Evaluation of Early Growth Response of Wheat Genotypes Grown Under PEG-Mediated Water Stress Conditions

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Abstract

The yield of bread wheat (Triticum aestivum L.), an important staple food cereal crop, is constantly challenged by drought stress. The unexpected changes in rainfall and heat spells induced by climate change further aggravate wheat production. Strategies to fast-screen wheat genotypes for drought stress in a controlled environment are key to wheat breeding programs' success in developing drought-tolerant wheat cultivars. The current study was designed to evaluate the early growth response of wheat genotypes grown under PEG-mediated water stress conditions. Six wheat genotypes comprising local wheat cultivars, landraces and durum wheat advanced lines were grown in Petr-dishes and pots under PEG-mediated water stress conditions (5% and 10% PEG), using a Completely Randomized Design (CRD). Data collected for parameters related to early growth responses such as root, coleoptile and leaf length, root fresh and dry biomass, no. of tillers plant-1, and leaf total chlorophyll contents were subjected to R-package. Results showed significant differences among all early growth parameters genotypes in response to 5% and 10% PEG concentrations (p = 0.05). PEG concentration of 10% significantly reduced root length, root density, coleoptile length, root fresh and dry weight, and leaf total chlorophyll contents among all genotypes. Wheat landraces and advanced durum wheat lines showed better drought tolerance at 10% PEG concentration relative to two wheat cultivars AZRC Dera and Akbar-19 by maintaining optimal root length, coleoptile length, root density and root weight. Landrace acc#11239 and durum line IDN-733 were found to be most drought tolerant to 10% high PEG concentration in terms of root and coleoptile length and root fresh and dry weight. The findings of this study are important for breeders to evaluate wheat genotypes for drought stress tolerance and develop climate-resilient and drought-tolerant wheat cultivars for better yield production.

Keywords: PEG, Drought, AZRC Dera, Akbar-19, Wheat landrace, Durum wheat

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Introduction

Wheat (*Triticum aestivum* L.) belongs to the grass family, also known as Poaceae, and more than half of the global human population directly or indirectly depends on this important cereal crop. Wheat tremendously contributes to global food security owing to its staple food nature in many parts of the world (Mottaleb *et al.*, 2022). The fast-growing global human population necessitates to enhance wheat productivity. The Green Revolution in 1960-1970 introduced semi-dwarf and pest-resistant wheat varieties that hugely contributed to boosting wheat yield to feed the hungry world. However, the pace of wheat productivity is static post-green revolution. Different biotic and abiotic stresses are drastically affecting current wheat productivity. The ongoing global climate change is expected to further aggravate the dilemma of static wheat productivity in different wheat-growing regions of the world. Thus, future wheat productivity enhancement will be achieved through breeding efforts to develop climate-smart wheat varieties better adapted to abiotic stress such as drought, salinity and heat stress, and biotic stresses such as rust, powdery mildew and scab (Shiferaw *et al.*, 2013).

The productivity of wheat is severely affected by drought stress, a major abiotic constraint of wheat yield, at different growth stages such as germination, seedling, tillering, booting, heading, anthesis and grain-filling. Seed germination and early growth are important transition stages for crop plants and contribute to overall crop productivity. Seed germination, vigour and coleoptile length are essential for the success of stand establishment of the crop. The semi-arid regions of the world experience low moisture availability during seed germination of wheat crops (Farooq *et al.*, 2019). Low moisture availability during seed germination followed by seedling and subsequent growth stages of wheat crops declines both production and maturity time (Bayoum *et al.*, 2008; Rauf *et al.*, 2007). The seedling stage of crop plants is highly vulnerable to the water deficit. The coleoptile length (protective sheath that covers the shoot during emergence) assisting the early growth of seedlings is important in achieving optimum height and establishment of a crop, particularly when the seed is planted deep to reach moisture in dry soils (Dilday *et al*. 1990). Thus, there is a need to improve the tolerance of crops at the seedling stages.

