Acta Nat. Sci. (2026) 7(1): 85-95
DOI: 10.61326/actanatsci.v7i1.479
e-ISSN: 2718-0638

Original Research Article
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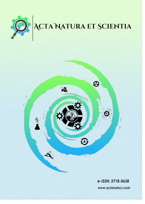

ACTA NATURA ET SCIENTIA

Growth and Chlorophyll Responses of Wheat Seedlings to Putrescine Under PEG-Induced Drought Stress

Pelin Şengül Toraman[1]✉ • Nuray Ergün[1] • Berna Çalıcı[1]

[1] Hatay Mustafa Kemal University, Faculty of Science, Department of Biology, 31000, Hatay, Türkiye; sengultoramanpelin@gmail.com, ergun.nuray@gmail.com, berna_kulac82@hotmail.com

✉ Corresponding Author: sengultoramanpelin@gmail.com

Please cite this paper as follows:
Şengül Toraman, P., Ergün, N., & Çalıcı, B. (2026). Growth and Chlorophyll Responses of Wheat Seedlings to Putrescine Under PEG-Induced Drought Stress. *Acta Natura et Scientia*, *7*(1), 85-95. https://doi.org/10.61326/actanatsci.v7i1.479

ARTICLE INFO

Article History
Received: 06.04.2026
Revised: 19.06.2026
Accepted: 24.06.2026
Available online: 27.06.2026

Keywords:

Gacer
Chlorophyll
PEG-6000
Putrescine
Triticum aestivum

ABSTRACT

Wheat is one of the most important cereal crops worldwide due to its broad cultivation area, diverse uses, and economic importance. This study aimed to determine the shoot length, shoot fresh and dry weight, root length, root fresh and dry weight, chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll (chl a+b) amounts of wheat seedlings. The interactions of 5% polyethylene glycol (PEG-6000) and different putrescine (PUT) doses (0.5 mM and 1 mM) were examined in wheat (*Triticum turgidum* L. var. *dicoccum*, Gacer, *Triticum aestivum* L., Konya-2002) seedlings. Under the drought stress effect caused by PEG, two wheat varieties were negatively affected and a decrease in shoot length, fresh weight, dry weight and chlorophyll amounts was observed. In Gacer wheat, an increase in fresh root weight was observed under drought stress treated with PEG, while a decrease was observed in Konya-2002. Compared to the control, it was determined that the root length of Gacer wheat was less affected by the presence of PEG than that of Konya-2002. Compared to PEG, under the effect of PUT, increases were observed in shoot length, fresh weight, root length and fresh weight, chlorophyll a and total chlorophyll content in both wheat varieties. The applied 5% PEG-6000 created a drought stress effect in wheat and it was observed that PUT reduced this stress effect. When the parameters we used in our study are examined together, these findings suggest that Gacer wheat is more resilience to drought than Konya-2002.

INTRODUCTION

Cereals provide a significant portion of the protein and carbohydrate needs of humans. Therefore, wheat ranks first among the cultivated cereals all over the world (Doğru & Ergün, 2021). Türkiye, particularly Central Anatolia, has suitable agroecological conditions for wheat cultivation due to its geographical location, soil structure, and water resources, making the region an important center for crop production. Wheat is a vital part of rural life and remains the main cereal crop in the region. While

Türkiye ranks among the top wheat-producing countries globally, durum wheat is especially important as it is widely used for both human consumption and livestock feed, making its agricultural and industrial production highly significant (Pandey et al., 2015).

The conservation of local wheat genetic resources is important for agricultural sustainability, biodiversity, and food security (Özberk et al., 2016). Bread wheat varieties may be sensitive or resistant to various stresses with their differences (Atak, 2017). As a result of genetic changes of *Triticum turgidum* L., the ancestor of Emmer wheat, which was cultivated in the past, and tetraploid wheat used today, was formed. Today, it is cultivated as bulgur wheat in Germik, Çatalca, Develi, and given names belonging to the region where it is found. Gacer, known as emmer wheat (*Triticum turgidum* L. var. *dicoccum*) (2n=28), which is a wheat that is on the verge of being forgotten has a high nutritional value, especially with its very low gluten content, protein content varying between 17-20%, and high carotenoid content, is a wheat species that grows in Anatolia and whose population is decreasing (Bulut, 2016). Gacer wheat has recently started to be intensively cultivated again in Develi district of Kayseri province by our farmers and scientists who know its value. The Konya-2002 variety of *Triticum aestivum* (2n=42), another wheat species we used in our study, is known to be drought-sensitive, lodging-resistant, and resistant to winter and cold (Balkan & Gençtan, 2023). Drought stress is observed at the highest level in the world and will negatively affect plant production in the future. In addition, there will be a decrease of up to 80% in cereal production (Kadıoğlu, 2006; İlyas et al., 2021). The most important issue in breeding studies aimed at drought tolerance in plants is knowing the morphological and physiological response mechanisms that plants possess and use to overcome water deficiency and drought (Hussain et al., 2011).

