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POLYETHYLENE GLYCOL MEDIATED OSMOTIC STRESS ON GERMINATION, SEEDLING TRAITS AND SEED METABOLIC EFFICIENCY OF WHEAT

S.K. Pramanik, S. Sikder and M.A. Hasan

Department of Crop Physiology and Ecology, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

Corresponding E-mail: pramaniksk100@gmail.com

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Abstract

Germination characteristics, early seedling growth and seed metabolic efficiency of four wheat genotypes (BARI Gom 28, BARI Gom 29, BAW 1177 and ESWYT 29) were evaluated under 15% polyethylene glycol **(**PEG-6000) induced osmotic stress (-3 bar). Germination characteristics (germination rate, co-efficient of germination and germination vigor index), shoot and root length, shoot and root dry weight, and seed metabolic efficiency decreased under osmotic stress induced by PEG than control treatment. But the degree of reduction was different for various wheat genotypes. Genotype BAW 1177 showed the highest performance in respect to germination and early seedling traits at both control and PEG induced osmotic stress than other three genotypes. So, genotype BAW 1177 can be considered as relatively drought tolerant genotype.

Introduction

In Bangladesh wheat is the second most important cereal crop after rice (Barma *et al.*, 2019) grown over an area of 0.33 million hectares with an annual production of about 1.03 million metric tons (BBS, 2020). Though wheat is an important cereal crop in Bangladesh but its average yield is low (3.09 t ha^{-1}) (BBS, 2020) as compared to that of the advanced countries. At present, the domestic production of the country can only encounter around 20% of total wheat demand (USDA, 2018). However, wheat productivity has been declined due to various abiotic stresses over the last two decades particularly drought stress (Shao *et al.*, 2008). Rising temperature and changing in precipitation pattern leads to increasing incidence and intensity of drought events in the country like Bangladesh (Khan *et al.*, 2019) where drought employs expressively adverse effects on wheat production in northern and central part of the country (Abhinandan *et al.*, 2018). Drought stress is responsible for either inhibition or delayed seed germination or seedling establishment (Balkan and Gençtan, 2013). It has negative effects on the morphological, physiological, and biochemical attributes of the wheat crop (Chachar *et al.*, 2016) and it results in a significant reduction in overall production (Bilal *et al.*, 2015 and Abid *et al.*, 2018).

Seed germination is a prerequisite and important transition stage for crop plants from seeds to seedlings. The semi-arid regions of the world experience low moisture availability during seed germination of wheat crop (Farooq *et al.*, 2019). Low moisture availability during seed germination and subsequent growth stages of wheat crop declines final production of wheat as seedling stage of crop plants is highly vulnerable to the water deficit stress (Rauf *et al.*, 2007).

Seed germination and seedling emergence and/or establishment are important criteria for testing the tolerance of wheat genotypes to various abiotic stresses, particularly, drought stress (Hubbard *et al.*, 2012, Rauf *et al.*, 2007). Evaluation of seed germination under polyethylene glycol (PEG-6000) induced drought stress is the most common screening method used to test the drought tolerance of different crop plants during seed germination and early seedling establishment (Awan *et al.*, 2021). The use of osmotic substances of high molecular weight such as PEG is a common method to test the drought tolerance of crop plants during seed germination and seedling establishment (Zarei *et al.*, 2007). Several investigations indicated that in vitro screening using PEG is one of the reliable approaches to select drought-tolerant genotypes based on germination indices (Kocheva and Georgiev 2003, Shahbazi 2012).

Therefore, the present study was carried out to test the drought tolerance of four wheat genotypes at early seedling stage through PEG-induced osmotic stress.

Materials and Methods

Experimental site and period

The experiment was conducted at Crop Physiology and Ecology Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh during November, 2021.

Experimental design and treatments

Four wheat genotypes were evaluated under two growing conditions control and 15% PEG induced drought stress following completely randomized design with three replications. PEG solution was prepared by dissolving calculating amount of PEG (151.5 g / 1000 ml) in tap water as described by Michel (1983). Normal tap water was used as control treatment. Characteristics of the wheat genotypes:

BARI Gom 28: High yielding variety, duration: 105-110 days, plant height: 95-100 cm, 1000-grain weight: 35-40 g, grain yield: 3.5-5.4 t/ha, tolerant to terminal heat stress, resistant to leaf rust and Ug99 race of stem rust, moderately tolerant to bipolaris leaf blight, moderately susceptible to wheat blast and fit to the rice-wheat cropping system