To develop drought-tolerant wheat cultivars, there is a need to perform lab and field-based screening of wheat germplasm for drought tolerance. However, breeding efforts to develop drought-tolerant wheat varieties have been limited due to the availability of drought-screening techniques. The lab-based screening requires a potent chemical agent to induce drought stress and ultimately cause morphological and biochemical changes in wheat. Such changes are used as markers/indicators to record the response of a wheat genotype to drought stress (Meneses *et al.*, 2011). Polyethylene glycol (PEG), a drought-inducing chemical, is frequently used to screen drought-tolerant wheat germplasm at an early stage of seedlings under laboratory conditions. PEG is non-ionic and inert and induces drought in vitro without physically entering plant cells. Previous studies have documented the application of PEG with varying concentrations to induce drought under control conditions (Jatoi *et al.*, 2014; Nawaz *et al.*, 2013). The current study was designed to evaluate different wheat genotypes grown under varying concentrations of PEG-mediated water stress for early growth responses.

Material and Method

The current research was conducted in the post-graduate research lab of the Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University Dera Ismail Khan.

Material

A total of six different wheat genotypes were subjected to varying concentrations of PEG. Genetically diverse germplasm, comprising varieties, landraces and durum wheat, was selected to check their response to drought stress. Detailed information on the wheat genotypes is given in Table 1.

Table 1 Germplasm used in this study

S. No.	Genotype name	Nature of the genotype
1	AZRC Dera	Wheat variety
2	Akbar-19	Wheat variety
3	Acc#011214	Wheat landrace
4	Acc#011239	Wheat landrace
5	IDN-720	Advanced durum wheat line
6	IDN-733	Advanced durum wheat line

Research Design

The research design constituted two drought treatments of varying concertation of PEG (5% w/v PEG in H_2O and 10% w/v PEG in H_2O) in Completely Randomized Design (CRD) using three replications. The experiment was conducted both in Petri plates and pots.

PEG treatment in pots

The seeds of six genotypes were sown in pots of uniform size (30 x 30 cm). Each Pot was filled with 10 kg of air-dried soil (soil + farm yard manure in a 3:1 ratio). A recommended dose of a mixture of fertilizers (2N: 1P: 1K) in the form of urea, single super phosphate (SSP) and potash mutate, respectively was applied to each pot. Normal irrigation was applied to each plot. Eight seeds of each genotype were sown in each pot. Later on, only three plants of uniform size were left after thinning in each pot before PEG treatment. PEG treatments were applied 42 days after sowing (DAS) according to the method of Kumari *et al*., (2014). All genotypes were grown at optimum temperature (25°C/20 °C day/night) and light period (16-h/8-h light/dark cycle).

PEG treatment in Petri-dishes

Germination, root and seedling growth experiments were performed in Petri-dishes. Each petri dish comprised five seeds of each genotype soaked in 0.1 % $HgCl_2$ for one minute, followed by rinsing with running tap water and finally with double distilled water. Each Petri dish was lined with Whatman No. 1 filter paper soaked with Hoagland's solution. A total of nine Petri dishes were used for each genotype, three for each of the control with normal watering, three for 10% PEG-6000 and three for 10% PEG-6000 treatment. Thus, a total of fifty-four Petri dishes were used for six genotypes in CRD. All genotypes were grown at optimum temperature (25°C/20 °C day/night) and light period (16-h/8-h light/dark cycle). All genotypes were exposed to PEG-mediated drought stress for eight days. Seedlings were harvested and processed 14 days after soaking (SAD) for scoring data related to germination, root and seedling growth *etc*.

Parameters

Early growth-related parameters

Three seedlings per pot were harvested for storing data related to shoot, root, coleoptile and tillers-related growth. Fresh weights of these seedlings were also measured instantly and dry weights were taken after oven drying for 72 h at 60°C. Data recorded for early growth-related parameters include germination%, coleoptile length, leaf length, no. of tillers, root length, shoot length, vigour index = shoot length X germination%), root fresh weight, root dry weights, shoot fresh weight, shoot dry weight, leaf fresh weight and leaf dry weight.

