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WHEAT GENOTYPES RESPOND DIFFERENTLY UNDER POLYETHYLENE GLYCOL (PEG) INDUCED DROUGHT STRESS DURING GERMINATION AND EARLY SEEDLING GROWTH STAGES

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Abstract

Water scarcity has emerged as one of a critical environmental constriction that negatively affect wheat growth, development and yield in Bangladesh. Identification of drought tolerant genotypes is crucial at drought sensitive initial stages of plant growth especially germination and seedling growth stages. To cater this issue, 30 wheat genotypes were subjected to polyehelene glycol (PEG 6000) induced drought stress levels of 0, -2.0 and -4.0 Mpa (osmotic stresses) and their response was tested at the germination and seedling growth stages. The experimental set up was Completely Ramdomized Design (CRD) with five replications. The results revealed that the rate of germination (RG) among the genotypes varied significantly with the different water potential levels, and the RG decreased with the advancement of stress. The stress tolerance index (STI) values based on RG were > 0.900 in wheat cultivars of Shatabdi, BARI Gom 25, BAW 1118, BAW 1151, BAW 1161, E 2, E 18, E 30, E 34 and E 38under severe water deficit stress, while the values were < 0.800 in wheat genotypes BARI Gom 26, BARI Gom 27, BARI Gom 28, BAW 1130, BAW 1140, BAW 1143, BAW 1168, E 28 and E 42. The co-efficient of germination (COG) was significantly decreased with increasing water potential stresses (-2 & -4 bars), and the genotypes Sourav, BARI Gom 25, BAW 1118, BAW 1135, BAW 1151, BAW 1157, BAW 1161, BAW 1163, BAW 1170, BAW 1171, E 2, E 18, E 23, E 29, E 34 and E 38showed > 0.980 STI values whereas, BARI Gom 26, BARI Gom 27, E 24, E 28, E 42, BAW 1130, BAW 1140 and BAW 1168 produced < 0.970 STI values under higher water potential. Higher STI values (> 0.920) regarding the germination vigour index (GVI) under higher water stress were observed in BARI Gom 25, E 18, E 23, E 34, E 38, BAW 1118, BAW 1161 and BAW 1170, and very lower STI values (< 0.800) were obtained in the Shatabdi, BARI Gom 26, BARI Gom 27, BAW 1130, BAW 1140, BAW 1168, E 3, E 24, E 28 and E 42 genotypes. The genotypes Shatabdi, BARI Gom 25, E 18, E 38, BAW 1118, BARI Gom 27, E 24, E 34, BAW 1143 and BAW 1170 showed higher STI values (> 0.550) indicating tolerant genotypes and the genotypes Sourav, BARI Gom 26, BARI Gom 28, BAW 1130, BAW 1151, BAW 1168, E 2, E 3, E 28 and E 29demonstrated lower STI values (< 0.450) designating susceptible genotypes. As water deficit stress increased, the shoot weight (g) of wheat genotypes decreased. Notably, genotypes Shatabdi, BARI Gom 25, E 18, E 34, E 38, and BAW 1118 exhibited Stress Tolerance Index (STI) values greater than 0.700. In contrast, genotypes BARI Gom 26, E 2, E 23, E 29, BAW 1130, BAW 1140, BAW 1151, BAW 1157, and BAW 1161 displayed STI values lower than 0.550. The genotypes showing higher and lower STI values denoted water stress tolerant and susceptible genotypes, respectively.

Key words: Germination; Wheat; Drought stress; Polyethylene glycol.

Introduction

The global human population reached 8.0 billion in mid-November 2022, compared to just 2.5 billion in 1950. Projections suggest the world's population will rise to 9.7 billion by 2050 and could peak at nearly 10.4 billion by the mid-2080s (United Nations, 2022). This population growth, coupled with increased consumption, poses a significant challenge for global food security amidst climate change and land-use scenarios (Islam *et al*., 2022). Food insecurity has been exacerbated by the COVID-19 pandemic since 2020 and the ongoing Russia-Ukraine war. Meeting the growing demand for food requires inevitable increases in crop productivity. Wheat (*Triticum aestivum* L.), a staple of human diets for millennia, plays a vital role in global food security (Yıldırım *et al*., 2018; El Sabagh *et al*., 2019, 2021; Hossain *et al*., 2023; Hafeez *et al*., 2024). As a member of the Poaceae family, it provides a significant portion of human calories, serving as a primary source of carbohydrates and essential nutrients (Akhter *et al*., 2017, 2019; Ahmad *et al*., 2021). Wheat-based products such as bread, pasta, and cereals are dietary staples for billions, especially in regions where it forms the cornerstone of nutrition (Barutcular *et al*., 2016a,b; Azam *et al*., 2018). Its versatility in cultivation across diverse climates and its adaptability to various culinary preparations make wheat indispensable. With its high yield potential and widespread consumption, wheat remains crucial in combating hunger and ensuring food stability for populations globally.

Water scarcity is the most critical factor negatively affecting at all stages of the life cycle drastically reducing wheat production (Chowdhury *et al*., 2021; Abbas *et al*., 2023). Assessing the relative yield performance of genotypes under drought stress and normal conditions serves as a primary step in identifying traits associated with drought tolerance and selecting genotypes for breeding in dry environments (Chowdhury *et al*., 2021). Thus, selection of best performed cultivars at water deficit environment is prime importance to increase the yield specially in semi-arid and arid regions. Seed germination, vigour of seedling, growth rate, mean time for emergence and tolerance to dehydrations are the most serious factors critically determining the yield (Noorka *et al*., 2007; Kamran *et al*., 2021). Hence, seed germination and seedling vigor are highly impacted by soil moisture deficits, particularly during the vulnerable seedling stage of crop plants.

Seed germination rate and seedling vigour of plants are extremely susceptible to soil moisture deficit. Hence, ealy growth attributes like seed germination, seedling emergence and seedling vigour might assist to screen out drought tolerant wheat cultivars. Furthermore, root traits such as root length and volume, root/shoot ratio, adventitious root length have been identified as useful traits connected with yield that can utilize for screening wheat genotypes at water deficit conditions (Shahbazi, 2012; Ahmed *et al*., 2024). Screening genotype pools of staple crops by imposing DS through polyethylene glycol (PEG) to evaluate drought sensitivity at seed germination and seedling emergence has been widely practiced (Awan *et al*., 2021; Islam *et al*., 2024). The PEG is a non-ionic high molecular inert water-soluble substance that can decrease water potential and increase osmotic potential creating an artificial water deficit environment suitable to simulate drought in experiments. The drought responses of PEG induced water deficit environments are genotypic dependent, and wheat genotypes showed significant differences at different seedling traits using diverse concentrations of PEG 6000 (Singh *et al*., 2008).

However, research gaps exist regarding drought tolerance potential of local elite genotypes of wheat in Bangladesh and wheat growers in the region have been in dire need to have research-based information on this aspect. Thus, we have hypothesized that different elite genotypes of wheat might respond differently to varying levels of imposed drought based on their genetic make-up differences. Therefore, identification drought tolerant potential of wheat genotypes is the vital issue to boost the wheat production in drought and saline prone areas Bangladesh and other regions having similar agro-climatic conditions. Thus, the experiment was designed to screen out 30 wheat genotypes by inducing PEG induced DS conditions to assess their drought tolerance potential based on germination and seedling traits.

Material and Method

Study site and materials: The research took place at the Laboratory within the Department of Crop Physiology and Ecology at Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during September 2014. Thirty wheat genotypes including six existing varieties and 24 advanced lines were acquired from Wheat Research Centre (WRC) of BARI, Dinajpur, Bangladesh were used as planting material for the present study (Table 1).

Study procedure and design: Two factors were considered: Factor A involved three levels of water potential, specifically i) Control (Tap water), and ii) Moderate stress (-2 bars) induced by PEG (Polyethylene Glycol 6000), and iii) higher stress (-4 bars) induced by PEG, whereas the other investigation factor was thirty wheat genotypes including existing popular varieties, advanced lines and entries were adopted in the experiment. The experimental layout was completely randomized design (CRD) with five replications. Wuest & Lutcher (2012) methodology was put into practice for recording the normal germination of wheat seed up to -1.1 Mpa soil water potential, but the value is slower from -1.3 to 1.6 Mpa and hampered greatly with below -1.6 Mpa.

Placement of seed for germination: The seeds underwent surface sterilization through immersion in a 0.1% mercuric chloride solution for 2 minutes, followed by extensive rinsing with sterilized water (REFF). Three levels of water potential corresponding to treatments (0, -2 bars and -4 bars) were achieved by dissolving calculated amount of PEG in tap water, in accordance with the procedure outlined by Michel (1983). The amount of 119.5 g and 178.4 g PEG were used for preparing -2 and -4 bars water potential, respectively through dissolving 1 litre of water. Seventy seeds of each genotype were placed in each petri-dish (12 cm diameter) for germination on sand bed and irrigated in accordance with the respective treatment. Afterwards, the petri-dishes were than irrigated daily with 5ml of respective solution. Five replicates were for each treatment. The seedlings were given 10 days to grow following placement of seeds for germination.

Data recorded: The number of germinated seed was counted daily. When the plumule and radicle had attained length of >2 mm, then a seed was counted as germinated. The germination indices were determined by using formula as given below:

The rate of germination (RG) was rcorded by following the equation of Krishnasamy & Seshu (1990).

$$RG\ (\%) = \frac{\text{Germinated seeds number at 48 h}}{\text{Germinated seed at 168 h}} \times 100$$

The co-efficient of germination and vigour index were computed utilizing the outlined in Copeland (1976).

$$COG = \frac{100\ (A_1 + A_2 + \text{---------} + A_n)}{A_1T_1 + A_2T_2 + \text{---------} + A_nT_n}$$

$$VI = \frac{A_1}{T_1} + \frac{A_2}{T_2} + \frac{A_n}{T_n}$$

Where, A and T present germinated seeds number and days taken to corresponding A recording respectively, while n denote days taken to final count.

At 10th days after placement seedling related traits viz., shoot and root length with their dry weight were recorded. To determine dry weight, the samples were subjected to drying at 70ºC for 72 h in an electric oven (Model- E28# 03-54639, Binder, Germany). Subsequently, the weights were recorded using an electrical balance (AND Model EK-300i). Mean lengths (measured in centimeters) and mean dry weights (measured in milligrams) were computed for every treatment combination.

Stress tolerance index (STI): The stress tolerance index was determined utilizing the formula established by Goudarzi & Pakniyat (2008).

$$STI = \frac{\text{Variable measured under DS conditions}}{\text{Variable measured without imposition of DS}}$$

Table 1. Genotypic and varietal information of wheat used in the study.

