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Short Communication

Genetic Characterization of Wheat (*Triticum aestivum* L.) Germplasm for Moisture Stress Tolerance Using *in-vivo* and *in-vitro* Techniques

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Moisture stress is one of the most critical environmental factors limiting wheat (*Triticum aestivum* L.) productivity worldwide, particularly in semi-arid regions where drought during the grain filling stage drastically reduces yield stability. The increasing frequency and intensity of drought due to climate change (Khaled *et al.,* 2015; Altaf *et al.,* 2021) necessitate the development of drought-tolerant wheat varieties with improved adaptability and yield potential. Breeding for drought tolerance requires identification of reliable morpho-physiological and seedling traits that correlate with yield under stress, as selection based solely on yield is often confounded by high genotype × environment interaction (Dhanda *et al.,* 2004). *In-vitro* screening offers a rapid, controlled, and reproducible method for identifying drought-tolerant genotypes by simulating moisture deficit through polyethylene glycol (PEG-6000)-induced osmotic stress, which mimics field drought conditions (Meneses *et al.,* 2011). Considering these aspects, the study was undertaken during Rabi 2023–24 at the Rice and Wheat Research Centre, Malan, to genetically characterize wheat germplasm for moisture stress tolerance under both field and laboratory conditions, to evaluate the relative efficiency of in vivo and *in-vitro* screening methods, and to identify superior genotypes and selection traits associated with drought tolerance. It was hypothesized that significant genetic variability exists among wheat genotypes for drought tolerance, that *in-vitro* PEG-induced screening can effectively predict field performance, and that specific morphological and seedling traits such as germination percentage, coleoptile length, seedling vigor index, and yield-contributing traits can serve as reliable selection criteria. The experimental material comprised forty wheat genotypes representing diverse genetic backgrounds including landraces, mutant lines, recombinant inbred lines, and released cultivars (Table 1). Field experiments were conducted under rainfed (E1) and irrigated (E2) conditions in α-design with three replications, while laboratory studies were carried out using PEG-6000 at four osmotic stress levels (C1: control, C2: -3 bar, C3: -6 bar, C4: -9 bar). Data were recorded for agronomic, morphological, and seedling parameters, and analyzed for variability, heritability, genetic advance, correlation, path analysis, and rank correlation to compare the in vivo and *in-vitro* performance. Under field conditions, the experiment was conducted in a randomized block design (RBD) with three replications in two contrasting environments: rainfed (E1) and irrigated (E2).

The analysis of variance revealed that the mean squares due to genotypes were significant for all studied traits under both the environments (Table 2), indicating the presence of considerable genetic variability among the genotypes for traits related to yield and drought tolerance. This variability provides a strong basis for effective selection in the breeding program. The mean performance

