|----------------------|----------|---------|---------------|---------------|
| | T0 | 0.75 MPa | 0.5 MPa | RD1 | RD2 | T0 | 0.75 MPa | 0.5 MPa | RD1 | RD2 |
| TJ-83 | 0.182 | 0.101 | 0.125 | -0.081 | -0.058 | 0.813 | 0.113 | 0.44 | -0.696 | -0.373 |
| Sarsabz | 0.199 | 0.136 | 0.156 | -0.063 | -0.043 | 0.115 | 0.032 | 0.093 | -0.083 | -0.022 |
| TD-1 | 0.237 | 0.151 | 0.174 | -0.086 | -0.063 | 0.44 | 0.074 | 0.153 | -0.366 | -0.287 |
| NIA-Sarang | 0.207 | 0.110 | 0.170 | -0.097 | -0.037 | 0.157 | 0.052 | 0.120 | -0.105 | -0.037 |
| Kiran-95 | 0.213 | 0.119 | 0.157 | -0.094 | -0.056 | 0.335 | 0.071 | 0.097 | -0.264 | -0.238 |
| TJ-83 × Sarsabz | 1.410 | 0.147 | 0.197 | -1.263 | -1.213 | 0.287 | 0.156 | 0.2447 | -0.131 | -0.042 |
| TJ-83 × TD-1 | 1.796 | 0.103 | 0.94 | -1.693 | -0.856 | 0.963 | 0.31 | 0.61 | -0.653 | -0.353 |
| TJ-83 × NIA-Sarang | 0.789 | 0.130 | 0.527 | -0.399 | -0.5763 | 0.581 | 0.25 | 0.410 | -0.331 | -0.171 |
| TJ-83 × Kiran-95 | 1.585 | 0.431 | 0.886 | -1.15 | -0.699 | 1.816 | 0.62 | 1.076 | -1.196 | -0.074 |
| Sarsabz × TD-1 | 1.640 | 0.773 | 1.103 | -0.867 | -0.537 | 1.25 | 0.298 | 0.985 | -0.952 | -0.265 |
| Sarsabz × NIA-Sarang | 1.290 | 0.093 | 0.293 | -1.97 | -0.997 | 0.867 | 0.22 | 0.767 | -0.647 | -0.10 |
| Sarsabz × Kiran-95 | 0.280 | 0.027 | 0.117 | -0.253 | -0.1633 | 0.383 | 0.157 | 0.0567 | -0.226 | -0.063 |
| TD-1 × NIA-Sarang | 0.2967 | 0.097 | 0.147 | -0.10 | -0.15 | 0.977 | 0.1267 | 0.323 | -0.850 | -0.654 |
| TD-1 × Kiran-95 | 0.676 | 0.067 | 0.317 | -0.61 | -0.359 | 0.873 | 0.011 | 0.130 | -0.862 | -0.43 |
| NIA-Sarang × Kiran-95 | 0.656 | 0.023 | 0.120 | -0.633 | -0.536 | 0.817 | 0.133 | 0.533 | -0.684 | -0.284 |

Table 4. Mean squares of seedling traits under water stress treatments and interactions

| Variation | DF | SL | RL | SFW | RFW | SDW | RDW |
|-------------------------------------|----|-----------|-----------|-----------|-----------|-----------|-----------|
| Replication | 2 | 0.3828ns | 0.412ns | 0.0021ns | 0.003ns | 0.0103ns | 0.003ns |
| Treatment | 2 | 83.3508** | 398.168** | 0.98736** | 0.20424** | 7.30521** | 14.1238** |
| Replication × Treatment | 4 | 8.1538** | 8.853** | 0.55358** | 0.57199** | 0.182** | 0.3039** |
| Genotypes | 14 | 13.5825** | 38.845** | 0.12063** | 0.25** | 9.22776** | 0.1697** |
| Treatment × Genotype | 28 | 9.6807** | 12.302** | 0.3647** | 0.174** | 0.2689** | 0.460** |
| Replication × Treatment × Genotypes | 86 | 2.1162** | 2.538** | 0.0401 | 0.01 | 0.02977 | 0.022 |

Note: DF – degrees of freedom; SL – shoot length; RL – root length; SFW – shoot fresh weight; RFW – root fresh weight; SDW – shoot dry; RDW – root dry weight

Correlation matrix

The data in (Figure 5) revealed that the correlation matrix was performed among the shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight of 5 wheat cultivars and their 10 F2 lines under normal watering, PEG-6000 at 0.5 MPa and PEG-6000 at 0.75 MPa treatments. The data indicated that the root fresh weight was significantly correlated with shoot fresh weight under normal watering treatment (Figure 5a). The root dry weight was significantly correlated with shoot dry weight under PEG-6000 at 0.5 MPa treatment (Figure 5b). The positive correlation was

found among root dry weight and shoot dry weight under PEG-6000 at 0.75 MPa treatment (Figure 5c). Khan et al. (2013) stated that the fresh root weight had showed the considerable correlation with fresh shoot weight and dry shoot weight of wheat. Awad et al. (2018) stated that root length was negatively associated with average root diameter under water stress condition. The obtained results are coincide with reported by (Panhwar et al., 2021; Panhwar et al., 2022), who stated highly positive, and negative correlations in between traits under study. Basheer et al. (2021), and Ozturk et al. (2021) noted highly significant differences in traits studied under water stress.