S. No.	Genotypes	Pedigree/Selection history
1.	Sourav (BAW 897)	Variety, NAC/VEE CM 64224-5Y-1M-1Y-2M-0Y
2.	Shatabdi (BAW 936)	Variety, MRNG/BVC//BLO/PVN/3/PJB-81 CM98472-1JO-0JO-0O-1JO-0JO-0R2DI
3.	BARI Gom 25 (BAW 1059)	Variety, ZSH 12/HLB 19//2*NL 297
4.	BARI Gom 26 (BAW1064)	Variety, ICTAL123/3/RAWAL87//VEE/HD2285 BD(JOY) 86-0JO-3JE-010JE-010JE-HRDI-RC5DI
5.	BARI Gom 27 (BAW 1120)	Variety, (FRANCOLIN#1) WAXWING*2/VIVITSICGSS01B00056T-099Y-099M-099M-099Y-099M-14Y-0B
6.	BARI Gom 28 (BAW 1141)	Variety, CHIL/2*STAR/4/BOW/CROW//BUC/PVN/3/2*VEE#10 CMSS95Y00624S-0100Y-0200M-17Y-010M-5Y-0M
7.	E 2	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3 /CROC_1AESQUARROSA (244)//OPATA/7/PASTOR//MILAN/KAU Z/3/BAV92CMSA04M01201T-050Y-040ZTP0M-040ZTY-040ZTM-040SY-6ZTM-01Y-0B
8.	E 3	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3 /CROC_1AESQUARROSA (244)//OPATA/7/PASTOR//MILAN/KAU Z/3/BAV92 CMSA04M01201T-050Y-050ZTP0M-040ZTY-040ZTM-040SY-6ZTM-03Y-0B
9.	E 18	PBW343*2/KUKUNA//WBLL1*2/KUKUNA CMSA05M00118S-0100ZTM-029(LR34HOM+HET) ZTY-040ZTM-040SY-2ZTM-0Y-0B
10.	E 23	KS85W663.42/MONARCAF2007//WBLLI*2/TUKURU CMSA06Y00889T-040ZTM-040ZTPOY-040ZTM-040SY-8ZTM-0Y-0B
11.	E 24	FRTL//ATTILA/3*BCN, PTSS02Y00011S-099B-099Y-099B-0Y-0B-17Y-0ZTB-0SY-0B-0Y
12.	E 28	PUB94.15.1.12/WBLL1 PTSS02Y00027S-011Y-0B-0Y-0B-7Y-0M-0SY-0Y-0Y
13.	E 29	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA CMSS93Y01001S-12Y-1KBY-010M-010Y-1M-0KBY-0M-2KBY-0Y-0HTY-0Y-0Y
14.	E 30	PBW343*2/KUKUNA//WBLL1*2/KUKUNA CMSA05M00118S-0100ZTM-029(LR34HOM+HET) ZTY-040ZTM-040SY-45ZTM-0Y-0B
15.	E 34	MEX94.2.19-0Y-0Y-0Y
16.	E 38	SOKOLL//W15.92/WBLL1, PTSS02B00088T-0TOPY-0B-0Y-0B-5Y-0M-0SY-0B-0Y
17.	E 42	SOKOLL/WBLL1 PTSS02Y00021S-099B-099Y-030ZTM-040SY-040M-31Y-0M-0SY-0B-0Y
18.	BAW 1118	BAW969/SHATABDIBD(DI)1319S-0DI-6DI-1DI-DIRC7
19.	BAW 1130	GOURAB/PAVON76NCD99-04-0DI-1DI-0DI-0DI-0DI-0DI-22DI-DIRC4
20.	BAW 1135	BAW969/SHATABDIBD(DI)1319S-0DI-6DI-1DI-DIRC6
21.	BAW 1138	CHEN/AE.SQ(TAUS)//BCN/3/2*PASTORCMSS98Y00844S-040Y-0B-0MXI-3DI-010DI-010DI-1DI-DIRC6
22.	BAW 1140	SOURAV/GOURABBD(JE)959S-0DI-5DI-010DI-010DI-010DI-1DI-DIRC8
23.	BAW 1143	BAW923/BAW1004BD(DI)1207S-0DI-4DI-010DI-010DI-0DI-DIRC7
24.	BARI Gom 29 (BAW 1151)	SOURAV/KLAT/SOREN//PSN/3/BOW/4/VEE#5.10/5/CNO 7/MFD//MON/3/ SERI/6/NL297 BD(DI)112S-0DI-030DI-030DI-030DI-9DI
25.	BAW 1157	BAW923/BAW1004BD(DI)1207S-0DI-4DI-010DI-010DI-0DI-DIRC6
26.	BARI Gom 30 (BAW 1161)	BAW677/BIJOYBD(JA)1365S-0DI-15DI-3DI-HR12R3DI
27.	BAW 1163	SHATABDI/BAW824BD(JE)1176S-0DI-11DI-010DI-010DI-8DI-HR27R8DI
28.	BAW 1168	BAW923/BIJOYBD(DI) 1327S-0DI-3DI-1DI-DIRC4
29.	BAW 1170	CHIR7/CBRD//GOURABBD(DI) 1327S-0DI-3DI-1DI-DIRC4
30.	BAW 1171	CHIR7/CBRD//GOURABBD(DI) 1335S- 16DI-010DI-010DI-010DI-1DI-DIRC4

Statistical analysis

The recorded data pertaining to all response variables under investigation were analyzed by partitioning the total variance with the help of computer using MSTAT program. The treatment means were compared using Least Significant Difference (LSD) Test at 5% probability level. Cluster analysis was done for grouping the wheat genotypes based on stress tolerance index to classify the 30 wheat genotypes subjected to water stress tolerance index of each character was subjected to multivariate analysis (Mahalanobis, 1936; Digby *et al*., 1989).

Results and Discussion

The response variables including seed germination characteristics and seedling traits of thirty wheat genotypes were recorded by inducing DS conditions through poly-ethylene glycol (PEG 6000). 3.1.

Germination characters

Germination ability (%): The analysis of variance showed that the RG was influenced significantly by wheat genotypes and DS level created by PEG. The rate of germination was directly correlated to the interaction effect of degree of water stress and wheat cultivars during seed germination (Table 2). The water scarcity positively correlated to the rate of germination by reducing germination rate with decreasing the water potential (Table 3). Under well water condition the rate of germination varied from 82.43 to 98.67 with a mean of 94.34 while moderate stress condition showed germination values from 75.45 to 94.84 with a mean of 87.75 and under severe stress condition it was varied from 61.19 to 92.33 with a mean of 80.47.

The PEG induced osmotic stress significantly decreased the germination percentages and mean germination time of 96 diverse wheat genotypes Dodig *et al*., (2015). Almaghrabi (2012) depicted that PEG 6000 at different concentrations remarkably inhibited many germinations related parameters in eight wheat genotypes. Similar results have been reported by Ashraf *et al*., (1996), Kaydan & Yagmur (2008), Khayantnezhad *et al*., (2010), Raza *et al*., (2012), and Jahanbin *et al*., (2012) explaining the correlation between PEG level and moisture uptaken by seeds, leading to declined seed germination percentage (GP). (Fig. 1) presented the stress tolerance index (STI) of 30 wheat cultivars at moderate water deficit stress.

Wheat genotypes- BARI Gom 25, E 2, E 3, E 18, E 30, E 34, E 38 and BAW 1118 showed more than 0.96 STI, while the wheat genotypes- BARI Gom 26, BARI Gom 28, E 42 and BAW 1140 provided less than 0.88 STI. The other wheat genotypes showed STI in between 0.88 and 0.96. The STI of thirty wheat genotypes based on rate of germination (%) at lower water potential is presented in (Fig. 2). The STI values of thirty wheat genotypes showed a broad range in response to drought tolerance. The genotypes Shatabdi, BARI Gom 25, E 2, E 18, E 30, E 34, E 38, BAW 1118, BAW 1151 and BAW 1161 showed larger than 0.90 STI while wheat genotypes including BARI Gom 26, BARI Gom 27, BARI Gom 28, E 28, E 42, BAW 1130, BAW 1140, BAW 1143 and BAW 1168 showed less than 0.80 STI and the other wheat genotypes sprayed STI in between 0.80 and 0.90.

Table 2. Mean square and co-efficient of variation of different quantitative characters as influenced by water stress level and wheat genotypes.

Plant characters	Mean square			CV (%)
	Stress level (A)	Wheat genotypes (B)	Interaction (A× B)	
Rate of germination (%)	4331.072**	302.459**	52.806 **	4.24
Co-efficient of germination	5.936 **	0.718**	0.090 NS	1.82
Vigour index	680.046**	35.909**	11.231 **	5.39
Shoot length (cm)	2667.724 **	12.301**	2.748 **	5.66
Root length (cm)	103.931 **	7.341 **	0.941 **	6.04
Shoot dry weight (mg)	742.559**	11.896 **	4.627**	7.86
Root dry weight (mg)	97.971 **	32.097**	1.922**	7.63
Seedling dry weight (mg)	1358.584 **	40.229**	9.091*	5.37

Table 3. Range and mean of quantitative characters of 30 wheat genotypes under well water, moderate water stress (-2 bars) and higher water stress (-4 bars) **conditions.**

Plant Characters	Well water		Moderate stress (-2 bars)		Higher stress (-4 bars)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Rate of germination (%)	82.43-98.67	94.34 ± 4.12	75.45-94.84	87.75 ± 5.93	61.19-92.33	80.47 ± 9.16
Co-efficient of germination	21.61-22.99	22.12 ± 0.30	20.73-22.21	21.86 ± 0.30	20.55-22.07	21.61 ± 0.34
Vigour index	34.04-43.32	38.42 ± 2.54	33.21-38.79	35.93 ± 1.71	27.33-38.22	32.93 ± 3.18
Shoot length (cm)	17.63-23.89	21.39 ± 1.33	13.78-18.88	16.06 ± 1.18	6.93-13.39	10.50 ± 1.66
Root length (cm)	9.99-13.67	11.69 ± 0.83	9.19-12.55	10.72 ± .95	7.41-11.97	9.55 ± 1.21
Shoot weight (mg)	11.12-19.40	14.39 ± 1.84	8.80-14.97	11.17± 1.25	6.87-13.25	8.66 ± 1.45
Root weight (mg)	5.74-17.90	9.37 ± 2.53	5.33-13.63	8.34 ± 1.84	3.87-10.20	7.29 ± 1.47
Seedling weight (mg)	17.18-35.10	23.76 ± 3.35	14.67-22.90	19.51 ± 2.03	12.07-20.07	15.95 ± 2.00

