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Impact of Polyethylene Glycol-Induced Drought Stress on *In Vitro* Shoot Regeneration in Wheat (*Triticum aestivum L.*) Genotypes

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Abstract

The research was conducted to determine the regeneration ability of ten wheat (*Triticum aestivum L.*) genotypes using callus clumps as ex-plant source. The experiment had six levels of drought stress as treatments (0%, 5%, 10%, 15%, 20% and 25% PEG levels) including control, fortified with BAP, Kinetin and 5mg/2,4-Dichlorophenoxy Acetic Acid (2,4-D) for the period of four weeks, with each treatment replicated three times, the data on days to mean callus shoot regeneration recorded the shortest mean days to regeneration of 14 in genotype 28 under 10% stress level, while the lengthiest was 31 days in genotype 21 under stress level 25%, also the highest number of regeneration was 5 with genotype 8 under 0% stress level, while the lowest was 0 in genotypes 21 and 22 under stress level 25%, the highest percentage shoot regeneration was 100% in genotype 8 under 0% stress, while the lowest percentage shoot was 0 in genotype 21 and 22 under 25% stress level. Meanwhile ANOVA was significantly different for both days to regeneration and number of regenerations between the treatment mean ($p< 0.05$), while Pearson correlation coefficient among the genotypes presented a positive correlation except between 10% and 0%, 15% and 5%, 15% and 10%. The result of this work can be used to recommend species that should be use for drought prone areas.

Keywords: Polyethylene glycol, *in vitro*, drought, regeneration, Wheet

Introduction

Wheat (*Triticum aestivum L.)* is a crucial global crop cultivated across approximately 200 million hectares in diverse environments, yielding over 600m metric tons annually [1]. The frequency and intensity of environmental extremes are expected to increase with climate change [2]. About 32% wheat cultivation areas of underdeveloped nations experience varying degrees in drought stress throughout the crop's planting season [3]. Rainfed wheat crops typically encounter more frequent and severe soil water deficits, but changing weather patterns and water shortages may also impact irrigated wheat, leading to increased risk of inadequate water [4]. Earth warming exacerbates stress conditions, posing limitations on plant production, which holds significant implications for human nutrition and socio-economic stability [5]. Developing and understanding of plant responses to drought is a fundamental part of developing stress-tolerant varieties [6]. A potential strategy at addressing future food demands amidst a growing population involves optimizing water usage by developing crop varieties with enhanced drought tolerance and reduced water requirements [7]. However, *in vitro* selection for tolerance to abiotic stress depends on the development of efficient and reliable callus induction and plant regeneration systems. In wheat species, various explants sources have been used for embryogenic callus formation and plant regeneration [8]. However, achieving high yields in water-limited environments remains challenging as a result of the complex drought nature and genetic regulation in plant effects [9]. Drought poses substantial threat to yield stability, making the enhancement of drought tolerance a critical focus for geneticists and breeders [10]. For drought stress induction, however, one of the most popular approaches is to use high molecular weight osmotic substances, such as polyethylene glycol [11].

Developing drought-tolerant cultivars through selective breeding solely guided by grain yield poses significant challenges, primarily due to the low heritability observed under drought stress conditions. Screening genotypes for early drought tolerance and inferring their root attributes at seedling stage has witnessed significant progress [12]. The genotypes with higher root volume combined with longer seminal and adventitious root length has been suggested as useful candidates for increasing grain yield [13]. *Invitro* selection techniques was utilized to improve tolerance to various environmental stresses, including cold, salinity, and drought [14].

With increasing drought conditions affecting wheat production, developing drought-tolerant genotypes is critical. This study aims to assess the effect of varying levels of PEG-induced drought stress on the shoot regeneration of ten wheat genotypes, to identify those with the highest regeneration potential under drought conditions.

Materials and Methods

Place of Research

The Research was conducted at the Department of Botany Laboratory, University of Agriculture Makurdi, Benue State.

Seed source

Ten wheat accessions (genotypes) of germplasm were obtained from Lake Chad Research Institute (A National Agriculturally based Research Institute), Maiduguri, Nigeria.

