# Effects of PEG-6000 Simulated Drought Stress on Germination of Five Derivative Lines of wheat-Leymus mollis

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# Abstract

To explore the drought tolerance of five wheat materials (H9, H24, H29, H30 and H47) and provide a basis for breeding drought-tolerant wheat. 5%, 7.5% and 10% Polyethylene glycol (PEG-6000) solutions were used to simulate drought environments.The results indicated that under drought stress, growth indices-including germination energy (GE), germination rate (GR), germination index (GI), germination drought resistance index (GDRI), root length (RL) and seedling length (SL)-exhibited a downward trend. Chlorophyll (Chl) content decreased with increasing stress concentration, among the materials, H47 showed the smallest decrease compared with the corresponding control group under different drought stresses (3.07%, 14.40% and 25.82% respectively). Malondialdehyde (MDA) and proline (Pro) contents generally increased with an higher PEG-6000 concentrations. Using the membership function method for comprehensive evaluation, the drought tolerance ranking of the five wheat materials was determined as H47 > H9 > H24 > H29 > H30, with H47 showing the highest comprehensive drought resistance score and the strongest drought tolerance. In conclusion, this study can provide excellent germplasm resources and basic research materials for breeding drought-resistant wheat varieties and mining drought-resistant genes.


# INTRODUCTION

In 2024, The *United Nations Convention to Combat Desertification* released a report pointing out that climate change has caused approximately three-quarters of the global land to gradually become arid, and in the 30 years prior to 2020, about 77.6% of the global land has become more arid than before. Wheat (*Triticum aestivum* L.), as one of the most important food crops in the world, provides the main carbohydrates for more than 1/3 of the world's population, and its growth, yield, and quality are closely related to food security. In recent years, drought has turned into a key factor restricting wheat yield, quality, and geographical distribution [1]. Globally, drought causes wheat yield reduction by 20%-50% annually, bringing huge losses to agricultural production [2–4]. As one of the major agricultural nations globally, arid and semi-arid areas in China account for 52.5% of the country's total land area, with drought disasters occurring frequently [5]. Breeding drought-tolerant wheat germplasm materials is not only a core task for ensuring global food security but also a crucial strategy to achieve sustainable agricultural development under extreme climate conditions. In recent years, scholars both at home and abroad have conducted out extensive research on wheat drought resistance identification, which is mainly carried out at different growth stages such as germination stage, seedling stage and adult plant stage. Compared with the adult plant stage, the identification at germination and seedling stages is less affected by the environment and easier to control conditions. Seed germination is a key period for seedling growth and the starting point of the whole growth period, which is very sensitive to water deficiency and a critical stage for evaluating crop drought resistance[6, 7]. Water deficiency under drought stress often leads to seedling shortage and ridge breaking, which further affects yield. Therefore, the germination and seedling stages are often used for identifying and evaluating the drought resistance of wheat and its germplasm resources [8–10].Polyethylene glycol (PEG-6000) has been widely used as an osmotic agent to simulate drought stress during the germination and seedling stages of crops such as wheat [11–14], rice (*Oryza sativa* L.) [15–17], maize (*Zea mays* L.) [18], soybean (*Glycine max* (L.) Merr.) [19], millet (*Setaria italica* (L.) Beauv) [20], barley (*Hordeum vulgare* L.) [21,22], and sorghum (Sorghum) [23] due to its advantages of non-toxicity to plants, strong operability, and high efficiency.

Crop drought resistance is a quantitative trait controlled by multiple genes [24, 25]. A single drought resistance index can hardly evaluate drought resistance comprehensively and accurately, and principal component analysis and fuzzy membership function method are often used for comprehensive evaluation of multiple drought resistance indices. Zhang et al. [26] used principal component analysis to conduct a comprehensive evaluation of 8 strong-gluten wheat varieties at the germination stage; Wang et al. [27] evaluated the drought resistance of Xinjiang winter wheat varieties at the germination stage by means of principal component analysis; Wei et al. [28] combined principal component analysis with membership function analysis to screen 5 drought-resistant varieties at the seedling stage, such as Shannong 24; Yang et al. [29] carried out comprehensive evaluation of drought resistance on 71 winter wheat varieties by using principal component analysis, membership function method and cluster analysis; Feng et al. [30] identified and classified the drought resistance of 119 spring wheat varieties at the germination stage through principal component analysis and cluster analysis; Chang et al. [31] comprehensively evaluated the drought resistance of *Agropyron cristatum*-wheat derivatives at the germination stage and adult plant stage by combining principal component analysis, membership function, and correlation analysis. Previous studies on wheat drought resistance mainly focused on the evaluation of drought resistance of wheat varieties, while there are few studies on the identification and exploration of related germplasm materials. Relatives of wheat possess excellent traits such as disease resistance, drought resistance, and cold resistance, which are valuable gene resource pools for wheat genetic improvement [32]. Exploring and utilizing these germplasm resources, as well as mining the excellent drought-resistant genes they contain, is of great significance for promoting wheat drought-resistant breeding and genetic research [33]. Yang et al. [34] and Zhao et al. [35] transferred chromosome segments of *Leymus mollis* into common wheat through distant hybridization techniques such as hybridization and backcrossing, creating a series of stable stripe rust-resistant *Leymus mollis*-wheat derivative germplasm materials; Du et al. [36] identified materials with resistance to Fusarium head blight, but there are few studies on the screening and identification of related drought-resistant materials. This study aimed to explore excellent drought-tolerant wheat germplasm materials. Five *Leymus mollis*-wheat derivative materials (numbered H9, H24, H29, H30 and H47) were selected. Different degrees of drought stress environments (0, 5%, 7.5% and 10%) were simulated by PEG-6000. The changes of growth indices (GE, GR, GI, GDRI, RL and SL) and physiological indices (Chl content, MDA content and Pro content) at the germination stage of the five materials were determined. Combined with principal component analysis and fuzzy membership function method, it comprehensively evaluates the drought tolerance of the 5 germplasm materials under different degrees of drought stress, aiming to screen out excellent drought-resistant germplasm materials and provide a solid foundation for wheat drought-resistant variety breeding and genetic research.

