Research Article

Polyethylene Glycol Mediated Osmotic Stress Impacts on Growth and Biochemical Aspects of Wheat Under Artificial Osmotic Stress Condition

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Abstract | Drought is a significant issue because of the worldwide effects of climate change and the negative effects it has on wheat output. For improving production in water-scarce locations, screening drought-tolerant wheat cultivars is crucial. At the Plant Physiology Division of NIA, Tando Jam, the current experiment was conducted during the Rabi year of 2021. At both the P0.01 and P0.05 probability levels, the growth measurements at the seedling stage revealed extremely significant variations for all characteristics among genotypes, treatments, and interactions. The results demonstrated a stronger osmotic impact when using -0.5 MPa PEG. At the seedling stage, the genotype SDW-3 had higher decreases in a number of growth indices, such as shoot length (-11.3), root length (-72.6), and shoot fresh weight (-4.4), with an increase in PEG (-0.5 MPa) level. According to the results of the current study, all six wheat genotypes' seed incubation shoot, root length, and shoot fresh weight drastically decreased under laboratory conditions due to osmotic stress. The genotype SDW-3 showed the greatest decrease under increased osmotic stress brought on by PEG-6000 (-5.0 MPa). As a result, the genotypes AST-1(V1), SDW-1, and SDW-2 may be employed in future breeding programmes and are drought resistant.

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1. Introduction

Being a worldwide restriction, drought has shown to have a significant influence on productivity (Li *et al*., 2020; Abro *et al*., 2020). Each stage of plant growth is eventually impacted by the impacts of drought on crop output. Additionally, there is a reduction in germination potential, coleoptile length, and vigour (Mahpara *et al*., 2022; Abro *et al*., 2019). The primary limiting variables for germination are semi-arid locations and low moisture places. The production of seedlings and their rate can be used to predict the maturity and yield of H. hirsutum (Sharma *et al*., 2022).

A popular in vitro selection technique is polyethylene glycol (PEG), which is used to weed out cultivars that can withstand drought (Tabatabai *et al*., 2022). It causes osmotic stress in plants and has a big impact on how quickly their roots and shoots grow (Latif *et al*., 2022). The nonionic, almost impermeable chains of the PEG molecules (PEG 6000) maintain the homogeneity of water potential throughout the experiment without harming people's health (Vuković *et al*., 2022). The research on identifying drought-resistant wheat genotypes have demonstrated that various features respond differently to varying amounts of PEG-6000 (Batool *et al*., 2022; Abro *et al*., 2021). According to (Abro *et al*., 2022; Hussain *et al*., 2022) decrease in the length of vegetative organs (such as the stem, plumule, and coleoptile) and a drop in the proportion of seeds that germinate as a result of the severity of moisture stress may not be a reliable signal for choosing resistant cultivars. Coleoptile lengths and leaf surface areas decline early in wheat development (Lei *et al*., 2021; Abro *et al*., 2021). Low germination rates would ensue as a result, which would decrease the likelihood of planting in damp soil (Manoj *et al*., 2021).

The osmotic stress brought on by the drought brought on by the depletion of subterranean water (PEG) influences ion transport and absorption throughout the plant's life cycle, from growth to crop production (Loutfy *et al*., 2022). An efficient method for modifying physiological characteristics and genetic processes that result in the breeding of superior cultivars is *in-vitro* drought resistance screening (Mahpara *et al*., 2022). Scientists are concentrating on increasing the output capacity of wheat through developing new varieties of bread wheat that have a favourable genetic makeup (Pandey *et al*., 2022). Increasing productivity and crop yield per unit area was the goal in primitive times. Because hybridization can best use the gene, genetically modified wheat can be very successful. Genetic modification is the best method for improving wheat production (Sharma *et al*., 2022). The only way to select wheat genotypes is if there is strain heterogeneity. Earlier researchers looked at the phenotypic correlation coefficients of different grain yield and its components with crop yield in terms of genetic improvement through a variety of experiments. Othmani *et al*. (2021) discovered that grain weight in the 1000-grain range had an effect on grain yield (Bijalwan *et al*., 2022). Assert that tillers plant-1 significantly contributed to grain yield. The objective of the current study is to filter for wheat genotypes that is drought resistant using polyethylene glycol (PEG-6000) liquid in a laboratory environment. The same genotypes were also examined in the field to further support the laboratory research. Our research compared the performance of different wheat genotypes based on morpho-physiological characteristics in both in vitro and in vivo tests. The wheat (*Triticum aestivum* L.) genotypes that were stressed at the seedling stage were also removed using PEG-6000. Our research compared the performance of different wheat genotypes based on morpho-physiological characteristics in both *in vitro* and *in vivo* tests. Additionally, we eliminated wheat (*Triticum aestivum* L.) genotypes that were stressed at the seedling stage using PEG-6000.

