Ahmed *et al. BMC Plant Biology* (2025) 25:390
https://doi.org/10.1186/s12870-025-06345-9

BMC Plant Biology

RESEARCH **Open Access**

Exploring drought tolerance for germination traits of diverse wheat genotypes at seedling stage: a multivariate analysis approach

Kashif Ahmed[1*], Ghulam Shabbir[2] and Mukhtar Ahmed[2]

Abstract

Wheat holds crucial global importance as a staple food in many regions. Drought stress significantly impedes seed germination of wheat. The lack of drought-tolerant wheat varieties hampers wheat production, especially in arid regions of the world. This study investigated seed germination and seedling growth in eighty wheat genotypes under moisture stress stimulated by polyethylene glycol (PEG^{6000}). The study included two osmotic potentials induced by PEG-20% (ψ: -0.491 MPa) and PEG-25% (ψ: -0.735 MPa), as well as a control set without PEG. The data showed that dehydration caused by polyethylene glycol generally had an adverse effect on the morphological characteristics of wheat seedlings by causing substantial losses during the early germination stage. The results acquired from analysis of variance explained highly significant variances ($p < 0.01$) across genotypes (G), PEG-Treatments (T^{PEG}), and interactions between genotypes and PEG-Treatments (G x T^{PEG}) for all observed variables. Moisture deficit radically affected all studied seedling traits of bread wheat under rising osmotic stress, with germination percentage (GP), shoot length (SL), root length (RL), coleoptile length (CL), seedling length (SDL), root fresh weight (FRW), root dry weight (DRW), shoot fresh weight (FSW), shoot dry weight (DSW), seedling biomass (SBM) and seedling vigor index (SVI) reduced by about 30–95% compared to control (PEG-0%) to the maximum induced osmotic stress at -0.735 MPa. The coefficient of relative inhibition (CRI) rose in response to osmotic stress, demonstrating growth inhibition. Boxplots demonstrated a considerable decline under stress, although scatter plots and correlation matrices revealed significant positive associations for most seedling traits, except CRI. The histograms for most variables showed a wider value range and more diversified distribution patterns. Principal component analysis (PCA) and genotype by trait biplot emphasized that PC1 represented 94.29% of the cumulative variation, with an eigenvalue of 11.31 out of 12 components. The heatmap displayed diverse genotype and trait characteristics, indicating higher values for drought resistance and lower values for susceptibility in genotype performance. As indicated by a range of multivariate analyses, the wheat lines NR-499, NARC-2009 and Pakistan-2013 stood out as the most drought-tolerant among the genotypes; whereas Borlaug-2016, NR-514 and NR-516 were found to be highly susceptible, whereas SBM, SDL, SVI and CRI have been found key indicators for subsequent screening. These tolerant wheat lines offer promising potential for developing drought-tolerant varieties that could thrive in arid regions, thereby strengthening wheat production in water-stressed environments.

---

*Correspondence:
Kashif Ahmed
kashif_ahmed01@yahoo.com

Full list of author information is available at the end of the article

Integrating these findings into breeding programs is crucial for realizing the potential of drought-tolerant wheat varieties in transforming global wheat production.

Keywords Phenotyping, Osmotic stress, Bread wheat, Stress tolerance index, Multivariate analysis

Introduction

Wheat, scientifically known as *Triticum aestivum* L., is a globally cultivated cereal crop, providing essential nutrients like carbohydrates, proteins, lipids, vitamins, and minerals as a significant food and feed to both humans and animals. Domesticated approximately 10,000 years ago in tetraploid and hexaploid varieties [1, 2], hexaploid bread wheat plays a pivotal role in meeting global nutritional needs. Despite ranking as the third most produced cereal after maize and rice [3, 4], the global demand for wheat is projected to surge by 60% due to an estimated population growth of 10 billion by 2050. Meeting this demand requires an annual yield growth of at least 1.6%, surpassing the current 1% growth rate [1, 5]. As environmental pressures escalate, focus intensifies on the gap between wheat production and consumption [6].

Climate change is anticipated to adversely affect agricultural productivity due to rapid fluctuations in temperature and precipitation, causing drought stress [7, 8]. Among abiotic stresses, drought stands out as a significant factor reducing overall productivity [9, 10]. Drought can lead to a 50–90% reduction in wheat yield [9]. Rainfed wheat yields are notably lower than irrigated regions due to the scarcity of rainwater. Assessing wheat genotype drought tolerance often relies on important measures like seed germination and seedling emergence [11], as seedling growth plays crucial roles in production anticipation, as observed by various studies [5, 12]. Drought stress significantly hinders seed germination and leads to notable changes in seedling physiology and biochemistry [13, 14].

Agricultural drought poses a substantial environmental challenge, hindering crucial physiological processes necessary for crop growth due to inadequate water supply [1]. Despite these challenges, many plants have evolved physiological adaptations to withstand water stress [14, 15]. Inadequate moisture during germination and subsequent growth stages reduces wheat crop productivity and delays maturity [16]. Germination percentage and seedling establishment notably decrease when soil osmotic potential approaches – 1.5 MPa [17]. Optimal microclimatic conditions and seed quality play critical roles in successful plant establishment [1, 18]. Bread wheat genotypes displaying strong early seedling establishment in challenging conditions can be identified and selected by screening diverse plant materials on a large scale [2, 9].

Polyethylene glycol (PEG) has long been used to test young plants for drought tolerance by inducing dehydration and restricting plant tissue conductivity during the seedling stage [19, 20]. Drought tolerance in diverse crop varieties is frequently evaluated by examining seed germination under PEG-induced stress, as PEG molecules with more than 3000 molecular weights cannot penetrate the cell wall [9]. PEG^{6000}, a commonly used variant, possesses a molecular weight of approximately around 6,000 g/mol [4]. Its ability to simulate drought stress in plants is attributed to its property as a non-permeating osmotic agent [21]. When applied, it restricts water availability in the growth medium, mimicking drought conditions without causing direct physical damage to the plants. High molecular weight osmotic chemical, like PEG^{6000}, is often used to assess agricultural-plant drought-resistance during early growth stages due to their capacity to induce severe water stress [4, 22]. The lower water potential of PEG affects both seed germination and growth, with more noticeable effects on shoots than primary roots [23]. PEG also enhances the transfer of ions and nonionic compounds such as mannitol, raffinose, and inulin [12, 24]. Several studies have demonstrated the reliability of in vitro screening with PEG to identify drought-tolerant genotypes, particularly based on germination indices [12, 25].

Historically, plant breeding primarily focused on above-ground plant traits, neglecting the study of root characteristics [4, 26, 27]. Recent studies, however, showed that PEG-induced drought has a significant impact on wheat seedling traits [6, 27]. Progress in screening for drought resistance and evaluating root traits has been made [18, 19], with selection of wheat genotypes featuring larger roots and extended seminal and adventitious roots showing promise for improved grain yield, especially in arid and semi-arid regions [9, 23, 24]. Root growth influences a plant's ability to endure stress under bad situations [21, 28]. Seedlings from drought-resistant genotypes have stronger root systems and more biomass during the germination process compared to other genotypes [8]. The coleoptile length, which protects the budding shoot, is an important predictor of seed germination and plant height. Researchers have also linked seed germination to the length of the radical, plumule, and seedling biomass. Under PEG-induced drought, decreased osmotic potential reduces shoot and root length, as well as biomass [29, 30].

Wheat germplasm that demonstrates a higher stress tolerance index (STI) is widely recognized as drought tolerant [31]. The use of STI-based screening offers significant advantages for crop cultivars in both stress and non-stress conditions [32]. Extensive research has consistently shown that drought-tolerant wheat genotypes generally exhibit higher STI values compared to susceptible ones. Therefore, STI serves as a valuable criterion for selecting superior crop cultivars that are effective under various climate conditions [32].

The vulnerability of wheat seedlings to drought stress during its early growth stages poses a critical challenge in agricultural productivity, impacting germination, growth, and overall crop development. Understanding the specific effects of drought stress on wheat seedlings is imperative for devising strategies to enhance crop resilience and mitigate potential yield losses. The precise mechanisms, genetic factors, and key traits associated with drought tolerance in wheat seedlings remain relatively understudied. This study aims to bridge this gap by focusing on the often-overlooked seedling stage and identifying pivotal markers for drought tolerance in wheat at this critical growth phase. Enhancing the drought tolerance at the seedling stage through selective breeding for these traits can facilitate the development of future drought-tolerant wheat genotypes under changing climatic conditions.

Materials and methods

Plant material

A collection of eighty (80) distinct genotypes of bread wheat consisting of cultivars and advance lines, were acquired from various reputable institutes, including the Plant Genetics Resource Institute, National Agricultural Research Centre Islamabad (PGRI-NARC), Wheat Program, National Agricultural Research Centre Islamabad (WP-NARC), Barani Agricultural Research Institute (BARI), Chakwal, and Directorate of Agriculture Research (Cereal Crops) Agriculture Research Institute (ARI), Quetta, for present study as tabulated in the Supplementary Table S1.

Experimental description

The experiment was arranged in a two-factor complete randomized design (CRD) with three replicates. These factors encompassed eighty (80) wheat genotypes and three levels of osmotic potential. Uniform-sized wheat genotype seeds were selected and surface-sterilized for 10 min using a 1% sodium hypochlorite solution, then rinsed several times with sterile distilled water. Subsequently, the seeds were soaked in distilled water for 10 min and then sown in three sets of 11 cm diameter petri plates filled with sterile sand, which was moistened with distilled water. The germination process took place over five days in a controlled environment chamber (Model: GC-560 H, Firstek Scientific, Taiwan), maintaining a constant temperature of 25 ± 1 °C for day and night. The relative humidity (RH) was maintained between 75% and 80%, with a photoperiod of 16 h of light and 8 h of darkness. Cool-white fluorescent lights emitting a photosynthetic photon flux density (PPFD) of 400 mol m^2 s^{-1} were used in this setup. After the initial five days, seedlings were shifted to moistened germination paper, i.e., Whatman's Filter Paper [16].

Stress treatment application

Moisture stress was induced by altering osmotic potentials using polyethylene glycol (PEG^{6000}; Sigma-Aldrich, St. Louis, MO, USA), a high molecular weight polymer commonly employed to evaluate seedling performance during the germination stage in various crops. Water potential (ψ, MPa) was triggered for drought-induced stress by ψ = -0.491 MPa and ψ = -0.735 MPa, induced by PEG-20% and PEG-25% respectively, whereas a control set without polyethylene glycol (PEG-0%; ψ = -0.033 MPa) was established using pure distilled water [19]. In this study, 5 ml of distilled water was added to each control petri dish every two days to counteract moisture evaporation, while 5 ml of PEG solution (weight/volume) was administered to each plate under both osmotic stress conditions. The experiment was concluded after 10 ± 2 days, at which point, fully germinated wheat seedlings were harvested, and data for randomly selected seedling were recorded [33].