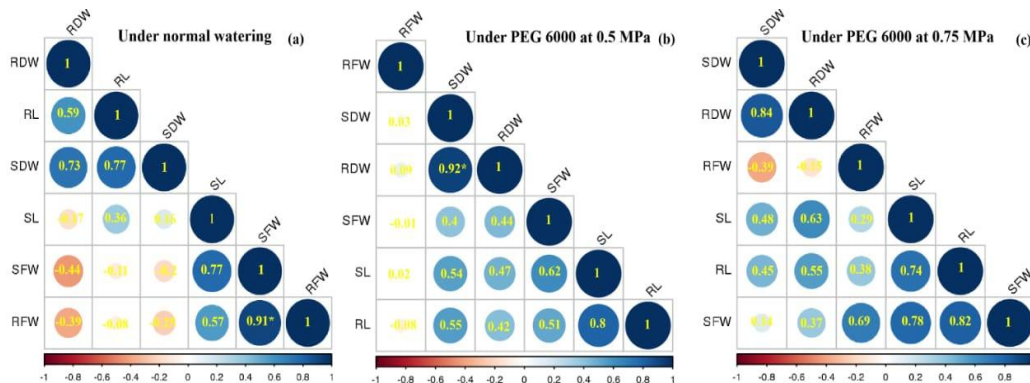


Figure 5. Correlation matrix of seedling traits of wheat parents and F2 lines under normal watering, PEG 6000 at 0.5 MPa and PEG 6000 at 0.75 MPa: SL – shoot length; RL – root length; SFW – shoot fresh weight; RFW – root fresh weight; SDW – shoot dry weight; RDW – root dry weight

CONCLUSION

Present study concluded that the maximum shoot length (cm) was observed in TJ-83 × NIA-Sarang F2 line, shoot fresh weight (g) in NIA-Sarang × Sarsabz F2 line, and shoot dry weight (g) in TJ-83 × NIA-Sarang F2 line followed by other varieties under normal watering treatment. The maximum shoot length (cm) was observed in TJ-83 × NIA-Sarang F2 line, shoot fresh weight (g) in TJ-83 × NIA-Sarang wheat F2 line, and shoot dry weight in TJ-83 × Kiran-95 wheat F2 line followed by other varieties under PEG-6000 at 0.5 MPa treatment. The highest shoot length (cm) was recorded in TD-1 × NIA-Sarang wheat F2 line, shoot fresh weight (g) in TD-1 × NIA-Sarang F2 wheat line, and shoot dry weight (g) in TJ-83 × TD-1 wheat F2 line than other varieties under PEG-6000 at 0.75 MPa treatment. The correlation matrix results revealed that the root fresh weight was significantly associated with shoot fresh weight of wheat varieties under normal watering treatment. The root dry weight was significantly correlated with shoot dry weight under PEG-6000 at 0.5 MPa treatment, and the positive correlation was observed among root dry weight and shoot dry weight under PEG-6000 at 0.75 MPa treatment. The present study recommended that this screening technique of drought tolerant at seedling stage in addition future studied must focus

onto evaluate the highly performing wheat varieties based on molecular level, impact of PEG-6000 on soil health, water uptake in plant, interaction of PEG-6000 with nutrients and, co-application PEG-600 with biochar and minerals under field conditions for development of drought tolerant breeding material.

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Author's statements

Contributions

Conceptualization: N.A.P., Z.A.S.; Data curation: N.A.P., Z.A.S.; Formal Analysis: A.H.L.; Investigation: A.H.L.; Methodology: N.A.P.; Project administration: Z.A.S., M.A.S.; Resources: N.A.P., M.A.S.; Software: A.H.L.; Supervision: Z.A.S., W.A.J., M.A.S.; Validation: A.H.L.; Visualization: A.H.L.; Writing – original draft: N.A.P.; Writing – review & editing: A.H.L. All authors have read and agreed to the published version of the manuscript.

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The authors declare no competing interests.

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No data were used for the current study.

AI Disclosure

The authors declare that generative AI was not used to assist in writing this manuscript.

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