Polyamines are widely found in living organisms (Kusano et al., 2008). It was initially thought that the main role of polyamines was that of direct protective molecules under stress conditions, and it was stated that they are compounds that are involved in a complex signaling system and have an important role in the regulation of stress tolerance. Some of these studies aim to prevent or eliminate the harmful effects of stresses that negatively affect plant development and yield in agricultural production through external applications (Hussain et al., 2011; Shu et al., 2012; Shi et al., 2013). However, it is stated that the physiological significance of this increase and the role of polyamines are still unclear, and that studies in this direction are necessary to understand the functions of polyamines in stress tolerance (Alcázar et al., 2006). Stress and its responses in plant adaptation are still a very important research topic. The study aimed to determine the effects of drought stress induced by 5% PEG-6000 on Gacer (*Triticum dicoccum* L.) and Konya-2002 (*Triticum aestivum* L.) wheat seedlings, to evaluate the role of different putrescine (PUT) doses on physiological parameters in mitigating stress conditions, and to reveal the responses of the Gacer variety, which has been researched to a limited extent in the literature, to drought stress.

MATERIAL AND METHODS

Two wheat genotypes were used in the experiment: Gacer (*Triticum turgidum* L. var. *dicoccum*) and Konya-2002 (*Triticum aestivum* L.). Gacer seeds were obtained after harvest from KAÇEM (Women Farmers Ecological Education and Production Center) in Kayseri, Türkiye, whereas Konya-2002 seeds were supplied by the Bahri Dağdaş International Agricultural Research Institute, Türkiye.

Before germination, seeds were surface-sterilized in 2% sodium hypochlorite for 20 min, rinsed thoroughly with distilled water, and soaked in distilled water for approximately 1 h. Seedlings were grown in 2-L plastic containers containing full-strength Hoagland nutrient solution (Arnon & Hoagland, 1940) adjusted to pH 5.7. The nutrient solution was renewed on the fifth day of growth.

When seedlings reached 10 days of age, polyethylene glycol and putrescine treatments were applied through the nutrient solution (Hellal et al., 2017). The experiment consisted of six treatment groups for each genotype: control, 5% PEG-6000, 0.5 mM putrescine, 1 mM putrescine, 5% PEG-6000 + 0.5 mM putrescine, and 5% PEG-6000 + 1 mM putrescine

(Money, 1989; Alobaidy, 2013; Çömlekçioğlu & Arıkan, 2017). At the end of the treatment period, the pots were divided into twelve different groups and shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight were measured. Dry weights were determined after oven-drying the plant material at 80°C for 48 h.

Chlorophyll a, chlorophyll b, and total chlorophyll contents were determined spectrophotometrically according to Porra et al. (1989).

Data Evaluation

Data were analyzed using three-way factorial analysis of variance (ANOVA), with genotype, PEG-6000 treatment, and putrescine concentration as fixed factors. When significant differences were detected, means were compared using Tukey post hoc test. Statistical significance was accepted at p≤0.05.

RESULTS

When plant growth parameters were examined, a decrease in shoot length was observed in both PEG-treated wheat varieties compared to the control (Figure 1). While both wheat varieties showed a decrease in stem length under PEG-induced drought stress, the Gacer wheat variety was more affected in terms of stem elongation compared to the Konya-2002 variety. Putrescine doses increased stem lengths in both PEG-treated wheat varieties.

When plant growth parameters were examined, a decrease in root length was observed in both PEG-treated wheat varieties compared to the control (Figure 2). When comparing root length to the control with PEG application, it was observed that Gacer wheat was less affected than Konya-2002.

The decrease in the presence of PEG in Konya-2002 wheat compared to the control showed that it was more affected than Gacer. The pairwise comparison results of PUT doses were found to be significant (p<0.001). The significant difference between the doses was that the greatest increase in fresh weights in both wheats was observed in Konya-2002 with the 0.5 mM PUT application (Figure 3).