Measurement of seedling traits

Ten seedlings from all PEG-induced drought stress (control, -0.491 and − 0.735 MPa) were assessed for various parameters, viz. seed germination percentage (SGP), coleoptile length (CL), shoot length (SL), root length (RL), seedling length (SDL), root fresh weight (FRW), root dry weight (DRW), shoot fresh weight (FSW), shoot dry weight (DSW), seedling biomass (SBM), seedling vigor index (SVI) and coefficient of relative inhibition (CRI). Germination was measured by counting the germinated seedlings when seedlings exhibited radical growth exceeding 3 mm. Coleoptile length was determined by assessing the length of the protective sheath encasing the emerging shoot. Furthermore, shoot length was measured from the tip of the longest leaf, while root length was measured from the tip of the longest root. Dry weights of root and shoot were measured after drying at 80 °C for 24 h, then these values were summed to estimate the seedling biomass. The seedling vigor index was estimated following the methodology [34]:

$$SVI = Seed\,Germination\,Percentage - Seedling\,Length$$

Additionally, the coefficient of relative inhibition (CRI), a measure of growth inhibition determined by the reduction in overall plant biomass accumulation due to stress treatments, was calculated following the method:

$$CRI = \frac{(Biomass\,of\,unstressed\,plants - Biomass\,of\,stressed\,plants\,)}{Biomass\,of\,unstressed\,plants}$$

Determination of stress tolerance index (STI)

The mean data for all seedling traits (SDT) were used to determine the Stress Tolerance Index (STI) under PEG-induced drought stress at -0.491 MPa and – 0.735 MPa compared to the control treatment. This calculation was performed using the following formula [35].

$$STI = \frac{(Seedling\,Trait\,under\,Control \times Seedling\,Trait\,under\,Stress)}{(Overall\,Mean\,value\,of\,Seedling\,Trait\,under\,Stress)^2}$$

Statistical analysis

The replicated data underwent Two-Factorial CRD Analysis of Variance (ANOVA) using the General Linear Model (GLM) in 'IBM Statistical Program for Social Science (SPSS Version 22) software [36]. This analysis included eighty wheat genotypes (G) and three PEG-Treatments (T^{PEG}), i.e., PEG-0% (control), PEG-20% (-0.491 MPa), and PEG-25% (-0.735 MPa) as fixed factors, assessing seedling traits as dependent variables. Additionally, the GLM model employed UNI-ANOVA to evaluate the Genotypes × PEG-Treatments (T^{PEG}) interaction, followed by a *post-hoc* Tukey's Honestly Significant Difference (HSD) test at a 5% probability level. Furthermore, mean seedling trait values underwent various multivariate statistical analyses, including Boxplot, Scatter-Pairplot, Phenotypic Correlation Matrix, and Principal Component Analysis (PCA). These analyses aimed to identify distinctive drought-tolerant and drought-susceptible bread wheat genotypes during the germination stage under varying osmotic potentials using R-Studio (Version: 3.3.0+) software. Additionally, cluster-based heatmapping was utilized for phenotyping based on the stress tolerance index (STI) using R-Studio Desktop Pro-2023.

Results and discussion

Phenotyping plays a crucial role in screening breeding germplasm, concentrating on drought-adaptive and constitutive morphological traits under osmotic stress conditions [2, 6, 8]. Osmotic stress significantly constrains plant growth, particularly during its juvenile stage, posing a serious threat to agricultural production [8, 14].

Genetic variability in wheat seedling attributes

Osmotic stress is an indicator of cellular drought stress [24]. The analysis of variance revealed highly significant variations ($p < 0.01$) across genotypes (G), PEG-Treatments (T^{PEG}), and their interactions between G × T^{PEG} for all recorded traits, including SGP, CL, RL, SL, SDL, FRW, FSW, DRW, DSW, SVI, SBM, and CRI (Table 1). These findings indicate substantial genetic heterogeneity in osmotic stress tolerance among the studied wheat genotypes. Moreover, both

Table 1 Analysis of variance (ANOVA) mean squares of Eighty bread wheat genotypes at the seedling stage under normal and drought conditions

Variables	**Genotypes**	**Treatments^{PEG}**	**Interaction**	**Error**	**Tukey's HSD (0.05)**			**C.V. (%)**
	(G)	**(T^{PEG})**	**(G x T^{PEG})**					
	(d.f: 79)	**(d.f: 2)**	**(d.f: 158)**	**(d.f. 480)**	**G**	**T^{PEG}**	**G x T^{PEG}**	
SGP	103.3**	46,027**	53.10**	17.6	*8.384*	*0.9*	*16.14*	*5.31*
CL	0.319**	270.9**	0.240**	0.064	*0.506*	*0.054*	*0.974*	*10.26*
RL	5.170**	4059**	2.850**	0.38	*1.226*	*0.131*	*2.362*	*7.87*
SL	3.590**	4833**	1.980**	0.11	*0.674*	*0.072*	*1.297*	*5.43*
SDL	13.90**	17,689**	5.300**	0.50	*1.423*	*0.152*	*2.741*	*5.08*
FRW	2819**	18,503**	2403**	1.00	*2.129*	*0.228*	*4.1*	*1.82*
FSW	2415**	31,224**	1776**	1.00	*1.992*	*0.214*	*3.835*	*1.67*
DRW	14.30**	12,546**	10.30**	0.30	*1.153*	*0.123*	*2.22*	*6.27*
DSW	66.687**	52,017**	20.763**	0.343	*5.991*	*0.125*	*2.252*	*1.15*
SVI	126,618**	18,810**	56,450**	8513	*184.2*	*19.8*	*354.8*	*7.78*
SBM	103.0**	69,328**	29.0**	1.00	*1.66*	*0.178*	*3.196*	*1.38*
CRI	***(d.f: 79)***	***(d.f: 1)***	***(d.f: 79)***	***(d.f: 320)***				
	0.0007**	23.91**	0.0004**	0.0001	*0.017*	*0.001*	*0.026*	*1.01*

Note: Here, SGP = Seed Germination Percentage, CL = Coleoptile Length, RL = Root Length, SL = Shoot Length, SDL = Seedling Length, FRW = Fresh Root Weight, DRW = Dry Root Weight, FSW = Fresh Shoot Weight, DSW = Dry Shoot Weight, SVI = Seedling Vigor Index, Seedling Biomass = SBM, CRI = Coefficient of Relative Inhibition.

PEG treatments displayed statistically distinct effects on seedling traits compared to the control. The significant interaction between G × T^{PEG} highlighted the differential performance of various genotypes under different osmotic potentials. Previous research has demonstrated the impact of drought stress on early growth-related morphological attributes in various crops [4, 12, 37, 38]. Consequently, these traits have been valuable in selecting extensive germplasm for drought tolerance during early growth stages, thereby saving time and labor in the field [28].

Relative effects of osmotic stress with polyethylene glycol

As expected, diverse bread wheat genotypes demonstrated significant variations in their resistance to PEG-induced drought stress. Previously, the remarkable reduction was reported in seed germination and growth traits under – 0.491 MPa (induced by PEG-20%) and – 0.735 MPa (induced by PEG-25%), reflecting a consistent declining trend across various seedling-related growth traits with increasing osmotic potential the [3, 18, 39]. Drought stress notably affected the efficiency and performance of all studied seedling attributes during early germination, showing substantial decreases in SGP, CL, RL, SL, SDL, FRW, FSW, DRW, DSW, SVI and 3by 29.85%, 60.78%, 67.64%, 78.71%, 72.94%, 78.79%, 85.99%, 84.90%, 95.14%, 80.96% and 93.63%, respectively (Fig. 1), compared to the control set (PEG-0%) to the maximum induced osmotic stress at -0.735 MPa induced by PEG-25%. Polyethylene glycol (PEG^{6000}) significantly hindered emergence and development, leading to considerable reductions in seedling attributes and the demise of wheat seedlings during early germination stages. Similar patterns of reductions in wheat seedling attributes due to osmotic stress were also observed in previous studies [15].

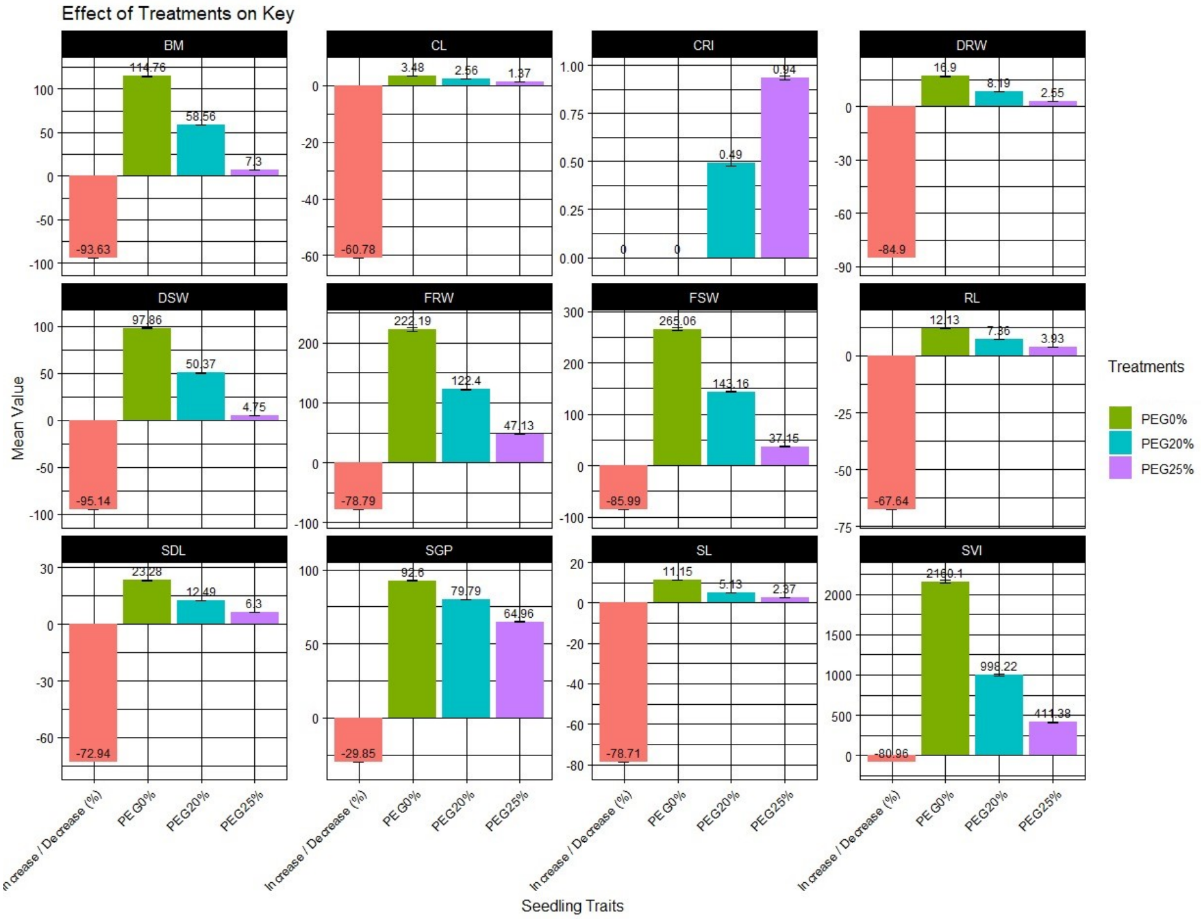

Fig. 1 Multivariate bar-graph represents mean performance and relative effects of wheat seedling traits under various PEG-induced osmotic potentials (Here, SGP = Seed Germination Percentage, CL = Coleoptile Length, RL = Root Length, SL = Shoot Length, SDL = Seedling Length, FRW = Fresh Root Weight, DRW = Dry Root Weight, FSW = Fresh Shoot Weight, DSW = Dry Shoot Weight, SVI = Seedling Vigor Index, Seedling Biomass = SBM, CRI = Coefficient of Relative Inhibition

Effect of osmotic stress on seed germination and coleoptile growth

Seed germination percentage (SGP) stands as a pivotal stage in the early growth and productivity of a plant's life cycle, significantly affected by drought stress. Among the studied genotypes, the control treatment exhibited the highest mean SGP at 92.6%, followed by 79.8% and 64.7% under the stress treatments of PEG-20% and PEG-25%, respectively (Fig. 1). Notably, the NR-499 genotype displayed the highest seed germination percentage overall, recording an impressive 86.7% as presented in Supplementary Table S2. Conversely, Borlaug-2016 exhibited the lowest seed germination at 67.2%. Examining individual genotypes revealed that wheat genotype NR-499 demonstrated maximum SGP at 98.5%, 88.3%, and 73.3% under PEG-0% (control), PEG-20% (-0.491 MPa), and PEG-25% (-0.735 MPa) osmotic stress levels, respectively (Table S2). In contrast, Borlaug-2016 displayed the minimum SGP at 81.7%, 71.7%, and 48.3% under the same stress levels. Osmotic water stress can lead to the degradation and inactivation of essential hydrolytic enzymes required for germination, inhibiting not only germination characteristics but also the extension growth of seedlings. This reduction in germination percentage highlights the sensitivity of wheat plants to drought stress [6, 40].

