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Microbial alleviation of PEG-induced moisture stress in wheat: Genotypic responses of germination and early seedling traits

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Abstract
Moisture stress during early seedling growth severely limits wheat establishment and productivity, particularly under rainfed and drought-prone environments. The present investigation evaluated the effect of polyethylene glycol (PEG)-induced moisture stress and microbial treatment on germination and seedling growth traits in twenty wheat genotypes under controlled conditions. Moisture stress was simulated using PEG at 10% and 15% concentrations, while microbial treatment was applied to assess its potential in mitigating stress effects. Germination percentage, shoot length, and root length were recorded to assess seedling performance under control, stress, and microbe-treated conditions. Results revealed a progressive decline in germination and seedling growth with increasing PEG concentration, confirming the inhibitory impact of osmotic stress. However, microbial treatment significantly improved all measured traits under both stress levels, indicating enhanced seedling vigor and tolerance to moisture stress. Root growth showed comparatively greater resilience under microbial inoculation, suggesting improved water acquisition and adaptive capacity under stress conditions. Genotypic differences were evident, highlighting the presence of genetic variability for moisture stress tolerance at the seedling stage. Overall, the study demonstrated that microbial inoculation effectively alleviates PEG-induced moisture stress and can serve as a sustainable strategy to enhance early seedling establishment and drought resilience in wheat, with promising implications for stress-resilient breeding and crop management practices.

Keywords: Wheat, moisture stress, polyethylene glycol, microbial inoculation

Introduction
Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide and serves as a primary source of calories and protein for a large proportion of the global population. In India and other semi-arid regions, wheat productivity is increasingly constrained by moisture stress due to erratic rainfall patterns and climate change. Frequent drought episodes during crop establishment stages adversely affect seed germination, seedling vigor, and plant stand, ultimately leading to significant yield losses. Among different growth stages, germination and early seedling establishment are highly sensitive to water deficit, and poor seedling performance under drought conditions often results in weak crop establishment and reduced productivity (Farooq *et al.*, 2009; Passioura, 2012) [2, 9].

Polyethylene glycol (PEG) is widely used to simulate moisture stress under controlled conditions, as it induces osmotic stress without causing ionic toxicity and effectively mimics drought-like environments. PEG creates a low water potential in the growth medium, thereby restricting water uptake by seeds and seedlings. PEG-induced stress has been reported to delay germination, reduce germination percentage, and suppress seedling growth by limiting cell elongation, enzyme activation, and metabolic processes. Several studies have demonstrated that increasing PEG concentrations significantly reduce shoot and root growth in wheat and other cereals, making PEG a reliable tool for screening genotypes for drought tolerance at early growth stages (Michel and Kaufmann, 1973; Mwale *et al.*, 2003; Khakwani *et al.*, 2011) [5, 6, 3].

In recent years, the use of beneficial microorganisms has emerged as a sustainable and eco-friendly approach to enhance plant tolerance to abiotic stresses, including drought. Plant growth-promoting microorganisms improve plant performance under moisture stress through multiple mechanisms such as enhanced nutrient solubilization, production of phytohormones like auxins and gibberellins, modulation of ethylene levels, improvement of antioxidant activity, and stimulation of root system development. Microbial inoculation has been shown to improve seed germination, seedling vigor, biomass accumulation, and root architecture under water-limited conditions, thereby enhancing drought adaptation in crops (Nadeem *et al.*, 2014; Vurukonda *et al.*, 2016; Kumar *et al.*, 2020) [7, 10, 4].

The interaction between plant genotype and microbial inoculants plays a critical role in determining the extent of stress tolerance. Wheat genotypes differ in their inherent drought tolerance as well as in their responsiveness to microbial associations, resulting in variable performance under moisture stress. Understanding genotype-specific responses under PEG-induced stress with and without microbial treatment provides valuable insight into the combined role of genetic potential and microbial intervention in improving early-stage drought tolerance (Ngumbi and Kloepper, 2016; Dimkpa *et al.*, 2009) [8, 1].