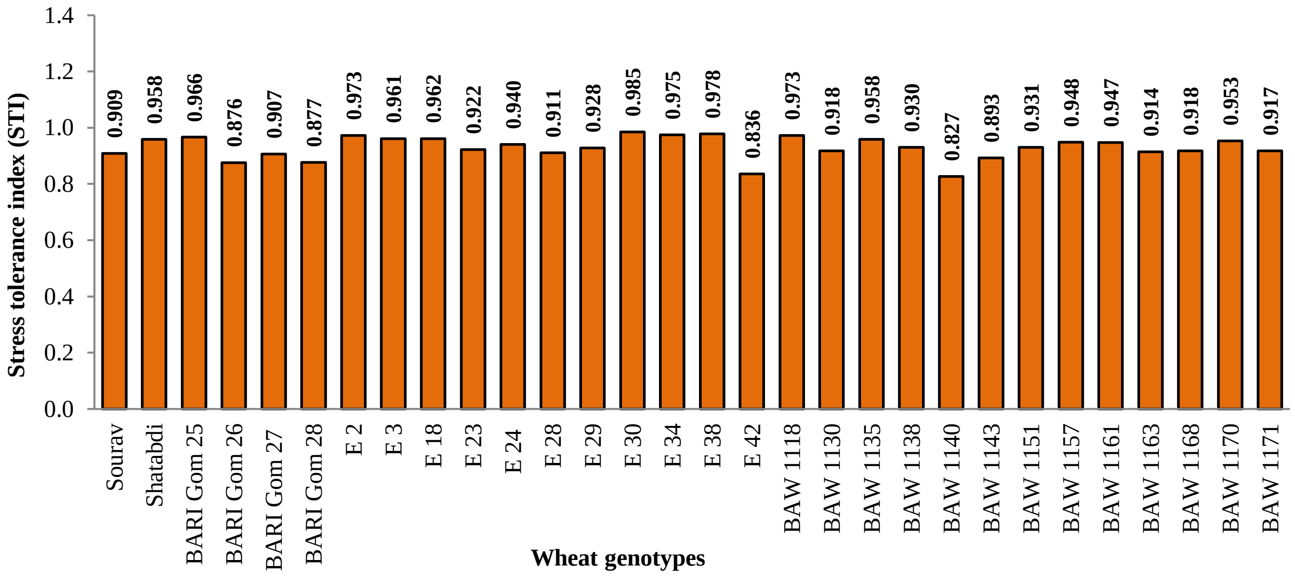

Fig. 1. Stress tolerance index evaluated of 30 wheat genotypes for rate of germination (%) under moderate water stress (-2 bars).

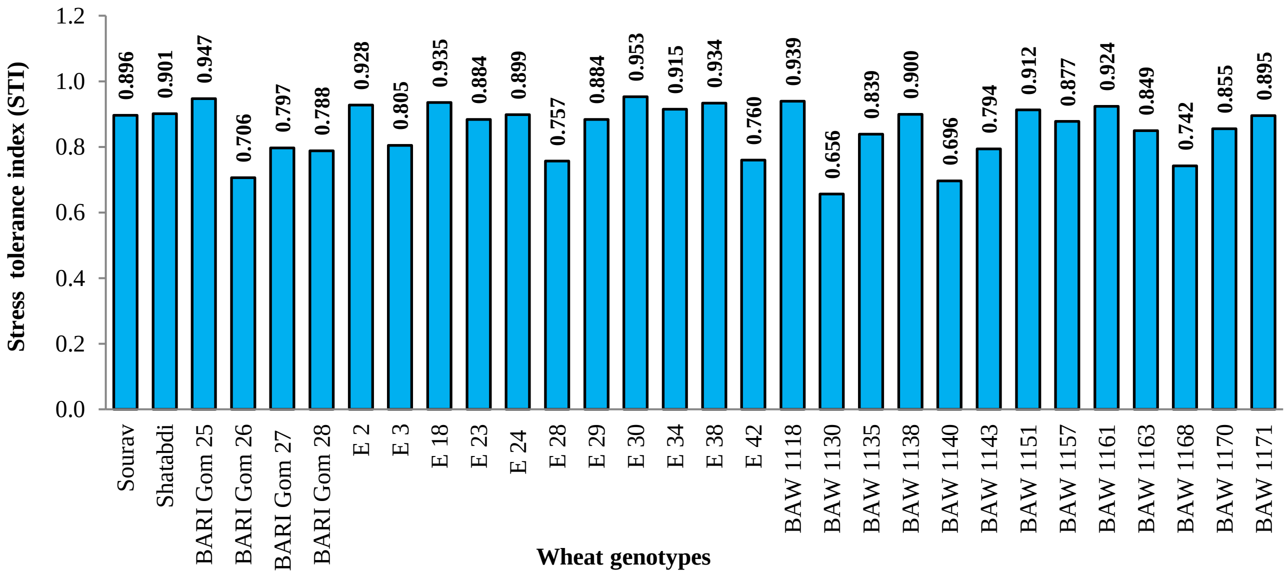

Fig. 2. Stress tolerance index evaluated of 30 wheat genotypes for rate of germination (%) under higher water stress (-4 bars).

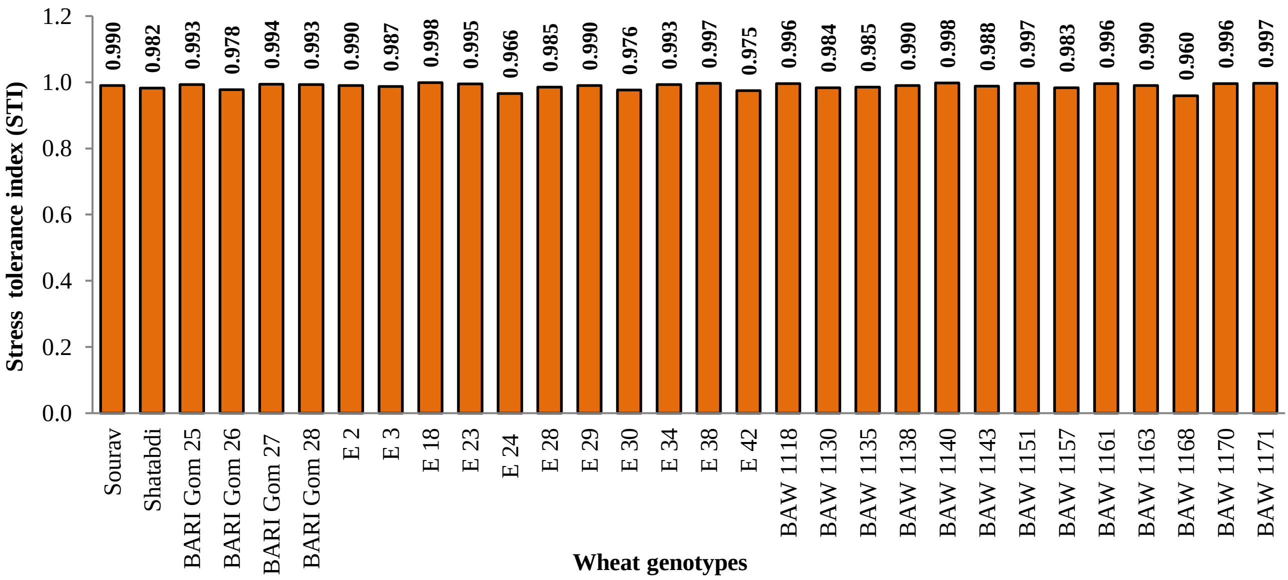

Fig. 3. Stress tolerance index evaluated of 30 wheat genotypes for co-efficient of germination under moderate water stress (-2 bars).

Co-efficient of germination (COG): The co-efficient of germination (COG) reflects the speed at which seeds germinate, and it was significantly varied by water stress levels and wheat genotypes during germination. While water stress levels and wheat genotypes significantly impacted COG, their combined effect (interaction effect) during germination was not statistically significant (Table 2). The results also revealed that the COG values decreased with decreasing water potential levels from 0 to -4 bars. The COG was found lower (ranging from 20.55 to 22.07 with a mean of 21.61), moderate (ranging from 20.73 to 22.21 with a mean of 21.86), and higher (ranging from 21.61 to 22.99 with a mean of 22.12) at higher water deficit stress, moderate water deficit stress and well-watered conditions, respectively (Table 3). Jajarmi (2009) and Jahanbin *et al*., (2012) stated that PEG induced moisture stress reduced the coefficient of germination and the reduction depends on the nature of wheat genotypes (Fig. 3).

Germination vigour index: Similar to the COG, the germination vigour index (GVI) reflects the speed of seed germination (Table 2). Our ANOVA revealed significant differences in GVI not only based on water stress levels and individual wheat genotypes, but also due to their combined influence (interaction effect). The range GVI among the wheat genotypes was 34.04 to 43.32 with a mean of 38.42 at well water condition, 33.21 to 38.79 with a mean of 35.93 at moderate water stress level and 27.33 to 38.22 with a mean of 32.93 at higher water stress level (Table 3). The GVI results exhibited reducing quickness of germination when increasing the moisture deficit stress (Fig. 4).

STI value indicates tolerance capability of genotypes against water deficit stress. At moderate water stress (-2 bars), the STI values of thirty wheat genotypes based on GVI are presented in (Fig. 5). Thirteen wheat genotypes, *viz*., BARI Gom 25, E 2, E 18, E 29, E 38, BAW 1118, BAW 1138, BAW 1140, BAW 1151, BAW 1161, BAW 1163, BAW 1170 and BAW 1171 demonstrated superior stress tolerance and yield stability based on their STI values exceeding 0.960. Conversely, four other genotypes, including Shatabdi, BARI Gom 27, E 3 and E 28 showed STI values less than 0.880. In the middle level of STI values ranging from 0.96 to 0.880 were observed in the other wheat genotypes. (Fig. 6), additionally presents the STI of 30 wheat genotypes assessed under severe water deficit stress (-4 bars) based on their GVI. Nonetheless, higher STI values (>0.920) were observed in BARI Gom 25, E 18, E 23, E 34, E 38, BAW 1118, BAW 1161 and, BAW 1170 whereas moderate STI ranged from 0.920 to 0.800 were recorded in Sourav, BARI Gom 28, E 2, E 29, E30, BAW 1135, BAW 1138, BAW 1143, BAW 1151, BAW 1157, BAW 1163 and BAW 1171, and very lower STI values less than 0.800 were obtained in the other (Shatabdi, BARI Gom 26, BARI Gom 27, E 3, E 24, E 28, E 42, BAW 1130, BAW 1140 and BAW 1168) wheat genotypes. The maximum, minimum and in-between STI value providing genotypes indicating their higher tolerant, susceptible and moderate tolerant to water deficit stresses, respectively (Figs. 5 & 6).