BARI Gom 29: High yielding variety, duration: 102-108 days, plant height: 95-100 cm, 1000-grain weight: 44-48 g, grain yield: 4.0-5.0 t/ha, tolerant to terminal heat stress, and moderately susceptible to wheat blast

BAW 1177: Advanced line of Bangladesh Wheat and Maize Research Institute (BWMRI)

ESWYT 29: Advanced line of BWMRI. Spring bread wheat adapted to Mega-environment 1 i.e. the optimally irrigated, low rainfall areas with an average minimum temperature in the coolest quarter between 3 and 11 C. (ME1):

Seed placement and data collection on germination and seedling traits

Twenty-five seeds of each genotype were placed on filter paper soaked by treatment solution and distilled water according to treatment in 11 cm diameter sterilized Petri dish. The 10 ml treatment solution or distilled water was poured on the filter paper and afterwards the solution or distilled water was given according to the needs. Seedlings were allowed to grow up to 7 days after placement of germination. Germination was counted at 24-hours interval starting from 72h after of seed set and continued up to 7th day. A seed was considered germinated as plumule and radicle came out and was larger than 2 mm long. The rate of germination was calculated according to Krishnasamy and Seshu, (1990) and co-efficient of germination and vigor index were calculated using the formulae (Copeland, 1976). At 7th days after seed placement for germination, five seedlings from each Petri dish were sampled for shoot and root length. Then shoot, root and remaining seeds were dried separately at 70°C for 72h in an electric oven (Model- E28# 03-54639, Binder, Germany) and weight were recorded with an electrical balance (Model- AND EK- 300i). The mean length and dry weight were calculated for each treatment combination.

Calculation of amount of seed material respired (SMR) and seed metabolic efficiency (SME)

SMR was calculated as: SMR = SDW – (SHW + RTW + RSW), Where, SDW = Seed dry weight before germination, SHW = Shoot dry weight, RTW = Root dry weight and RSW = Remaining seed dry weight. Seed metabolic efficiency was calculated using the formula (Rao and Sinha, 1993).

The relative performance regarding different germination and seedling traits was calculated as described by Asana and William (1965) using the following formula-

Relative performance (%) = (Variable measured under stress condition ÷ Variable measured under normal condition) × 100

Calculation of drought tolerance/resistance indices

The drought tolerance/resistance indices based on different traits were calculated using the following formulas:

i) Tolerance (TOL) = Yp-Ys (Rosielle and Hamblin 1981); where Yp and Ys are the mean values of genotypes under non-stress and stress conditions, respectively; the genotypes with low values of this index are more stable in two different conditions.
ii) Drought tolerance index (DTI) = Ys ÷ Yp (Goudarzi and Pakniyat 2008); the genotypes with high value of this index will be more tolerant to stress.
iii) Drought sensitivity index (DSI) = (Yp -Ys) ÷ Yp (Farshadfar and Javadinia 2011); the genotypes with low value of this index will be more desirable.
iv) Mean productivity (MP) = (Ys + Yp) ÷ 2 (Rosielle and Hamblin 1981); the genotypes with high value of this index will be more desirable.

Statistical analyses of data

The experimental data were analyzed by partitioning the total variance using STATA program (Small Stata 12.0) and the means were compared by Tukey's Test at 5% level of probability.

Results and Discussion

Germination characteristics

The interaction effect of growing conditions and wheat genotypes significantly influenced the germination rate and germination vigor index but insignificant in co-efficient of germination (Table 1). All the genotypes showed higher germination rate at control (84.83 to 98.48%) as compared to PEG induced osmotic stress condition (64.11 to 79.12%). Under stress condition, BAW 1177 attained the highest germination rate (79.12%), whereas ESWYT 29 had the lowest germination rate (64.11%). In relative performance, BARI Gom 29 showed the highest performance (87.49%) while ESWYT 29 had the lowest performance (70.33%) and rest two genotypes BARI Gom 28 and BAW 1177 showed moderate performance (77.12 and 80.34%, respectively).

Co-efficient of germination was found higher at control with a range from 35.49% in BARI Gom 29 to 36.44% in BAW 1177 as compared to PEG induced osmotic stress condition with a range from 33.73% in BARI Gom 29 to 34.91% in BAW 1177. Considering relative performance highest performance was found in BAW 1177 (95.80%), while BARI Gom 28 had the lowest performance (94.35%).