Coleoptile length (CL) serves as a crucial protective sheath, safeguarding the tissue surrounding the stem of seedlings [41]. In this study, the maximum mean CL was observed to be 3.5 cm in the control treatment (PEG-0%). Under moisture-deficient conditions of PEG-20% and PEG-25%, the CL decreased to 2.6 cm and 1.4 cm, respectively compared to control (Fig. 1). Among all moisture treatments, genotypes Pakistan-2013 and NR-516 exhibited the longest (3.2 cm) and shortest (0.45 cm) coleoptile lengths, respectively (Table S2). Furthermore, Pakistan-2013 displayed the longest coleoptile lengths (4.4 cm, 3.4 cm, and 1.9 cm) under PEG-0% (control), PEG-20% (-0.491 MPa), and PEG-25% (-0.735 MPa), respectively, while NR-516 exhibited the shortest lengths (2.8 cm, 1.9 cm, and 0.9 cm) under the same moisture conditions (Table S2). It's important to note that heightened stress during germination and early seedling phases negatively impacts various parameters, including germination rate, seedling vigor, and coleoptile length [42].

Effect of osmotic stress on root growth and biomass characteristics

Roots play a vital role in meeting transpiration needs and significantly contribute to providing moisture to plants [13, 43]. Root length (RL) serves as a key indicator of plant resilience in drought-prone environments. In the control treatment (PEG-0%), the mean RL reached the highest value of 12.1 cm, followed by stress treatments with PEG-20% (7.4 cm) and PEG-25% (3.9 cm), as depicted in Fig. 1. Across all treatments, NR-499 exhibited the longest root length of 10.3 cm, while Borlaug-2016 displayed the shortest at 5.8 cm (Table S2). NR-499 demonstrated superior root lengths (15.7 cm, 9.3 cm, and 5.8 cm) at osmotic stress levels of -0.491 MPa and – 0.735 MPa, whereas NR-528 (9.4 cm) at PEG-0% and Borlaug-2016 (5.7 cm and 2.2 cm) depicted the shortest RL at PEG-20% and PEG-25% concentrations, respectively. The reduction in root length of the bread wheat germplasm studied here under osmotic pressure aligns with previous findings [6]. Additionally, wheat genotypes displayed extended root length under drought stress could indicate an adaptable response, potentially enhancing the root's capacity to access deeper soil water [14, 44].

The fresh weight of roots (FRW) significantly influences seedling biomass. The highest average FRW was 222.2 mg under PEG-0%, followed by 122.4 mg and 47.1 mg under PEG-20% and PEG-25% stress conditions, respectively (Fig. 1). Across all PEG^{6000} levels studied, NR-499 displayed the highest FRW at 172.0 mg, while Borlaug-2016 showed the lowest at 92.3 mg (Table S2). Specifically, NR-499 and Borlaug-2016 exhibited maximum FRW (311.2 mg, 148.8 mg, and 55.9 mg) and minimum FRW (151.2 mg, 90.7 mg, and 35.1 mg) at osmotic potentials of PEG-0% (control), PEG-20% (-0.491 MPa), and PEG-25% (-0.735 MPa), respectively. These preceding studies also demonstrated a significant decrease in shoot and root length, as well as shoot and root fresh weight in wheat [30].

The dry weight of an individual root (DRW) is assessed after complete moisture removal. In Fig. 1, the control treatment (PEG-0%) demonstrated the highest average DRW (16.9 mg), followed by stress treatments with PEG-20% (8.2 mg) and PEG-25% (2.6 mg). NR-499 and Borlaug-2016 exhibited the heaviest (12.2 mg) and lightest (6.5 mg) DRW, respectively, across all osmotic potentials (Table S2). Specifically, under water stress at PEG-0% (control), PEG-20% (-0.491 MPa), and PEG-25% (-0.735 MPa), wheat advance line NR-499 produced the highest dry weights (22.8 mg, 10.5 mg, and 3.3 mg), however, Borlaug-2016 yielded the lowest (11.6 mg, 6.1 mg, and 2.0 mg). This study's outcomes highlight that dry mass was notably affected by moisture stress [3, 40]. Moreover, the dry weight of water-stressed wheat seedlings increased in comparison to conditions without water stress, potentially due to a surplus of stored nutrients in the stressed seedlings. The limited water availability might have resulted in the accumulation of underutilized nutrients, potentially due to reduced hydrolysis and/or decreased nutrient uptake by the less developed radicle and plumule [5].

Effect of osmotic stress on shoot growth and biomass characteristics

Shoot length (SL) is a critical trait in young wheat seedlings, significantly impacting their overall development [4]. Among the genotypes studied, the control treatment showed the highest mean SL of 11.1 cm, followed by 5.1 cm and 2.4 cm under the PEG-20% and PEG-25% stress treatments, respectively (Fig. 1). Notably, NR-499 displayed the longest shoot length (8.1 cm) across all treatments, while Borlaug-2016 showed the shortest (4.4 cm). Wheat line NR-499 exhibited maximum SL, measuring 13.3 cm and 7.6 cm under control and – 0.491 MPa of osmotic potentials, respectively (Table S2). Conversely, NARC-2009 displayed the longest shoot length (3.5 cm) under – 0.735 MPa of osmotic potential, whereas Borlaug-2016 had the smallest shoot length (8.5 cm, 3.6 cm, and 1.2 cm) under PEG-0% (control), PEG-20% (-0.491 MPa), and PEG-25% (-0.735 MPa), respectively (Table S2). The reductions in shoot length may relate to cell division hindrances and tuberization processes induced by moisture stress, causing developmental pauses in growth [45].

Fresh shoot weight (FSW) is crucial in evaluating wheat seedlings. Our study assessed FSW under control (PEG-0%) and stress treatments (PEG-20% and PEG-25%). Results showed the highest mean FSW in the control treatment at 265.1 mg, followed by PEG-20% (143.2 mg) and PEG-25% (37.1 mg). NARC-2009 displayed the heaviest FSW (180.8 mg), whereas wheat line ARI10 exhibited the lightest (110.3 mg) across all treatments (Table S2). Specifically, NARC-2009 consistently showed the heaviest FSW (330.6 mg, 165.4 mg, and 46.4 mg) at osmotic stress levels of PEG-0% (control), PEG-20% (-0.491 MPa), and PEG-25% (-0.735 MPa), however, ARI10 consistently had the lightest FSW (188.6 mg, 115.6 mg, and 26.7 mg) under the same levels (Table S2). As previously also reported the adverse effects of drought stress on wheat lines and cellular dehydration in shoot tissue [5].

Dry shoot weight (DSW) is an effective method for estimating plant biomass. In our study, the highest mean DSW was 97.9 mg under the control treatment (PEG-0%), followed by 50.4 mg and 4.8 mg under PEG-20% and PEG-25% drought conditions, respectively (Fig. 1). NARC-2009 and SAWYT-36 exhibited the heaviest (57.0 mg) and lightest (44.4 mg) DSW, respectively (Table S2). Particularly, NARC-2009 showed the highest DSW (105.7 mg and 58.2 mg) under control and – 0.491 MPa conditions, while Pakistan-2013 displayed the highest DSW (7.4 mg) under – 0.735 MPa. Conversely, wheat line SAWYT-36 had the lowest DSW (88.7 mg, 41.7 mg, and 2.9 mg) under PEG-0% (control), PEG-20% (-0.491 MPa), and PEG-25% (-0.735 MPa), respectively (Table S2). The decrease in shoot dry biomass at higher drought stress levels is due to reduced moisture availability and hindered transport of photosynthate from source to sink. This phenomenon has been documented by several studies, indicating distinct genetic influences on plant biomass among different genotypes [6, 30].

Effect of osmotic stress on seedling growth and vigor index

Seedling length (SDL) serves as a valuable stage for assessing drought resistance during development in laboratory conditions [41]. The highest average SDL among all wheat genotypes was recorded at 23.3 cm in the control treatment (PEG-0%), followed by 12.5 cm and 6.3 cm under moisture-deficient conditions of PEG-20% and PEG-25%, respectively (Fig. 1). Wheat lines NR-499 and Borlaug-2016 displayed the longest (18.4 cm) and shortest (10.2 cm) seedling lengths across all treatments (Table S2). Under osmotic stress of PEG-0% (control), PEG-20% (-0.491 MPa), and PEG-25% (-0.735 MPa), wheat genotype NR-499 exhibited higher SDL (29.0 cm, 16.9 cm, and 9.2 cm), whereas Borlaug-2016 displayed lower SDL (18.0 cm, 9.3 cm, and 3.4 cm) at 0%, 20%, and 25% PEG^{6000} concentrations, respectively. Similar outcomes were observed that reduction in seedling elongation due to drought stress could be attributed to the impact on the seedling's meristem cells, disrupting the cell division and elongation process [46]. Insufficient water conditions affect cell water uptake, leading to decreased turgor pressure essential for cell expansion, consequently hindering growth.

The seedling vigor index (SVI) is notably sensitive to drought stress, displaying varying dominance levels across different moisture conditions. The highest average SVI value among all wheat genotypes reached 2160.1 in the control treatment (PEG-0%), followed by 998.2 and 411.4 under PEG-20% and PEG-25% stress levels, respectively (Fig. 1). Among the examined PEG^{6000} levels, NR-499 and Borlaug-2016 showcased the highest (1672.3) and lowest (767.6) seedling vigor indexes (Table S2). Similarly, under various osmotic stress levels, wheat line NR-499 displayed higher values (2851.7, 1492.5, and 672.6), whereas Borlaug-2016 showed lower values (1470.0, 669.0, and 163.8) for SVI. Similarly, superior traits in wheat genotypes, including increased seed germination percentage, enhanced root and shoot length, and higher seedling vigor index [25]. Elevated PEG intensity during seedling development suppressed growth and resilience. Drought stress consistently diminishes germination rate, shoot and root length, and dry biomass. There was a clear reduction in SVI with increased stress intensity in preceding studies as well [40].

Seedling biomass (SBM) represents the total dry weight of an individual seedling encompassing both root and shoot components, serving as a fundamental seedling characteristic. The control treatment (PEG-0%) exhibited the highest mean SBM (114.8 mg), trailed by stress treatments of PEG-20% (58.6 mg) and PEG-25% (7.3 mg) among the studied wheat genotypes (Fig. 1). The wheat lines NR-499 and Borlaug-2016 displayed the heaviest (69.2 mg) and lightest (50.9 mg) seedling biomass across all osmotic potentials (Table S2). Likewise, wheat line NR-499 displayed the greatest SBM values (128.5 mg, 68.7 mg, and 10.4 mg), while Borlaug-2016 displayed the lowest (100.3 mg, 47.7 mg, and 4.8 mg) under osmotic stress of PEG-0% (control), PEG-20% (-0.491 MPa), and PEG-25% (-0.735 MPa), respectively (Table S2). The influence of PEG^{6000} stimulated water stress restrained seed reserve utilization and sugar solubilization during germination, consequently resulting in reduced seedling dry weight. Several researchers have observed a decline in seedling dry weight, illustrating the significant impact of drought conditions on plant dry mass [7, 10].