Greater and early seeding vigour of wheat cultivars can increase the water use efficiency up to 25% (Siddique *et al*., 1990), and accepted to evaluate wheat yield characteristics at moisture deficit environments (Botwright *et al*., 2002; Richards *et al*., 2002). Ahmad *et al*., (2013) observed positive correlation between germination rate index (GI) and seedling parameters such as root and coleoptile lengthalong with seedling vigour. The results are in line with findings of Jahanbin *et al*., (2012) who reported that germination indices like GI, and germination rate index (GRI) of 12 wheat cultivars were remarkably decreased with increasing PEG induced osmotic potentials (0, -4/0, -8/0 and -2/1 mp), and noted significant differences among the genotypes considering the stress tolerance index (STI), and also they declared the genotype S2 as the most resistant type and the genotype S9 as the most sensitive one.

Seedling growth parameters

Shoot length (cm): The ANOVA revealed significant disparities among water deficit stress levels, wheat genotypes, and their interaction effect concerning the shoot length of 10 days old seedlings (Table 2). The maximum and minimum shoot lengths recorded under well water conditions were 23.89 cm and 17.63 cm, respectively, with a mean of 21.39 cm. In moderate water deficit stress, the maximum and minimum shoot lengths were 18.88 cm and 13.78 cm, respectively, with a mean of 16.06 cm. Under higher water deficit stress, the maximum and minimum shoot lengths observed were 13.39 cm and 6.93 cm, respectively, with a mean of 10.50 cm (Table 3). The results revealed that the shoot length was decreased with the decreased of water potentials. Under moderate water stress (-2 bars), the STI based on shoot length were measured for 30 wheat genotypes. Among these, genotypes E 18, BAW 1135, BAW 1138, and BAW 1171 exhibited the higher STI values (>0.80), indicating greater stress tolerance. Conversely, genotypes Sourav, BARI Gom 26, E3, E 28, E 34, BAW 1140, BAW 1143, BAW 1151, BAW 1157, BAW 1163, BAW 1168, and BAW 1170 demonstrated lower STI values (< 0.75), suggesting lower stress tolerance. The remaining genotypes fell within the range of 0.80 and 0.75 STI (Fig. 7). On the contrary, under higher water deficit stress the higher STI (>0.55) generating genotypes were Shatabdi, BARI Gom 25, E 18, E 38 and BAW 1118, BARI Gom 27, E 24, E 34, BAW 1143, BAW 1170, and lower STI (< 0.450) producing genotypes were Sourav, BARI Gom 26, BARI Gom 28, E 2, E 3, E 28, E 29, BAW 1130, BAW 1151, and BAW 1168 and the other genotypes provide moderate STI (0.55 to 0.450) (Fig. 8). Higher STI value indicates higher tolerant to water stress deficit, moderate STI value shows moderate tolerant to water deficit stress and lower STI value designates susceptible to water deficit stress. Dodig *et al*., (2015) tested 96 wheat genotypes at germination and seedling growth stages under PEG induced osmotic stresses (-0·2, -0·4 and -0·6 MPa) and the shoot length was reduced with increasing stresses. Poly-ethylene glycol (PEG) 6000 induced water stress reduced the shoot length of eight wheat genotypes (Almaghrabi, 2012) and plumule length of 12 wheat genotypes (Jahanbin *et al*., 2012).

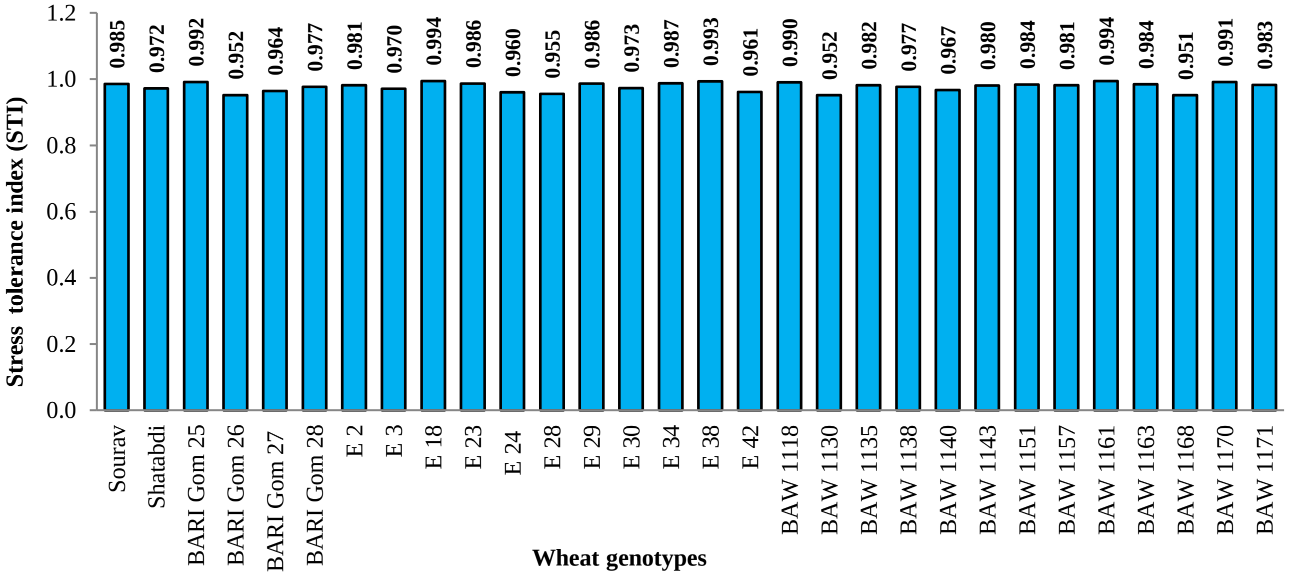

Fig. 4. Stress tolerance index evaluated of 30 wheat genotypes for co-efficient of germination under higher water stress (-4 bars).

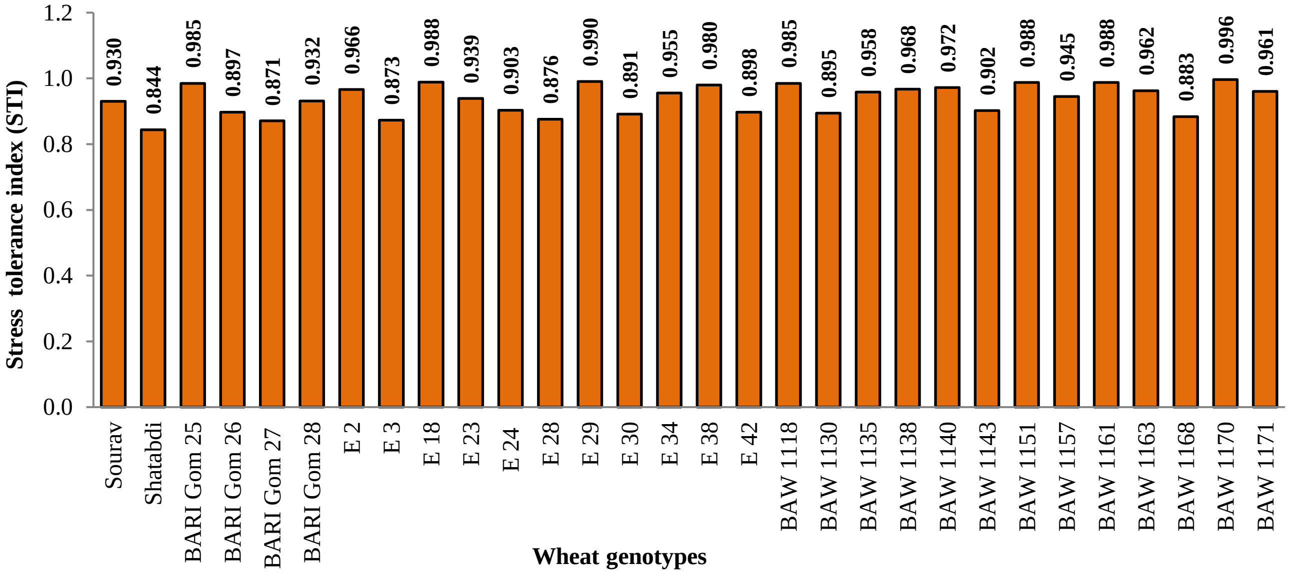

Fig. 5. Stress tolerance index evaluated of 30 wheat genotypes for vigour index under moderate water stress (-2 bars)

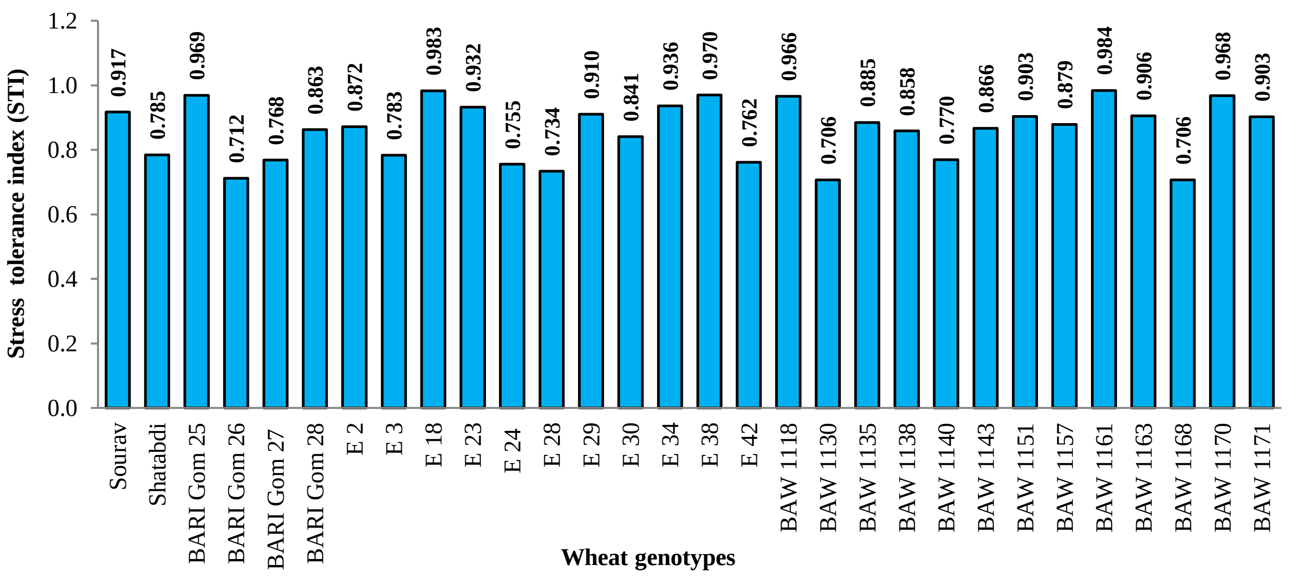

Fig. 6. Stress tolerance index evaluated of 30 wheat genotypes for vigour index under higher water stress (-4 bars).