2. Materials and Methods

The current study was carried out in the Rabi year of 2021 by the Tandojam Plant Physiology Division of the Nuclear Institute of Agriculture (NIA). 06 wheat genotypes were excluded from the experiment under control conditions (1/4th Hoagland solution) in addition to a simulated drought of 0.5 MPa caused by Polyethylene glycol-6000. These genotypes included V1 AST-1, V2 AST-1, V3 SDW-1, V4 SDW-2, V5 SDW-3, V6, and Khirman (PEG-6000). The Plant Breeding and Genetics Division (PBGD) of the Nuclear Institute of Agriculture (NIA) in Tandojam provided the different wheat germplasm samples (the advanced lines). The study took place in a growth cabinet with a 25/25°C daytime and 12°C nighttime temperature control. It was factorially set up in a fully randomised design (CRD), and it was duplicated three times (irradiance: 22 Wm-2). The seedlings in each treatment were cultivated on plastic moulded nets and put in 8.5 cm diameter bowls after being surface sterilised with 0.5 percent NaCl (sodium hypochlorite) on 30 wheat seeds from each genotype (Abro *et al.*, 2021, 2020). For 72 hours, the bowls were kept in the dark to guarantee a successful germination process. The seeds were exposed to a 12-hour photoperiod for 11 days (irradiance: 22 Wm-2). 06 wheat genotypes were discarded from the study under control circumstances (1/4th Hoagland solution), and polyethylene glycol-6000 was used to mimic a drought of 0.5 MPa (PEG-6000). When necessary, Ca (OH) 2 and/or H2SO4 were used to assess the nutritional solution's pH and adjust it to 5.5 + 0.2. The plants were removed carefully ten days after planting, separated into shoots and roots, thoroughly cleansed with filtered water, and blotted dry. After being weighed dry for the shoot and root, the materials were thoroughly treated in a wiley mill to pass through with a 1 mm (40 mesh) filter. Uniformly ground materials were digested with a di-acid mixture of nitric (HNO3) and per chloric (HClO4) at a 3:1 ratio. Using a flame photometer (Jenway PFP-7) to calculate the potassium content in the shoot and root (mg g-1dry wt) in accordance with (Abro *et al.*, 2020, 2021; Wei et al., 2013).

2.1 Statistical analysis

Data were examined by Gomez and Gomez using the statistical method of analysis of variance (Gomez and Gomez, 1984). When the F-test was significant, a multiple mean comparison analysis incorporating the variety and stress therapy was performed. Tukey's significant difference was then utilised to determine the treatment combinations.

3. Results and Discussion

In this work, an *in-vitro* environment was used to assess the ability of the six wheat cultivars to survive water stress induced by PEG (6000) at the early seedling stage. Six distinct wheat cultivars had varying performances under the various PEG treatments, according to statistical analysis data. There was a substantial two-way interaction between cultivars and the amount of drought for all seedling measures (P 0.01). The mean square of analysis at the vegetative stages for the nutrient uptake, including shoot (length), root (length), shoot (fresh wt), root (fresh wt), shoot (dry wt), and potassium (K) percent, revealed highly significant difference at P0.01 and P0.05 probability level for all traits for genotypes, treatments, and interaction (Table 1).

Table 1: *In vitro* effect of drought condition on shoot length (cm) of wheat genotypes.

