

# In-vitro screening of Iranian Landraces (*Triticumaestivum* L.) at the seedling stage for water stress tolerance using Polyethylene glycol (PEG 6000)

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**Abstract**— To understand, the parameters in wheat which can be used as criteria for drought tolerance, the present investigation was carried out on eight genotypes to an optimum dose of Polyethylene glycol (PEG6000) for water stress tolerance at germination and seedling stage. The experiment was conducted in the Department of Plant Breeding and Genetics with three replications under completely Randomized Design, 7 different concentration of Polyethylene glycol that were 0, 6%, 8%, 10%, 12%, 14%, 16% and 18% used in this experiment which were prepared according to weight by volume method. There was a significant reduction in seedling parameters with an increasing concentration of Polyethylene glycol. Based upon the vigor index low treatment of polyethylene glycol decreased the seedling parameters to a lesser extent than high concentration. The maximum difference in all seedling parameters was observed at 14% but as the concentration of polyethylene glycol increased (16% and 18%) there was a greater reduction in the root, shoot length and vigor index as compared to 14% of Polyethylene glycol which had an intermediate effect on seedling parameters. Identifying the drought-tolerant wheat genotypes during the seedling stage by in-vitro screening is a physiological approach which assists breeder for rapid selection of genotypes.

## Highlights

- Reduction in seedling parameters with increasing concentration of Polyethylene glycol
- Water stress-tolerant landraces have maximum vigor index
- For in-vitro screening, the moderate concentration of Polyethylene glycol should be used

**Keywords**— Water stress, Polyethylene glycol, Iranian wheat landraces, screening, and seedling growth.

## I. INTRODUCTION

Wheat is the staple food contributing 20% calories to the world's population, with a total harvest area of 2.1 million km<sup>2</sup> and global production of 700 million tonnes (Shiferaw et al 2013 and Lobel and Gourdjji 2012). Water deficit is one of the major constraints in agricultural production including wheat, which has devastated the economy of many countries. Water is also becoming a scarce commodity and its severity has been forecast further in this area as well, in the years to come. Water stress affect plant at any stage but germination and seedling growth are severely affected however these traits plays a vital role in determining yield (Raufet al 2007). Roots are more sensitive to drought stress because regulation of water mechanism occurs inside the apoplast of the root (Ghafoor 2013). Root and shoot length are important traits

for screening water tolerance in the selection of drought tolerance at the early seedling stage is frequently accomplished by using drought-induced chemical Polyethylene glycol (PEG 6000). Underwater stress osmotic adjustment is very necessary for plants to cope up with stress. Polyethylene glycol (PEG 6000) is a neutral osmotically group of polymers having high molecular weight used to induce water deficit in plants by modifying the osmotic potential of nutrient solution in a controlled manner. Screening of seedling traits using PEG is the most simple and cost-effective method for screening maximum germplasm. Identification of wheat genotypes that can tolerate water stress conditions is vital to boost wheat production and also helpful for understanding yield-limiting factors. To date, most of the Iranian germplasm has not been characterized and used in plant breeding

(Hoisington et al 2010). Therefore assessing genetic variation and differentiation of Iranian wheat landraces and cultivars will facilitate the effective use of these valuable genetic resources in future breeding to broaden genetic diversity. The present study was carried to pinpoint the optimum dose of Polyethylene glycol 6000 to create water stress at the seedling stage so that the response of Iranian landraces for stress tolerance elucidate and landraces screened efficiently.