Therefore, screening wheat genotypes at the seedling stage under simulated moisture stress, coupled with microbial application, offers an efficient strategy for identifying drought-resilient genotypes. In this context, the present study was undertaken to evaluate the effect of PEG-induced moisture stress and microbial treatment on germination percentage and early seedling growth traits in twenty wheat genotypes, with the objective of identifying genotypes exhibiting superior seedling performance and assessing the potential of microbial inoculation as a sustainable approach to improve early seedling establishment under moisture stress conditions.

Experimental material

The experimental material consisted of twenty wheat genotypes, including both bread and durum wheat types (Table 1). Actinobacteria isolates (AUDT 545 and AUDT 862) were obtained from the Department of Microbiology, UAS, Dharwad. The wheat seeds were surface-sterilized using 0.1% sodium hypochlorite solution for 10 minutes, followed by 2–3 washings with sterile water. The genotypes were screened under moisture stress using polyethylene glycol (PEG-6000). This screening was conducted to evaluate the effect of different PEG concentrations (0%, 10% and 15%) on seed germination and seedling growth under two conditions: microbe-treated seeds inoculated with Actinobacteria and microbe-untreated seeds without inoculation. Observations were recorded after seven days of incubation for seed germination percentage, shoot length, and root length. The analysis of variance for germination per cent, shoot length and root length under a completely randomized design (CRD) showed significant differences among the genotypes across treatments (Table 2) (Table 3) (Table 4).

The present investigation was carried out under laboratory conditions to evaluate the effect of PEG-induced moisture stress and microbial treatment on germination and early seedling traits in wheat (*Triticum aestivum* L.). The experimental material consisted of twenty genetically diverse wheat genotypes, and healthy, uniform seeds were selected to ensure consistency in germination and seedling growth. Moisture stress was simulated using polyethylene glycol (PEG 6000), which creates osmotic stress by reducing water potential without entering plant tissues. Three moisture regimes were imposed, comprising a control (distilled water), PEG 10%, and PEG 15%, with PEG solutions freshly prepared using distilled water.

Germination was recorded daily, and final germination percentage was calculated based on the number of seeds that produced normal seedlings. After a specified growth period, randomly selected seedlings from each treatment were used for recording seedling traits. Shoot length was measured from the base of the seedling to the tip of the longest leaf, while root length was measured from the base to the tip of the longest root, and the observations were expressed in centimeters. The recorded data were subjected to analysis of variance appropriate for a factorial completely randomized design to determine the significance of genotypic effects,

Table 1: List of twenty durum and bread wheat genotypes used for study

G1	UAS 446
G2	Amruth
G3	DWR-2006
G4	AKDW-2997-16
G5	Bijaga yellow
G6	GDP 40
G7	UAS-428
G8	UAS-347
G9	UAS-375
G10	HD-2888
G11	UAS-334
G12	UAS-3020
G13	UASBW 11421
G14	UASBW 12982
G15	UASBW 13039
G16	UASBW 12380
G17	UASDW 30805
G18	UASDW 31156
G19	UASDW 30820
G20	UASDW31138

Table 3: Estimates of ANOVA for germination response of wheat genotypes under PEG induced stress and microbial interventions

Source of Variation	DF	Mean Sum of Square (Germination%)					
		Control	PEG 10%	PEG 15%	Microbe	M+10%	M+15%
Treatment	19.00	2.34*	15.61*	45.29*	0.94*	4.81*	55.39**
Error	20.00	0.95	5.25	8.85	0.43	2.05	14.43
CV		1.01	2.49	3.44	0.66	1.49	4.08

Table 4: Estimates of ANOVA for shoot growth response of wheat genotypes to PEG induced stress and microbial interventions

Source of Variation	DF	Mean Sum of Square (Shoot Length)					
		Control	PEG 10%	PEG 15%	Microbe	M+10%	M+15%
Treatment	19.00	15.96**	15.10**	15.91**	26.48**	22.44**	21.30**
Error	20.00	0.69	0.46	0.19	0.53	0.53	0.30
CV		4.24	4.20	3.56	3.41	3.85	3.25

Table 5: Estimates of ANOVA for root growth response of wheat genotypes to PEG induced stress and microbial interventions

Source of Variation	DF	Mean Sum of Square (Root Length)					
		Control	PEG 10%	PEG 15%	Microbe	M+10%	M+15%
Treatment	19.00	7.23**	13.70**	17.90**	9.71**	10.56**	14.14**
Error	20.00	0.54	0.31	0.31	0.47	0.71	0.62
CV		3.64	3.19	3.99	3.06	4.08	4.15

Abbreviation- PEG- Polyethylene Glycol

moisture stress levels, microbial treatment, and their interactions. Mean comparisons were performed using standard statistical procedures to ensure accurate interpretation of the results.