S. No	Genotypes	Control		Drought		Mean		R. Dec (%)
1	AST-I(V1)	16.58	AB	15.53	BC	16.055	AB	6.4
2	AST-I(V6)	15.85	ABC	13.04	DE	14.443	C	17.7
3	SDW-1	17.56	A	16.04	ABC	16.8	A	8.7
4	SDW-2	16.96	AB	14.59	CD	15.778	AB	14.0
5	SDW-3	11.87	E	13.21	DE	12.543	D	-11.3
6	Khirman	16.06	ABC	15.18	BC	15.622	B	5.5
	Mean	15.82	A	14.60	B			7.7

HSD (0.05); Treat =0.44; Geno = 1.15; T x G = 1.90

3.1 Shoot and Root length

Under control conditions, SDW-1 (17.56 cm) had the longest shoots on average while SDW-3 (11.87 cm) had the shortest. Whereas, in drought conditions, the SDW-1 maximum shoot length was 16.04 cm, and the AST-I minimum was V6 (13.04 cm). However, Khirman showed the lowest reduced R.D (5.5%) and AST-I showed the most decreased (17.7 %%). (V6). In an *in vitro* investigation, wheat genotypes were examined to see how their root length responded to drought circumstances (moisture stress). The results (Tables 2, 3) revealed that the tested wheat genotypes responded differently to drought conditions. SDW-3 (7.05 cm) had the shortest root length while AST-I (V1) had the longest root length at the maximum

Table 1: Mean square of analysis for different growth parameters under osmotic stress using PEG (6000).

Source	DF	Shoot (length)	Root (length)	Shoot (fresh wt)	Root (fresh wt)	Shoot (dry wt)	Root (dry wt)	Potassium (K) %
Replication	2	0.1008	0.0910	0.0227	0.04110	0.00049	0.00064	4.6978
Genotypes	5	13.7201**	13.6548**	0.1220**	0.06717ns	0.1310**	0.00794**	16.5458*
Treatment	1	13.3103**	10.2187*	17.6931**	0.58303**	0.03277**	0.05505**	25.3344*
Genotype x treatment	5	3.1948**	8.2668**	1.0429**	0.22056**	0.01150**	0.00351**	22.8318**
Error	22	0.4096	1.6852	0.0718	0.02764	0.00091	0.00029	5.7472

**: significant at <0.01probability level; *: Significant at P<0.05probability level and ns.: non-significant.

Table 2: ***In vitro*** **effect of drought condition on root length (cm) of wheat genotypes.**

S. No	Genotypes	Control		Drought		Mean		R. Dec (%)
1	AST-I(V1)	13.71	A	11.78	AB	12.75	A	14.1
2	AST-I(V6)	10.38	ABC	11.38	AB	10.88	AB	-9.6
3	SDW-1	8.62	BC	10.46	ABC	9.54	B	-21.3
4	SDW-2	12.22	AB	12.27	AB	12.24	A	-0.4
5	SDW-3	7.05	C	12.17	AB	9.61	B	-72.6
6	Khirman	12.64	A	12.96	A	12.80	A	-2.5
	Mean	10.77	B	11.84	A			-9.9

HSD (0.05); Treat =0.90; Geno = 2.33; T x G = 3.85

Table 3: ***In vitro*** **effect of drought condition on shoot fresh weight (g) of wheat genotypes.**

S. No	Genotypes	Control		Drought		Mean		R. Dec (%)
1	AST-I(V1)	3.93	B	2.69	C	3.31	A	31.6
2	AST-I(V6)	4.67	AB	2.47	C	3.57	A	47.0
3	SDW-1	4.06	AB	2.50	C	3.28	A	38.5
4	SDW-2	4.74	A	2.61	C	3.68	A	44.8
5	SDW-3	2.46	C	2.57	C	2.52	B	-4.4
6	Khirman	4.34	AB	2.93	C	3.64	A	32.3
	Mean	4.03	A	2.63	B			34.8

HSD (0.05); Treat =0.19; Geno = 0.48; T x G = 0.80

under control conditions. However, in Khirman, the greatest root length measured under drought conditions was 12.96 cm, while the minimum SDW-1 (10.46 cm). However, the AST-I test showed a relative reduction of 14.1%. (V1). The main factors for choosing wheat cultivars that are drought-resistant relate to plant growth, such as root and shoot length, seedling fresh weight, etc. (Abro *et al*., 2020, 2021; Foito *et al*., 2009). It is also obvious that different cultivars' shoot lengths are considerably impacted by water stress caused by PEG. Because cell division and shoot elongation both require artificial osmotic stress. The researcher found that PEG had an effect on the shoot length of numerous wheat varieties in his study experiment and came to similar conclusions (Almaghrabi, 2012). This explanation states that the root length reduces with increasing PEG concentrations, possibly as a result of the inhibition of cell division and elongation (Doneva *et al*., 2021). The reduction in root length during drought stress may be due to a limitation of cell division and elongation that results in soft tuberization, claim Chachar *et al.* (2014).