Seedling growth inhibition index by coefficient of relative inhibition

The coefficient of relative inhibition (CRI) serves as a key indicator of plant growth limitation [14], reflecting a higher degree of inhibition as the osmotic stress from PEG^{6000} intensifies, indicating greater tolerance in genotypes with lower levels. The stress treatments using PEG-20% and PEG-25% displayed the lowest (0.490) and highest (0.490) mean CRI values, as depicted in Fig. 1. Among the wheat genotypes, Pakistan-2013 exhibited the most tolerance with the lowest CRI value (0.690), while Borlaug-2016 showed the highest vulnerability with the highest CRI value (0.738). Furthermore, in terms of individual performance, NARC-2009 and Pakistan-2013 emerged as tolerant genotypes, displaying lower CRI values (0.459 and 0.918) under PEG-20% and PEG-25%, respectively (Table S2). Conversely, NR-514 and Borlaug-2016 demonstrated sensitivity, exhibiting higher CRI values (0.530 and 0.952) under osmotic stress levels of -0.491 and – 0.735 MPa, respectively. The incremental rise in the relative inhibition coefficient reflects a gradual hindrance in plant development, aligning with the previous findings that showed the decrease in plant growth correlated with the overall stress level [6]. Similarly, previous findings also initiated a positive correlation between the

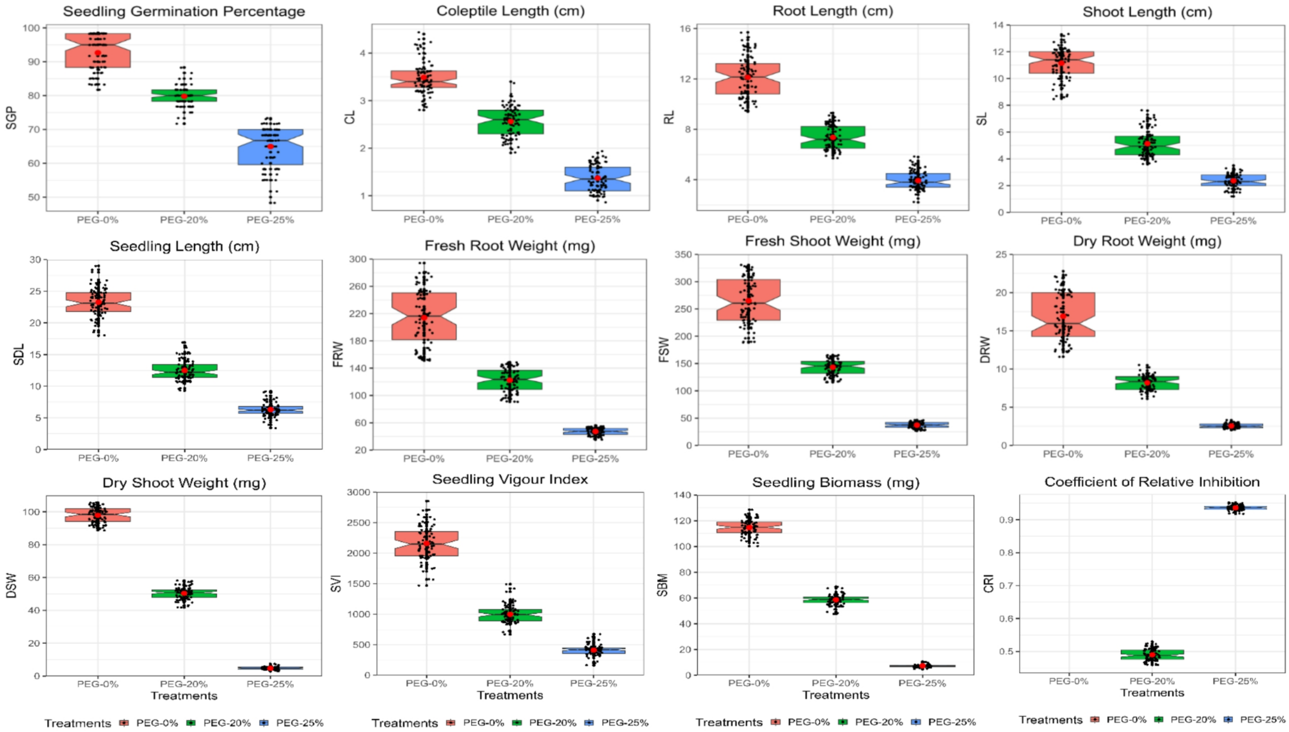

Fig. 2 Boxplot showing the descriptive statistics of the seedling traits of bread wheat genotypes under control and PEG-induced drought stress (Here SGP = Seedling Germination Percentage, CL = Coleoptile Length, RL = Root Length, SL = Shoot Length, SDL = Seedling Length, FRW = Fresh Root Weight, DRW = Dry Root Weight, FSW = Fresh Shoot Weight, DSW = Dry Shoot Weight, SVI = Seedling Vigor Index, SBM = Seedling Biomass and CRI = Coefficient of Relative Inhibition.)

coefficient of relative inhibition and growth reduction under PEG-induced osmotic stress [4].

Multivariate analysis of wheat seedling traits

Multivariate analysis, including boxplots, scatter-pair plots, phenotypic correlation matrices, principal component analysis (PCA), and clustered heatmapping, were conducted to assess various seedling attributes in bread wheat. Mean values of seedling traits were employed to construct boxplots, scatter-pair plots, correlation matrices, and PCA, whereas clustered heatmapping specifically visualized the stress tolerance index (STI) across all seedling traits under PEG-20% (ψ = -0.491 MPa) and PEG-25% (ψ = -0.735 MPa).

Boxplot analysis

Boxplots are valuable graphical representation, used to visually represent data distribution and compare different groups effectively. They provide a concise overview of multiple seedling characteristics across various environments, treatments, or genotypes in the context of wheat seedling attribute research. These plots present key statistical information such as the median, quartiles, and potential outliers [29], offering insights into data distribution. Figure 2 illustrates the descriptive statistics of seedling traits, revealing notably lower trait values in PEG-treated seedlings. Interestingly, PEG^{6000} treatments led to a more significant decrease in shoot attributes compared to root features (Fig. 2). Within the boxplot, the horizontal line represents the median, while the red dot signifies the mean of the analyzed seedling attributes. The boxplot's lower and upper limits, along with the whiskers, denote Q1 (first quartile or 25th percentile), Q3 (third quartile or 75th percentile), (Q1-1.5IQR), and (Q3 + 1.5IQR), respectively. The black dots scattered in the boxplot depict the distribution of 80 wheat genotypes, showing a medium to large spread for most seedling attributes. However, SBM and CRI exhibit a narrower distribution range. Notably, shoot and root length, along with fresh weight, displayed significant impacts under PEG-induced drought stress (Fig. 2). These findings are consistent with prior research indicating decreased root and shoot length, as well as fresh and dry weight in wheat under similar conditions of moisture stress [3, 9, 29].

Scatter-pairplot and phenotypic correlation matrix

Scatter-pair plots serve as a rapid method to explore distributions and associations within a dataset [47]. The correlation coefficients among the features determine the extent of their relationships [30]. In Fig. 3, Pearson correlation analysis revealed significant associations among observed seedling features. Except

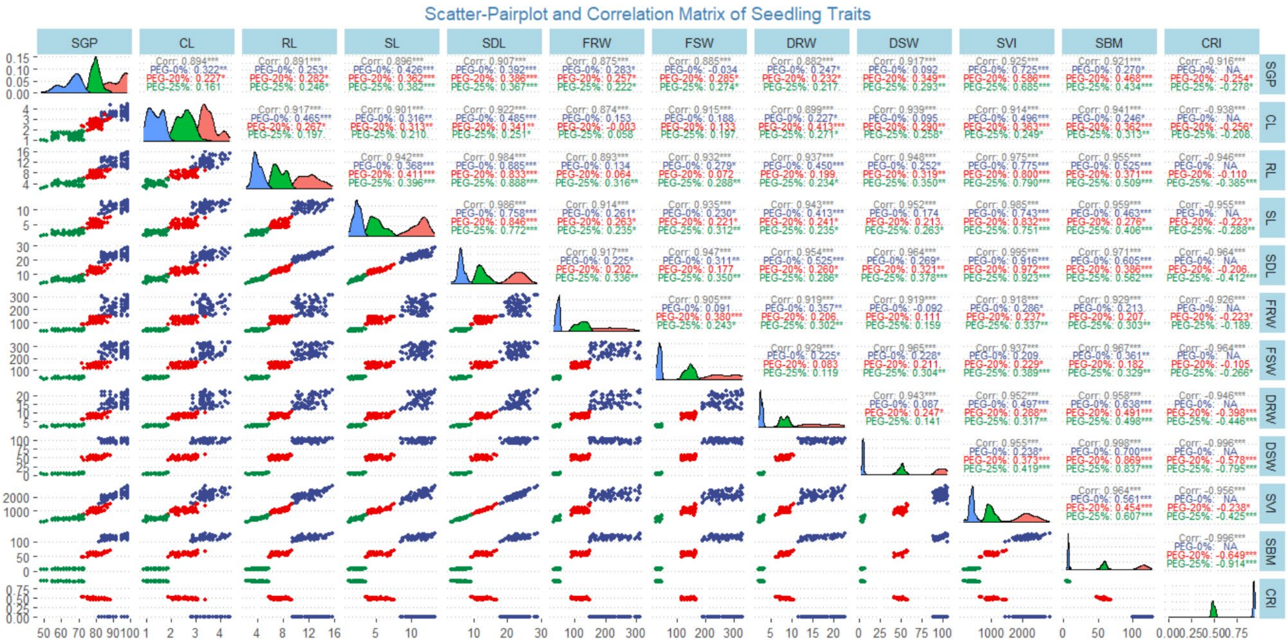

Fig. 3 Scatter-pairplot with correlation matrix and distribution histogram of the studied seedling traits of 80 wheat genotypes grown under control and PEG-induced drought stress. In the upper panel, black colored values overall or average positive and negative correlation of all three osmotic potentials, while blue, red and green values indicate positive and negative correlation of PEG-0% (control), PEG-20% and PEG-25% respectively. The diagonal panel indicates the distribution histogram of correlated traits with similar color trends. The lower panel indicates a scatter-pair plot of the correlated traits (Here SGP = Seed Germination Percentage, CL = Coleoptile Length, RL = Root Length, SL = Shoot Length, SDL = Seedling Length, FRW = Fresh Root Weight, DRW = Dry Root Weight, FSW = Fresh Shoot Weight, DSW = Dry Shoot Weight, SVI = Seedling Vigor Index, SBM = Seedling Biomass and CRI = Coefficient of Relative Inhibition.)

FSW and CRI, which exhibited notably negative correlations with most traits and non-significant negative associations with a few studied traits under both control and PEG-induced drought stress, the majority of shoot and root traits displayed significant positive correlations among themselves. The correlation study highlighted that SGP, CL, RL, SL, and SDL demonstrated positive correlations with all other seedling attributes under control and PEG-induced drought treatments, except for CRI, which displayed a negative association. Greater RL has been linked to drought resistance as it enables plants to access deeper soil water [1, 9]. Similarly, CL exhibited positive relationships of varying significance with most seedling variables under control and PEG-induced drought treatments, except for FRW and CRI, where it displayed a negative pattern. Correspondingly, FRW demonstrated positive associations with most seedling variables under control and PEG-induced drought treatments, except for DSW and CRI, where it indicated a negative pattern. Moreover, under both control and PEG-induced drought treatments, FSW exhibited positive correlations of varying significance with most seedling traits, except for SGP and CRI, where it displayed a negative pattern (Fig. 3). Furthermore, except for CRI, DRW, DSW, SVI, and SBM revealed positive associations with all examined variables at different significance levels [46].