Results and Discussion

Moisture stress imposed through polyethylene glycol (PEG) significantly influenced seed germination across all twenty wheat genotypes. Under non-stress (control) conditions, germination percentage was high, with an overall mean of 96.75%, indicating good seed viability and uniform germination potential. However, increasing PEG concentration resulted in a consistent decline in germination. The mean germination decreased to 91.90% at PEG 10% and further to 86.40% at PEG 15%, confirming the inhibitory effect of osmotic stress on water uptake and metabolic activation during germination. The minimum germination (68.5%) was recorded in UASDW-31156 at PEG 15%, highlighting the severe impact of high moisture stress on sensitive genotypes.

Microbial treatment markedly alleviated the adverse effects of PEG stress across genotypes. The overall mean germination percentage increased from 91.68% (non-microbial mean) to 96.15% under microbial treatment. Under control conditions, microbial inoculation improved germination from 96.75% to 99.13%, while under PEG stress, germination increased from 91.90% to 96.30% at PEG 10% and from 86.40% to 93.03% at PEG 15%. This clearly demonstrates the protective role of microbial inoculation in sustaining germination under moisture-limited conditions. Several genotypes showed consistently superior germination under microbial treatment. Bijaga yellow recorded the highest mean germination (98.67%), followed by UAS 446 (98.0%), Amruth (97.0%), AKDW-2997-16 (97.67%), and UAS-428 (97.67%). The maximum germination of 100% was observed in Amruth and AKDW-2997-16 under microbial control conditions, whereas the lowest microbial mean germination (87.5%) was recorded in UASDW-31156, indicating genotype-specific variability in microbial responsiveness.

The enhancement of germination due to microbial inoculation may be attributed to improved water relations, synthesis of growth-promoting substances, enhanced osmotic adjustment, and activation of antioxidant defense mechanisms, which collectively support early seed metabolic processes under stress. These findings corroborate earlier reports that beneficial microbes mitigate drought stress during germination by improving seed vigor and physiological resilience (Vurukonda *et al*., 2016; Nadeem *et al*., 2014) [10, 7] (Table 6).

Table 6: Effect of PEG induced stress and microbial application on germination percentage of twenty wheat genotypes

Sl. No.	Genotypes	Germination Per cent							
		Control	PEG 10%	PEG 15%	Mean	Microbe	M+PEG 10%	M+PEG 15%	Mean
1	UAS 446 (c)	97.50	95.00	90.00	94.17	99.50	98.50	96.00	98.00
2	Amruth (c)	96.00	90.50	89.00	91.83	100.00	96.00	95.00	97.00
3	DWR-2006 (c)	96.00	92.00	90.00	92.67	99.50	96.00	95.00	96.83
4	AKDW-2997-16 (c)	97.50	95.00	90.00	94.17	100.00	97.00	96.00	97.67
5	Bijaga yellow (c)	98.50	95.00	88.50	94.00	100.00	98.50	97.50	98.67
6	GDP 40	95.60	90.00	86.50	90.67	98.50	97.00	96.00	97.17
7	UAS-428 (sc)	96.00	95.00	89.00	93.33	99.50	97.50	96.00	97.67
8	UAS-347 (c)	96.00	91.00	84.50	90.50	98.50	96.50	92.50	95.83
9	UAS-375 (c)	96.00	89.00	84.50	89.83	98.50	96.00	93.50	96.00
10	HD-2888 (c)	97.50	88.50	86.50	90.83	100.00	95.60	94.00	96.50
11	UAS-334 (sc)	97.50	95.00	86.00	92.83	99.00	97.00	91.00	95.67
12	UAS-3020	98.50	95.00	85.00	92.83	99.50	97.50	94.00	97.00
13	UASBW 11421	98.50	90.00	88.50	92.33	99.50	95.60	93.00	96.00
14	UASBW 12982	95.00	90.00	88.50	91.17	99.50	96.00	93.50	96.33
15	UASBW 13039	96.00	90.00	87.50	91.17	98.50	96.50	93.50	96.17
16	UASBW 12380	96.00	89.00	87.50	90.83	98.50	94.50	93.00	95.33
17	UASDW 30805	96.00	95.00	90.00	93.67	97.50	98.00	96.00	97.17
18	UASDW 31156	97.50	87.00	68.50	84.33	98.50	92.00	72.00	87.50
19	UASDW 30820	97.50	91.00	82.00	90.17	99.00	94.00	91.00	94.67
20	UASDW31138	96.00	95.00	86.00	92.33	99.00	96.50	92.00	95.83
	Mean	96.75	91.90	86.40	91.68	99.13	96.30	93.03	96.15
	Min	95.00	87.00	68.50	84.33	97.50	92.00	72.00	87.50
	Max	98.50	95.00	90.00	94.17	100.00	98.50	97.50	98.67