## II. MATERIALS AND METHODS

The experiment was carried out in a completely randomized design with three replications and plant material was procured from International Maize and Wheat Improvement (CIMMYT). The study was carried out in the Research Laboratory of wheat section Plant Breeding and Genetics, Punjab Agricultural University Ludhiana. Five randomly selected Iranian landraces along with 3 checks were included in the standardization experiment. The solution of Polyethylene glycol (PEG 6000) was prepared according to weight by volume method (Bayoumi et al 2008). The different concentration of Polyethylene glycol (PEG 6000) (6%, 8%, 10%, 12%, 14%, 16% and 18%) was prepared in the present study. To prepare T1 (6%) 60 g of Polyethylene glycol (PEG 6000) was dissolved in distilled water to raise the volume to one liter and similarly all treatment T2 (8%) 80g, T3 (10%) 100g, T4 (12%) 120g, T5 (14%) 140g, T6 (16%) 160g, T7 (18%) 180g of Polyethylene glycol (PEG 6000) was dissolved in distilled water to raise the volume to one liter. Polyethylene glycol (PEG 6000) was used to induce drought stress during the germination and seedling stage by using a cigar roll method (Zhu et al 2005). After surface sterilization of seeds with 0.1 % mercuric chloride solution for 2 minutes, the seeds were washed with water and kept for germination on germination paper in distilled water (control) and PEG 6000 solution of different concentrations (6%, 8%, 10%, 12%, 14%, 16%, and 18%) for water-stress studies. Rolls were placed in a growth chamber in darkness at 25°C for 3 days under a photoperiod of 16/8 hours. This experiment was conducted on check varieties to standardize the concentration of Polyethylene glycol (PEG 6000) which affects germination and seedling growth. The data on germination percent, root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight was recorded from different treatments and vigor index was calculated by using formula

Vigor index =  $\frac{\text{Germination percentage} \times \text{seedling dry weight}}{\text{Root} + \text{shoot dry weight in g}}$

## III. RESULTS AND DISCUSSIONS

### Analysis of Variance

Analysis of variance for all the seedling traits for water stress tolerance was conducted. ANOVA revealed significant differences among cultivars water stress and the interaction effect between the landraces and Polyethylene glycol (PEG) concentration. The mean square due to treatment, genotype, and interaction between treatment and genotype were highly significant for all the seedling traits. (Table 2)

### Germination percentage

Maximum germination percentage (100%) was observed under control conditions in Iranian landraces and commercial check varieties followed by (96.2) in T1 and (90.4) in T2 were 6% and 8% PEG applied respectively (Table 1). With the increasing concentration of Polyethylene glycol (PEG 6000), there was a decrease in the rate of germination. Minimum germination percentage i.e. was 35.5 and 24.7 recorded at 16% and 18% of peg 6000. Iranian landraces and wheat cultivars showed 70% germination at 140 g/l Polyethylene glycol (Table 1). A similar result of the reduction in germination percentage with increasing peg concentration was reported by Ghodsi (2004) and Rauf et al (2007).

### Root length

Mean values regarding root length are presented in (Table 1). The root length decreased significantly with increasing moisture stress in Iranian landraces and wheat cultivars. Maximum root length (21.0) was observed under control conditions followed by 17.9 and 17 in T1 and T2 respectively whereas minimum (3.35) root length was recorded at 18%. Root length at T4 and T5 showed a difference of 1.4 cm (Table 1). The result of the present study consisted of the findings of Rauf et al (2007), Singh et al (1994) and Jajarmi et al (2009).

### Shoot length

With increasing stress levels there was a reduction in shoot length in Iranian landraces and wheat cultivar. The decrease in shoot length was maximum at 18% Polyethylene glycol (4.86 cm) followed by 16% (6.88 cm) and 14% (11.0) peg respectively (Table 1). Under control condition, maximum shoot length was recorded that was 17.4 cm followed by T1 (15.3 cm) and T2 (14.2 cm) respectively. (Table 1). Reduction in shoot length was similar to the findings of Kamran et al (2009).