At control condition, the highest germination vigor index was found in BAW 1177 (33.83), whereas the lowest in BARI Gom 29 (30.71). The moderate germination vigor index (32.44) was found in both BARI Gom 28 and ESWYT 29. Under stress condition, again BAW 1177 attained the highest germination vigor index (27.15), while ESWYT 29 had the lowest (23.87) which was statistically similar with BARI Gom 28 (24.02) and BARI Gom 29 (23.94). In relative performance, genotype BAW 1177 showed the highest performance (80.25%), whereas ESWYT 29 the lowest performance (73.58%).

Table 1. Effects of polyethylene glycol induced osmotic stress (-3 bar) on germination characteristics of wheat genotypes

Genotypes	Growing conditions	Germination rate		Co-efficient of germination		Germination vigor index	
		%	RP (%)	-	RP (%)	-	RP (%)
BARI Gom 28	Control Stress	96.57a 74.47b	77.12	36.10 34.06	94.35	32.44a 24.02d	74.04
BARI Gom 29	Control Stress	84.83a 74.22b	87.49	35.49 33.73	95.04	30.71b 23.94d	77.96
BAW 1177	Control Stress	98.48a 79.12b	80.34	36.44 34.91	95.80	33.83a 27.15c	80.25
ESWYT 29	Control Stress	91.15a 64.11b	70.33	36.19 34.56	95.50	32.44a 23.87d	73.58
CV (%)		1.34	-	1.96	-	1.44	-

In a column, means followed by the same letter(s) did not differ significantly at P ≤ 5% level by Tukey Test. RP = Relative performance.

A decline in germination percentage under moisture stress has been reported in wheat by Sharma *et al.* (2022). They observed that the germination rate was reduced with the increment of water deficit stress but the degree of reduction in rate of germination was not similar for all wheat genotypes. Development at the germination stage have been adopted a suitable growth stage for testing the drought stress tolerance in wheat. It could be assumed that the presence of increased concentrations of osmotic potential during the growth of germination stage inhibits the developmental traits and survival of wheat. Differential degree of sensitivity in germination characteristics to PEG induced water stress among the wheat genotypes may be due to genetic variability of wheat to water stress condition. Mahpara *et al.* (2022) Bilgili *et al.* (2019) and Rana *et al.* (2017) similarly detected significant differences in germination characteristics of wheat genotypes under PEG induced water deficit stress.

Early seedling growth

Early seedling growth (shoot length, root length, shoot dry weight, and root dry weight) of 7 days old seedling was significantly influenced by the interaction effect of growing conditions and wheat genotypes (Table 2 and Fig. 1). The longest shoot length was found at control with a range from 3.44 cm in BARI Gom 28 to 3.82 cm in BAW 1177 as compared to stress condition with a range from 1.36 cm in BARI Gom 29 to 2.72 cm in BAW 1177. At control condition, the longest shoot length (3.82 cm) was found in BAW 1177 followed by 3.79 cm in ESWYT 29 and 3.52 cm in BARI Gom 29, whereas the shortest shoot length (3.44 cm) was found in BARI Gom 28. Under stress condition, BAW 1177 produced the highest shoot length (2.72 cm) followed by BARI gom 28 (2.19 cm), while BARI Gom 29 had the lowest shoot length (1.36 cm) which was statistically identical with ESWYT 29 (1.37 cm).

Normal tap water **PEG solution**

Fig. 1. Seven days old seedlings of wheat genotype grown under normal tap water and PEG solution.

Considering relative performance, BAW 1177 performed highest (71.20%), whereas ESWYT 29 performed lowest (36.14%) followed by BARI Gom 29 (38.63%). Moderate performance (63.66%) was found in BARI Gom 28. At control condition, BAW 1177 produced the longest root (7.28 cm) followed by BARI Gom 29 (7.06 cm) and ESWYT 29 (7.21 cm), whereas BARI Gom 28 produced the shortest root (6.18 cm).