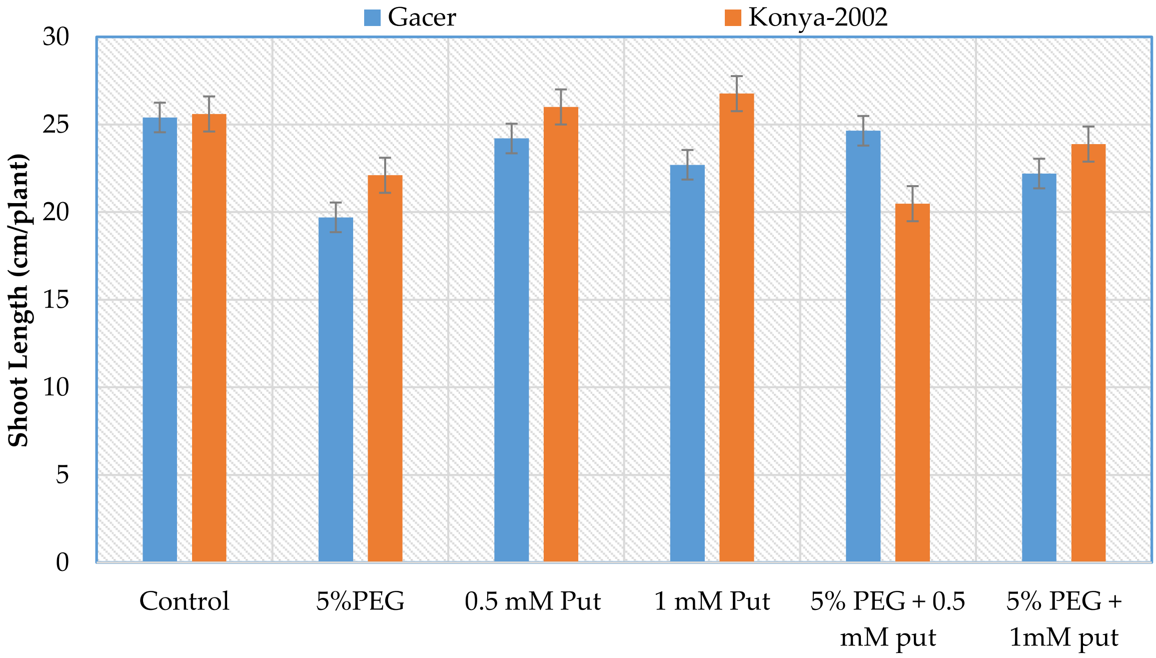

Figure 1. Changes in average shoot length of *Triticum dicoccum* 2n=28(Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) seedlings grown under PEG and PUT conditions **(**Genotype = *p = 0.042), PEG (***p < 0.001), Putrescine (* p = 0.024), Genotype × PEG (**p < 0.001), Genotype × Putrescine (** p < 0.001), PEG × Putrescine (p = 0.063), Genotype × PEG × Putrescine (*p = 0.007).

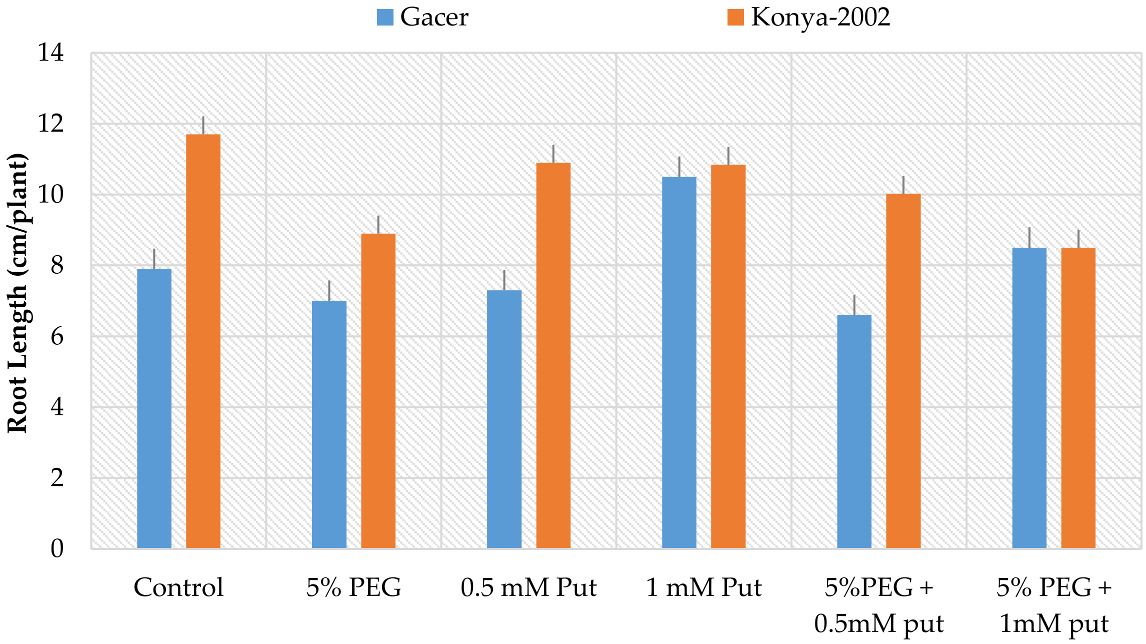

Figure 2. Changes in average root length of *Triticum dicoccum* 2n=28(Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) seedlings grown under PEG and PUT conditions. Genotype (**$p < 0.001$), PEG ($p^{**} < 0.001$), Putrescine (**$p = 0.001$), Genotype × PEG (* $p = 0.020$), Genotype × Putrescine (*$p = 0.003$), PEG × Putrescine ($p = 0.490$), Genotype × PEG × Putrescine ($p = 0.795$).