Fig. 7. Stress tolerance index evaluated of 30 wheat genotypes for shoot length (cm) under moderate water stress (-2 bars).

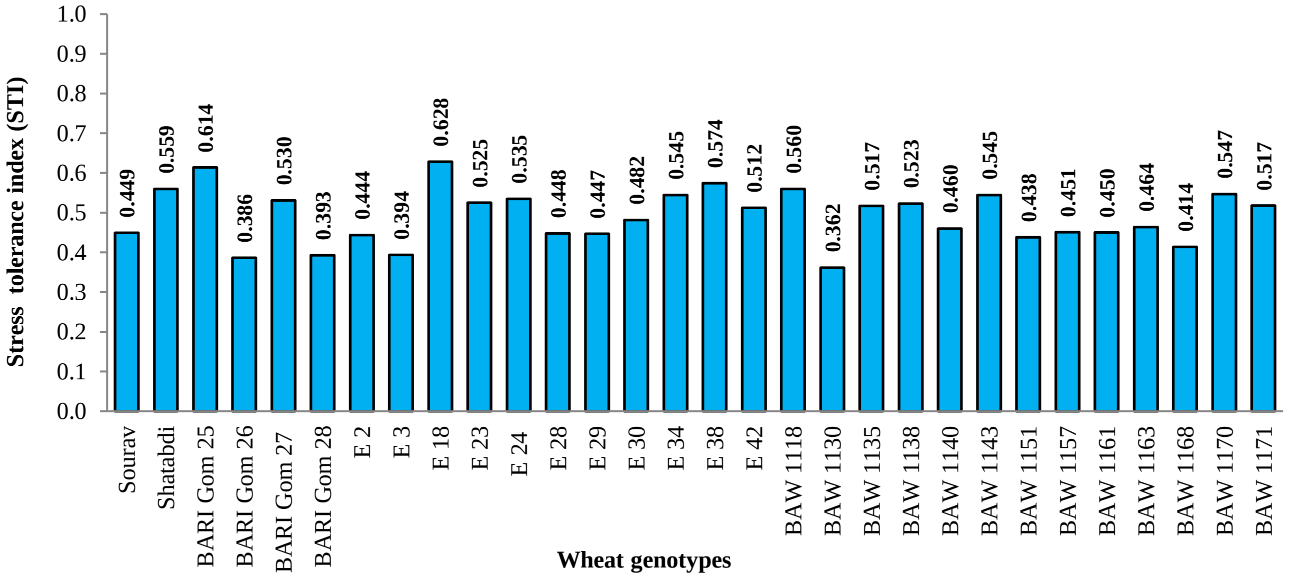

Fig. 8. Stress tolerance index evaluated of 30 wheat genotypes for shoot length (cm) under higher water stress (-4 bars).

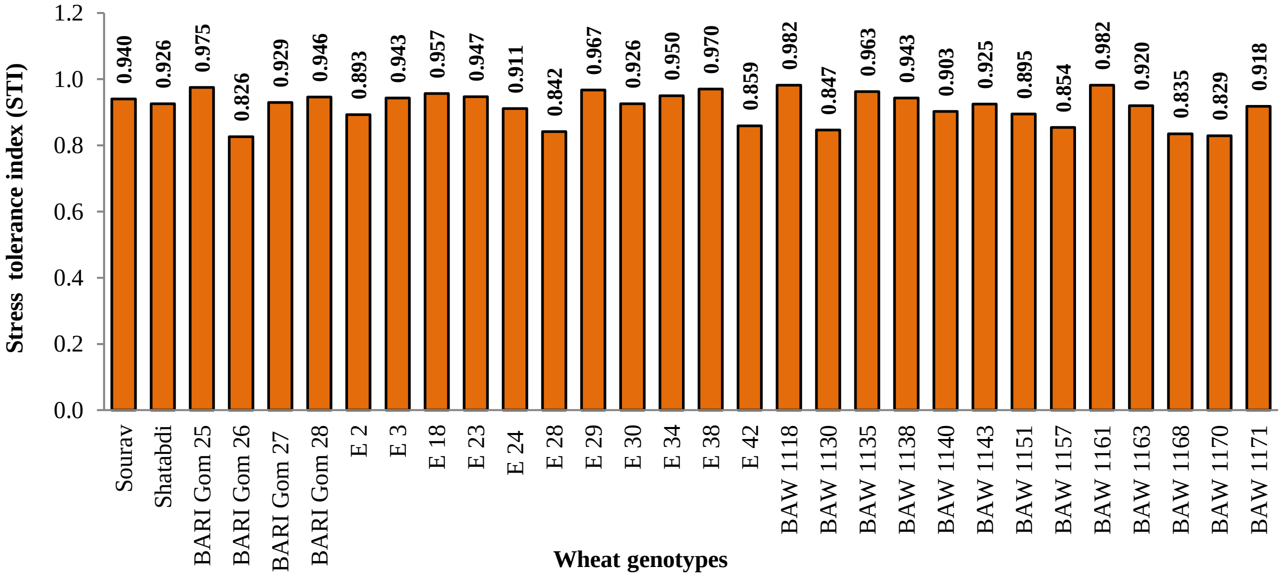

Fig. 9. Stress tolerance index of 30 wheat genotypes based on root length (cm) under moderate water stress (-2 bar).

Root length (cm): The seedling root length (10 days old) was significantly influenced by wheat genotypes and water potential levels induced by PEG and their interaction effects (Table 2). A wide range of variability was observed among genotypes against water deficit conditions. The results showed that the root lengths range with a mean at well-watered, moderate water potential level and higher water potentials level were 9.99 to 13.67 cm with a mean of 11.69 cm; 9.19 to 12.55 cm with a mean of 10.72 cm; 7.41 to 11.97 cm with a mean of 9.55 cm, respectively (Table 3). The root length was reduced significantly with the increment of water deficit stress from 0 to -4 bars. However, the highest root length was recorded by control treatment while the lowest corresponding value was exhibited by -4 bars water stress (Table 3).

The STI values based on the root length were measured against water deficit stress (Figs. 9 & 10). In Figure 9, the STI based on root length of 30 wheat genotypes under moderate water stress (- 2 bars) is depicted. Nevertheless, the genotypes of BARI Gom 25, BARI Gom 28, E 3, E 18, E 23, E 29, E 34, E 38, BAW 1118, BAW 1135, BAW 1138, and BAW 1161 exhibited STI values exceeding 0.94, while genotypes including BARI Gom 26, E 28, E 42, BAW 1130, BAW 1157, BAW 1168 and BAW 1170 displayed STI values less than 0.88. Other genotypes demonstrated intermediate STI values ranging between 0.940 and 0.88 (Figs. 9 & 10). The STI considering the root length at higher water deficit stress was also measured (Fig. 10). However, the STI values ranged from 0.97 to 0.87 in the genotypes of BARI Gom 25, E 18, E 23, E 24, E 29, E 34, E 38, BAW 1118, BAW 1135 and BAW 1161, indicating water deficit stress tolerant genotypes, while the moderate STI values ranged from 0.86 to 0.76 in the genotypes of BARI Gom 27, E 2, E 3, E 30, BAW 1140, BAW 1143, BAW 1157, BAW 1163 and BAW 1171, and rest of the genotypes used in this experiment contained lower STI values less than from 0.76. From the results it is indicated that the higher STI value containing genotypes under water deficit stress can be treated as higher tolerant genotypes to water stress deficit, and lower STI value containing genotypes signifying as susceptible to water deficit stress. Root length and seedling dry weight serve as primary selection criteria for evaluating genotypes under drought stress conditions, and these parameters are crucial indicators of a genotype's ability to withstand water scarcity (Dhanda *et al*., 2004; Qayyum *et al*., 2012). Deep roots and ability to accumulate dry biomass are considered typical characteristics of drought tolerant genotypes (Zhao *et al*., 2004). Root length, or radical length, of wheat genotypes was observed to significantly decrease with increasing levels of PEG-induced water stress, according to studies conducted by Shahbaz *et al*., (2011), Almaghrabi (2012), Jahanbin *et al*., (2012), Raza *et al*., (2012) and Dodig *et al*., (2015).

The STI based on seedling growth traits is a good selection criterion of wheat genotypes under drought stress, and higher values of STI indicates higher drought stress tolerance (Dadbakhsh *et al*., 2011; Jahanbin *et al*., 2012, Fard and Sedaghat, 2013). Raza *et al*., (2012) tested eight wheat (*Triticum aestivum* L.) genotypes (viz. Pasban-90, Inqalab-91, Auqab-2000, AS-2002, Sahar-2006, Shafaq-2006, Lasani-2008, and FSD-2008) and reported that water deficit stress significantly diminished seedling growth properties across all genotypes, and the variety Lasani-2008 exhibited the highest root length STI and water content, indicating its drought-tolerant nature, while the lowest was recorded in Auqab-2000, indicating its susceptibility to drought stress.

Shoot dry weight (mg): The analysis of variance showed that a highly significant differences among the different levels of water stress induced by PEG, among wheat genotypes, and the interaction effect of water stress × wheat genotype for producing the shoot dry weight (mg) was observed (Table 2).