Table 1: Showing 10 Wheat Genotypes used for the Research

S/N	Genotype NO	Name /Wheat Pedigree
1	2	INQALAB9I*2/TUKURU//WHEAR
2	6	WBLLI/4/BOW/NKT/CBRD/3/CBRD/5/WBLLI*2……
3	7	HUBARA-2/QAFZAH-2I//DONIN-2
4	8	ATTILA 50Y//ATTILA/BCN/3/STAR*3/MUSK-3
5	9	ATTILA*/PBW 65*2/4/BOW/NTK/CBRD/3/CBRD
6	21	ATTILA GAN ATTILA
7	22	WBLLI/4/BOW/NKT/CBRD /3/CBRD/5/WBLLI*2/…
8	23	HOOSAM-8//CHAM-6/FLORKWA-2
9	27	FRET2/TUKURU/FRET2/3/MONIA/CHTO/AMEL/4/…
10	28	WEAVER/WL3928//SW89.3064/3/KAUZ//MON/CROW'S'

The genotypes/pedigree were identified and supplied by Lake Chad Research Institute, Maiduguri.

Sterilization

Explants plant from 10 wheat genotypes obtained from Lake Chad Research Institute, Maiduguri, were subjected to sequence of surface sterilization procedures. Initially, they were washed five times with detergent and two drops of polyoxyethylene (20) sorbitan monolaurate (tween 20) with pipe borne water, and rinsed five times using distill water, and the sterile seeds were placed in clean containers filled with a 20% solution of benlete, derived from a stock of hexaconozole 5%, a commercially available systemic fungicide, for 30 minutes. Afterward, the seeds were removed from the solution and washed again with distill water five times. They were immersed in 70% ethanol for a period ranging between 3 to 5 minutes, then rinsed with distill water five times, and the seeds were immersed in a 20% chlorox solution (commonly referred to as bleach), and subsequently washed with autoclaved distilled water (double distilled water). After sterilization, the seeds underwent an overnight soaking in sterile water. The embryos were carefully removed from the caryopsis using surgical knives and forceps under laminar air flow hood **[15]**.

Embryo Removal

Sterile seeds were transferred from the containers with the aid of a sterile forceps and placed on a sanitized petri dish, under laminar airflow conditions, the embryos were carefully excised using sterile surgical knives [16].

Inoculation of Embryo

The excised embryos were transferred using forceps sterilized with spirit burner into a sterile cultured media in a 500ml bottles. Five embryos of each genotype were contained in a bottle replicated three times in a treatment **[16]**.

Callusing

50ml of sterile culture media were introduced into 500ml bottles and five sterile embryos were inoculated on each replicate under laminar air flow hood, and were kept in a dark room for duration of three weeks, under a temperature of 25°C ±10°C for calli formation. Calli induction media (CIM) comprised Murashige and Skoog mineral salts **[17]**, fortified with 30g/L sucrose. Various levels of 2, 4-D (0, 1, 2, 3, 4, & 5mg/l) were incorporated as treatments, each comprising three bottles containing five embryos per genotype [15].

Proliferation of Callus

The genotypes demonstrating response in callogenesis were subsequently sub-cultured twice at the intervals of two weeks on fresh MS medium to facilitate their proliferation.

In Vitro Drought Tolerance Selection:

50mg clumps of the proliferated calli (about 7weeks old) of wheat genotypes were transferred to drought selection medium under sterile condition, which contained MS salts, the promising 5mg/L 2, 4-D in mg/L from callogenesis (similar to CIM) and polyethylene glycol (PEG) 6,000 of six levels of concentrations (0%, 5%, 10%, 15%, 20% and 25%)

were used including a control (0%) as treatment for osmoticum. The calli were incubated at 25±1°C in continuous darkness for a stress period of 4 weeks. However, to avoid deficiency of mineral components in selection media during stress period, the calli were shifted to fresh selection media after 15 days interval. Fifteen replicates each of the ten i.e. (genotype numbers 2, 6, 7, 8, 9, 21, 22, 23,27and 28,) genotypes from callus induction with 2, 4-D (in three 500ml bottles) were made for each of the five PEG treatments **[15]**.