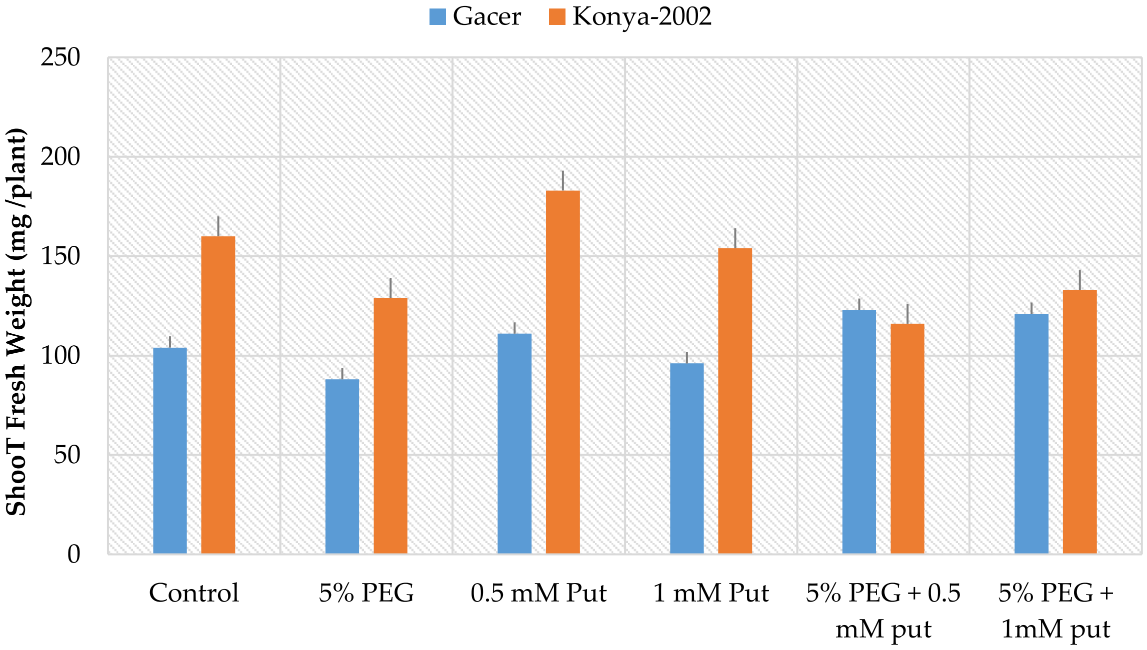

Figure 3. Changes in average shoot fresh weight of *Triticum dicoccum* 2n=28(Gacer) and *Triticum aestivum* 2n=42(Konya-2002) seedlings grown under PEG and PUT conditions. Genotype ($p < 0.001$), *PEG* (**$p < 0.001$), Putrescine (**$p < 0.001$), Genotype × PEG (*$p = 0.022$), Genotype × Putrescine ($p = 0.491$), PEG × Putrescine (*$p = 0.03$), Genotype × PEG × Putrescine (*$p = 0.003$).

PEG application resulted in a decrease in shoot dry weight in Gacer and Konya-2002 seedlings. PUT applications increased shoot dry weight in both wheat varieties. Increased PUT doses led to further increases in shoot dry weight. In shoot dry weight data, a decrease was observed in drought-affected Gacer and Konya-2002 wheat varieties with PEG application, while the combined application of PEG and Putrescine resulted in an increase in wheat ($p < 0.05$) (Figure 4).

Comparing the presence of PEG in the wheat to the control, an increase in root fresh weight was observed in Gacer, while a decrease was observed in Konya-2002. Root fresh weight increased proportionally with increasing PUT doses applied to Konya-2002. The greatest increase in root fresh weight in Gacer was observed with the 0.5 mM PUT application (Figure 5). The lowest average weight was observed in both wheat varieties with the PEG + 0.5 mM PUT application.

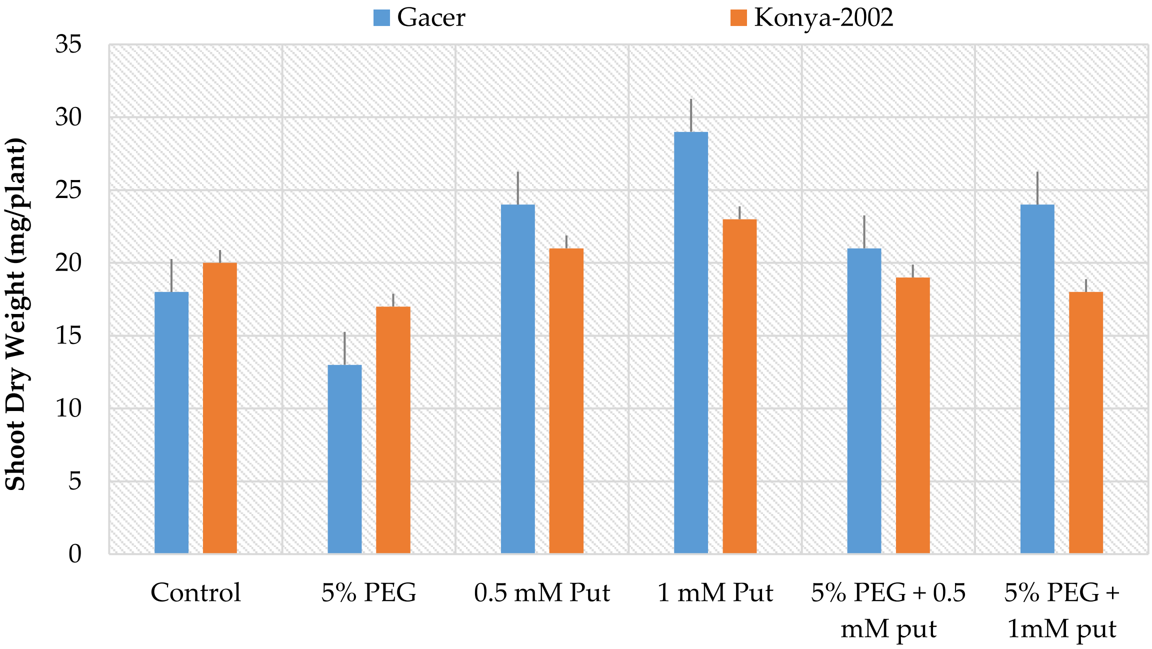

Figure 4. Changes in average shoot dry weight of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) seedlings grown under PEG and PUT conditions. Genotype (p = 0.513), PEG (*p = 0.009), Putrescine (**p < 0.001), Genotype × PEG (*p = 0.041), Genotype × Putrescine (p = 0.053), PEG × PUT (p = 0.542), Genotype × PEG × Putrescine (**p < 0.001)).