Phenotypic correlations revealed that CRI displayed an adverse association with all seedling traits due to its inherent growth-inhibiting nature under both PEG-20% (ψ = -0.491 MPa) and PEG-25% (ψ = -0.735 MPa) drought conditions. These findings align with previous observations, indicating similar association patterns across different osmotic levels [15, 41]. With few exceptions in the correlation analysis, all examined traits in this study exhibited strong interrelationships (Fig. 3), indicating that alterations in any of these traits could impact others. This underscores the crucial role of these wheat seedling characteristics in determining the response to PEG-induced drought stress, suggesting that selecting one reliable feature under such conditions would influence other seedling traits [30]. These findings emphasize the significance of seedling

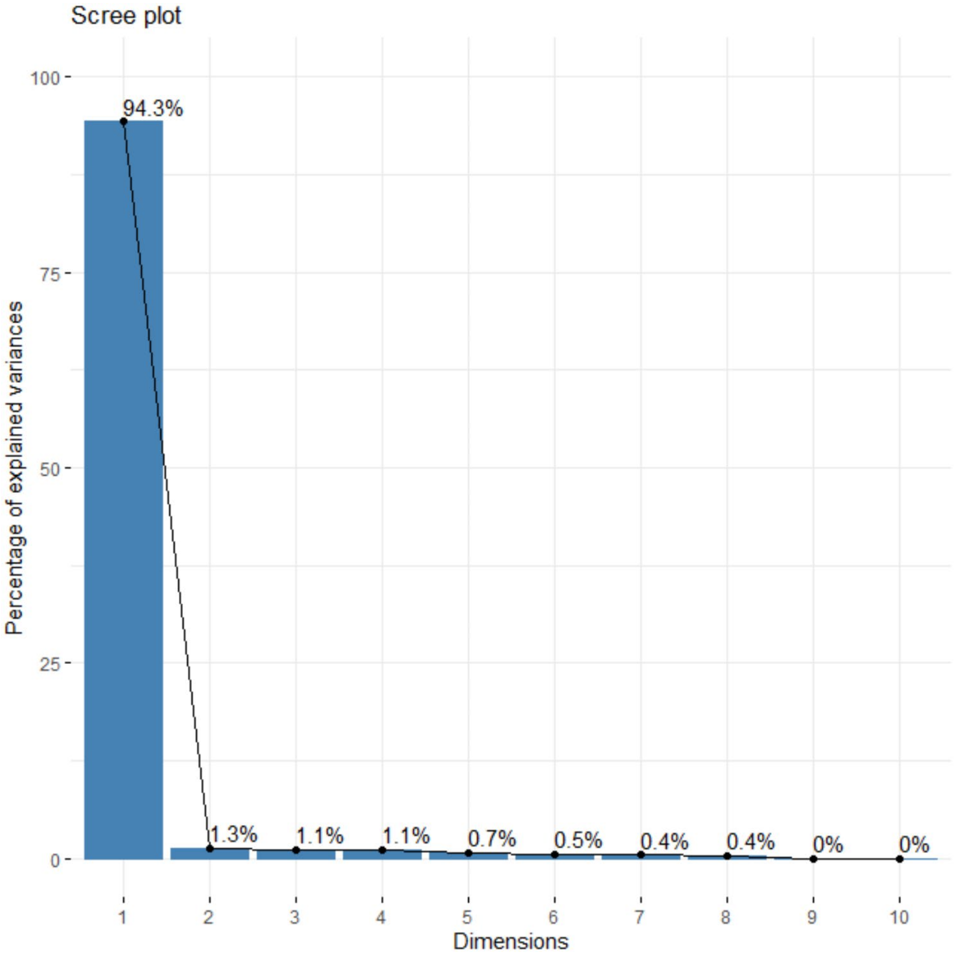

Fig. 4 Scree plot of principal component for seedling traits of bread wheat genotypes

and physiological attributes in aiding plants' survival and adaptation to water-stressed environments, thereby sustaining growth and productivity [2, 9]. Consequently, they advocate for the inclusion of these attributes in breeding programs to identify and select tolerant genotypes.

Principal component analysis

Principal Component Analysis (PCA) serves as a robust multivariate statistical method used to explore the genetic diversity within wheat germplasm and identify potential genotypes and significant genetic characteristics [48]. This analytical technique was employed to examine the variability patterns among wheat genotypes and assess their diversity and associations with observed traits based on trait correlations and derived clusters. Out of the total 12 principal components (PCs) identified, the first PC exhibited eigenvalues > 1, contributing to over 94% of the total variability. Hence, the first component was considered highly significant, as depicted in the scree plot (Fig. 4).

The non-significant PCs (eigenvalue < 1) were considered not worth further exploration. Typically, the sum of eigenvalues corresponds to the number of traits under consideration. In the scenarios of water stress and control treatment, the first factor encapsulates information representing 11.31% and 0.16% of the original traits, respectively. The PC values elucidated the comprehensive control of all features, encompassing approximately 94% of the genotypic variability related to PEG stress tolerance within the first component. Moreover, the first two PCs collectively explained 95% of the variability (Table 2). Furthermore, SBM, SDL, CRI, DSW, SVI, SL, and RL exhibited the highest variability within PC1, whereas SGP, CL, and SL showcased substantial variance within PC2 (as indicated in Table 2; Fig. 5). These attributes displayed notable fluctuations across the first two principal components, encompassing over 95% of the cumulative variance.

PCA biplot analysis has been widely and effectively used by other researchers to screen drought-tolerant wheat cultivars [29]. PCA Biplot I & II (Fig. 6) illustrates the relationships between various characteristics and wheat genotypes under moisture-deficient conditions induced by PEG^{6000}. In the PCA biplot, positive and negative loadings indicate positive and negative connections between the components and measured variables. Traits with high positive or negative loading significantly contributed to the observed diversity among the 80 confirmed wheat genotypes. For instance, CRI exhibited a positive association with PC1 and negative associations with the remaining traits, particularly SBM, SDL, and SVI, showing notably strong negative loadings (Table 2; Fig. 6). The second PC contributed approximately 1% of the total variation, with SL, SDL, and SVI mainly positioned on the positive side, while SGP and CL made significant contributions on the negative side. The loading of seedling characteristics represented the association pattern. Genotypes positioned around the vertex of the geom-polygon are considered to exhibit greater diversity compared to others [38].

The length and orientation of primary vectors (variables) represent variance and co-variance, respectively. An acute angle (90°) signifies a negative relationship between two characteristics [9, 48]. It's important to note that the angles between vectors of features in

Table 2 Rotated component matrix of seedling traits of 80 wheat genotypes assessed under various osmotic potentials

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
SGP	-0.278	-0.595	0.300	0.606	-0.225	-0.136	0.103	0.103	-0.122	0.078	-0.012	0.000
CL	-0.282	-0.485	0.086	-0.361	0.681	0.067	-0.229	0.159	0.025	-0.016	0.000	0.001
RL	-0.289	0.228	0.353	-0.116	0.212	0.057	0.642	-0.238	-0.204	0.101	-0.015	-0.398
SL	-0.290	0.318	0.160	0.103	-0.116	0.246	-0.608	0.186	-0.253	0.235	-0.008	-0.425
SDL	-0.294	0.279	0.258	-0.002	0.043	0.155	-0.003	-0.020	-0.221	0.181	-0.019	0.813
FRW	-0.280	0.104	-0.679	0.479	0.328	0.298	0.143	-0.047	0.008	0.029	-0.013	0.000
FSW	-0.288	-0.026	-0.220	-0.327	-0.354	0.110	0.325	0.719	0.011	-0.033	-0.009	0.001
DRW	-0.287	0.266	-0.150	0.042	0.146	-0.877	-0.082	0.111	-0.052	-0.003	-0.103	-0.001
DSW	-0.293	-0.147	-0.129	-0.219	-0.268	0.029	-0.061	-0.368	0.350	0.331	-0.619	-0.009
SVI	-0.293	0.234	0.306	0.177	0.024	0.098	-0.055	0.053	0.668	-0.521	0.024	-0.003
SBM	-0.295	-0.084	-0.129	-0.164	-0.196	-0.084	-0.045	-0.280	0.231	0.281	0.777	0.001
CRI	0.294	0.123	0.165	0.184	0.246	-0.034	0.119	0.352	0.452	0.658	0.011	-0.009
Standard Deviation	3.364	0.399	0.368	0.365	0.294	0.250	0.230	0.216	0.065	0.061	0.030	0.005
Eigenvalue	11.31	0.16	0.14	0.13	0.09	0.06	0.05	0.05	0.00	0.00	0.00	0.00
Proportion of Variance	94.29	1.33	1.13	1.11	0.72	0.52	0.44	0.39	0.04	0.03	0.01	0.00
Cumulative Proportion	94.29	95.61	96.75	97.85	98.58	99.10	99.54	99.93	99.96	99.99	100.00	100.00

(Here, SGP = Seed Germination Percentage, CL = Coleoptile Length, RL = Root Length, SL = Shoot Length, SDL = Seedling Length, FRW = Fresh Root Weight, DRW = Dry Root Weight, FSW = Fresh Shoot Weight, DSW = Dry Shoot Weight, SVI = Seedling Vigor Index, Seedling Biomass = SBM, CRI = Coefficient of Relative Inhibition.)

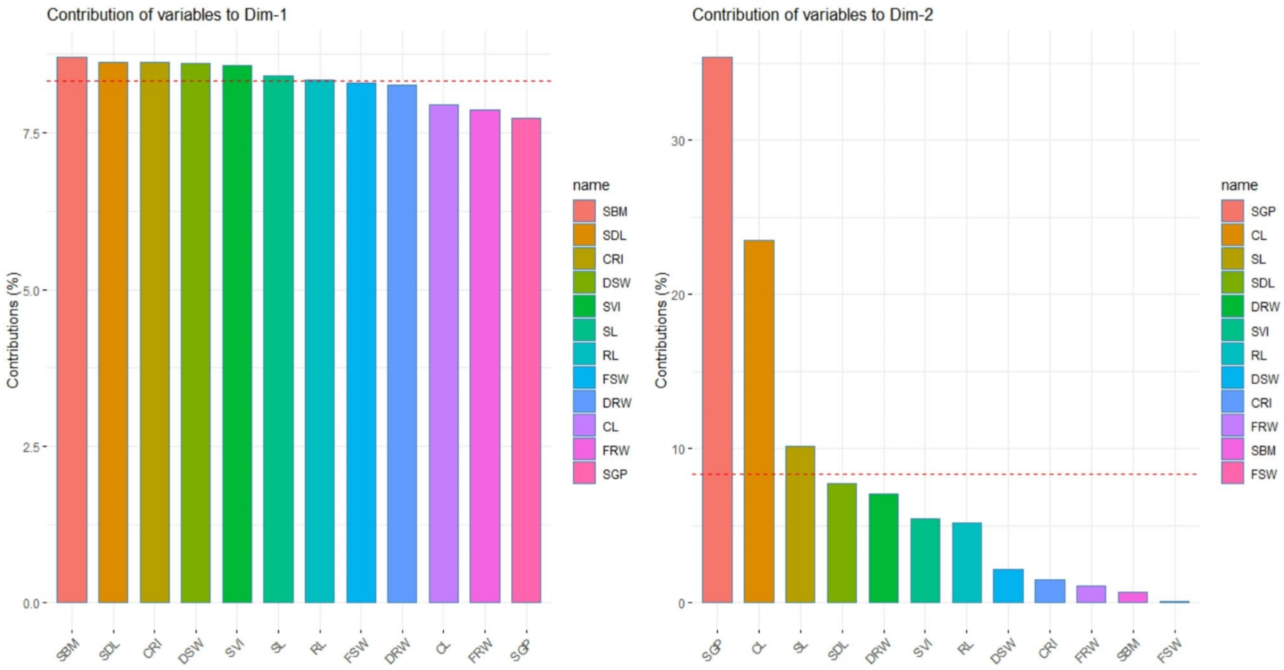

Fig. 5 Contribution of variance of Wheat Seedling Traits to PC1 and PC2 of principal components
(Here SGP = Seed Germination Percentage, CL = Coleoptile Length, RL = Root Length, SL = Shoot Length, SDL = Seedling Length, FRW = Fresh Root Weight, DRW = Dry Root Weight, FSW = Fresh Shoot Weight, DSW = Dry Shoot Weight, SVI = Seedling Vigor Index, SBM = Seedling Biomass and CRI = Coefficient of Relative Inhibition.)