Physiological parameter

Total leaf chlorophyll contents

Accumulation of total chlorophyll contents in a leaf in response to PEG is a remarkable physiological parameter depicting a genotype drought tolerance. Leaf total chlorophyll contents were measured using a digital SPAD meter (SPAD 502, UK).

Statistical analysis

Data collected was subjected to R-package (https://www.r-project.org/) for measuring means and Least Significant Differences (LSDs), and graphical presentation.

RESULTS

Effect of PEG on root length

Results showed significant differences in the mean root length of all genotypes in response to both PEG treatments (p = 0.05). Control of the AZRC Dera genotype showed extensive root density and root length while the maximum decrease in root length and root density was induced by higher PEG concentration (10%), followed by optimum PEG concentration of 5% PEG (Fig. 1). Similar trend of decrease in root length by both PEG treatments were observed in Akbar-19, the two landraces and durum lines. Root length was more severely affected in AZRC Dera, Akbar-19 and landrace acc#11214, especially by the application of 10% PEG. On the contrary, durum line IDN-733 was the most PEG-tolerant genotype followed by IDN-702 and landrace acc#11239 in terms of root length (Fig. 2).

Fig. 1 Response of root length of AZRC Dera to varying concentrations of PEG. **A:** 5% PEG, **B:** 10% PEG, **C:** control.

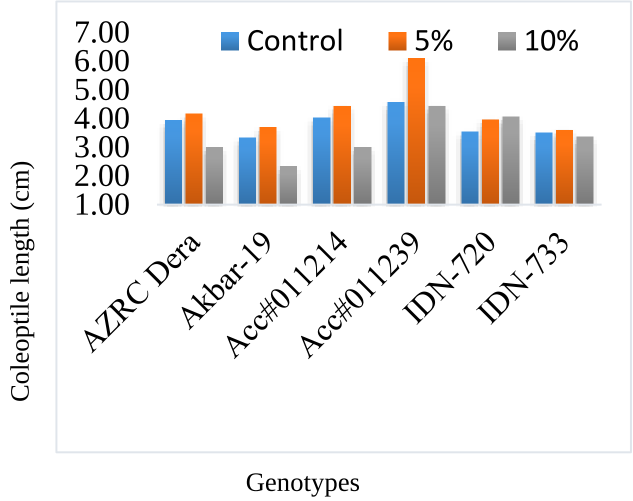


Fig. 2 Response of the root length of wheat genotypes varying concentration of PEG.

Effect of PEG on coleoptile length

Coleoptile lengths were more severely reduced by Akbar-19, followed by AZRC Dera by the application of 10% PEG. Both durum lines and landraces showed moderate to high levels of tolerance to both PEG treatments in terms of coleoptile length. Interestingly, landrace acc#1239 exhibited an increase in coleoptile length at 5% PEG level as compared to both control and 10% PEG treatment (Fig. 3)

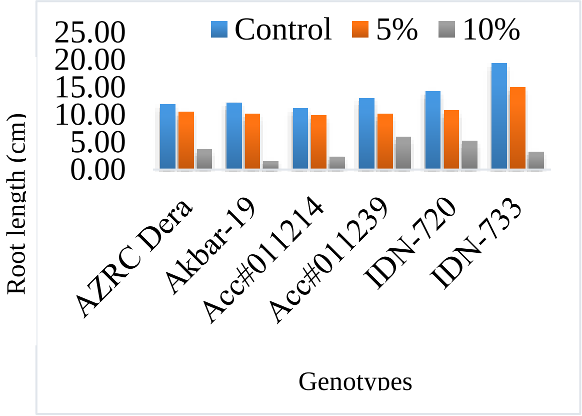


Fig. 3 Response of the coleoptile length of wheat genotypes to varying concentrations of PEG. (Below).