Plant Regeneration under PEG Treatments:

The ten genotypes whose performance cut across the different levels of PEG were further transferred to media fortified with 30g/L sucrose, 8g/L agar and 2.0mg/L BAP (6-benzyloaminopurine) and 0.5mg/l kinetin, and incubated at 25±2°C under the photoperiod of 16 hours light and 8 hours darkness. The media were refreshed between every 15-21 days in four weeks, with greenish colorations as signs of regeneration. Data on days to callus shoot regeneration and Mean Numbers of Regenerated Shoots from Callus were recorded **[18]**.

Statistics

Data were analyzed using ANOVA and IBM SPSS Statistical Package, version 26 to determine the significance of treatment effects on callus shoot regeneration, followed by Pearson correlation to assess the relationships among genotypes. A p-value of < 0.05 was considered statistically significant.

Results and Discussion

Genotypes 8 had the shortest mean days (14 days) to regeneration at 0% PEG level, while the longest mean days (31 days) to regeneration was recorded with genotype 21 at 25% PEG level (Table 2). ANOVA was significantly different ($P > 0.05$) between treatments, while Pearson correlation coefficient was positively correlated among genotypes 5% & 0% and 25% & 20% PEG levels, though there was a decrease in the mean number of regenerations from 0% PEG level to 25% PEG level (Table 4). This work corroborates the findings of **[7]**, on *in vitro* Callus Induction Potentials of Wheat Genotypes using Matured Embryo as ex-plant source under different Levels of 2,4-Dichlorophenoxyacetic Acid (2,4-D), in which it was observed that the highest mean values for days to callus was at varieties 5 (6.60), with the least mean values for days to callus at genotype 8 (5.40) **[19]**, in the study of *in vitro* screening of durum wheat against water-stress mediated through polyethylene glycol, with the results showing all the regenerated plant genotypes, decreased significantly with increasing osmotic stress in selective media.

Table 2: Effect of varying Levels of Drought Stress (PEG) on Days to Callus Regeneration for 10 Wheat genotypes

Variety	0%	5%	10%	15%	20%	25%	Mean
2	17	21	23	27	28	30	19.3
6	18	22	24	27	29	29	20.0
7	17	22	24	25	28	29	19.3
8	16	20	22	26	27	28	18.5
9	18	21	24	26	28	30	19.5
21	20	22	24	27	30	31	20.5
22	19	22	25	27	29	30	20.3
23	21	23	25	27	27	29	20.8
27	20	23	23	25	28	29	19.8
28	20	21	14	27	28	29	19.8
Mean	11.62	13.56	14.87	16.50	17.62	18.37	

F= 4.560, d.f.= 9, (P>0.05)

Table 3: ANOVA of varying Concentrations of Drought Stress (PEG) on Days to Regeneration for 10 Wheat genotypes.

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	20.758	5	4.152	4.560	.083[b]
	Residual	3.642	4	.910		
	Total	24.400	9			

a. Dependent Variable: PEG 0%

Table 4: Correlation matrix of varying Concentrations of Drought Stress (PEG) on Days to Regeneration for 10 Wheat genotypes

		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%	PEG 25%
PEG 0%	Pearson Correlation	1					
	Sig. (2-tailed)						
	N	10					
PEG 5%	Pearson Correlation	.697*	1				
	Sig. (2-tailed)	.025					
	N	10	10				
PEG 10%	Pearson Correlation	-.121	.414	1			
	Sig. (2-tailed)	.738	.234				
	N	10	10	10			
PEG15%	Pearson Correlation	.288	-.111	-.131	1		
	Sig. (2-tailed)	.420	.760	.719			
	N	10	10	10	10		
PEG 20%	Pearson Correlation	.206	.204	.165	.315	1	
	Sig. (2-tailed)	.569	.572	.649	.375		
	N	10	10	10	10	10	
PEG 25%	Pearson Correlation	.288	.167	.278	.375	.746*	1
	Sig. (2-tailed)	.420	.645	.437	.286	.013	
	N	10	10	10	10	10	10

*. Correlation is significant at the 0.05 level (2-tailed).