Table 1: List of experimental wheat genotypes used in the study

S. No.	Genotype	Pedigree
1.	Black Wheat	28H129/ 2*PBW621
2.	Blue Wheat	28H129/ 2*PBW621
3.	Purple wheat	28H129/ 2*PBW621
4.	CL 5	-
5.	DBW 187	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/KACHU/6/KACHU
6.	Dharwad Dry	DWR-163/DHARWAD DRY//DWR225
7.	HPW 89	INTERMEDIORODS/HD2248
8.	HPW 349	OASIS/SKUAZ114*BCN/3/PASTOR/4/KAUZ*2/YACO//KAUZCMSS98Y01808T-040M-0100M-040Y-040M-030Y-1M-2Y-0M
9.	HPW 373	MILAN//BUC/CHRC/3/METSO
10.	HPW 481	IC557720 ×HPW 184-P_2
11.	HPW 484	PBW 677/GW322//BAJ#1
12.	HPW 489	HPW 386/VL973
13.	HPW 490	HPW 386/PW 1046
14.	HPW 491	VL 829/FLW4
15.	HPW 492	HPW296/HPW 381
16.	HPW 493	TAW142/HPW249
17.	HPW 494	HS490/HPW249
18.	HS 490	HS 364/HPW114//HS240//HS 346
19.	Kanku	Local landrace
20.	PBW 725	PBW621/GLUPRO/3*PBW568/3/PBW621
21.	PBW 752	PBW621/4/PBW343//YR10/6*AVOCET/3/3*PBW343/5/PBW621
23.	PBW 872	MUTUS*2/MUU//2*MUCUY
24.	PW 2201	HPW 249 × VL 925
25.	RIL 45	HPW 360× VL 892
26.	RIL 174	HPW 360× VL 892
27.	RIL 175	HPW 360× VL 892
28.	Sharbati	Local landrace
29.	SKUAW 102	Selection from HS634
30.	SKW 368	HD3070/DPW621-50
31.	TYRM 2	Mutant of HD2967
32.	VL 2053	VW 0956/ MONARCHA* 2/MV 17
33.	VL 2054	VL 2012/ VORONA/ HD2402/3/TRICHMIR/ ICO//SABALAN
34.	VL 3031	VL 930/DBW 50// RSK/CA8055//CHAM6/ 4/NWT/3/TAST/SPRW//TAW 12399.75
35.	WW 9912	-
36.	WW 9934	-
37.	HPW 368 (C)	NAC/THAC//3*PVN/3/MIRLO/BUC/4/2/*PASTOR
38.	HS 507 (C)	KAUZ/MYNA/VUL/BUC/FLK/4/MILAN
39.	HS 562 (C)	OASIS/SKUAZ//4*BCN/3/2*PASTOR
40.	VL 2041 (C)	NESSER/SAULSKU32/MACS6240//HS507
41.	VL 892 (C)	WH542/PBW226

of genotypes for various traits exhibited a broad range of variation under both rainfed and irrigated conditions. The significant variability observed among genotypes for agronomic, physiological, and seedling traits confirms the hypothesis that substantial genetic diversity exists for drought tolerance in wheat germplasm, which is consistent with earlier and recent reports (Altaf *et al.,* 2021; Sallam *et al.,* 2022). For days to 50% flowering, the genotypes took 93 to 114 days under E1 and 109 to 123 days under E2, suggesting that moisture stress in rainfed conditions shortened the phenological period. Early flowering genotypes such as SKUAW 102 and RIL 45 were found suitable under stress conditions, as early flowering often allows the crop to escape terminal drought, whereas HPW 492 was among the late flowering genotypes. Similarly, for days to 75% maturity, the range varied from 136 to 155 days under rainfed and 153 to 168 days under irrigated conditions, with Kanku and SKUAW 102 identified as early maturing, and PBW 725 as a late maturing genotype. These results suggest that phenological plasticity plays an important role in drought adaptation, as early maturity under limited moisture enables plants to complete their life cycle before severe stress sets in. Similar findings have been reported by Blum (2018) and Bhatta *et al.* (2020), who emphasized that early phenology enables wheat plants to avoid terminal drought stress, particularly in rainfed systems. Although days to flowering and maturity exhibited high heritability, the moderate genetic advance indicates the influence of environmental modulation and non-additive gene effects, a trend also observed by Mwadzingeni *et al.* (2016) and ElBasyoni *et al.* (2018).

Table 2: Analysis of variance for different traits under rainfed (E1) and irrigated (E2) conditions in wheat

Characters	Replications Mean squares in E1 (Rainfed)	Blocks within replication	Genotypes	Error	Replications Mean squares in E2 (Irrigated)	Blocks within replication	Genotypes	Error
df	**2**	**12**	**39**	**66**	**2**	**12**	**39**	**66**
Days to 50% flowering	11.32	0.60	26.84*	4.71	11.39	5.40	62.67*	1.50
Days to 75% maturity	3.39	4.19	44.46*	5.95	0.21	4.94	85.57*	4.11
Plant height	27.95	20.05	112.40*	10.00	79.00	16.66	116.14*	10.00
Peduncle length	8.67	1.39	15.59*	1.83	0.65	0.17	21.16*	0.21
Biological yield/plant	0.01	0.21	3.74*	0.29	36.01	1.66	14.86*	1.00
Grain yield/ plant	0.04	0.17	0.85*	0.20	6.23	0.20	2.25*	0.11
1000-grain weight	0.44	0.79	26.39*	1.16	13.28	1.50	48.54*	1.72
Harvest index	2.25	12.55	56.38*	14.98	2.18	0.95	87.55*	3.71