Abbreviation- PEG- Polyethylene Glycol.

Influence of PEG stress and microbial inoculation on shoot length

Shoot length decreased progressively with increasing PEG concentration across all genotypes, indicating high sensitivity of early seedling growth to moisture stress. The overall mean shoot length under control conditions was 19.51 cm, which declined to 16.19 cm at PEG 10% and further to 12.38 cm at PEG 15%, representing a substantial reduction under severe osmotic stress. This reduction can be attributed to PEG-induced osmotic stress that restricts cell elongation, reduces turgor pressure, and suppresses photosynthetic efficiency, ultimately limiting shoot growth. The pronounced reduction at PEG 15% confirms that shoot growth is highly vulnerable to moisture stress during early seedling stages.

Microbial inoculation significantly enhanced shoot length under both stress and non-stress conditions. Across genotypes, the mean shoot length increased from 16.03 cm (non-microbial mean) to 19.01 cm under microbial treatment. Under control conditions, microbial inoculation increased shoot length from 19.51 cm to 21.29 cm, while under PEG stress, shoot length improved from 16.19 cm to 18.82 cm at PEG 10% and from 12.38 cm to 16.91 cm at PEG 15%. This clearly demonstrates the protective role of microbial inoculation in mitigating drought-induced growth inhibition.

Among the genotypes, Amruth (25.67 cm), HD-2888 (23.86 cm), UAS-375 (23.78 cm), and Bijaga yellow (23.52 cm) recorded the highest mean shoot length under microbial treatment, indicating strong genotype-specific responsiveness. The maximum shoot length (28.0 cm) was observed in Amruth under microbial control conditions, while the minimum shoot length (9.0 cm) occurred under PEG 15% without microbial inoculation.The improvement in shoot growth due to microbial inoculation may be attributed to microbial production of phytohormones (auxins and gibberellins), enhanced nutrient mobilization, improved root–shoot signaling, and reduction of stress-induced ethylene levels. Similar enhancement of shoot elongation and biomass accumulation under drought stress due to microbial inoculants has been reported earlier (Ngumbi and Kloepper, 2016; Kumar *et al*., 2020) [8, 4] (Table 7).

Table 7: Effect of PEG induced osmotic stress and microbial application on shoot length of twenty wheat genotypes