Effect of PEG on Leaf Length

The effect of 5% PEG treatment was minimal on the leaf length of all six genotypes, however, the application of 10% PEG drastically reduced leaf length in all genotypes. IDN-733 was found to be the most tolerant genotype to both PEG concentrations in terms of leaf length, followed by landrace acc#11239. The most drastic decrease in leaf length was observed in IDN-733 at 10% PEG concentration, followed by landrace acc#11214 (Fig. 4).

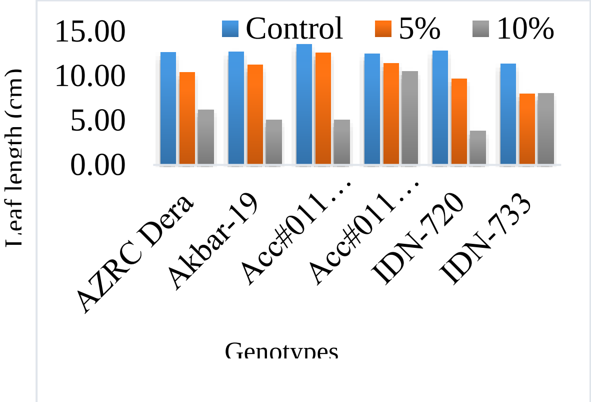


Fig. 4 Response of the leaf length of wheat genotypes to varying concentrations of PEG.

Effect of PEG on fresh root weight

Significant differences were observed in the fresh root weight of all genotypes in response to both PEG treatments (p = 0.05). Both concentrations of PEG treatments severely reduced fresh root weight, 10% PEG being the most effective to reduce fresh root weight in all genotypes except Akbar-19 and landrace acc#11214 where the effect was less than that of 5% PEG treatment. Based on fresh root weight, landrace acc#11214 was found the most tolerant genotype in response to 10% PEG treatment (Fig. 5).

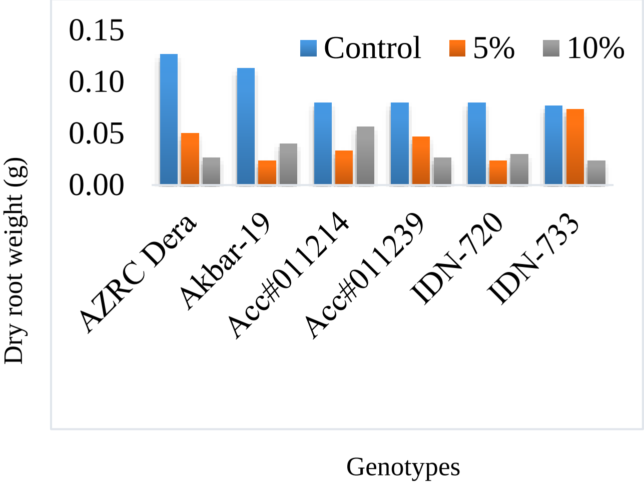


Fig. 5 Response of the fresh root weight of wheat genotypes to varying concentrations of PEG.

Effect of PEG on dry root weight

Significant differences were observed in the dry root weight of all genotypes in response to both PEG treatments (p = 0.05). Both concentrations of PEG treatments severely reduced dry root weight, 10% PEG being the most effective to reduce dry root weight in all genotypes except Akbar-19 and landrace acc#11214 where the effect was less than that of 5% PEG treatment. Based on dry root weight, landrace acc#11214 was found the most tolerant genotype, followed by Akbar-19 in response to 10% PEG treatment (Fig. 6).

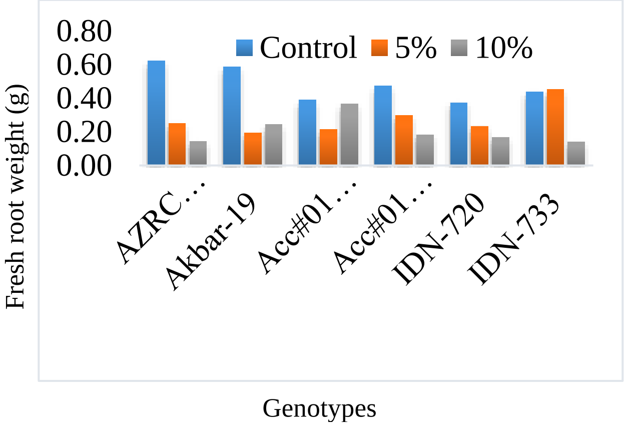


Fig. 6 Response of the fresh root weight of wheat genotypes to varying concentrations of PEG.