* Significant at $P \leq 0.05$; E2: Irrigated; E1: Rainfed

Plant height varied between 75 to 110 cm under rainfed and 75 to 117 cm under irrigated conditions (Table 3). Drought stress caused a reduction in plant height in most genotypes due to decreased cell expansion and elongation under water deficit conditions. Among the genotypes, Kanku and PBW 782 recorded greater plant height, whereas the shorter ones were generally more stress adapted. Shorter genotypes generally exhibited better stress adaptation, corroborating earlier observations that reduced stature minimizes transpiration demand and lodging risk under drought (Khakwani *et al.,* 2019).

Peduncle length is an important trait that contributes to efficient translocation of assimilates to developing grains during grain filling, especially under water stress. The peduncle length ranged from 9.33 to 21 cm under E1 and 9.5 to 22 cm under E2. The genotypes HPW 349, DBW 187, and Blue wheat exhibited higher peduncle length and may possess superior translocation efficiency under drought conditions. The heritability for this trait was high (98% and 85% under E1 and E2, respectively) with moderate to high genetic advance, indicating that additive gene effects govern its expression, and hence, selection for this trait could be effective. These results matched with the findings of Ehdaie *et al.* (2016) and Ayalew *et al.* (2020), who reported that peduncle reserves significantly contribute to grain yield under drought conditions.

The biological yield per plant varied from 8 to 13.74 g under rainfed and 9 to 20 g under irrigated conditions, while grain yield per plant ranged between 2.22 and 5.8 g under E1 and 3.08 to 7.9 g under E2 (Table 3). As expected, grain yield and biological yield were significantly reduced under stress conditions; however, certain genotypes such as VL 2053, HPW 89, HPW 494, and WW 9912 maintained relatively high yield levels under both conditions, suggesting their resilience to drought stress. High heritability and high genetic advance observed for grain yield, biological yield, and peduncle length indicate the predominance of additive gene action, which favors direct selection for improvement. The 1000-grain weight ranged between 30 and 44 g under E1 and 35 to 54 g under E2 (Table 3). Heavier grains were recorded in VL 2054 and PBW 752, which performed better under both conditions. The harvest index ranged between 28.7% and 47.7% under rainfed and 20.6% to 48% under irrigated conditions, with HPW 484 and HPW 493 recording higher values. Since harvest index reflects the efficiency of partitioning of assimilates into the grain, genotypes with higher values are considered physiologically more efficient and potentially drought tolerant. These findings are in close agreement with Kumar *et al.* (2021) and Sallam *et al.* (2019), who emphasized that yield stability under drought is often associated with strong additive genetic control of yield components.

The magnitude of heritability and genetic advance are key indicators of the extent of genetic control and the type of gene action involved. In the present study, traits such as peduncle length, biological yield, grain yield, and 1000-grain weight exhibited high heritability coupled with high genetic advance as percent of mean, indicating that these are largely governed by additive genes and thus, selection based on these traits would be effective. On the other hand, traits such as days to flowering and maturity, though having high heritability, showed moderate genetic advance, suggesting some degree of environmental influence and non-additive gene effects. The results are in agreement with earlier findings in wheat where additive gene effects were predominant for yield-contributing traits under drought stress. Similar associations between harvest index and drought tolerance have been reported by Reynolds *et al.* (2020) and Borrell *et al.* (2014).