Sl. No.	Genotypes	Shoot length							
		Control	**PEG 10%**	**PEG 15%**	**Mean**	**Microbe**	**M+PEG 10%**	**M+PEG 15%**	**Mean**
1	UAS 446 (c)	18.83	17.67	10.00	15.60	19.50	17.80	17.00	18.10
2	Amruth (c)	24.17	22.83	20.67	22.56	28.00	25.00	24.00	25.67
3	DWR-2006 (c)	24.17	12.22	10.05	15.48	25.60	17.67	16.17	19.81
4	AKDW-2997-16 (c)	17.83	15.67	10.67	14.72	18.17	17.33	15.33	16.94
5	Bijaga yellow (c)	20.23	18.00	15.60	17.91	25.00	24.73	20.83	23.52
6	GDP 40	17.80	14.90	9.00	13.90	18.00	15.00	12.73	15.24
7	UAS-428 (sc)	21.00	13.77	11.33	15.37	22.17	18.33	15.67	18.72
8	UAS-347 (c)	18.50	17.33	11.33	15.72	21.00	19.83	16.23	19.02
9	UAS-375 (c)	23.20	20.50	16.20	19.97	25.00	24.33	22.00	23.78
10	HD-2888 (c)	22.67	19.50	14.67	18.95	25.67	23.90	22.00	23.86
11	UAS-334 (sc)	17.80	13.20	9.40	13.47	25.80	19.20	15.20	20.07
12	UAS-3020	18.00	14.60	12.10	14.90	18.80	16.00	14.60	16.47
13	UASBW 11421	16.40	15.08	12.30	14.59	17.00	16.42	14.00	15.81
14	UASBW 12982	17.83	16.50	11.00	15.11	18.40	17.33	15.69	17.11
15	UASBW 13039	24.33	18.40	14.67	19.13	24.97	21.33	20.83	22.38
16	UASBW 12380	19.71	14.43	12.28	15.47	20.29	18.17	16.67	18.38
17	UASDW 30805	16.80	15.20	12.40	14.80	17.60	16.33	14.60	16.18
18	UASDW 31156	15.40	12.20	9.60	12.40	16.20	13.50	12.20	13.97
19	UASDW 30820	19.00	16.83	11.33	15.72	20.30	17.33	16.16	17.93
20	UASDW31138	16.47	15.03	13.00	14.83	18.40	16.83	16.39	17.21
	Mean	19.51	16.19	12.38	16.03	21.29	18.82	16.91	19.01
	Min	15.40	12.20	9.00	12.40	16.20	13.50	12.20	13.97
	Max	24.33	22.83	20.67	22.56	28.00	25.00	24.00	25.67

Abbreviation- PEG- Polyethylene Glycol

Response of root length to PEG stress and microbial treatment

Root length exhibited comparatively greater tolerance to PEG-induced moisture stress than shoot length, although a clear declining trend was observed with increasing PEG concentration. The overall mean root length under control conditions was 20.21 cm, which decreased to 17.46 cm at PEG 10% and further to 13.90 cm at PEG 15%. This reduction indicates that while roots are relatively more resilient to osmotic stress, severe moisture deficit still restricted root elongation. The minimum root length of 7.42 cm was recorded under PEG 15% without microbial treatment, whereas the maximum of 23.2 cm occurred under control conditions.

Microbial inoculation significantly enhanced root length across genotypes under both stress and non-stress conditions. The mean root length increased from 17.19 cm (non-microbial mean) to 20.67 cm with microbial treatment. Under control conditions, microbial inoculation increased mean root length from 20.21 cm to 22.47 cm, while under PEG stress, root length improved from 17.46 cm to 20.58 cm at PEG 10% and from 13.90 cm to 18.96 cm at PEG 15%, demonstrating the strong ameliorative effect of microbial inoculation under moisture stress.

Among the genotypes, UAS-375 recorded the highest mean root length under microbial treatment (26.26 cm), followed by Amruth (22.54 cm), HD-2888 (22.24 cm), and UASDW-30805 (21.58 cm). Notably, UAS-375 exhibited the maximum root length of 28.0 cm under microbial control conditions, highlighting its superior root growth potential and strong responsiveness to microbial inoculation even under stress. In contrast, UASDW-31156 showed the lowest mean root length (16.52 cm) under microbial treatment, indicating genotype-specific variation in stress tolerance and microbial response.

Enhanced root growth under microbial inoculation is particularly advantageous under drought conditions, as it facilitates deeper soil exploration and improved water acquisition. The beneficial effects of microbes on root elongation may be attributed to improved hormonal balance (auxins and cytokinins), stimulation of root cell division, enhanced nutrient availability, and increased accumulation of osmoprotectants. These findings are in agreement with earlier reports demonstrating improved root architecture and drought tolerance in crops inoculated with beneficial microbes under water-limited environments (Dimkpa *et al*., 2009; Vurukonda *et al*., 2016) [1, 10].