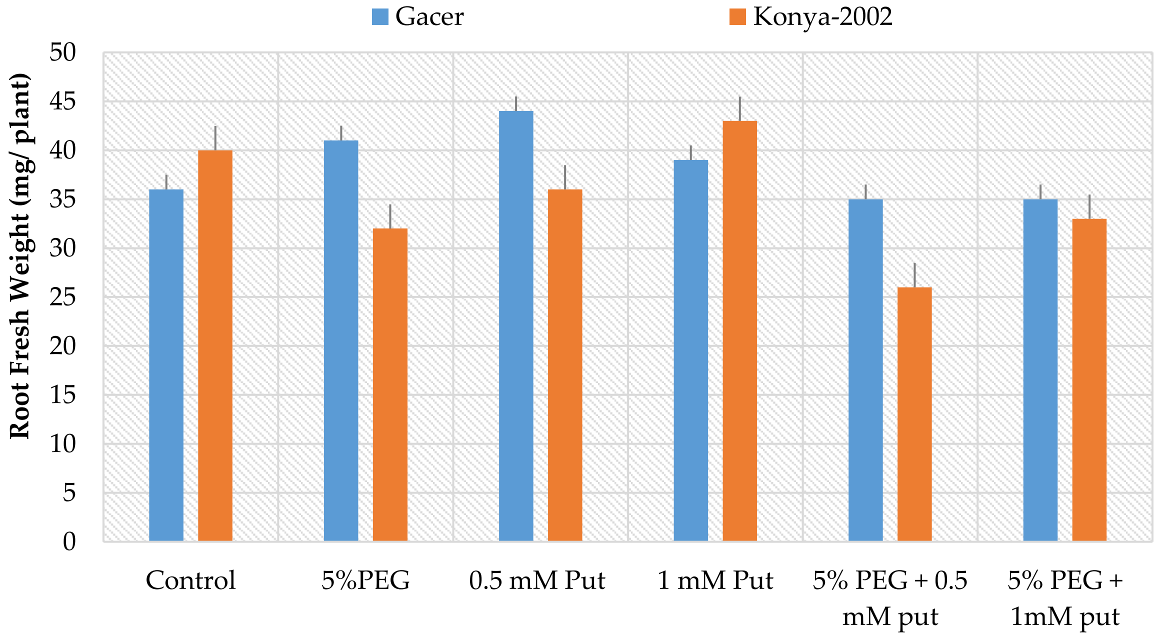

Figure 5. Changes in average root fresh weight of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) seedlings grown under PEG and PUT conditions. Genotype (*p = 0.034), PEG (p= 0.087), PUT (p < 0.001), Genotype × PEG (p = 0.298), Genotype × PUT (**p = 0.001), PEG × PUT (*p = 0.037), Genotype × PEG × Putrescine (p = 0.054).

It was observed that the presence of PEG in wheat increased the dry root weight in Gacer and Konya-2002 compared to the control. The greatest increase was observed in Gacer. The least dry root weight was observed in Konya-2002 with the 0.5 mM PUT application. In Gacer, the greatest increase in root weight was observed with the 0.5 mM PUT application (Figure 6). In both wheats, the least average weight was observed with the control application.