Table 2. Effects of polyethylene glycol induced osmotic stress (-3 bar) on early seedling growth of 7 days old seedlings of wheat genotypes

Genotypes	Growing conditions	Shoot length		Root length		Shoot dry weight		Root dry weight	
		cm	RP (%)	cm	RP (%)	mg	RP (%)	mg	RP (%)
BARI Gom 28	Control	3.44a	63.66	6.18b	78.64	0.030bc	76.66	0.040b	70.00
	Stress	2.19b		4.86c		0.023d		0.028d	
BARI Gom 29	Control	3.52a	38.63	7.06a	64.44	0.033ab	72.72	0.035c	71.42
	Stress	1.36c		4.55c		0.024d		0.025e	
BAW 1177	Control	3.82a	71.20	7.28a	82.14	0.035a	77.14	0.045a	73.33
	Stress	2.72b		5.98b		0.027cd		0.033c	
ESWYT 29	Control	3.79a	36.14	7.21a	64.07	0.031abc	58.06	0.038bc	52.63
	Stress	1.37c		4.62c		0.018e		0.020e	
CV (%)		6.61	-	6.16	-	6.14	-	2.99	-

In a column, means followed by the same letter(s) did not differ significantly at P ≤ 5% level by Tukey Test. RP = Relative performance.

Under stress condition, BAW 1177 produced the longest root (5.98 cm), whereas BARI Gom 29 had the shortest root (4.55 cm). But under stress condition, all the genotypes produced statistically similar root length. In relative performance, it was found that genotype BAW 1177 showed higher relative root length (82.14%) compared to others genotypes (78.64% in BARI Gom 28, 64.44% in BARI Gom 29 and 64.07% in ESWYT 29).

Results showed that shoot dry weight was decreased at water stress condition. Minor variations among the wheat genotypes both under control (0.030 mg to 0.035 mg) and PEG induced water deficit (0.018 mg to 0.027 mg) conditions were observed. Under control condition, BAW 1177 attained the highest shoot dry weight (0.035 mg) followed by BARI Gom 29 (0.033 mg) and ESWYT 29 (0.031 mg), whereas BARI Gom 28 had the lowest shoot dry weight (0.03 mg).

Under water stress condition, ESWYT 29 showed the lowest shoot dry weight (0.018 mg) and highest shoot dry weight was found in BAW 1177 (0.027 mg) followed by BARI Gom 28 (0.023 mg) and BARI Gom 29 (0.024 mg). In relative performance, it was found that the genotype BAW 1177 and BARI Gom 28 showed the higher relative value (77.14 and 76.66%), whereas ESWYT 29 attained the lowest relative value (58.06%) in shoot dry weight. BARI Gom 29 showed moderate performance (72.72%) compared to others.

Results showed that root dry weight was decreased at stress condition. There existed minor variations among the wheat genotypes both at control (0.035 mg to 0.045 mg) and PEG induced osmotic stress (0.024 mg to 0.033 mg) conditions. Under control condition, BAW 1177 showed the highest root dry weight (0.045 mg), whereas BARI Gom 29 had the lowest (0.035mg) and it was at par with ESWYT 29 (0.038 mg). Under osmotic stress condition, ESWYT 29 showed the lowest root dry weight (0.024 mg) followed by BARI Gom 29 (0.025 mg) and highest root dry weight was found in BAW 1177 (0.033 mg). In relative performance, genotype BAW 1177 showed the higher relative value (73.33%) and ESWYT 29 lower relative value (52.63%). Moderate performance was found in BARI Gom 29 (71.42%) followed by BARI Gom 28 (70.00%).

Reduction in seedling growth is the result of restricted cell division and enlargement, as drought stress directly reduces growth by decreasing cell division and elongation (Kramer, 1983). Reduction in shoot and root length might be due to less water absorption and decrease in external osmotic potential created by PEG (Kaydan and Yagmur, 2008). Significant reduction in term of shoot length, root length and seedling dry weight among the genotypes might be attributed to their differential response in term of tolerance level to moisture stress. These results of the present study are parallel to the findings of Faisal *et al.* (2019) Mahpara *et al.* (2022) and Sharma *et al.* (2022).

Seed metabolic efficiency (SME)

The significant variation of SME of four wheat genotypes at different growing conditions is presented in Table 3. At control condition, genotype BAW 1177 attained the maximum SME (3.36 g g^{-1}) which was statistically identical with ESWYT 29 (2.91 g g^{-1}), whereas lowest SME in BARI Gom 29 (1.41 g g^{-1}) followed by BARI Gom 28 (1.47 g g^{-1}). At PEG induced osmotic stress, genotype BAW 1177 attained the highest SME (2.4 g g^{-1}), whereas ESWYT 29 the lowest SME (0.65 g g^{-1}) followed by BARI Gom 29 (0.72 g g^{-1}) and BARI Gom 28 (0.96 g g^{-1}). Stress condition reduced the SME in all genotypes but the magnitude of reduction in SME was not similar in all genotypes. In relative performance, highest performance was performed by BAW 1177 (71.43%) and lowest performance by ESWYT 29 (22.33%).