The lowest total mean regeneration of (0.57) according to treatments was at 25% PEG stress level, while the highest total mean regeneration was (4.27) according to treatments was scored at 0% PEG level (Table 5). Also genotype 8 had the highest mean number (5.00) of callus regeneration recorded at 0% PEG level, while there were no callus regeneration in genotypes 21 and 22 at 25% PEG level (Table 5), with ANOVA showing significant difference ($P < 0.05$) between treatments, when $F = 25.922$ (Table 6) while Pearson correlation coefficient was positively correlated among genotypes at PEG levels 0% & 10%, 5%, 10% & 15% and 15%, 0% & 10 (Table 7).

Table 5: Effect of varying Levels of Drought Stress (PEG) on Mean Numbers of Regenerated Shoots from Callus of Wheat Genotypes

Variety	0%	5%	10%	15%	20%	25%	Means
2	4.7	4.0	3.0	2.7	1.7	1.0	8.7
6	4.0	3.0	2.3	2.3	1.0	0.6	6.7
7	4.7	4.0	3.3	2.7	2.0	1.0	8.8
8	5.0	4.7	3.7	3.0	2.3	1.7	10.2
9	4.0	2.7	2.3	2.0	1.0	0.3	6.2
21	3.7	2.7	2.3	1.3	0.3	0.0	5.2
22	4.0	2.7	2.0	1.3	0.7	0.0	5.3
23	4.0	3.0	2.7	2.7	0.7	0.3	6.3
27	4.3	3.0	3.0	1.7	1.0	0.3	6.7
28	4.3	3.7	3.0	2.7	1.3	0.7	7.8
Means	4.27	3.35	2.76	2.24	1.20	0.57	

$F = 25.922$, d.f. = 9, ($P<0.05$)

Table 6: ANOVA of varying Levels of Drought Stress (PEG) on Mean Numbers of Regenerated Shoots from Callus of 10 Wheat Genotypes.

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.475	5	.295	25.922	.004[b]
	Residual	.046	4	.011		
	Total	1.521	9			

a. Dependent Variable: PEG 0%

b. Predictors: (Constant), PEG 5%, PEG 10%, PEG 15%, PEG 20%, PEG 25%

Table 7: Correlation matrix of varying Levels of Drought Stress (PEG) on Mean Numbers of Regenerated Shoots from Callus of 10 Wheat Genotypes

		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%	PEG 25%
	Pearson Correlation	1		*			*
PEG 0%	Sig. (2-tailed)						
	N	10					
	Pearson Correlation	.946**	1	*	*		
PEG 5%	Sig. (2-tailed)	.000					
	N	10	10				
	Pearson Correlation	.895**	.905**	1			*
PEG 10%	Sig. (2-tailed)	.000	.000				
	N	10	10	10			
	Pearson Correlation	.716*	.800**	.748*	1		
PEG 15%	Sig. (2-tailed)	.020	.005	.013			.
	N	10	10	10	10		
	Pearson Correlation	.970**	.940**	.853**	.765**	1	
PEG 20%	Sig. (2-tailed)	.000	.000	.002	.010		
	N	10	10	10	10	10	
	Pearson Correlation	.921**	.964**	.858**	.826**	.953**	1
PEG 25%	Sig. (2-tailed)	.000	.000	.001	.003	.000	
	N	10	10	10	10	10	10

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Also the effect of PEG on percentage shoot regeneration showed the highest percentage (100%) at genotype 8 under 0% PEG level, while the lowest percentage regeneration of (0.00%) were witnessed at genotypes 21 and 22 under PEG level 25% respectively (Table 8), this result is in line the work of [20], titled the impact of PEG induced drought stress on seed germination and seedling growth of different bread wheat, with the highest decrease in seed germination percentage, root and shoot length, dry root weight, fresh root weight, fresh shoot weight, dry shoot weights and chlorophyll index recorded for -1.2 MPa osmotic potential compared to the control treatment of the study, whereas the lowest decrease was recorded for -0.6 MPa osmotic potential