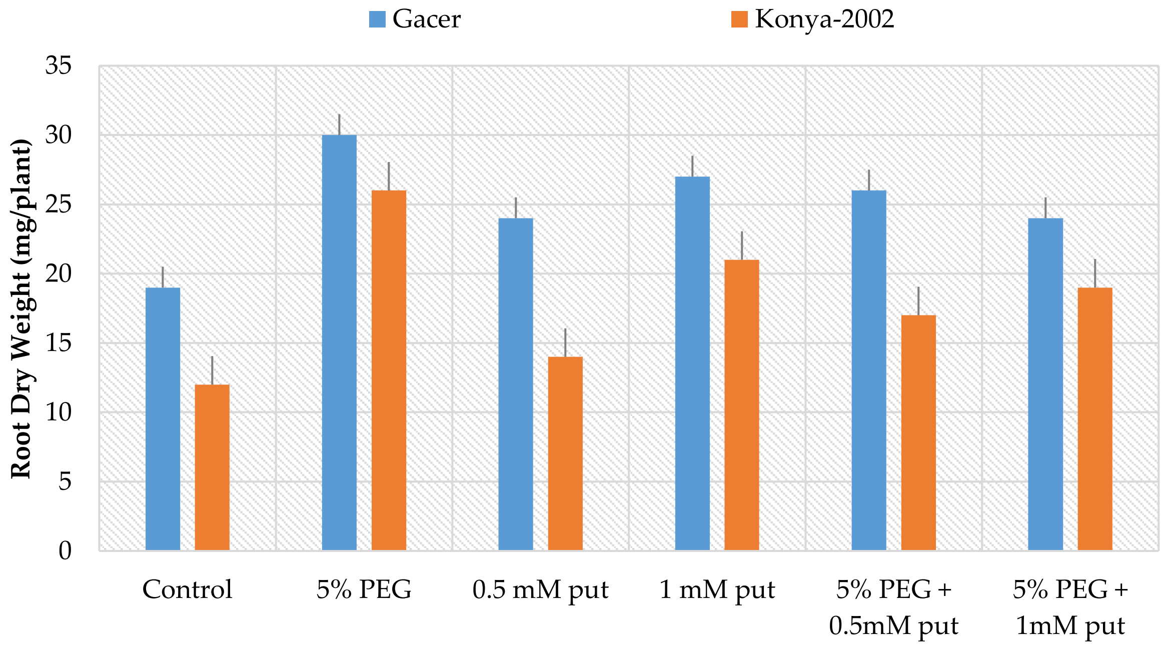

Figure 6. Changes in average root dry weight of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42(Konya-2002) seedlings grown under PEG and PUT conditions. (Genotype ($p < 0.001$), PEG ($p = 0.154$), PUT (**$p < 0.001$), Genotype × PEG ($p = 0.154$), Genotype × PUT ($p = 0.313$), PEG × PUT(**$p < 0.001$), Genotype × PEG × PUT (**$p < 0.001$).

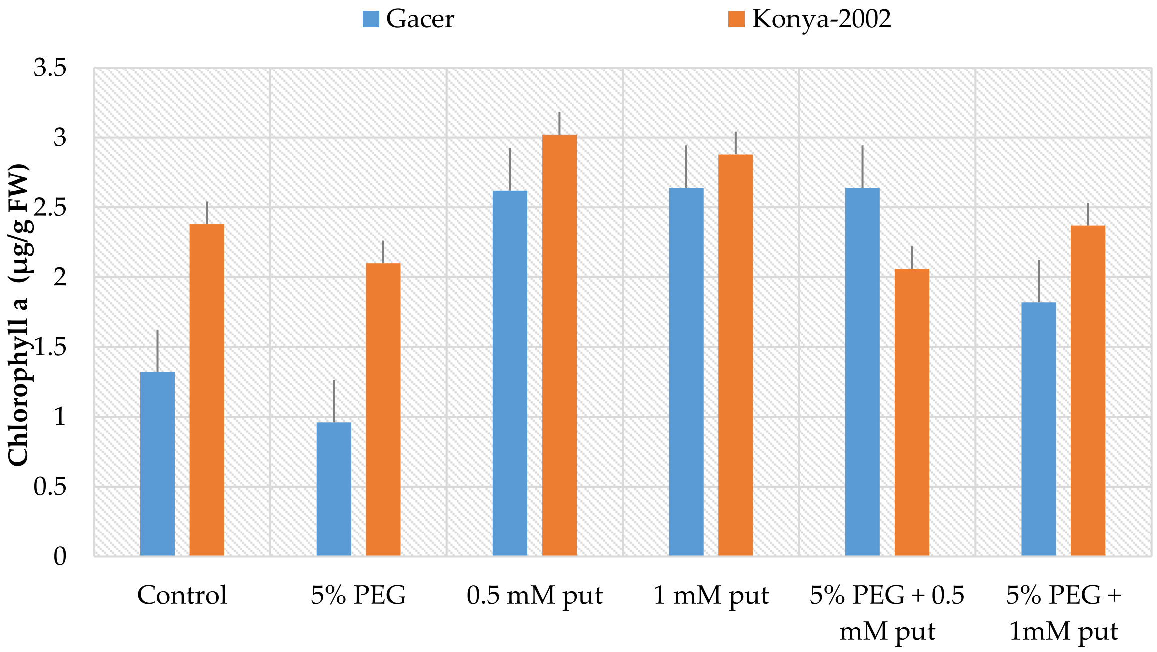

Figure 7. Changes in the average chlorophyll a content of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) grown under PEG and PUT conditions. (PEG (**, $p = 0.010$), Genotype ($p = 0.149$), PUT (**, $p = 0.009$).

Significant differences were found between the groups in terms of mean chlorophyll a (chl a) of Gacer and Konya-2002 wheat (p≤0.05). Compared to the control, PEG application resulted in a decrease in chlorophyll a and this decrease was most pronounced in Gacer (p≤0.05). The PUT application was found to significantly increase the amount of chlorophyll a in wheat. In the pairwise comparison, it was statistically significant that the greatest increase in chlorophyll a level (150%) was observed with the applied 0.5 mM PUT. (p≤0.05) (Figure 7).

Compared to the control, PEG application caused a decrease in chl b content in Gacer and Konya-2002 wheat varieties, and this decrease was statistically significant (p<0.001). The greatest decrease was observed in Konya-2002, at 37. 5%. In Gacer, PUT applications resulted in an increase in chl b content compared to the control, while in Konya-2002, a decrease was observed (Figure 8).