This result revealed that there was remarkable difference among the genotypes on producing shoot dry weight. However, the highest and lowest shoot weight (mg) was 13.25 and 6.87 mg with a mean of 8.66 in higher water stress, and from 14.97 to 8.80 with a mean of 11.17 in moderate water stress levels and from 19.40 to 11.12 with a mean of 14.39 in well watered condition (Table 3). From Table 3, it was evident that shoot dry weight (mg) decreased with increasing water deficit stress. Fig. 11 presented the STI of thirty wheat genotypes based on the shoot dry weight (mg) at moderate water deficit stress. STI was calculated by comparing the shoot dry weight of stressed plants to that of non-stressed plants, revealing considerable variations in water deficit stress tolerance among the wheat genotypes. Among the wheat genotypes, Sourav, Shatabdi, BARI Gom 25, E 18, E 38, BAW 1118, and BAW 1168 exhibited STI values exceeding 0.85. Conversely, BARI Gom 26, E 23, E 29, BAW 1130, BAW 1157, and BAW 1161 displayed STI values lower than 0.72. The remaining wheat genotypes, including BARI Gom 27, BARI Gom 28, E 2, E 3, E 24, E 28, E 30, E 34, E 42, BAW 1135, BAW 1138, BAW 1140, BAW 1143, BAW 1163, BAW 1170, and BAW 1171, showed STI values ranging between 0.85 and 0.72. Figure 12 depicted the STI of 30 wheat genotypes based on shoot weight under higher water deficit stress, indicating substantial differences in water deficit stress tolerance among the wheat genotypes. Shatabdi, BARI Gom 25, E 18, E 34, E 38, and BAW 1118 exhibited STI values exceeding 0.700, while BARI Gom 26, E 2, E 23, E 29, BAW 1130, BAW 1140, BAW 1151, BAW 1157, and BAW 1161 displayed STI values lower than 0.550. The remaining wheat genotypes showed STI values ranging between 0.700 and 0.550. Observations indicated that genotypes with higher STI values were more tolerant to water stress, those with lower STI values were susceptible, and those with moderate STI values exhibited moderate-level water stress tolerance. Dodig *et al*., (2015) reported that shoot growth rate was reduced with increasing PEG induced osmotic stresses (-0·2, -0·4 and -0·6 MPa) at germination and seedling emergence of 96 diverse wheat genotypes. The results in this study are also consisted with the findings of Almaghrabi (2012) and El Sabagh *et al*., (2017) who reported that seedling growth traits like shoot length, shoot fresh weight and shoot dry weight were reduced with the imposition of drought stresses (0.0, 60, 120, 180, 240 and 300 g/L PEG).

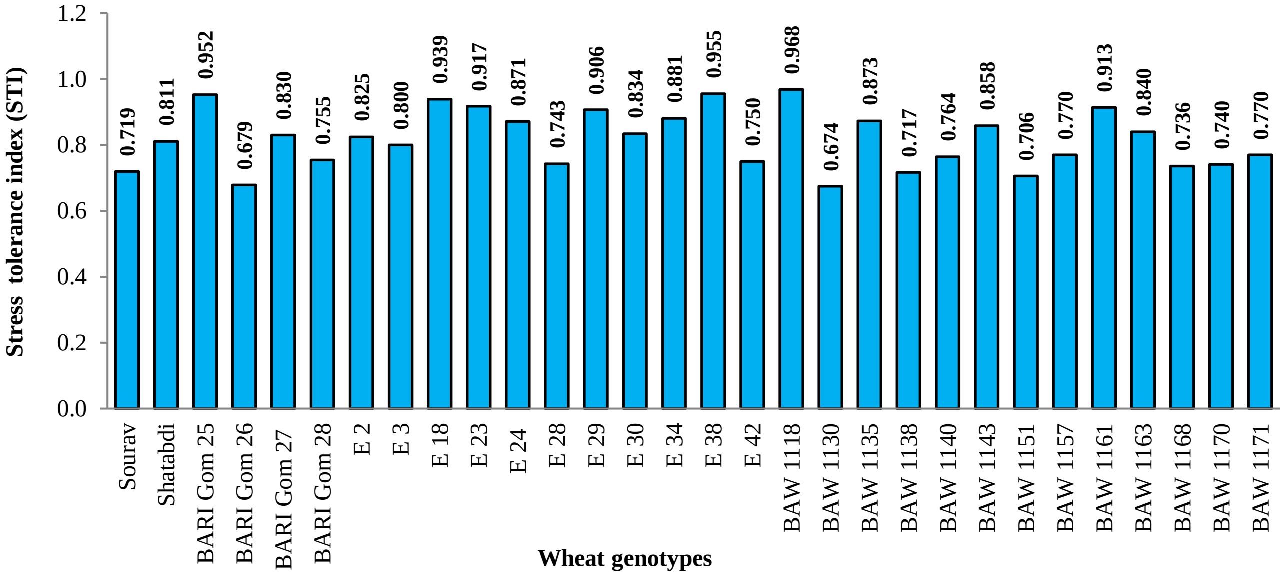

Fig. 10. Stress tolerance index of 30 wheat genotypes based on root length (cm) under higher water stress (- 4 bar).

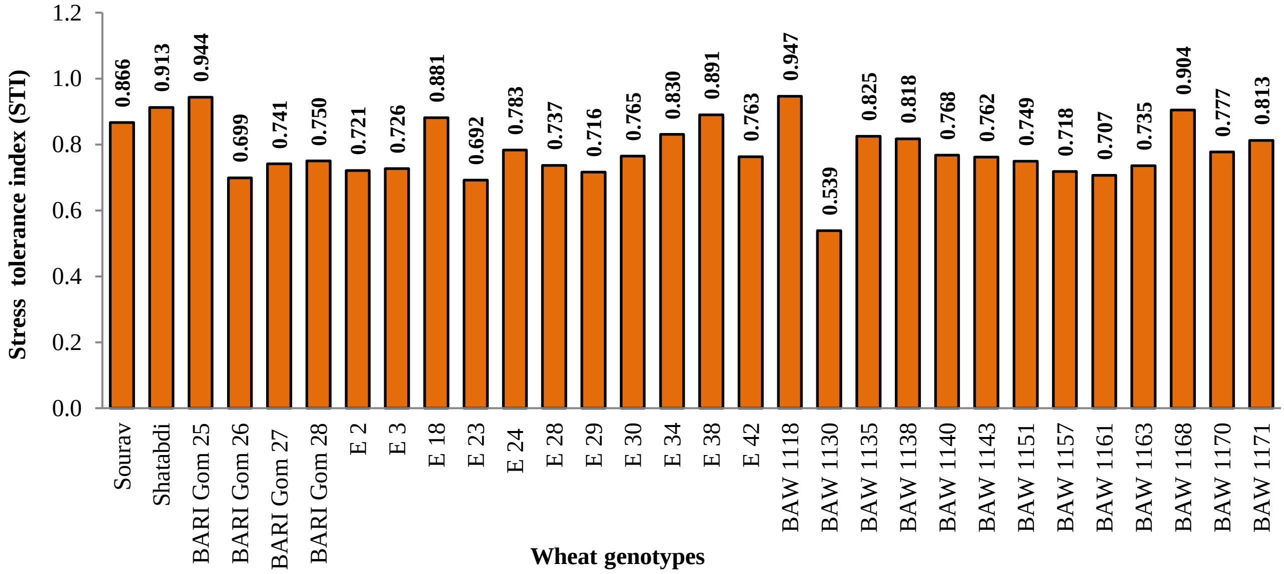

Fig. 11. Stress tolerance index of 30 wheat genotypes based on individual shoot weight (mg) under moderate water stress (-2 bars).

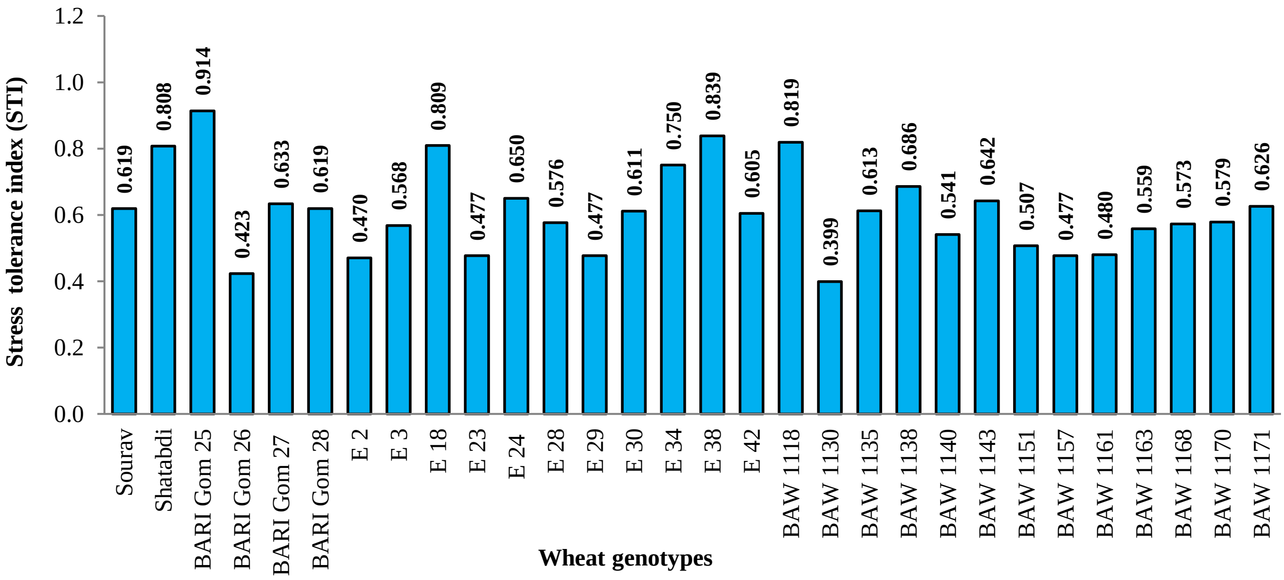

Fig. 12. Stress tolerance index of 30 wheat genotypes based on individual shoot dry weight (mg) under higher water stress (-4 bars).

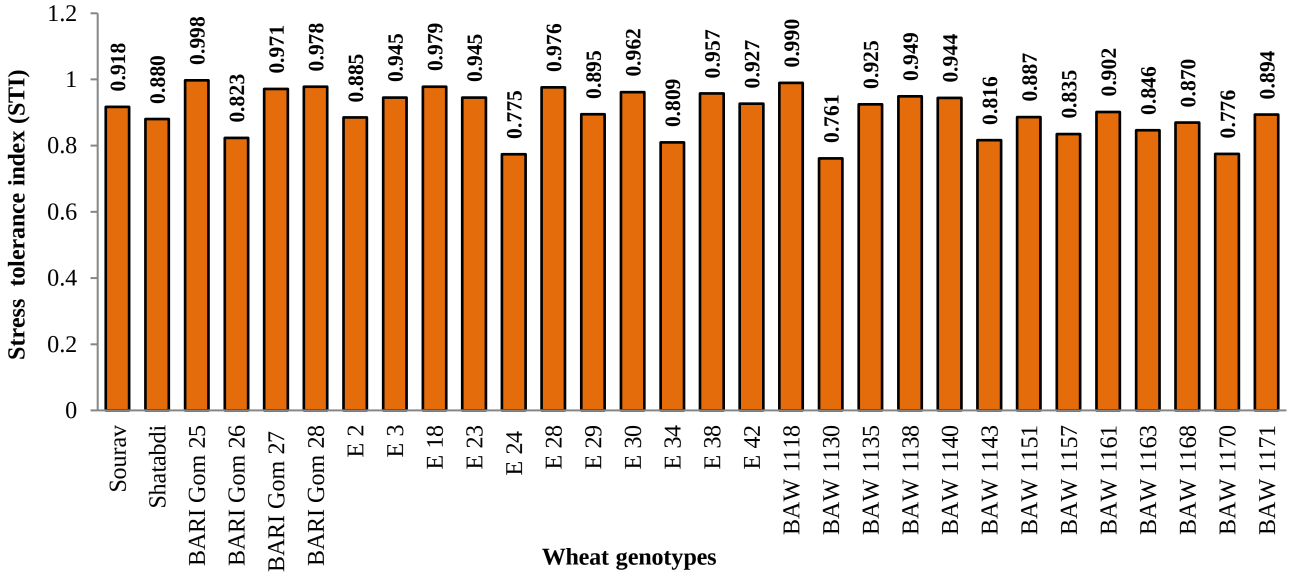

Fig. 13. Stress tolerance index of 30 wheat genotypes based on individual root dry weight (mg) under moderate water stress (-2 bars).