### Root and shoot fresh weight

Maximum root (0.13g) and shoot (0.30g) fresh weight were recorded in control conditions followed by T1 and T2 respectively whereas minimum root fresh weight was recorded at 16% and 18% that was 0.05 respectively

(Table 1). A reduction of 0.04g was observed in T5 (14%) as compared to T4 (12%) peg (Table 1). Minimum shoot fresh weight was observed at 18% peg (0.03) followed by 14% peg (0.06) respectively. There was a reduction of 0.03g and 0.04 in T4 (12%) and T5 (14%) as compared to T3 (10%) (Table 1). The result of the present is similar to the findings of Bayoumi et al (2008) who reported that there was a reduction in root and shoot biomass with increasing concentration of Polyethylene glycol

#### Root and shoot dry weight:

Maximum root dry weight (0.05g) was recorded in control conditions, T2, T3, and T4, whereas in T7 (18%) minimum root dry weight (0.02g) was observed. 0.04 g was recorded at 14% peg followed by (0.03) in 12% of peg (Table 1). The maximum and minimum shoot dry weight was recorded in all treatments were 0.03 and 0.02 g respectively. The decreasing trend in shoot and root dry weight with increasing concentration of polyethylene glycol were reported by Kamran et al (2009) and Ahmad et al (2013) in wheat which is consistent with the present study.

#### Vigor index

The highest value (3724) of seedling vigor was observed in control conditions in Iranian landraces and commercial check varieties followed by 3063 at 6% peg and 2734 at 8% peg respectively, however, the seedling vigor decrease with increasing concentration of polyethylene glycol (Table 1). The least value of vigor index was recorded at 18% peg that was 290. There was a difference of 140 and 746 in T3 and T4 as compared to the vigor index in T2 (2754). There was a reduction in vigor index with increasing concentration of Polyethylene glycol (PEG) as reported by Cokkizgin (2013) in pea, the similar result had been reported by Moras et al (2005) in the bean. Reduction in vigor index in wheat with increasing concentration of Polyethylene glycol (PEG 6000) reported by Tamiru and Ashagerin (2014).

#### Correlation studies

The correlation coefficient was calculated among all the characters (Table 3). Germination percentage showed a

positive relation with root length, shoot length, root fresh weight, shoot fresh weight and seedling vigor. It means an increase in germination percentage would also increase in these traits. Root length is strongly correlated with shoot length and vigor index. These results are following the findings of Khan et al (2002). Correlation analysis showed that shoot length exhibited a positive association with root length and vigor index which means an increase in shoot length will also cause an increase in these parameters. These results are supported by Akram et al (1998). Root and Shoot fresh weight were correlated with root length whereas the vigor index showed a strong positive correlation with root length and shoot length.

#### IV. CONCLUSION

Generally from the present study, it is concluded that there is a reduction in germination and seedling growth with increasing concentration of polyethylene glycol. The low treatment of Polyethylene glycol (6% and 8%) decreased the seedling parameter to a lesser extent than moderate treatment (10%, 12%, and 14%) and high concentration (16% and 18%) of Polyethylene glycol. Germination percentage, root length, shoot length and vigor index of wheat genotypes showed lesser reduction till 12% of Polyethylene glycol. The maximum difference in all seedling parameters observed at 14% of Polyethylene glycol while at higher concentration of polyethylene glycol there was a major reduction in germination percentage, root length, shoot length and vigor index of wheat cultivars. Based upon the vigor index all the genotypes respond better at 14% of Polyethylene glycol (PEG 6000). Vigor index can increase the crops seasonal water use efficiency by as much as 25% and is recognized as a trait to select for improving yield under water stress (Richards et al 2002) and Botwright et al (2002). The genotypes which have higher seedling vigor index also have better germination percentage, root length and shoot length (Hafid et al 1998)

Table 1: Effect of different drought levels on growth parameters on wheat genotypes