Overall, the combined results clearly demonstrate that PEG-induced moisture stress adversely affected seedling growth traits in wheat, with severity increasing at higher PEG concentrations. However, microbial treatment consistently mitigated the negative effects of moisture stress by significantly improving shoot length and root length across genotypes. The magnitude of response varied among genotypes, indicating differential genetic potential and microbial responsiveness. Genotypes exhibiting stable and superior performance under microbial treatment, particularly UAS-375, Amruth, HD-2888, and UASDW-30805, may be exploited in breeding programs aimed at enhancing early seedling establishment and drought resilience in wheat (Table 8).

Table 8: Effect of PEG induced osmotic stress and microbial application on root length of twenty wheat genotypes

Sl. No.	Genotypes	Root length							
		Control	PEG 10%	PEG 15%	Mean	Microbe	M+PEG 10%	M+PEG 15%	Mean
1	UAS 446 (c)	19.50	18.58	13.50	17.19	23.33	20.80	22.33	22.15
2	Amruth (c)	22.17	19.67	18.50	20.11	23.67	21.17	22.79	22.54
3	DWR-2006 (c)	20.00	13.42	7.42	13.61	20.80	16.50	17.08	18.13
4	AKDW-2997-16 (c)	20.33	17.67	15.00	17.67	21.33	20.17	16.50	19.33
5	Bijaga yellow (c)	17.72	17.63	16.57	17.31	19.50	18.50	17.93	18.64
6	GDP 40	16.00	15.43	11.00	14.14	20.00	19.00	16.68	18.56
7	UAS-428 (sc)	20.00	16.33	14.33	16.89	24.00	20.50	19.82	21.44
8	UAS-347 (c)	21.00	18.33	14.00	17.78	23.67	18.87	15.77	19.44
9	UAS-375 (c)	22.50	21.20	17.50	20.40	28.00	26.79	24.00	26.26
10	HD-2888 (c)	21.33	17.57	13.22	17.37	23.42	22.63	20.67	22.24
11	UAS-334 (sc)	20.00	17.00	12.40	16.47	20.70	19.40	15.40	18.50
12	UAS-3020	23.20	15.18	12.70	17.03	23.62	23.20	20.72	22.51
13	UASBW 11421	19.82	18.80	16.20	18.27	20.80	19.67	18.00	19.49
14	UASBW 12982	19.83	19.50	10.00	16.44	23.00	20.18	19.34	20.84
15	UASBW 13039	21.52	19.50	13.80	18.27	23.50	21.83	16.00	20.44
16	UASBW 12380	22.83	17.17	15.67	18.56	24.00	21.50	17.83	21.11
17	UASDW 30805	20.80	20.23	18.27	19.77	23.15	20.93	20.67	21.58
18	UASDW 31156	16.83	10.90	9.77	12.50	17.65	16.60	15.30	16.52
19	UASDW 30820	18.33	14.33	11.50	14.72	23.40	22.17	21.17	22.25
20	UASDW31138	20.40	20.83	16.57	19.27	21.92	21.25	21.24	21.47
	Mean	20.21	17.46	13.90	17.19	22.47	20.58	18.96	20.67
	Min	16.00	10.90	7.42	12.50	17.65	16.50	15.30	16.52
	Max	23.20	21.20	18.50	20.40	28.00	26.79	24.00	26.26

Abbreviation- PEG- Polyethylene Glycol

Conclusion

The study conclusively revealed that PEG-induced moisture stress caused a marked reduction in germination percentage, shoot length, and root length in wheat seedlings, with greater suppression observed at higher PEG concentrations, indicating the sensitivity of early seedling traits to osmotic stress; however, microbial treatment effectively alleviated these adverse effects by significantly improving germination and promoting better shoot and root growth across genotypes under both moderate and severe stress conditions, reflecting enhanced seedling vigor and stress tolerance, particularly through improved root development for efficient water uptake; the differential response among genotypes further confirmed the presence of genetic variability for moisture stress tolerance, and overall, the findings emphasize the potential of microbial inoculation as a sustainable and eco-friendly approach to enhance early-stage drought resilience in wheat, while genotypes showing consistent performance under microbial treatment may serve as valuable resources for breeding and deployment in moisture-stressed environments.

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