3.2 Shoot and root fresh weight (g)

In an *in vitro* investigation, wheat genotypes were examined for how they responded to moisture stress and drought in terms of shoot fresh weight, and the results (Table 3) revealed that the tested wheat genotypes responded differently to drought. Under control conditions, SDW-2 showed the highest shoot fresh weight (4.74 g), while SDW-3 showed the lowest (2.46 g). While in a drought, Khirman had the highest fresh shoot weight (2.93 g) and the lowest AST-I (V6) levels (2.47 g). A lowest reduced R.D of 44.8 percent was found in SDW-2, whilst a maximum decreased R.D of 32.3 percent was found in Khirman. In an in vitro experiment, wheat genotypes were examined to see how drought circumstances (moisture stress) affected the root fresh weight of the plants. The results (Table 4) revealed that the tested wheat genotypes responded to drought in different ways. Under control conditions, AST-I (V6) (1.98 g) had the highest root fresh weight and SDW-3 (1.43 g) had the lowest. Whereas, in drought conditions, SDW-3 recorded the highest root fresh weight (1.84 g), while SDW-1 recorded the lowest (1.12 g). However, Khirman showed the lowest reduced R.D (12.7%) and SDW-1 showed the most decreased (38.6%). Our findings concur with those made by (Abro *et al*., 2020, 2021; Wei *et al*., 2013), who saw a trend of decline in the shoot and root's fresh/dry weight. However, it was noticed by multiple studies that the both variables of shoot/root fresh and dry weight greatly increased shoot and root masses in both cultivars under well-watered circumstances.

Table 4: ***In vitro*** **effect of drought condition on root fresh weight (g) of wheat genotypes.**

S. No	Genotypes	Control		Drought		Mean		R. Dec (%)
1	AST-I(V1)	1.65	ABC	1.40	BCD	1.52	A	15.3
2	AST-I(V6)	1.98	A	1.42	BCD	1.70	A	28.4
3	SDW-1	1.82	AB	1.12	D	1.47	A	38.6
4	SDW-2	1.52	ABCD	1.32	CD	1.42	A	13.5
5	SDW-3	1.43	BCD	1.84	AB	1.64	A	-28.3
6	Khirman	1.69	ABC	1.47	BCD	1.58	A	12.7
	Mean	1.68	A	1.43	B			15.1

HSD (0.05); Treat =0.12; Geno = 0.30; T x G = 0.49

3.3 Shoot and root dry weight (g)

In the *in vitro* experiments, wheat cultivars were tested for how drought-related moisture stress affected their shoot dry weight. The results (Table 5) showed that the tested wheat genotypes reacted to drought in different ways. Under control conditions, SDW-2 (0.51 g) had the highest shot dry weight while SDW-3 (0.29 g) had the lowest shoot dry weight. While under drought conditions, the highest shoot dry weight (SDW-3) measured was 0.41 g, and the lowest (AST-I) was V1 (0.31 g). However, Khirman showed the lowest reduced R.D (15.0%) and AST-I showed the most decreased (24.7%) (V6). In an in vitro experiment, wheat genotypes were examined to see how drought circumstances (moisture stress) affected the root dry weight of the plants. The results (Table 6) revealed that the tested wheat genotypes responded to drought in different ways. Under regulated conditions, SDW-3 had the highest root dry weight (0.34 g), whereas Khirman had the lowest (0.19 g). However, in times of dryness, SDW-1's highest root dry weight (0.19 g) and AST-lowest I's (V1) were also recorded (0.12 g). A minimum reduced R.D of 21.0 percent was found in SDW-1, whilst a maximum decreased R.D of 52.0 percent was found in SDW-3. Other studies that found that water stress had a significant influence on root and shoot dry matter production (Abro *et al.*, 2020, 2021; Ahmad *et al.*, 2013; Marciska *et al.*, 2013) also noticed a downward trend in root and shoot dry weight.

Table 5: *In vitro* effect of drought condition on shoot dry weight (g) of wheat genotypes.

S. No	Genotypes	Control		Drought		Mean		R. Dec (%)
1	AST-I(V1)	0.38	BCDE	0.31	DE	0.34	C	19.0
2	AST-I(V6)	0.45	AB	0.34	CDE	0.39	BC	24.7
3	SDW-1	0.50	A	0.39	BCD	0.44	AB	22.4
4	SDW-2	0.51	A	0.39	BCD	0.45	A	23.3
5	SDW-3	0.29	E	0.41	BC	0.35	C	-37.9
6	Khirman	0.41	BC	0.34	CDE	0.37	C	15.0
	Mean	0.42	A	0.36	B			14.3

HSD (0.05); Treat = 0.02; Geno = 0.05; T x G = 0.09

Table 6: *In vitro* effect of drought condition on root dry weight (g) of wheat genotypes.