Effect of PEG on no. of tillers plant^{-1}

Differences in the mean no. of tillers plant^{-1} were not significant among all genotypes at 5% PEG concentration while 10% PEG treatment significantly reduced no. of tillers plant^{-1} among all genotypes. Akbar-19 showed the maximum decrease in no. of tillers plant^{-1} at both PEG concentrations as compared to control. Surprisingly, AZRC Dera exhibited an increase in no. of tillers plant^{-1} at 5% PEG concentration (Fig. 7).

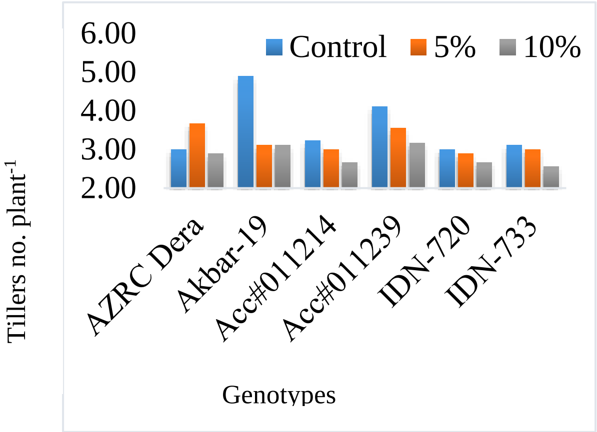
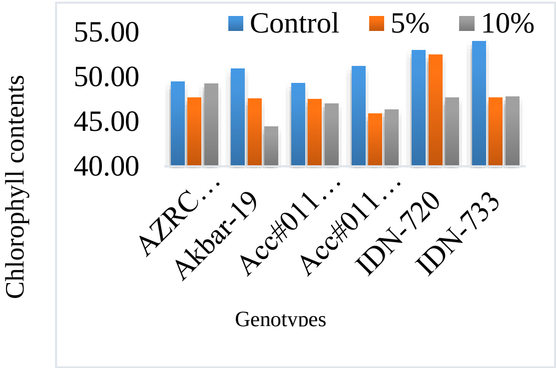


Fig. 7 Response of the no. of tillers plant^{-1} of wheat genotypes to varying concentrations of PEG.

Effect of PEG on leaf total chlorophyll contents

Significant differences were observed in the leaf total chlorophyll contents of all genotypes in response to both PEG treatments (p = 0.05). The reduction in the leaf's total chlorophyll contents in response to both concentrations (5% and 10%) of PEG was the same. The most tolerant wheat genotype in terms of the response of the leaf total chlorophyll contents to both PEG treatments was AZRC Dera while the least tolerant was Akbar-19 (Fig. 8).