PEG-induced osmotic stress effectively differentiated genotypes at the seedling stage, confirming its utility as a rapid and reproducible screening method. Progressive declines in germination percentage, shoot length, coleoptile length, and seedling vigour index with increasing PEG concentration reflect restricted water uptake and metabolic inhibition under osmotic stress, as also reported by Meneses *et al.* (2011) and Rauf *et al.* (2022). However, genotypes such as HPW 494, HPW 489, DBW 187, and Kanku maintained higher germination even at –6 and –9 bar (Table 4), indicating superior osmotic adjustment and membrane stability. Increased root-shoot ratio under moderate stress suggests preferential allocation of assimilates to root growth, enhancing water uptake–a well-documented adaptive response under drought (Comas *et al.,* 2013; Uga *et al.,* 2019). Genotypes like HPW 481, RIL 45, and Blue wheat exhibited this adaptive plasticity. Coleoptile length, a critical trait for seedling emergence under dry soils, showed high heritability and genetic advance. Genotypes such as HPW 493 and HPW 481 with longer coleoptiles may have improved establishment under moisture-limited conditions, supporting findings by Rebetzke *et al.* (2016) and Botwright *et al.* (202s). Seedling vigour index (SVI) emerged as a highly discriminating trait, integrating germination and seedling growth. Genotypes like HPW 489, VL 3031, and Dharwad Dry showed higher SVI under severe stress, highlighting their early-stage drought tolerance. High heritability and genetic advance for SVI and root traits indicate strong additive genetic control, reinforcing their suitability as early selection criteria (Sallam *et al.,* 2022).

Positive and significant correlations among germination percentage, root length, shoot length, and SVI across stress levels (Table 5) demonstrate the integrated nature of seedling traits in drought tolerance. Similar correlations have been reported by Dhanda *et al.* (2004), Ahmad *et al.* (2018), and Rane *et al.* (2023), emphasizing that selection

Table 3: Estimates of genetic parameters of variability for different traits under rainfed (E1) and irrigated (E2) conditions in wheat

Traits	Environments	Range	Mean ± S.E.	PCV (%)	GCV (%)	h² (bs) (%)	**GA (% of mean)**
Days to 50% flowering	E1	93.30–114.70	105.03 ± 0.81	5.12	5.09	99.00	10.45
	E2	109.00–123.00	117.51 ± 1.70	3.25	2.72	70.00	4.70
Days to 75% maturity	E1	136.00–154.67	145.70 ± 0.68	3.25	3.25	92.00	6.66
	E2	153.00–168.00	161.02 ± 2.01	2.90	2.47	72.22	4.32
Plant height	E1	75.00–110.00	97.17 ± 2.62	7.94	7.21	82.62	13.51
	E2	75.60–117.50	91.93 ± 0.58	8.85	8.82	99.25	18.10
Peduncle length	E1	9.33–21.00	13.81 ± 0.60	22.53	22.36	98.60	13.51
	E2	9.40–22.00	14.46 ± 0.88	19.44	17.96	85.37	34.19
Biological yield/plant	E1	8.09–13.74	11.83 ± 0.42	11.11	10.23	84.90	20.01
	E2	9.30–20.10	15.30 ± 0.73	17.70	16.72	89.22	32.52
Grain yield/plant	E1	2.22–5.80	4.43 ± 0.35	15.61	12.27	61.84	20.00
	E2	3.80–7.90	5.80 ± 0.29	18.04	16.95	88.23	32.79

S.E. = Standard error; PCV = Phenotypic coefficient of variation; GCV = Genotypic coefficient of variation; h²(bs) = Heritability (broad sense); GA = Genetic advance as percent of mean.

Table 4: Estimates of correlation coefficients among different traits at phenotypic level under rainfed (E1) and irrigated (E2) conditions in wheat