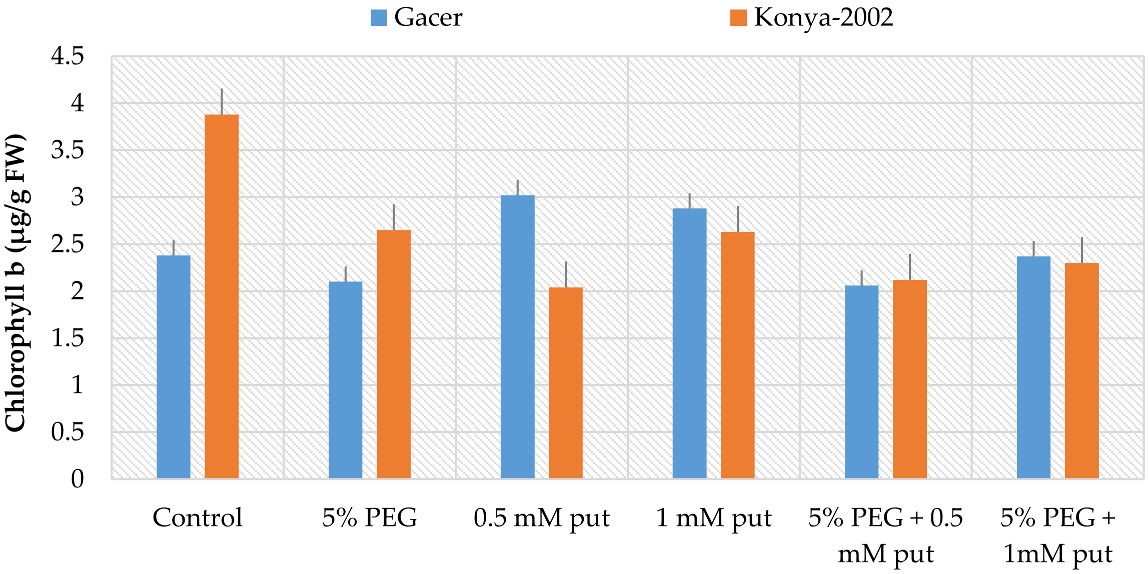

Figure 8. Changes in the average chlorophyll b content of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) grown under PEG and PUT conditions (PEG **, p < 0.001), Genotype (p = 0.298), Putrescine (p = 0.068).

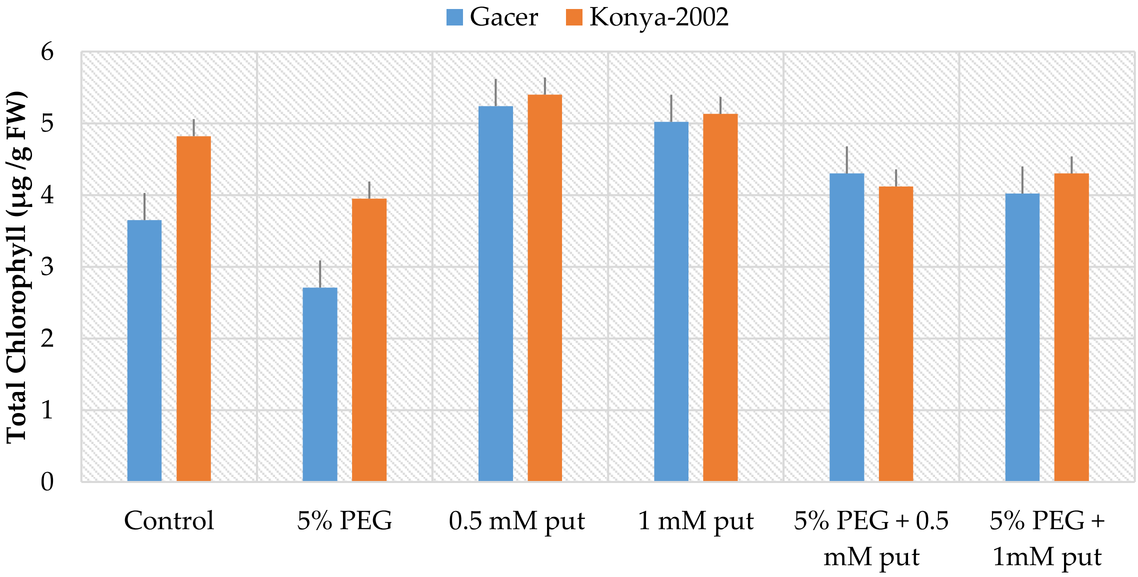

Figure 9. Changes in average total chlorophyll content of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) seedlings grown under PEG and PUT conditions. (**p < 0.001), Genotype (p = 0.098), PUT (*p = 0.048), Genotype × PUT (p = 0.065).

In Gacer wheat, the total chlorophyll content increased with the applied PUT doses, and no difference was observed between the doses. In Konya-2002 wheat, the greatest increase in total chlorophyll content was observed with 0.5 mM PUT and was found to be statistically significant ($p \leq 0.05$) (Figure 9).