Discussion

Drought is one of the key abiotic stresses that drastically affect wheat production at different stages of the life cycle of wheat. Wheat yield has declined by -5.5% since 1980-2010. The ongoing global climate change is causing a rising global temperature and manifesting itself into unexpected heat waves and unexpected rainfall changes. Such trends in drought and heat stress are projected to cause a further decline in wheat yield in the future (Lobell *et al.*, 2011; Rosenzweig *et al.,l* 2014; Asseng *et al.*, 2015). Breeding for climate-smart and drought-tolerant wheat cultivars is the most economical and environmentally safe strategy to combat future wheat yield losses incurred by drought. The pre-requisite of such breeding programs is the availability of diverse wheat germplasm to be screened for drought stress and select the most drought-tolerant genotypes as breeding parents for developing climate-resilient and drought-tolerant wheat cultivars. Wheat wild relatives, progenitor's species, landraces and durum wheat constitute a diverse gene pool that might contribute elite alleles for drought tolerance. Keeping this idea, we selected diverse germplasm comprising of two local elite wheat cultivars namely AZRC Dera and Akbar-19, two landraces and two advanced durum wheat lines, and to screen them for lab-based drought tolerance. As previously documented, wheat landraces (farmers 'traditional varieties abandoned post-green revolution due to low yield) and durum wheat act as a hidden source, and have contributed novel and elite alleles for drought tolerance (Dodig et al., 2012; Mohammadi 2016). As compared to two modern wheat varieties AZRC Dera and Akbar-19, the two landraces and durum wheat lines showed superior drought tolerance to lab-based drought stress induced by the application of medium (5%) and high (10%) levels of PEG. This trend is manifested in different early growth root, shoot, tillers and leaf-related parameters of two landraces and two durum lines that better performed in terms of PEG-mediated drought tolerance as compared to two modern cultivars used in this study (Fig. 1-7). Root length and root density are the primary and key components of wheat plants that are directly affected by drought stress. In our findings, both 5% and 10% levels of PEG treatments drastically reduced root length among all genotypes. However, durum line IDN-733 was the most PEG-tolerant genotype followed by IDN-702 and landrace acc#11239 in terms of root length (Fig. 2). Similarly, longer coleoptile length is a good indicator of better drought tolerance, especially at early growth stages. We also found landrace acc#11239 exhibited an increase in coleoptile length at 5% PEG level as compared to both control and 10% PEG treatment (Fig. 3). These findings infer that both root length and coleoptile lengths should be used to screen for lab-based, and hence field-based drought tolerance in wheat.

The success of breeding efforts is limited by the availability of better screening tools for drought tolerance in wheat. Among chemical agents, PEG is the most frequently used chemical agent to screen wheat genotypes for drought tolerance in a controlled lab environment. Such frequent application of PEG for in-vitro drought stress induction is attributed to the inert and non-ionic nature of PEG molecules. However, selecting the best concentration of PEG to induce lab-based drought stress in wheat and screening the induced changes in various morphological, physiological and biochemical attributes need to be addressed. Different reports have documented different concentrations of PEG to induce lab-based drought stress in wheat (Kumari *et al*., 2014; Pour-Aboughadare *et al*., 2020; Memon *et al*., 2023). We applied two different concentrations of PEG (5% and 10%) and selected the best optimum level that might induce visually significant changes in different morphological and physiological parameters of the early growth response of wheat to drought stress. Our findings showed that both concentrations of PEG induced significant changes in the form of reduction in root length, coleoptile length, leaf length, fresh and dry root weight, and accumulation of leaf total chlorophyll contents. Different genotypes responded differently to both concentrations of PEG, however, the effect of 10% PEG concentration was more severe on all parameters studies among all genotypes relative to 5% PEG level. Wheat landrace acc#11239 and durum line IDN-733 were found to be the most tolerant genotypes to 10% PEG drought stress (Fig. 2 and 3) by exhibiting maximum root and coleoptile length, thereby showing their potential used in wheat breeding programs aimed to develop drought-tolerant wheat cultivars. Moreover, we suggest that 10% PEG is the suitable optimal concentration to induce lab-based drought stress in wheat genotypes.

Conclusion

Lab-based drought stress induction and screening for drought-tolerant wheat genotypes are important to later on perform drought stress studies in the field. We showed that the application of 10% PEG concentration is an optimal concentration to induce lab-based drought stress in wheat genotypes and evaluate/screen them for drought stress. Contrary to the two wheat cultivars used in this study, the two landraces especially acc#11239 and two durum wheat advanced lines (especially IDN-733) showed better drought tolerance by maintaining better root length, root density and coleoptile length. The three early growth parameters (root length, root density and coleoptile length) are suggested to be selected for screening wheat genotypes to drought stress both in the lab and field. The findings of this study are important for breeders to evaluate wheat genotypes for drought stress tolerance and develop climate-resilient and drought-tolerant wheat cultivars for better yield potential.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors Contribution

Nasr Ullah Khan and Muhammad Mohibullah conceived the idea, designed the study and drafted the manuscript. Wasif Rasool and Asif Rasool conducted the experiments and collected and analyzed the data. Sundas Batool helped with data analysis and provided a critical evaluation of the manuscript.