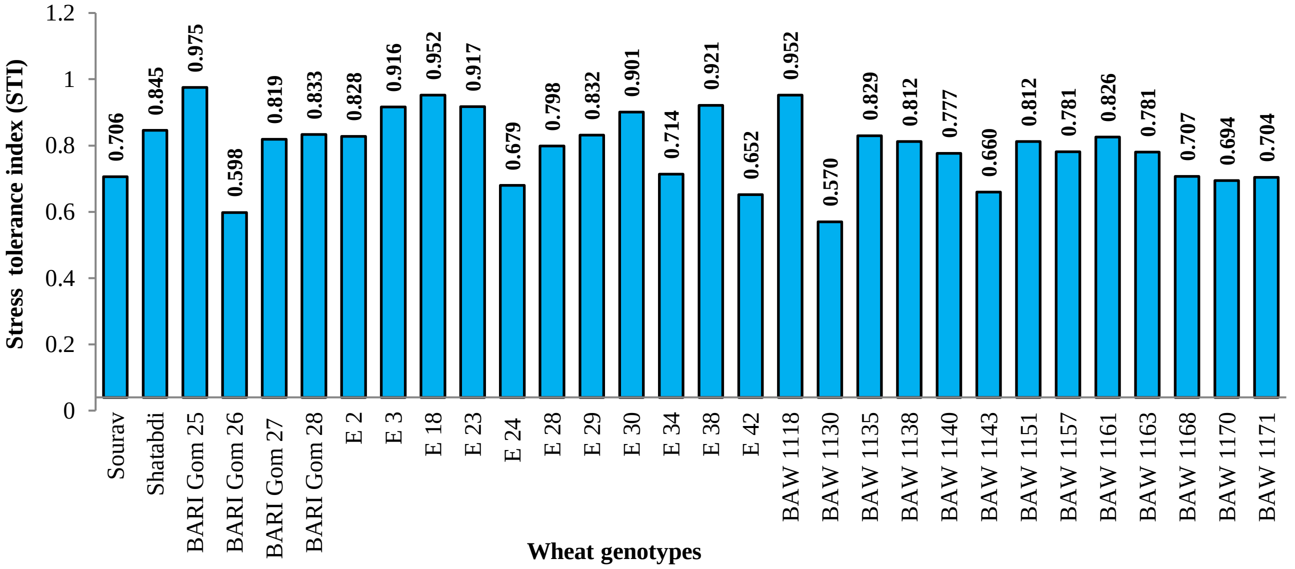

Fig. 14. Stress tolerance index of 30 wheat genotypes based on individual root dry weight (mg) under higher water stress (-4 bars).

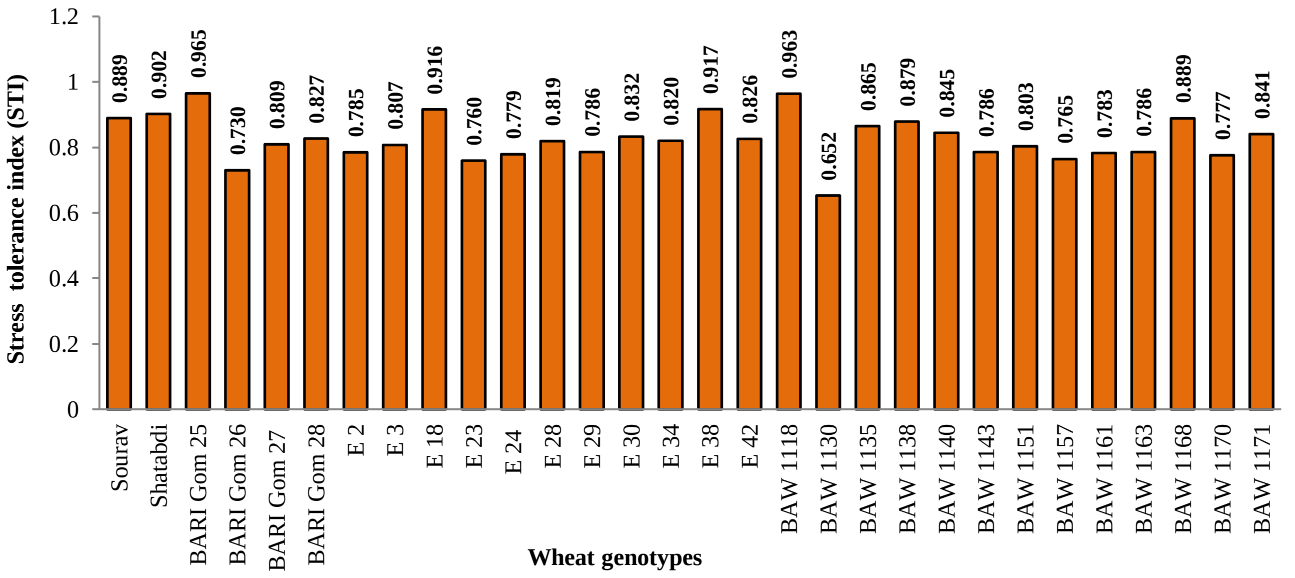

Fig. 15. Stress tolerance index of 30 wheat genotypes based on individual seedling dry weight (mg) under moderate water stress (-2 bar).

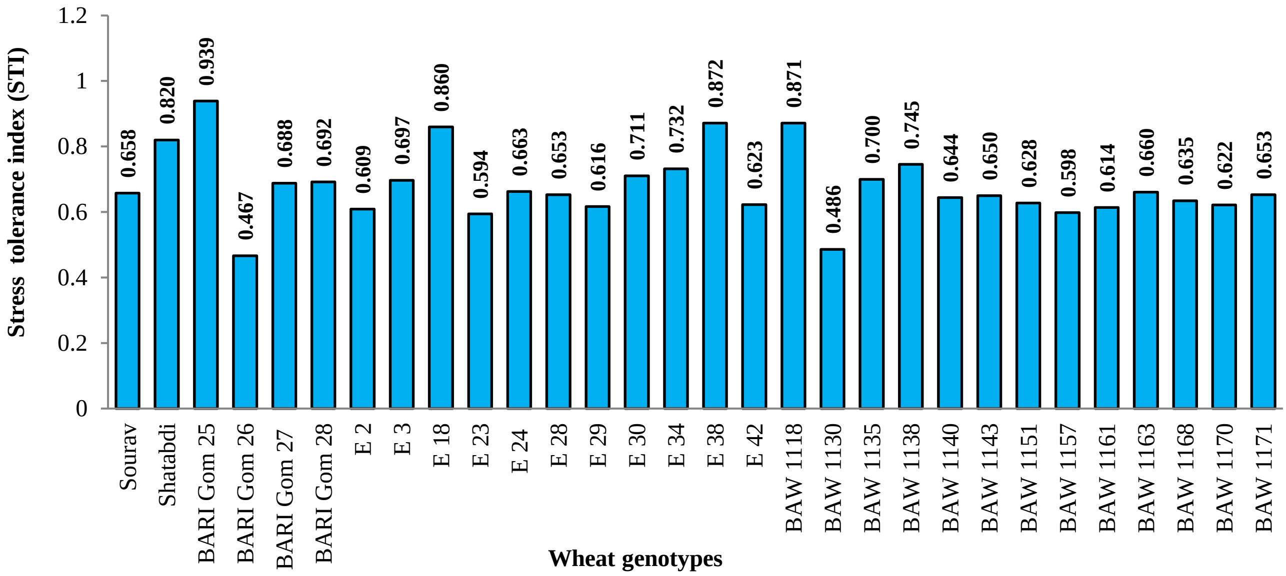

Fig. 16. Stress tolerance index of 30 wheat genotypes based on individual seedling dry weight (mg) under higher water stress (- 4 bar).

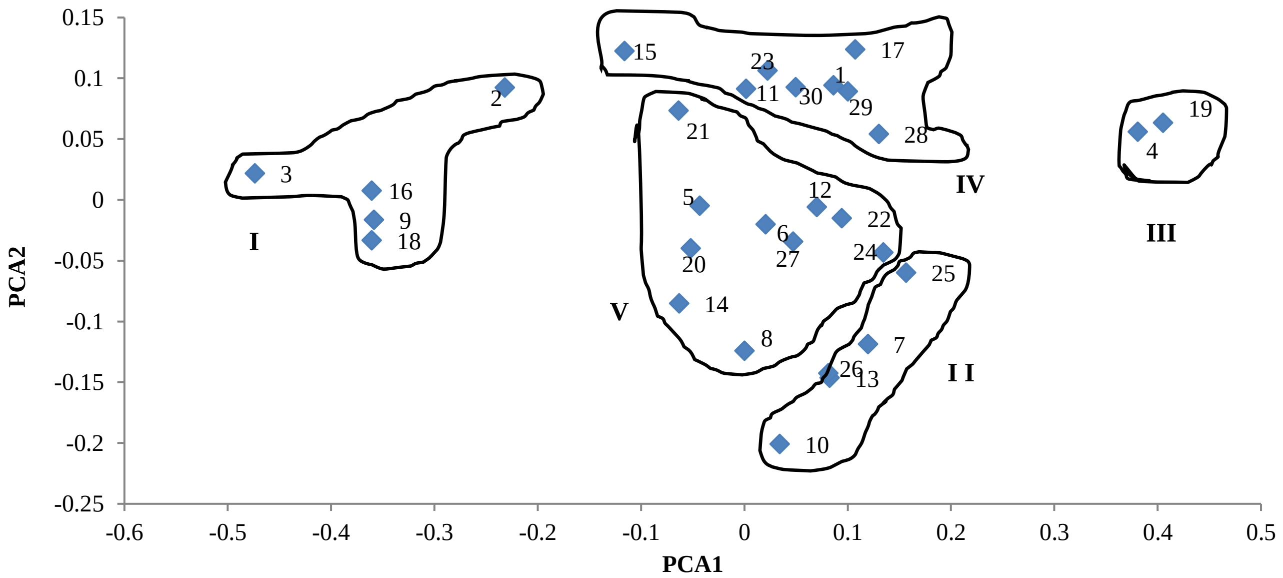

Fig. 17. Scatter distribution of 30 wheat genotypes based on their principal component scores superimposed with clustering.