biplot analysis don't perfectly correspond to correlation coefficients [5]. Examined features displayed higher magnitudes due to their longer vector lengths, significantly contributing to the overall variation (Fig. 6 - PCA Biplot I & II). Seedling traits with smaller or closer vector angles indicated a favorable correlation. For example, within Cluster-I, SL, SDL, SVI, RL, DRW, and SDL exhibited notably positive associations with each other. Similarly, FSW, SBM, and DSW displayed positive connections and were grouped into Cluster II. Additionally, CL and SGP showed a significant positive relationship within Cluster-III (Fig. 6). Members of Cluster-I demonstrated a negative association with members of Cluster-III due to the obtuse angle between them. Except for CRI, nearly all tested seedling traits were significantly linked with the initial PEG-0% cluster. The results indicated that correlations between pairs of traits aligned well with the estimation of vector angles and the contribution of the same trait pair in the PCA biplot. Typically, one variable is selected from these identified groups of primary components. Hence, CRI emerges as the optimal choice from stress treatment for the primary grouping, showing the most loadings from component one. Similar tendencies of highly contributing loadings of wheat seedling traits [12].

Basically, PCA biplot analysis helps identify the most diverse genotypes and classifies them into primary groups, exemplified by three distinct clusters (PEG-0%, PEG-20%, and PEG-25%) in our datasets. These clusters were determined based on the induced osmotic potentials of PEG^{6000}, considering both PC1 and PC2 simultaneously, as depicted in Fig. 6 (PCA Biplot-I & II). Genotypes within the first cluster (PEG-0%) showed a more extensive distribution compared to those in the other clusters. Additionally, the PCA biplot illustrates cluster centroids (the multidimensional average of the cluster) and their inter-distances, as seen in Fig. 6 PCA Biplot-I. The distance between a wheat genotype and the biplot origin signifies the genotype's distinctiveness concerning an average genotype [15]. Notably, wheat lines 30 (NARC-2009), 31 (Pakistan-2013), 36 (NR-499), 54 (33010), 77 (ARI13), 78 (Zardana-89), 79 (Zarghoon-79), 163 (18673), 172 (32821), 192 (Borlaug-2016), 199 (NR-514), and 200 (NR-516) exhibited extreme values for one or more attributes, as evident in Fig. 6 (PCA Biplot-II). While these genotypes may not necessarily be superior, they could serve as potential parental lines for certain drought-resistant seedling traits [41, 48].

Heatmapping of bread wheat seedling traits

The heatmap provides a comparative overview of genotype performance using the collected data. Presented in Fig. 7, the heatmap analysis considered the stress tolerance index (STI) under both PEG-20% (ψ

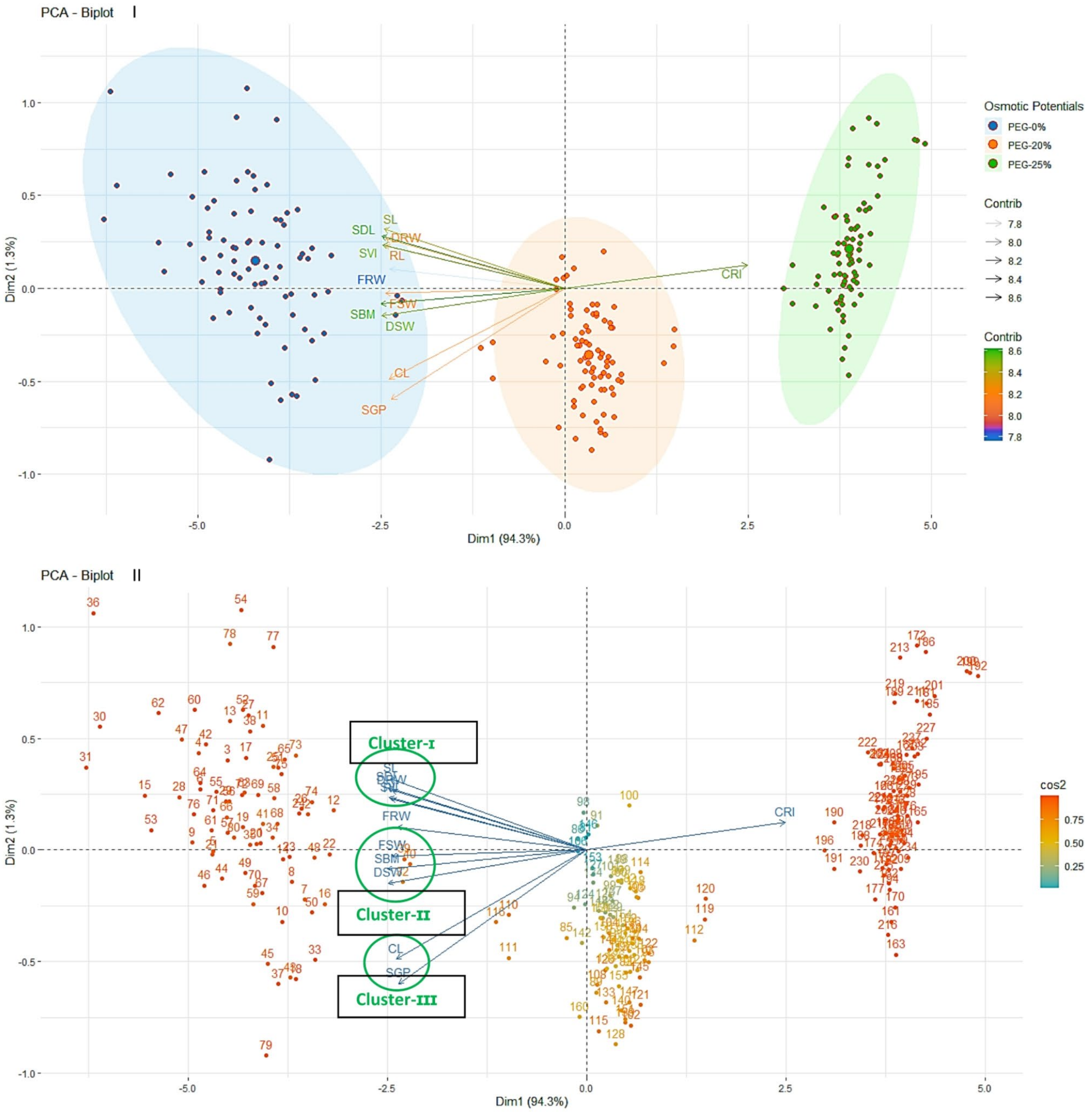

Fig. 6 Genotype by Trait Biplot of Principal Component Analysis of 80 wheat genotypes
(Here SGP = Seed Germination Percentage, CL = Coleoptile Length, RL = Root Length, SL = Shoot Length, SDL = Seedling Length, FRW = Fresh Root Weight, DRW = Dry Root Weight, FSW = Fresh Shoot Weight, DSW = Dry Shoot Weight, SVI = Seedling Vigor Index, SBM = Seedling Biomass and CRI = Coefficient of Relative Inhibition.)

= -0.491 MPa) and PEG-25% (ψ = -0.735 MPa) conditions. According to the color scale, the positive darker scale represents drought-resistant wheat genotypes for all seedling traits except CRI, as the lowest CRI genotypes are identified as drought-tolerant and vice versa [4]. Conversely, the darker negative stripes in the heatmap signify sensitive genotypes, excluding CRI (Fig. 7). Additionally, as the color intensity decreases, genotypes exhibit moderate performance within both positive and negative ranges under drought conditions induced by PEG^{6000} [9].

The analysis revealed that STI values for SGP were generally lower across most wheat genotypes under both osmotic levels, while a few showed an average drought potential. However, only a limited number of genotypes demonstrated an average capability to

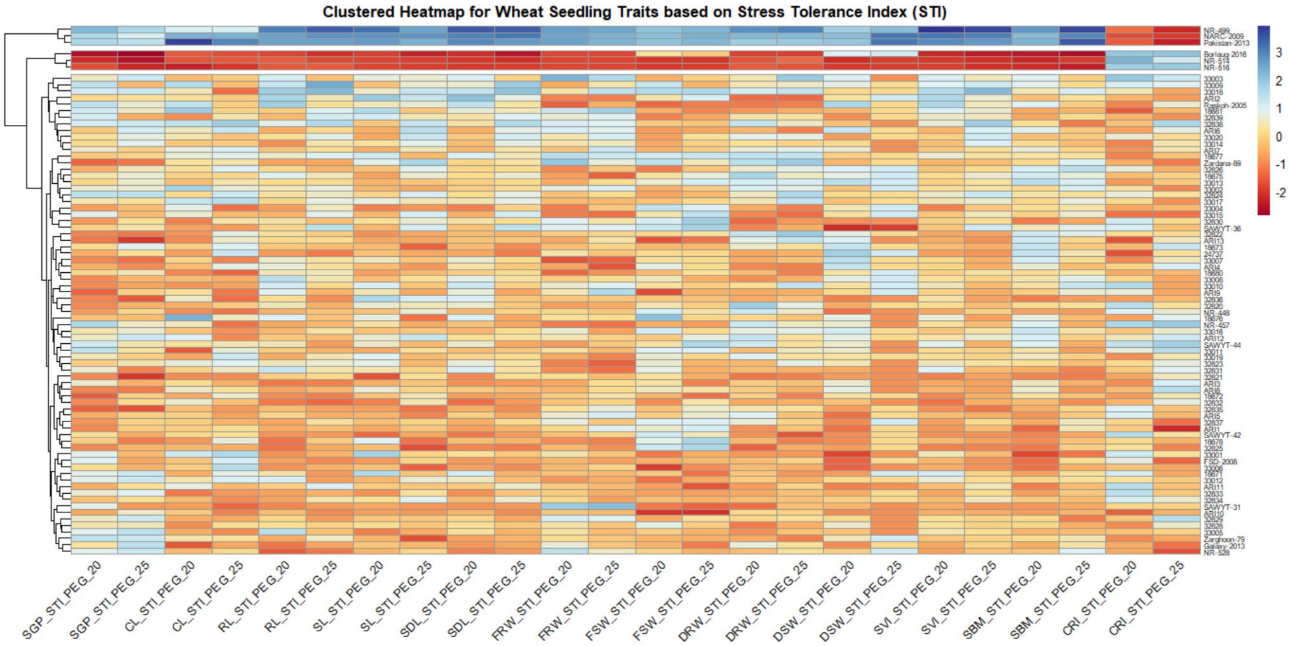

Fig. 7 Heatmap of various seedling traits of 80 bread wheat genotypes under PEG^{6000}-20% and PEG^{6000}-25% based on stress tolerance index (STI) (Here SGP = Seed Germination Percentage, CL = Coleoptile Length, RL = Root Length, SL = Shoot Length, SDL = Seedling Length, FRW = Fresh Root Weight, DRW = Dry Root Weight, FSW = Fresh Shoot Weight, DSW = Dry Shoot Weight, SVI = Seedling Vigor Index, SBM = Seedling Biomass and CRI = Coefficient of Relative Inhibition.)

withstand drought conditions. Notably, as stress levels increased, the performance of genotypes for this characteristic declined (Fig. 7). While CL genotypes showed significant differences between lower and higher stress levels, no notable distinctions were identified within the same genotype across both conditions. RL, SL, and SDL exhibited varying effects across different genotypes. Initially, all genotypes performed above the average line under minimal moisture stress. However, with increased stress, a reduction in these traits was observed across all genotypes. For most genotypes, fresh and dry root and shoot weights (FRW, FSW, DRW, and DSW) demonstrated medium to higher STI values. These four traits continuously varied by modifying the genotype, and their values were significantly impacted as drought conditions intensified from PEG-20% (ψ = -0.491 MPa) to PEG-25% (ψ = -0.735 MPa).