Traits	**Env.**	**Days to 75% maturity**	**Plant height**	**Peduncle length**	**Biological yield/plant**	**1000-grain weight**	**Harvest index**	**Drought susceptibility index**	**Grain yield/ plant**
Days to 50% flowering	E1	0.177	0.269*	-0.102	0.177	-0.318*	0.112	-0.143	0.237
	E2	-0.126	-0.083	-0.110	-0.133	-0.143	0.209*	0.070	0.041
Days to 75% maturity	E1	–	0.018	-0.177	-0.060	0.048	0.114	-0.239*	0.047
	E2	–	-0.510*	-0.052	0.112	-0.054	0.099	0.248*	0.189*
Plant height	E1	–	–	0.141	0.116	0.006	0.325*	-0.112	0.361*
	E2	–	–	-0.091	-0.156	-0.225*	-0.105	-0.434*	-0.226*
Peduncle length	E1	–	–	–	0.071	0.044	-0.156	-0.018	-0.093
	E2	–	–	–	-0.012	0.101	-0.125	0.073	-0.111
Biological yield/ plant	E1	–	–	–	–	-0.237*	-0.314*	-0.400*	0.512*
	E2	–	–	–	–	-0.001	-0.521*	0.520*	0.528*
1000-grain weight	E1	–	–	–	–	–	0.151	0.165	0.510*
	E2	–	–	–	–	–	0.135	0.297*	0.561*
Harvest index	E1	–	–	–	–	–	–	-0.282*	0.652*
	E2	–	–	–	–	–	–	0.229*	0.433*

* Significant at $P \leq 0.05$

Table 5: Mean performance and ANOVA for germination and seedling parameters under controlled conditions in wheat

Traits	Osmotic stress levels	Genotypes (df=39)	Error (df=78)
Germination percentage	C1 (Control)	100.34*	7.59
	C2 (–3 bar)	83.82*	4.15
	C3 (–6 bar)	44.98*	3.96
	C4 (-9 bar)	28.29*	2.17
Root length	C1 (Control)	21.10*	0.25
	C2 (–3 bar)	4.24*	0.10
	C3 (–6 bar)	1.16*	0.05
	C4 (-9 bar)	0.65*	0.12
Shoot length	C1 (Control)	13.22*	0.06
	C2 (–3 bar)	3.57*	0.07
	C3 (–6 bar)	2.98*	0.04
	C4 (-9 bar)	2.19*	0.02
Root–shoot ratio	C1 (Control)	0.20*	0.00
	C2 (–3 bar)	0.13	0.15
	C3 (–6 bar)	0.17*	0.06
	C4 (-9 bar)	0.14	0.01
Coleoptile length	C1 (Control)	0.62*	0.06
	C2 (–3 bar)	0.052*	0.05
	C3 (–6 bar)	0.30*	0.03
	C4 (-9 bar)	0.38*	0.03
Seedling vigour index	C1 (Control)	364317.86*	10623.29
	C2 (–3 bar)	101189.68*	1720.99
	C3 (–6 bar)	27115.29*	81118.16
	C4 (-9 bar)	30034.46*	8318.79

* Significant at $P \leq 0.05$

for these traits can indirectly enhance drought tolerance. Importantly, the strong correspondence between *in-vitro* and in vivo performance of genotypes such as HPW 489, HPW 490, HPW 493, HPW 494, VL 2053, DBW 187, and Kanku validates PEG-based screening as a reliable predictor of field performance. This concurrence supports earlier studies by Mwadzingeni *et al.* (2016) and El Hassouni *et al.* (2019), who advocated integrated screening approaches for drought tolerance breeding.

Conclusions

The study revealed significant genetic variability among wheat genotypes for traits related to drought tolerance under both field and laboratory conditions. High heritability and genetic advance for key yield and physiological traits such as peduncle length, biological yield, grain yield, 1000-grain weight, and seedling vigour index indicate predominance of additive gene action and scope for direct selection. The genotypes HPW 489, HPW 490, HPW 493, HPW 494, VL 2053, DBW 187, and Kanku emerged as promising candidates exhibiting superior performance under moisture stress. The seedling traits such as germination percentage, shoot length, and seedling vigour index proved to be reliable early indicators of drought tolerance and can serve as effective selection criteria in breeding programs. Thus, the integration of in vivo and *in-vitro* screening approaches offers a comprehensive and efficient strategy for identifying and utilizing drought-tolerant germplasm in wheat improvement programs aimed at enhancing productivity and resilience under changing climatic conditions.

Authors' contributions

KS- compiled and edited the manuscript; statistical analysis of the findings and in editing of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Approval

The article doesn't contain any study involving ethical approval.

Use of Generative AI or AI assisted technologies

Authors declare that no Generative AI or AI assisted technologies have been used in preparation of this manuscript.

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