Table 3. Effects of polyethylene glycol induced osmotic stress (-3 bar) on seed metabolic efficiency of wheat genotypes

Genotypes	Growing conditions	Seed metabolic efficiency	
		g g^{-1}	Relative performance (%)
BARI Gom 28	Control	1.47b	65.30
	Stress	0.96c	
BARI Gom 29	Control	1.41b	51.06
	Stress	0.72c	
BAW 1177	Control	3.36a	71.43
	Stress	2.40a	
ESWYT 29	Control	2.91a	22.33
	Stress	0.65c	
CV (%)		6.15	-

In a column, means followed by the same letter(s) did not differ significantly at P ≤ 5% level by Tukey Test.

The reduction in SME at water stress condition suggested that at water stress, substrate respiration was not linked to building useful plant parts (shoot and root) and could lead to thermal dissipation of respiratory energy by an alternate oxidase pathway or cyanide resistant pathway. Reduction in SME may be attributed to inability to accumulate respiratory product to wheat seedlings. The results are in accordance of findings of Sikder *et al.* (2010). They concluded that seed metabolic efficiency of wheat genotypes was affected to a greater extent under stress condition.

Correlation analysis among different germination and early seedling traits

Correlation analysis among different germination and early seedling traits in this investigation presented in Table 4 indicates that all the traits maintained a significant positive relation with each other.

Table 4. Correlations (Pearson) among the different germination and seedling traits of wheat genotypes

	Germination rate	Co-efficient of germination	Germination vigor index	Shoot length	Root length	Shoot dry weight	Root dry weight	Seed metabolic efficiency
Germination rate	1.000**							
Co-efficient of germination	0.926**	1.000**						
Germination vigor index	0.889**	0.972**	1.000**					
Shoot length	0.835**	0.902**	0.891**	1.000**				
Root length	0.833**	0.868**	0.880**	0.923**	1.000**			
Shoot dry weight	0.799**	0.780**	0.780**	0.872**	0.850**	1.000**		
Root dry weight	0.9311**	0.9210**	0.8795**	0.9014**	0.8345**	0.8855**	1.0000**	
Seed metabolic efficiency	0.768**	0.649**	0.556**	0.533*	0.561**	0.647**	0.756**	1.000**

** and * indicate significant at the 1% and 5% probability level, respectively.

Relationship of different seedling traits with seed metabolic efficiency

Different seedling attributes (shoot length, root length, shoot dry weight and root dry weight) of wheat genotypes maintained a positive linear relationship with seed metabolic efficiency (Fig. 2). The results revealed that the genotype with higher SME produced higher shoot length, root length, shoot dry weight and root dry weight, while the genotype with lower SME provided lower values of that attributes. It indicated that the seedling traits of wheat genotypes increased with the increment of SME and decreased with the decreasing of SME. The higher shoot length, root length, shoot dry weight and root dry weight resulted from higher SME probably due to the genotype with higher SME links adequate substrate respiration to build useful plant parts (shoot and root). It also might be due to the more ability of genotype with higher SME to accumulate respiratory product to wheat seedlings.

Drought tolerance/resistance indices

Drought tolerance, drought tolerance index, drought sensitivity index and mean productivity of wheat genotypes based on different germination and early seedling traits are presented in Table 5. Variation in different indices observed in different wheat genotypes indicating different levels of drought tolerance under PEG induced osmotic stress. The genotype with the least TOL and DSI and the highest DTI, and MP showed more tolerance to stress condition than the other genotypes. Farshadfar *et al.* (2013) and Hooshmandi (2019) used TOL, DTI, DSI and MP as a tolerance criterion for wheat genotypes in stress conditions.