References

Asseng S, Cao W, Zhang W, Ludwig F. Crop physiology, modelling and climate change: impact and adaptation strategies. Crop Physiol. 2009; 511-43

Bayoumi TY, Eid MH, Metwali EM. Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. African J Biotechnol. 2008; 7:2341–2352

Dilday RH, Mgonja MA, Amonsilpam SA, Collins FC, Wells BR. Plant height vs. Mesocotyl and coleoptiles elongation in rice: linkage or pleiotropism. Crop Science. 1990; 30:815–818

Dodig D, Zorić M, Kandić V, Perović D, Šurlan-Momirović G. Comparison of responses to drought stress of 100 wheat accessions and landraces to identify opportunities for improving wheat drought resistance. Plant Breeding. 2012; 131(3):369-739

Farooq S, Onen H, Ozaslan C, Baskin CC, Gunal H. Seed germination niche for common ragweed (*Ambrosia artemisiifolia* L.) populations naturalized in Turkey. South African J Bot. 2019;123: 361–371

Jatoi SA, Latif MM, Arif M, Ahson M, Khan A, Siddiqui SU. Comparative assessment of wheat landraces against polyethene glycol simulated drought stress. Sci. Tech. Develop. 2014; 33: 1-6

Kumari A, Sairam RK, Singh SK, Krishna GK. Early growth response: an indicator of subsequent growth and yield of wheat genotypes grown under simulated water stress conditions. Indian Journal of Plant Physiology. 2014; 19:94-100

Lobell DB, Burke MB. Why are agricultural impacts of climate change so uncertain? The importance of temperature relative to precipitation. Environmental Research Letters. 2008; 3(3):034007

Memon S, Abro AA, Jakhro MI, Farid A, Habib M, Ahmed M, Bhutto LA, Memon SA, Farooq M. Polyethylene glycol mediated osmotic stress impacts on growth and biochemical aspects of wheat under artificial osmotic stress condition. Journal of Innovative Sciences. 2023;9(1):44-50

Meneses CH, Bruno RD, Fernandes PD, Pereira WE, Lima LH, Lima MM, Vidal

MS. Germination of cotton cultivar seeds under water stress induced by polyethyleneglycol-6000. Scientia Agricola. 2011; 68:131-8

Mohammadi R. Efficiency of yield-based drought tolerance indices to identify tolerant genotypes in durum wheat. Euphytica. 2016; 211:71-89

Mottaleb KA, Kruseman G, Snapp S. Potential impacts of Ukraine-Russia armed conflict on global wheat food security: A quantitative exploration. Global Food Security. 2022; 35: p.100659

Nawaz S, Ahmed N, Iqbal A, Khaliq I. Optimization of regeneration protocols for wheat under drought and salt stress. Pak. J. Agri. Sci. 2013; 50: 663-670

Pour-Aboughadareh A, Etminan A, Abdelrahman M, Siddique KH, Tran LS. Assessment of biochemical and physiological parameters of durum wheat genotypes at the seedling stage during polyethene glycol-induced water stress. Plant Growth Regulation. 2020; 92(1):81-93

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. African J Biotechnol. 2007;6: 971–975

Rosenzweig C, Jones JW, Hatfield JL, Ruane AC, Boote KJ, Thorburn P, Antle JM, Nelson GC, Porter C, Janssen S, Asseng S. The agricultural model intercomparison and improvement project (AgMIP): protocols and pilot studies. Agricultural and forest meteorology. 2013; 170:166-82

Shiferaw B, Smale M, Braun HJ, Duveiller E, Reynolds M, Mauricio G. Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food security. 2013; 5:291-317