Parameters	Peg(g/l)	IWA 860005	IWA 8600303	IWA 8600542	IWA 8606753	IWA 8600824	C- 306	PBW175	PBW660	Mean	LSD
Germination Percentage	0%	100	100	100	100	100	100	100	100	100a	6.23
	6%	90	100	100	100	90	100	90	100	96.25b	
	8%	86.5	90.5	90.5	90.5	90	100	85.5	90	90.44c	
	10%	80.5	85.5	90.5	85.5	85.5	96.6	85.5	95.5	88.14d	
	12%	80	83.3	85.5	80	80	95.5	80	85.5	83.73e	
	14%	76.5	80.5	80	70.5	70	85.5	80	85.5	78.56f	

	16%	40.5	7.05	60.5	40.5	4.05	40	50.5	40.5	35.5g	
	18%	20.5	5.5	40.5	20	20.5	40	20	30.5	24.7h	
Root Length	0%	18.5	19	18	20.5	20.5	23.5	23.5	24.5	21a	1.33
	6%	17.5	15.5	18	15.5	18	20.5	17.5	21	17.9a	
	8%	17	14	15.5	14	17.5	19	19.5	19.5	17 a	
	10%	16.5	14	14	13.8	15.5	19	17.5	17.5	16.0 a	
	12%	15	13.5	12.5	12	14	16	12	15.5	13. b	
	14%	10.8	12	12	7.8	13.5	16.3	12	15	12. b	
	16%	6.5	9.8	7.8	4.5	4.5	7.8	9.8	7.8	7.31 c	
	18%	3.5	2.5	2.5	3.5	3.5	4.5	4.5	2.3	3.35d	
Shoot Length	0%	15	17	15	17	18.5	19.4	19	18.5	17.4a	1.79
	6%	15.5	13.9	18	13.5	14.5	15.5	15.5	16	15.3b	
	8%	15	13	12.5	12.8	14	16	14.5	15.5	14.2b	
	10%	13.5	13.7	12	13	13	14.5	14	15	13.6c	
	12%	14	12	12	11	12	15	12	14	12.8d	
	14%	7.8	11	9.8	7.5	11.5	15	12	13.5	11.0e	
	16%	5	7.5	6.5	4	4.5	9.5	7.5	10.5	6.88f	
	18%	5.5	3	3	2.9	4	7	5	8.5	4.86g	
Root Fresh weight	0%	0.12	0.14	0.13	0.12	0.12	0.13	0.14	0.14	0.14a	0.44
	6%	0.14	0.12	0.1	0.13	0.16	0.15	0.15	0.16	0.13a	
	8%	0.13	0.12	0.12	0.13	0.14	0.12	0.13	0.13	0.13a	
	10%	0.1	0.1	0.11	0.09	0.12	0.12	0.09	0.12	0.11a	
	12%	0.09	0.08	0.08	0.1	0.1	0.19	0.08	0.08	0.1a	
	14%	0.06	0.06	0.07	0.08	0.06	0.06	0.05	0.04	0.06a	
	16%	0.04	0.05	0.06	0.05	0.04	0.04	0.05	0.04	0.05a	
	18%	0.04	0.05	0.06	0.05	0.04	0.04	0.05	0.04	0.05a	
Shoot Fresh weight	0%	0.37	0.3	0.29	0.2	0.18	0.41	0.38	0.28	0.30a	0.48
	6%	0.24	0.25	0.22	0.18	0.18	0.16	0.2	0.2	0.20a	
	8%	0.15	0.14	0.16	0.16	0.14	0.15	0.15	0.14	0.15a	
	10%	0.1	0.12	0.14	0.1	0.1	0.11	0.09	0.08	0.11a	
	12%	0.1	0.07	0.06	0.08	0.09	0.08	0.08	0.07	0.08a	
	14%	0.07	0.06	0.09	0.08	0.06	0.06	0.05	0.05	0.07a	
	16%	0.06	0.05	0.08	0.06	0.06	0.05	0.04	0.04	0.06a	
	18%	0.05	0.03	0.02	0.02	0.03	0.02	0.02	0.03	0.03a	
Root Dry weight	0%	0.05	0.07	0.04	0.06	0.05	0.06	0.03	0.04	0.05a	0.30
	6%	0.05	0.02	0.04	0.06	0.05	0.03	0.04	0.06	0.04a	
	8%	0.05	0.05	0.06	0.04	0.05	0.03	0.05	0.05	0.05a	
	10%	0.05	0.06	0.03	0.03	0.05	0.04	0.05	0.05	0.05a	
	12%	0.04	0.05	0.04	0.05	0.07	0.05	0.04	0.05	0.05a	
	14%	0.06	0.07	0.04	0.05	0.03	0.03	0.04	0.03	0.04a	
	16%	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.04	0.03a	
	18%	0.04	0.02	0.03	0.02	0.01	0.02	0.02	0.01	0.02a	
Shoot dry weight	0%	0.04	0.03	0.04	0.03	0.04	0.03	0.03	0.03	0.03a	0.19
	6%	0.02	0.03	0.02	0.03	0.02	0.03	0.02	0.02	0.02a	