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Table 8: Effect of varying levels of drought stress (PEG) on percentage shoot regeneration of wheat genotypes

Genotype	0%	5%	10%	15%	20%	25%
2	93.3	80	60	53.3	53.3	20
s6	80	60	46.7	46.7	20	13.3
7	93.3	80	66.7	53.3	40	20
8	100	93.3	73.3	60	46.7	33.3
9	80	53.3	46.7	40	20	6.7
21	73.3	53.3	46.7	26.7	6.7	0.0
22	80	53.3	40	26.7	13.3	0.0
23	80	60	53.3	40	13.3	6.7
27	86.7	60	60	33.3	20	6.7
28	86.7	73.3	60	53.3	26.7	13.3

In another development plates showed that the highest number of callus shoot regeneration in replicates A_1, A_2 and B_3 (Plate 1) were recorded at 0% and 5% PEG levels respectively, while the lowest number of shoot regeneration in replicate F_1 (Plate 2) was recorded at 25% PEG level. This result corroborates the work of **[16]**, in the work titled, *Invitro* screening of durum wheat against water stress mediated through polyethylene glycol, in which was reported that, embryogenic calli regenerated at a high frequency on the control medium

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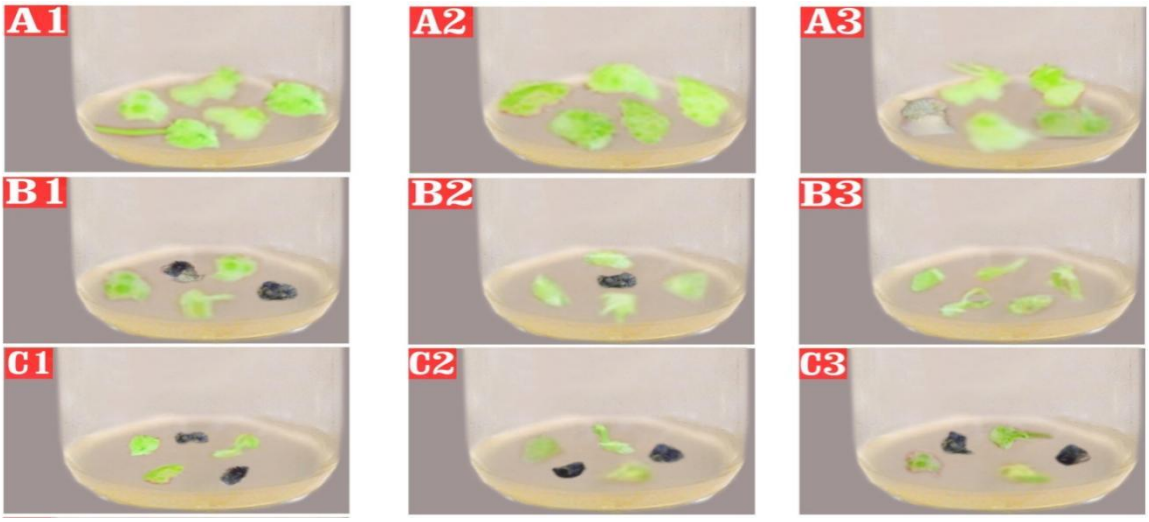

Plate1: Showing the effect of BAP, kinetin and varying Levels of Drought Stress (PEG) and 5mg/l 2, 4-Dichlorophynoxy acetic acid on shoot regeneration of genotype 2.