Fresh and dry weights of roots were more affected than shoots in the responses to moisture stress, as roots are largely important for absorbing water and nutrients from the soil. When moisture stress develops, the plant's root system becomes less effective in absorbing water. Roots may undergo a more dramatic decline in growth and biomass accumulation as a result of low water availability [41]. Furthermore, during a drought, plants may prioritize water conservation by reducing root growth, resulting in a greater loss in the fresh and dry weight of the roots than the shoots [2]. SVI displayed a declining trend under both stress conditions, with only a few genotypes showing positive STI values, indicating drought resistance. This trait is highly sensitive to drought stress due to its varying degree of dominance. SBM and CRI exhibited variable values among genotypes, remaining within a continuous range (Fig. 6). Most genotypes closely aligned with the mean value for these traits due to their mediocre performance. Overall, moisture stress significantly affected SBM and CRI across varying levels of osmotic stress. Furthermore, CRI demonstrated a diverse genotypic response under both levels of drought stress.

Wheat genotypes were segregated into three primary clusters, characterizing their distinct levels of tolerance, susceptibility, and intermediate performance [49]. Notably, the tolerant genotypes, such as NARC-09, Pakistan-2010, and NR-499, displayed favorable behavior across both stress conditions, exhibiting higher positive values for all seedling parameters except CRI, along with higher negative values for CRI (Fig. 7). These genotypes exhibited reduced susceptibility to stress phases compared to others, indicating their resilience to drought stress. Their overall performance trend, evident from the colored gradient strips, demonstrates their resilience. Conversely, the second cluster comprised three of the most sensitive wheat genotypes, Borlaug-2016, NR-514, and NR-516. The remaining wheat genotypes were placed in the third cluster, indicating an average response. Although some genotypes displayed significantly different responses under varying osmotic conditions, considering the collective outcomes, it can be concluded that NARC-09, Pakistan-2013, and NR-499 showcase drought resistance owing to their stable physiological metabolism. On the other hand, NR-514, NR-516, and Borlaug-2016 exhibit higher susceptibility to drought stress, as highlighted in Fig. 7. Consistent with recent findings, those emphasized that developing wheat ideotypes based on crucial seedling traits could significantly contribute to wheat improvement, bridging yield gaps, and unlocking crop production potential [9, 41, 49].

Selection based on multivariate analysis and stress tolerance index (STI)

The multivariate analysis was used to investigate the links and patterns between several wheat genotypes and seedling traits under drought stress. This approach was used to identify discrete genotype clusters, evaluate the diversity in stress response, and obtain a better knowledge of the underlying traits that contribute to drought tolerance. The STI values were employed as criteria for selecting the most drought-tolerant wheat genotypes, with higher STI values indicating drought tolerance, while lower STI values suggested susceptibility to drought. Among these findings, NR-499 emerged as a drought-tolerant wheat genotype by exhibiting highest STI values, followed by NARC-2009 and Pakistan-2013 (Table 3), demonstrating commendable performance across numerous seedling traits. In contrast, Borlaug-2016, NR-514, and NR-516 exhibited sensitivity to drought due to relatively lower STI values because of compromised physiological attributes during early germination under both moisture stress conditions. Regarding most diverse traits such as SBM, SDL, SVI, and CRI have been identified as promising indicators and are strongly associated with drought tolerance. These seedling traits can also be used as baseline markers for subsequent screening of drought-tolerant wheat varieties. It is also recommended to utilize these identified complementary wheat genotypes and seedling traits to assess their genetic, physiological, biochemical and molecular characterization that contribute to their resilience more broadly, possibly revealing novel pathways to improve drought tolerance under unpredictable climatic shifts.

Table 3 Selection of top five drought tolerant and Botton five susceptible wheat genotypes based on stress tolerance index (STI) under osmotic stress of PEG-25% (ψ = -0.735 MPa)

	Genotypes	**SGP**$^{(PEG-25\%)}$	**Genotypes**	**CL**$^{(PEG-25\%)}$	**Genotypes**	**RL**$^{(PEG-25\%)}$	**Genotypes**	**SL**$^{(PEG-25\%)}$	**Genotypes**	**SDL**$^{(PEG-25\%)}$	**Genotypes**	**FRW**$^{(PEG-25\%)}$
Top Five Drought Tolerant Genotypes	NR-499	0.842	Pakistan-2013	0.669	NR-499	0.621	NARC-2009	0.309	NR-499	0.491	NR-499	0.352
	33,005	0.822	NARC-2009	0.608	NARC-2009	0.563	NR-499	0.303	NARC-2009	0.466	NARC-2009	0.343
	32,824	0.821	32,838	0.564	Pakistan-2013	0.545	Pakistan-2013	0.287	Pakistan-2013	0.444	Pakistan-2013	0.341
	Galaxy-2013	0.821	33,001	0.544	33,009	0.536	Zardana-89	0.260	33,018	0.400	SAWYT-31	0.334
	Pakistan-2013	0.821	33,017	0.533	33,018	0.513	ARI6	0.254	33,009	0.390	18,681	0.306
	Genotypes	**FSW**$^{(PEG-25\%)}$	**Genotypes**	**DRW**$^{(PEG-25\%)}$	**Genotypes**	**DSW**$^{(PEG-25\%)}$	**Genotypes**	**SVI**$^{(PEG-25\%)}$	**Genotypes**	**SBM**$^{(PEG-25\%)}$	**Genotypes**	**CRI**$^{(PEG-25\%)}$
	NARC-2009	0.218	NR-499	0.261	Pakistan-2013	0.081	NR-499	0.411	NR-499	0.101	Pakistan-2013	0.918
	NR-499	0.215	NARC-2009	0.230	NARC-2009	0.079	NARC-2009	0.366	Pakistan-2013	0.099	ARI1	0.919
	Pakistan-2013	0.214	33,003	0.221	NR-499	0.073	Pakistan-2013	0.351	NARC-2009	0.093	NR-499	0.919
	32,830	0.200	32,822	0.220	33,015	0.062	33,018	0.320	32,826	0.078	NARC-2009	0.921
	18,678	0.198	Zardana-89	0.218	32,826	0.061	32,838	0.296	33,010	0.076	NR-528	0.923
Bottom Five Drought Susceptible Genotypes	**Genotypes**	**SGP**$^{(PEG-25\%)}$	**Genotypes**	**CL**$^{(PEG-25\%)}$	**Genotypes**	**RL**$^{(PEG-25\%)}$	**Genotypes**	**SL**$^{(PEG-25\%)}$	**Genotypes**	**SDL**$^{(PEG-25\%)}$	**Genotypes**	**FRW**$^{(PEG-25\%)}$
	32,821	0.534	18,680	0.262	NR-528	0.219	32,825	0.104	24,737	0.203	32,823	0.132
	ARI13	0.534	SAWYT-31	0.259	32,832	0.207	Zarghoon-79	0.098	32,825	0.201	ARI4	0.123
	NR-516	0.512	NR-514	0.244	NR-516	0.189	NR-516	0.087	NR-516	0.146	NR-516	0.113
	NR-514	0.486	Borlaug-2016	0.228	NR-514	0.171	NR-514	0.085	NR-514	0.136	NR-514	0.110
	Borlaug-2016	0.460	NR-516	0.204	Borlaug-2016	0.142	Borlaug-2016	0.069	Borlaug-2016	0.113	Borlaug-2016	0.108
	Genotypes	**FSW**$^{(PEG-25\%)}$	**Genotypes**	**DRW**$^{(PEG-25\%)}$	**Genotypes**	**DSW**$^{(PEG-25\%)}$	**Genotypes**	**SVI**$^{(PEG-25\%)}$	**Genotypes**	**SBM**$^{(PEG-25\%)}$	**Genotypes**	**CRI**$^{(PEG-25\%)}$
	SAWYT-31	0.092	33,015	0.100	32,838	0.036	32,825	0.133	32,838	0.052	32,838	0.949
	ARI11	0.086	ARI2	0.096	33,011	0.036	32,835	0.118	33,001	0.051	SAWYT-44	0.950
	NR-516	0.076	SAWYT-42	0.091	NR-514	0.031	NR-516	0.075	NR-514	0.044	NR-516	0.951
	NR-514	0.073	NR-516	0.091	NR-516	0.028	NR-514	0.066	NR-516	0.040	NR-457	0.951
	ARI10	0.072	Borlaug-2016	0.079	SAWYT-36	0.026	Borlaug-2016	0.052	Borlaug-2016	0.037	Borlaug-2016	0.952

(Here, SGP = Seed Germination Percentage, CL = Coleoptile Length, RL = Root Length, SL = Shoot Length, SDL = Seedling Length, FRW = Fresh Root Weight, DRW = Dry Root Weight, FSW = Fresh Shoot Weight, DSW = Dry Shoot Weight, SVI = Seedling Vigor Index, Seedling Biomass = SBM, CRI = Coefficient of Relative Inhibition.)

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06345-9.

Supplementary Material 1

Supplementary Material 2

Acknowledgements
Thankful to the Government of Balochistan to conduct this PhD based research.

Author contributions

All authors shares equal rights and have no conflict of interest.

Funding
Project was funded bythe Agriculture Department, Government of Balochistan, Pakistan.

Data availability

All primary and secondary data have been provided and uploaded as supplementary files in the submission manuscript.

Declarations

Ethics approval and consent to participate

No Ethical issues are related to this research article.

Consent for publication

No special consent is required as all authors are agreed to publish.

Competing interests

The authors declare no competing interests.

Clinical trial number

Not applicable.