Table 4. Distribution of 30 wheat genotypes into five different clusters subjected to water deficit stress.

Cluster	Number of genotypes	Per cent of total genotypes	Wheat genotypes
I	5	16.67	Shatabdi, BARI Gom 25, E 18, E 38, and BAW 1118
II	5	16.67	E 2, E 23, E 29, BAW 1157 and BAW 1161
III	2	6.67	BARI Gom 26 and BAW 1130
IV	8	26.66	Sourav, E 24, E 34, E 42, BAW 1143, BAW 1168, BAW 1170 and BAW 1171
V	10	33.33	BARI Gom 27, BARI Gom 28, E 3, E 28, E 30, BAW 1135, BAW 1138, BAW 1140, BAW 1151 and BAW 1163
Total	30	100	

Root dry weight (mg): The ANOVA revealed significant influences on the root dry weight (mg) of 10-day-old seedlings, with factors including wheat genotypes, different levels of water stress induced by PEG, and the interaction effect of moisture stress levels and wheat genotypes (Table 2). It was observed that root dry weight decreased with the reduction of water potential stress (Fig. 12). However, root dry weight varied from 5.74 to 17.90 with a mean of 9.37 recorded at control (well watered) condition, intermediate weight varied from 5.33 to 13.63 with a mean of 8.34 was found at moderate water potential (-2 bars) and the lowest was at lower water potentials stress (-4 bars) varied from 3.87 to 10.20 with a mean of 7.29 (Table 3).

Figure 13 illustrates the STI values of 30 wheat genotypes for root dry weight under moderate water potential stress (-2 bars). The STI was calculated based on root weight of stressed plants to that of non-stressed plants. The higher STI values in the wheat genotypes indicate higher tolerance in water deficit stress. The higher STI values (>0.950) were recorded in the BARI Gom 25, BARI Gom 27, BARI Gom 28, E 18, E 28, E 30, E 38 and BAW 1118 genotypes. On the contrary, lower STI (<0.85) producing wheat genotypes were BARI Gom 26, E 24, E 34, BAW 1130, BAW 1143, BAW 1157, BAW 1163 and BAW 1170. Rest of the genotypes provided moderate STI were in-between 0.850 and 0.950 (Fig. 13). The STI values of 30 wheat genotypes based on root dry weight under higher water potential stress (-4 bars) are depicted. The varying STI values among the genotypes indicate differing levels of tolerance to water stress (Fig. 14). Among these wheat genotypes, Shatabdi, BARI Gom 25, BARI Gom 28, E 3, E 18, E 23, E 30, E 38, and BAW 1118 exhibited STI values exceeding 0.830, indicating their higher stress tolerance. Conversely, Sourav, BARI Gom 26, E 24, E 34, E 42, BAW 1130, BAW 1140, BAW 1143, BAW 1168, BAW 1170, and BAW 1171 displayed STI values lower than 0.780, indicating their susceptibility to stress. The remaining wheat genotypes demonstrated moderate STI values falling between 0.830 and 0.780. The STI is a good indicator which notified the level of stress tolerance of the genotypes, and higher the value of the STI indicates higher stress tolerance genotypes.

In contrast, lower STI value indicates stress susceptible genotypes. Seedling growth traits such as root fresh weight, root dry weight, and root number were significantly influenced by varying stress levels (0.0, 60, 120, 180, 240, and 300 g/L PEG). Among the eight cultivars studied, Sakha 93 and Madini exhibited the highest values for most parameters, followed by Yamanei, Kaseemi, and Tabokei as reported by Almaghrabi (2012). Seedling growth parameters of our study can be adopted to differentiate tolerance and susceptible cultivars under moisture stressed environments.

Seedling dry weight (mg): Results of the analysis of variance showed highly significant difference among water potentials levels, wheat genotypes and also their interaction for the seedling dry weight (Table 2). The maximum seedling dry weight of 23.76 mg (mean) was recorded under well water condition (ranging from 17.18 to 35.10 mg), and the minimum of 15.95 mg was observed at lower water potential (-4 bars) ranging from 12.07 to 20.07 mg and moderate at intermediate seedling dry weight of 19.51 mg (ranging from 14.67 to 22.90 mg) (Table 3). The results also showed that the seedling dry weight was reduced with the reduction of water potentials stress (Table 3). Considering the seedling dry weight, the STI value was calculated which indicates the tolerance capability of genotypes against water deficit stress. The STI of thirty wheat genotypes based on seedling dry weight under moderate water stress (-2 bars) are shown in Fig. 15. Results indicated that genotypes such as Sourav, Shatabdi, BARI Gom 25, E 18, E 38, BAW 1118, BAW 1135, BAW 1138, and BAW 1168 exhibited higher STI values (more than 0.850), indicating their drought tolerance. Conversely, genotypes including BARI Gom 26, E 2, E 23, E 24, E 29, BAW 1130, BAW 1143, BAW 1157, BAW 1161, BAW 1163, and BAW 1170 displayed lower STI values (less than 0.800), indicating their susceptibility to drought stress. Other genotypes showed STI values falling between 0.850 and 0.800, suggesting moderate tolerance. Figure 16 illustrates the STI of 30 wheat genotypes under higher water deficit stress (-4 bars). Higher STI value (STI > 0.700) producing genotypes were Shatabdi, BARI Gom 25, E 18, E 30, E 34, E 38, BAW 1118 and BAW 1138 which can be enchanted as drought tolerant genotypes. Lower STI value (STI < 0.650) providing genotypes are BARI Gom 26, E 2, E 23, E 29, E 42, BAW 1130, BAW 1140, BAW 1151, BAW 1157, BAW 1161, BAW 1168 and BAW 1170 and other genotypes produce moderate STI value ranging from 0.650 to 0.700. Higher STI value indicates higher tolerant to water deficit stress, moderate STI value specifies moderate tolerant to water deficit stress, and lower STI value indicates susceptible to water deficit stress.

Seedling dry weight notably declined with the escalation of PEG-induced drought stresses, highlighting its potential as a promising selection criterion for assessing genotypic responses to moisture deficit challenges (Dhanda *et al*., 2004; Qayyum *et al*., 2012; Almaghrabi, 2012). The STI values regarding seedling weight decreased and higher values of seedling weight STI be a sign of higher drought tolerant genotype. Dodig *et al*., (2015) reported that the STI can serve as a valuable tool for selecting drought-tolerant genotypes during the seedling stage across various stress and non-stress environments. Prior research, including those in wheat (González & Ayerbe, 2011), as well as in other cereals such as barley (Moud & Maghsoudi, 2008), maize, and triticale (Grzesiak *et al*., 2012), have demonstrated that seedling growth under water deficit conditions can serve as a reliable indicator of drought tolerance in plants when extrapolated to field conditions.

Grouping of genotypes through cluster analysis and principal component analysis

Cluster analysis: In cluster analysis, 30 wheat genotypes were grouped into 5 clusters based on various germination characters and seedling traits subjected to multivariate analysis (Table 4). Thirty wheat genotypes were categorized in five clustering using rate of germination, co-efficient of germination, vigour index, shoot and root length and their dry weights as response variables. The cluster analysis results showed that first cluster comprised of five genotypes out of 30 wheat genotypes, these were Shatabdi, BARI Gom 25, E 18, E 38, and BAW 1118 and first cluster comprised 16.67% of the total genotypes, second cluster also included the same number of genotypes, the same as cluster 1 genotypes which represented 16.67% of the total genotypes, namely, E 2, E 23, E 29, BAW 1157 and BAW 1161. The minimum number of wheat genotypes (2), genotype no. 4 (BARI Gom 26) and 19 (BAW 1130) were grouped in cluster III which represented the least 6.67% of total genotypes. The highest genotypes number (10) was classified into cluster V followed by eight (8) in cluster IV. Cluster four enclosed eight genotypes-Sourav, E 24, E 34, E 42, BAW 1143, BAW 1168, BAW 1170 and BAW 1171, these were 26.67% of the total genotypes, and cluster five controlled ten genotypes these included 33.3% of the total genotypes viz. BARI Gom 27, BARI Gom 28, E 3, E 28, E 30, BAW 1135, BAW 1138, BAW 1140, BAW 1151 and BAW 1163 and this cluster also integrated the highest number of genotypes (Table 4). The genotypes included within a cluster had less diversity among themselves and the genotypes included in different clusters had more diversity among different clusters.

Principal component analysis (PCA): The first two principal components caused approximately 91.03% to the total variance (Fig. 17). There were five groups based on germination and seedling traits showing similarities within group and diversity among groups. The crossing between genotypes from different clusters would produce a wide range of diversity for germination characters and seedling traits facilitating more avenues in selection and improvement.

Conclusion

In this experiment, 30 wheat genotypes were screened under PEG induced three water potentials of 0, -2 and -4 bars. The rate of germination (RG), co-efficient of germination (COG), germination vigour index (GVI), length and dry weight of shoot and root decreased with the advancement of stress. However, the highest and the lowest RG of 92.33 and 61.19% were recorded under water stress conditions of -2 and -4 bars, respectively. Considering the results, it might be concluded that water deficit conditions delayed germination and reduced seedling growth traits of wheat genotypes. Based on these response variables, wheat genotypes especially E 3, BAW 1135, E 30, E 34, BAW 1138, Shatabdi, E 18, BAW 1118, E 38 and BARI Gom 25 might be inferred as the most drought tolerant, while BAW 1151, E 42, BAW 1170, E 29, BAW 1161, E 2, BAW 1157, E 23, BAW 1130 and BARI Gom 26 might be declared as drought sensitive genotypes. These findings might serve as baseline to conduct further in-depth studies to screen out the most performing wheat genotypes from the pool of these elite genotypes for general adaptation in the region and other areas having similar ago-climatic conditions.

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