DISCUSSION

The present study demonstrated that PEG-induced drought stress significantly affected growth and physiological traits in wheat, although the magnitude and direction of these effects varied depending on genotype and putrescine application. PEG treatment consistently reduced shoot growth, as well as chlorophyll content, confirming its effectiveness in simulating drought stress conditions. Similarly, İlhan et al. (2026) in their study determined that drought stress was induced in *Triticum dicoccum* Schrank and *Triticum aestivum* L. populations using different doses of PEG-6000, and that the measured values of shoot length, root length, shoot dry weight, root dry weight, shoot fresh weight, root fresh weight, and relative water content decreased as the PEG intensity increased. Zhong et al. (2025) supported our study that putrescine, used for therapeutic purposes and applied exogenously to the plant, reduced the stress effect in wheat. The decreased chlorophyll values of our drought-stressed wheat increased to high levels with putrescine. Bukhari et al. (2021) evaluated the effects of polyethylene glycol (PEG-6000) at osmotic potentials of −0.17, −0.32, −0.47, and −0.62 MPa on ten wheat genotypes. Their findings indicated that, compared with the control treatment, germination stress tolerance and root length tolerance were highest under the −0.62 MPa PEG treatment. Pekol et al. (2016) investigated the effects of drought and salinity stress on root and shoot growth in wheat species, including *Triticum turgidum* subsp., *Triticum aestivum*, and *Triticum monococcum* (einkorn wheat). According to the data obtained, they stated that *Triticum turgidum subsp. durum* wheat was more tolerant to salt and drought stresses than *T. aestivum* and *T. monococcum*.

With PEG application, a decrease in total chlorophyll content was observed in both wheat varieties, and Gacer wheat was the most affected with 64% ($p<0.001$). According to the pairwise comparison results, significant differences were found between control-PEG, control-PUT, PEG-PEG+PUT in Gacer and Konya-2002 wheat varieties ($p<0.001$). Rahman et al. (2024) reported that the application of 0.3 mM putrescine to wheat, rice and maize plants increased chlorophyll content and actual quantum yield in wheat, and this is consistent with our results. Balkan & Gençtan (2023) reported that chlorophyll content in the leaves of bread wheat varieties decreased with increasing levels of drought stress. Hebat-Allah et al. (2023) evaluated the interaction of different drought levels on wheat grains and the physiological characteristics of wheat treated with 3 doses (0.25, 0.5, and 1 mM) of putrescine. The study found that drought conditions reduced plant height, fresh and dry weights, while 1 mM putrescine application improved wheat growth performance under both control and drought conditions, which is consistent with our findings.

Alharby et al. (2021) reported that drought stress adversely affects plant growth, chlorophyll content, photosynthetic efficiency, and plant water status. They further demonstrated that seed pretreatment with polyamines and corn extract–enriched polyamines can enhance growth performance and yield-related parameters in wheat. Toraman et al. (2020) determined a decrease in chl a, chl b, chl a+b content, shoot length and dry weight in *Triticum aestivum* genotypes as a result of drought, flooding and salt stress applications. Abdelmoghny et al. (2020) found statistically significant differences in the morpho-physiological characteristics of *Gossypium hirsutum* under drought conditions. They stated that root length is very important in terms of cut leaf water loss and cell membrane permeability.

Importantly, the interaction effects revealed that the response to putrescine was not uniform across genotypes or stress conditions. Significant Genotype × PEG and Genotype × PEG × Putrescine interactions for several traits (shoot length, shoot dry weight, and root fresh weight) indicate that the effectiveness of putrescine is strongly genotype-dependent and modulated by stress intensity. This highlights that polyamine-mediated stress tolerance is a complex, multi-level physiological process rather than a simple additive effect.

The results indicate that Gacer wheat may be more drought-resistant than Konya-2002, as its root growth length is less affected by drought stress. The findings also confirm that putrescine plays a significant role in enhancing drought tolerance, although its effectiveness depends strongly on genotype and environmental stress context. These results have important implications for crop improvement strategies. The strong genotype-dependent response suggests that selection of drought-tolerant varieties such as Gacer, combined with exogenous application of polyamines, could be a promising strategy to improve wheat performance under water-limited conditions.

CONCLUSION

PEG-induced drought stress negatively affected growth and photosynthetic traits in both wheat genotypes; however, Gacer variety showed higher resilience to drought stress compared to the Konya-2002 variety. The fact that root growth of Gacer wheat was not significantly affected under PEG-induced drought stress conditions indicates that Gacer possesses more effective adaptation mechanisms to water deficiency. Putrescine application, particularly at 0.5 mM, effectively alleviated the detrimental effects of drought stress by improving growth parameters and chlorophyll content. These findings indicate that putrescine plays an important role in enhancing drought tolerance in wheat seedlings. In conclusion, the responses observed in this study demonstrate the superior drought resilience of Gacer and highlight the potential of putrescine as a stress-mitigating agent. Furthermore, the results support the conservation and wider utilization of Gacer, a nutritionally valuable local wheat genotype, in future wheat breeding and cultivation programs. Further molecular studies are required to elucidate the mechanisms underlying polyamine-mediated drought tolerance.

ACKNOWLEDGEMENTS

This article was derived from PhD thesis of the first author and funded by Scientific Research Projects Coordinator (BAP) of Hatay Mustafa Kemal University (21.D.028).

Compliance with Ethical Standards

Authors' Contributions

PŞT: Investigation, Data curation, Formal analysis, Project administration, Writing – original draft, Writing – review & editing

NE: Conceptualization, Project administration, Writing – review & editing

BÇ: Investigation, Formal analysis

All authors critically reviewed and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

This study was financially supported by Scientific Research Projects Coordinator (BAP) of Hatay Mustafa Kemal University (21.D.028).

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI Disclosure

The authors did not use any artificial intelligence technology in the writing of this article or in the creation of the images, tables, or graphs.

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