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Control 0% PEG = Number of Regenerated Shoot A_1: 5, A_2: 5 & A_3: 4
Number of non-Regenerated Shoots A_1: 0, A_2: 0 & A_3: 1
5% PEG = Number of Regenerated Shoot B1: 3, B_2: 4 & B_3:5
Number of non-Regenerated Shoots B_1: 2, B_2: 1 & B_3: 0
10% PEG = Number of Regenerated Shoot C1: 3, C_2: 3 & C_3:3
Number of non-Regenerated Shoots C_1: 2, C_2: 2 & C_3: 2

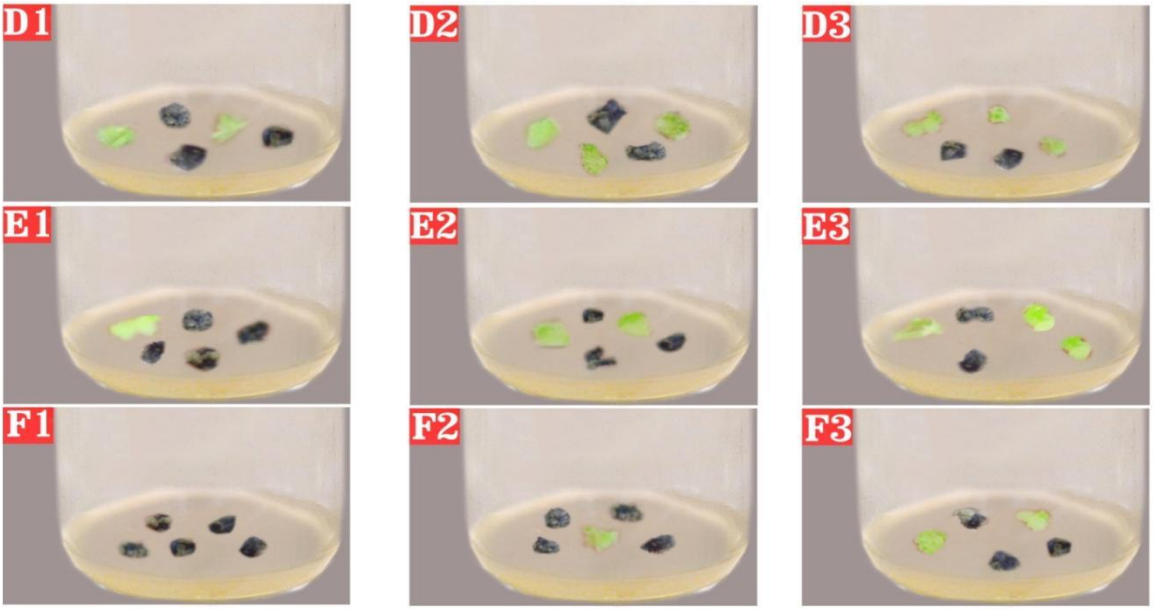

Plate2: Showing effect of BAP, kinetin and varying Levels of Drought Stress (PEG) and 5mg/l 2, 4-Dichlorophynoxy acetic acid on shoot regeneration of genotype 2.

15% PEG = Number of Regenerated Shoot D_1: 2, D_2: 3 & D_3:3
Number of non-Regenerated Shoots D_1: 3, D_2: 2 & D_3: 2
20% PEG = Number of Regenerated Shoot E_1: 1, E_2: 2 & E_3:3
Number of non-Regenerated Shoots E_1: 4, E_2: 3 & E_3: 2
25% PEG = Number of Regenerated Shoot F1: 0, F_2: 1 & F_3:2
Number of non-Regenerated Shoots F_1: 5, F_2: 4 & F_3: 3

Conclusion

After subjecting the 10 genotypes callus clumps to varying concentrations of polyethylene glycol, and shoot regenerated using six levels of drought stress including control (0%, 5%, 10%, 15%, 20% and 25% PEG levels) fortified with BAP, Kinetin and 5mg/l 2, 4-Dichlorophenoxy Acetic Acid for the period of four weeks, with each treatment replicated three times, the data on days to regeneration, mean number of shoot regeneration and percentage regeneration recorded showed a significant difference between treatments (Table 2 and 5), with genotype 28 (Table 2) having the lowest mean days to regenerating (14 days), While genotype 8 had the highest potential for regeneration under stress condition (Table 5), also the highest percentage regeneration (100%) was recorded in genotype 8 (Table 8). Based on the foregoing result it could be concluded that genotype 8 has the potential to withstand stress and should be recommend if such situation arise.

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