Author details
[1]Directorate of Cereal Crops, Agriculture Research Institute, Islamabad, Pakistan
[2]Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

Received: 21 October 2024 / Accepted: 4 March 2025

References

1. Ahmed K, Shabbir G, Ahmed M, Noor S, Mohi Ud Din A, Qamar M, Rehman N. Expression profiling of *TaARGOS* homoeologous drought responsive genes in bread wheat. Sci Rep. 2022;12(1):3595. https://doi.org/10.1038/s41598-022-07637-y
2. Guizani A, Askri H, Amenta ML, Defez R, Babay E, Bianco C, Gharbi F. Drought responsiveness in six wheat genotypes: identification of stress resistance indicators. Front Plant Sci. 2023;14. https://doi.org/10.3389/fpls.2023.1232583
3. Awan SA, Khan I, Rizwan M, Zhang X, Brestic M, Khan A, Huang L. Exogenous abscisic acid and jasmonic acid restrain polyethylene glycol-induced drought by improving the growth and antioxidative enzyme activities in Pearl millet. Physiol Plant. 2021;172(2):809–19. https://doi.org/10.1111/ppl.13247
4. Mustamu NE, Tampubolon K, Basyuni M, Al-Taey DK, Janabi HJ K. A, Mehdizadeh M. Drought stress induced by polyethylene glycol (PEG) in local maize at the early seedling stage. Heliyon. 2023;9(9). https://doi.org/10.1016/j.heliyon.2023.e20209.
5. Mickky BM, Aldesuquy HS. Impact of osmotic stress on seedling growth observations, membrane characteristics and antioxidant defense system of different wheat genotypes. Egypt J Basic Appl Sci. 2017;4(1):47–54. https://doi.org/10.1016/j.ejbas.2016.10.001
6. Mahpara S, Zainab A, Ullah R, Kausar S, Bilal M, Latif MI, Zuan ATK. The impact of PEG-induced drought stress on seed germination and seedling growth of different bread wheat (*Triticum aestivum* L.) genotypes. PLoS ONE. 2022;17(2):e0262937. https://doi.org/10.1371/journal.pone.0262937
7. Licaj I, Di Meo MC, Fiorillo A, Samperna S, Marra M, Rocco M. Comparative analysis of the response to polyethylene glycol-simulated drought stress in roots from seedlings of modern and ancient wheat varieties. Plants. 2023;12(3):428. https://doi.org/10.3390/plants12030428
8. Wood JD, Gu L, Hanson PJ, Frankenberg C, Sack L. The ecosystem wilting point defines drought response and recovery of a Quercus-Carya forest. Glob Change Biol. 2023;29(7):2015–29. https://doi.org/10.1111/gcb.16582
9. Ahmed K, Shabbir G, Ahmed M, Shah KN. Phenotyping for drought resistance in bread wheat using physiological and biochemical traits. Sci Total Environ. 2020;729:139082. https://doi.org/10.1016/j.scitotenv.2020.139082
10. Noorka IR. Effect of drought/water stress and adaptation of unintended consequences on wheat growth and development in Pakistan. Hand Book Plant Crop Physiol. 2014;(3):967–978. https://doi.org/10.1201/B16675-28
11. Rauf M, Munir M, ul Hassan M, Ahmad M, Afzal M. Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage. Afr J Biotechnol. 2007;6(8). https://doi.org/10.5897/AJB2007.000-2119
12. Hussain HA, Men S, Hussain S, Chen Y, Ali S, Zhang S, Wang L. Interactive effects of drought and heat stresses on morphophysiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. Sci Rep. 2019;9(1):1–12. https://doi.org/10.1038/s41598-019-40362-7
13. Hubbard M, Germida J, Vujanovic V. Fungal endophytes improve wheat seed germination under heat and drought stress. Botany. 2012;90(2):137–49. https://doi.org/10.1139/b11-091
14. Chunyan L, Xiangchi Z, Chao L, Cheng L. Ionomic and metabolic responses of wheat seedlings to PEG-6000-simulated drought stress under two phosphorus levels. PLoS ONE. 2022;17(9):e0274915. https://doi.org/10.1371/journal.pone.0274915
15. Khan A, Khaliq I, Ahmad M, Ahmed H, Khan AG, Farooq MS. Comparative performance of spring wheat (*Triticum aestivum* L.) through heat stress indices. Pak J Bot. 2018;50:4.
16. Bayoumi TY, Eid MH, Metwali EM. Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. Afr J Biotechnol. 2008;7(14). https://doi.org/10.5897/AJB2008.000-505981-488
17. Mérida-García R, Liu G, He S, Gonzalez-Dugo V, Dorado G, Gálvez S, Hernandez P. Genetic dissection of agronomic and quality traits based on association mapping and genomic selection approaches in durum wheat grown in Southern Spain. PLoS ONE. 2019;14(2):e0211718. https://doi.org/10.1371/journal.pone.0211718
18. Gasparovic K, Zivcak M, Brestic M, Hauptvogel P. Diversity of leaf cuticular transpiration and growth traits in field-grown wheat and aegilops genetic resources. Agronomy. 2021;11(3):522. https://doi.org/10.3390/agronomy11030522
19. Michel BE, Kaufmann MR. The osmotic potential of polyethylene glycol 6000. Plant Physiol. 1973;51:914–6. https://doi.org/10.1104/pp.51.5.914.
20. Farooq S, Onen H, Ozaslan C, Baskin CC, Gunal H. Seed germination niche for common ragweed (*Ambrosia Artemisiifolia* L.) populations naturalized in Turkey. South Afr J Bot. 2019;123:361–71. https://doi.org/10.1016/j.sajb.2019.03.031
21. Jiang Y, MacDonald SE, Zwiazek JJ. Effects of cold storage and water stress on water relations and gas exchange of white Spruce (*Picea glauca*) seedlings. Tree Physiol. 1995;15(4):267–73. https://doi.org/10.1093/treephys/15.4.267
22. Zarei L, Farshadfar E, Haghparast R, Rajabi R, Badieh MMS. Evaluation of some indirect traits and indices to identify drought tolerance in bread wheat (*Triticum aestivum* L). Asian J Plant Sci. 2007;6(8):1204–10. https://doi.org/10.3923/ajps.2007.1204.1210
23. Shahbazi F. A study on the seed susceptibility of wheat (*Triticum aestivum* L.) cultivars to impact damage. J Agricultural Sci Technol. 2012;14(3):505–12.
24. Afzal F, Reddy B, Gul A, Khalid M, Subhani A, Shazadi K, Rasheed A. Physiological, biochemical and agronomic traits associated with drought tolerance in a synthetic-derived wheat diversity panel. Crop Pasture Sci. 2017;68(3):213–24. https://doi.org/10.1071/CP16367
25. Turkan I, Bor M, Ozdemir F, Koca H. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Sci. 2005;168:223–31. https://doi.org/10.1016/j.plantsci.2004.07.032
26. Passioura JB. Phenotyping for drought tolerance in grain crops: when is it useful to breeders? Funct Plant Biol. 2012;39(11):851–9. https://doi.org/10.1071/fp12079

27. Richard CA, Hickey LT, Fletcher S, Jennings R, Chenu K, Christopher JT. High-throughput phenotyping of seminal root traits in wheat. Plant Methods. 2015;11(1):1–11. PMID: 25750658. https://doi.org/10.1186/s13007-015-0055-9
28. Gholami A, Sharafi S, Sharafi A, Ghasemi S. Germination of different seed size of Pinto bean cultivars as affected by salinity and drought stress. J Food Agric Environ. 2009;7(2):55.
29. Ghosh S, Shahed MA, Robin AHK. Polyethylene glycol induced osmotic stress affects germination and seedling establishment of wheat genotypes. Plant Breed Biotechnol. 2020;8(2):174–85. https://doi.org/10.9787/PBB.2020.8.2.174
30. Mohi-Ud-Din, M., Hossain, M., Rohman, M., Uddin, M., Haque, M., Ahmed, J. U.,… Mostofa,M. G. Multivariate analysis of morpho-physiological traits reveals differential drought tolerance potential of bread wheat genotypes at the seedling stage. Plants. 2021;10(5):879. https://doi.org/10.3390/plants10050879.
31. Mohammadi-joo S, Mirasi A, Saeidi-aboeshaghi R, Amiri M. Evaluation of bread wheat (*Triticum aestivum* L.) genotypes based on resistance indices under field conditions. Int J Bioscience. 2015;6(2):331–7. https://doi.org/10.12692/ijb/6.2.331-337
32. Nouraein M, Mohammadi SA, Aharizad S, Moghaddam M, Sadeghzadeh B. Evaluation of drought tolerance indices in wheat Recombinant inbred line population. Ann Biol Res. 2013;4(3):113–22.
33. Li, Y., Li, X., Zhang, J., Li, D., Yan, L., You, M.,… Yan, J. Physiological and proteomic responses of contrasting Alfalfa (Medicago sativa L.) varieties to high temperature stress. Frontiers in Plant Science. 2021;12. https://doi.org/10.3389/fpls.2021.7530115-558.
34. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria 1. Crop Sci. 1973;13(6):630–3. https://doi.org/10.2135/cropsci1973.0011183X001300060013x
35. Fernandez GC. Effective selection criteria for assessing plant stress tolerance. 1993:257–270.
36. Heinisch O. Steel, RGD, and JH Torrie: Principles and procedures of statistics. (With special reference to the biological sciences.) McGraw-Hill Book Company, New York, Toronto, London 1960, 481 S., 15 Abb.; 81 s 6 d. 1962.
37. Ashraf M. Inducing drought tolerance in plants: recent advances. Biotechnol Adv. 2010;28(1):169–83. https://doi.org/10.1016/j.biotechadv.2009.11.005
38. Wattoo FM, Rana RM, Fiaz S, Zafar SA, Noor MA, Hassan HM,…Amir RM. Identification of drought tolerant maize genotypes and seedling based morpho-physiological selection indices for crop improvement. Sains Malays. 2018;47:295–302. https://doi.org/10.17576/jsm-2018-4702-11
39. Monneveux P, Jing R, Misra SC. Phenotyping wheat for adaptation to drought. Drought Phenotyping Crops: Theory Practice: CGIAR Generation Chall Program. 2011. https://doi.org/10.3389/fphys.2012.00429
40. Faisal S, Mujtaba SM, Asma, Mahboob W. Polyethylene glycol mediated osmotic stress impacts on growth and biochemical aspects of wheat (*Triticum aestivum* L). J Crop Sci Biotechnol. 2019;22:213–23. https://doi.org/10.1007/s12892-018-0166-0
41. Qi Y, Ma L, Ghani MI, Peng Q, Fan R, Hu X, Chen X. Effects of drought stress induced by hypertonic polyethylene glycol (PEG-6000) on *Passiflora edulis sims* physiological properties. Plants. 2023;12(12):2296. https://doi.org/10.3390/plants12122296
42. Khadka K, Earl HJ, Raizada MN, Navabi A. A physio-morphological trait-based approach for breeding drought tolerant wheat. Front Plant Sci. 2020;11:715. https://doi.org/10.3389/fpls.2020.00715
43. Liu X, Huang B. Heat stress injury in relation to membrane lipid membrane maintenance in tall fescue. Crop Sci. 2000;32:251–6.
44. Radhouane L. Response of Tunisian autochthonous Pearl millet (*Pennisetum glaucum* (L.) R. Br.) to drought stress induced by polyethylene glycol (PEG) 6000. Afr J Biotechnol. 2007;6(9). https://doi.org/10.4314/AJB.V6I9.57121
45. Marmar A, Baenziger S, Dweikat I, El Hussein AA. Preliminary screening for water stress tolerance and genetic diversity in wheat (*Triticum aestivum* L.) cultivars from Sudan. J Genetic Eng Biotechnol. 2013;11(2):87–94. https://doi.org/10.1016/j.jgeb.2013.08.004
46. Hellal FA, El-Shabrawi HM, El-Hady AM, Khatab IA, El-Sayed SAA, Abdelly C. Influence of PEG induced drought stress on molecular and biochemical constituents and seedling growth of Egyptian barley cultivars. J Genetic Eng Biotechnol. 2018;16(1):203–12. https://doi.org/10.1016/j.jgeb.2017.10.009
47. Wasaya A, Zhang X, Fang Q, Yan Z. Root phenotyping for drought tolerance: a review. Agronomy. 2018;8(11):241. https://doi.org/10.3390/agronomy8110241
48. Abid MA, Malik W, Yasmeen A, Qayyum A, Zhang R, Liang C, Ashraf J. Mode of inheritance for biochemical traits in genetically engineered cotton under water stress. Annals Bot (AoB) Plants. 2016;8. https://doi.org/10.1093/aobpla/plw008
49. Huang B, Fu J. Photosynthesis, respiration, and carbon allocation of two cool-season perennial grasses in response to surface soil drying. Plant Soil. 2000;227(1):17–26. https://doi.org/10.1023/A:1026512212113

Publisher's note