	8%	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.03	0.02a	
	10%	0.02	0.02	0.02	0.03	0.03	0.02	0.02	0.04	0.03a	
	12%	0.04	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.03a	
	14%	0.02	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.03a	
	16%	0.02	0.03	0.03	0.04	0.03	0.02	0.03	0.02	0.03a	
	18%	0.03	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.02a	
Vigor Index	0%	3350	3600	3400	3200	3400	4290	4250	4300	3724a	338.4
	6%	2970	2940	3300	2900	2925	3600	2970	2900	3063b	
	8%	2768	2443	2400	2400	2300	3500	2907	3150	2734c	
	10%	2415	2368	2353	2291	2291	3236	2693	3103	2594d	
	12%	2320	2124	2094	2040	2080	1920	1900	1422	1988e	
	14%	1851	1744	1078	1750	2565	1920	1900	465	1659f	
	16%	1219	865	344	364.5	550	692	684.5	184.5	613g	
	18%	277	222	128	153	460	440	329	310.5	290h	

Values in mean column sharing the same letter are statistically non- significant at 5%

Table.2: Analysis of variance for seedling traits of Iranian landraces and check varieties under different drought level

Source of variation	DF	Germ%	RL	SL	RFW	SFW	RDW	SDW	VI
Genotypes	7	531.4*	51.9*	35.04*	0.123*	0.476*	0.345*	0.639*	1037059*
Drought levels	7	17727.3*	817.53*	386.7*	0.375*	0.141*	0.252*	0.777*	323295*
Cultivars× Drought levels	49	153.96*	7.011*	11.07*	0.797*	0.93*	0.378*	0.145*	45374*
Error	128	44.04*	1.01*	0.863*	0.255*	0.229*	0.13*	0.364*	13020.4*
Total	191								
Coefficient of variation		8.85	7.35	7.69	5.56	4.01	8.47	7.42	17.74

Abbreviations- germ%- Germination percentage, RL- Root length, SL- Shoot length, RFW- Root fresh weight, SFW- Shoot fresh weight, RDW- Root dry weight, SDW- Shoot dry weight and VI- Vigor index \*Significant at 5% level

Table 3: Correlation coefficient among various seedling traits of different wheat genotypes

	GP	RL	SL	SFW	RFW	RDW	SDW	VI
GP	1	0.87**	0.86**	0.89**	0.88**	0.53**	0.35**	0.85**
RL		1	0.93**	0.68**	0.82**	0.52**	0.43**	0.91**
SL			1	0.67**	0.37**	0.43**	0.42**	0.88**
SFW				1	0.68**	0.43**	0.32**	0.76**
RFW					1	0.5	0.18**	0.82**
RDW						1	0.15**	0.48**
SDW							1	0.34**
VI								1

Abbreviations- GP Germination percentage RL- Root length, SL- Shoot length, RFW- Root fresh weight, SFW- Shoot fresh weight, RDW- Root dry weight, SDW- Shoot dry weight and VI- Vigor index\*\* significant at 0.01

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