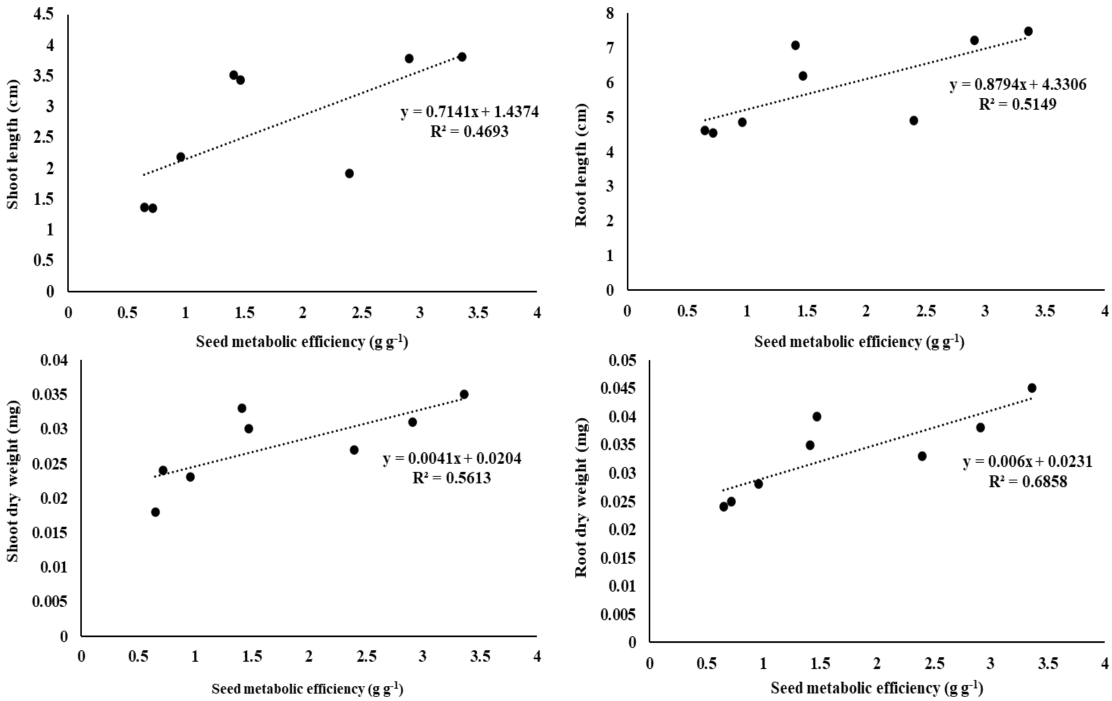

Fig. 2. Linear relationship of different seedling traits with seed metabolic efficiency.

Table 5. Drought tolerance /resistance indices of wheat genotypes based on different germination and seedling traits

Wheat genotypes	Tolerance/resistance indices	Germination and seedling traits							
		GR	CG	GVI	SL	RL	SDW	RDW	SME
BARI Gom 28	TOL	22.10	2.04	8.42	1.25	1.32	0.007	0.012	0.51
	DTI	0.77	0.94	0.74	0.64	0.79	0.770	0.700	0.65
	DSI	0.23	0.06	0.26	0.36	0.21	0.230	0.300	0.35
	MP	85.52	35.08	28.23	2.82	5.52	0.027	0.034	1.22
BARI Gom 29	TOL	10.61	1.76	6.77	2.16	2.51	0.009	0.010	0.69
	DTI	0.87	0.95	0.78	0.39	0.64	0.730	0.710	0.51
	DSI	0.13	0.05	0.22	0.61	0.36	0.270	0.290	0.49
	MP	79.53	34.61	27.33	2.44	5.81	0.029	0.030	1.07
BAW 1177	TOL	19.36	1.53	6.68	1.10	1.30	0.008	0.012	0.96
	DTI	0.80	0.96	0.80	0.71	0.82	0.770	0.730	0.71
	DSI	0.20	0.04	0.20	0.29	0.18	0.230	0.270	0.29
	MP	88.80	35.68	30.49	3.27	6.63	0.031	0.039	2.88
ESWYT 29	TOL	27.04	1.63	8.57	2.42	2.59	0.013	0.018	2.26
	DTI	0.70	0.95	0.74	0.36	0.64	0.580	0.530	0.22
	DSI	0.30	0.05	0.26	0.64	0.36	0.420	0.470	0.78
	MP	77.63	35.38	28.16	2.58	5.92	0.025	0.031	1.78

GR = Germination rate, CG = Co-efficient of germination, GVI = Germination vigor index, SL = Shoot length, RL = Root length, SDW = Shoot dry weight, RDW = Root dry weight, SME = Seed metabolic efficiency, TOL =Tolerance, DTI = Drought tolerance index, DSI = Drought sensitivity index, MP = Mean productivity

Conclusion

Based on different germination and early seedling traits evaluated as well as drought tolerance indices, genotype BAW 1177 was found comparatively drought tolerant, whereas ESWYT 29 was found drought susceptible and BARI Gom 29 and BARI Gom 28 were found moderately drought tolerant.

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