S. No	Genotypes	Control		Drought		Mean		R. Dec (%)
1	AST-I(V1)	0.18	DEF	0.12	G	0.15	D	31.2
2	AST-I(V6)	0.22	BCD	0.16	EFG	0.19	BC	26.8
3	SDW-1	0.24	BC	0.19	CDEF	0.21	B	21.0
4	SDW-2	0.26	B	0.18	DEF	0.22	B	29.6
5	SDW-3	0.34	A	0.16	EFG	0.25	A	52.0
6	Khirman	0.19	CDE	0.14	FG	0.17	CD	27.9
	Mean	0.16	B	0.24	A			-49.3

HSD (0.05); Treat =0.01; Geno = 0.03; T x G = 0.05

3.4 Potassium content (%)

In an in vitro experiment, the potassium content of wheat genotypes was screened to determine how they would react to moisture stress and drought. The results (Table 7) revealed that the tested wheat genotypes responded differently to drought. Under controlled conditions, SDW-3 had the greatest potassium content (22.20%), while AST-I had the lowest amount (V6) (18.0%). The lowest SDW-1 levels and maximum potassium concentration in AST-I (V1) (25.27%) were discovered during drought circumstances (17.73%). SDW-1 had the lowest reduced R.D (2.7%) and SDW-3 had the highest decreased R.D (18.6%). This might be explained by the fact that higher K+ concentrations in the soil provided roots with more possibilities to absorb K+ and that cellular membrane recovery increased K+ conservation in plant tissues. Study from (Abro *et al.*, 2020, 2021; Wei *et al.*, 2013) supports the findings of his study, which discovered that enough exogenous K+ significantly increased K+ contents in both the shoot and root of PEG6000-stressed plants.

Table 7: *In vitro* effect of drought condition on potassium content (%) of wheat genotypes.

S. No	Genotypes	Control		Drought		Mean		R. Dec (%)
1	AST-I(V1)	20.50	ABC	25.27	A	22.88	A	-23.3
2	AST-I(V6)	18.00	BC	24.97	AB	21.48	AB	-38.7
3	SDW-1	18.23	ABC	17.73	C	17.98	B	2.7
4	SDW-2	19.03	ABC	20.47	ABC	19.75	AB	-7.5
5	SDW-3	22.20	ABC	18.07	BC	20.13	AB	18.6
6	Khirman	20.07	ABC	21.60	ABC	20.83	AB	-7.6
	Mean	19.67	B	21.35	A			-8.5

HSD (0.05); Treat =1.66; Geno = 4.31; T x G = 7.12

Conclusions and Recommendations

The current study demonstrates that, both *in vitro* and *in vivo*, osmotic stress significantly lowered its seeds incubated, root length, and shoot fresh and dry weight throughout all six wheat cultivars. The greatest reduction was found in the genotype SDW-3 when PEG-6000-induced enhanced osmotic stress was present (-5.0 MPa). AST-1(V1), SDW-1, and SDW-2 as a consequence. The results demonstrated that the osmotic impact was larger when PEG was applied at a pressure of -0.5 MPa. With an increase in PEG (-0.5 MPa) level, the genotype SDW-3 reduced various growth parameters, including shoot length, root length, and shoot fresh weight, more during the seedling stage. It was suggested that these genotypes may be used in next breeding initiatives and taken into consideration as genotypes resistant to drought.

Novelty Statement

These wheat cultivars which were under observation of for study on the basis of our study we can strongly suggested that wheat cultivator can be utilize in drought stress areas.

Author's Contribution

Shabana Memon and Aamir Ali Abro: Conceptualization, methodology, for-mal analysis, writing-original draft.
Muhammad Iqbal Jakhro: Helped in methodology section
Aqsa Farid: Helped in introduction section.
Maliha Habib and Maqbool Ahmed: helped in analysis
Liaquat Ali Bhutto and Saba Ambreen Memon: helped in research
Muhammad Farooq: Conceptualization, review the manuscript and amp; editing

Conflict of interest
The authors have declared no conflict of interest.

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