# MATERIALS AND METHODS

**Experimental Materials.**The five wheat germplasm materials used in this experiment were all stable derivative materials from the cross between wheat and Leymus mollis, numbered H9, H24, H29, H30, and H47, which were provided by the Molecular Chromosome Engineering Laboratory of Northwest A&F University. The experimental reagent PEG-6000 was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. The experiment was conducted in the Plant Physiology and Biochemistry Laboratory of Weinan Normal University from September 2024 to January 2025.

Seed Treatment.In accordance with the *Rules for Crop Seed Testing - Germination Test* (GB/T 3543.4–1995), plump wheat seeds with basically uniform size, intact and free from mildew were selected. The seeds were disinfected by soaking in 5% sodium hypochlorite solution for 15 min, then rinsed with distilled water 3–4 times for later use. The treated seeds were placed with the ventral groove downward evenly in clean glass petri dishes (12 cm in diameter) lined with double layers of moist filter paper. The treatment solution was added to reach 2/3 of the seed height, and the petri dishes were placed in an environment with a 12 h light /12 h dark cycle and other suitable conditions. The filter paper and treatment solution were replaced regularly every day to maintain a constant concentration. The test included 4 treatment groups with 3 replicates each, specifically: 1. a control group (CK) cultured with distilled water; 2. a drought treatment group cultured with 5% PEG-6000 solution; 3. a drought treatment group cultured with 7.5% PEG-6000 solution; 4. a drought treatment group cultured with 10% PEG-6000 solution..

**Determination of Germination and Growth Indices.** The determination of wheat seed germination indices included germination energy (GE), germination rate (GR), germination index (GI), germination drought resistance index (GDRI), root length (RL) and seedling length (SL). Seed germination was defined as the plumule length reaching 1/2 of the seed length. Root length and seedling length were measured from the seed embryo to the root tip and leaf tip, respectively. The number of germinated seeds in each group was observed and recorded at regular intervals on the 2nd, 4th, 6th, and 8th days, with the plumule length reaching 1/2 of the seed length as the standard for germination. Germination was considered complete when no seeds germinated for 3 consecutive days. After germination, 10 seedlings were randomly selected from each treatment to measure RL and BL.

Germination energy = (Total number of germinated seeds on the 4th day of culture / Total number of tested seeds) × 100%;

Germination rate = (Number of germinated seeds on the 8th day of culture / Total number of tested seeds) × 100%;

Germination index = 1.00 × nd2 + 0.75 × nd4 + 0.50 × nd6 + 0.25 × nd8; where nd2, nd4, nd6, and nd8 are the germination rates on days 2, 4, 6, and 8, respectively.

Germination drought resistance index= (Germination index under stress / Germination rate of control material) × 100%.

Root length (RL) and bud length (BL) were measured with a ruler from the seed embryo to the tip of the longest root and plumule after germination, respectively. The specific operations were referred to the methods described by Zhang et al. [37].

**Determination of Physiological Indices.**After all growth indices were determined, the leaves of each treatment in each group were uniformly cut, frozen in liquid nitrogen, and stored in a -20℃ refrigerator for later use. Chlorophyll (Chl) content, malondialdehyde (MDA) content, and proline (Pro) content were determined by absorption spectrophotometry, thiobarbituric acid method, and sulfosalicylic acid-acidic ninhydrin method, respectively. The specific operations were referred to the methods of Zhang et al. [37].

**Data Processing and Analysis.**Microsoft Excel 2010 was used to organize the experimental data, and SPSS Statistics 27.0 was employed for significance analysis of differences ($P < 0.05$). Correlation analysis, principal component analysis, and fuzzy membership function method [38, 39] were adopted to comprehensively evaluate the drought tolerance of the five wheat materials under different treatments. Origin Pro 2022 was used as the graphing software.

# RESULTS

**Effects of Drought Stress on Wheat Seed Germination Energy (GE).**As shown in Fig. 1 (a), the GE of H9 under drought stress simulated by three concentrations of PEG-6000 decreased compared with CK, with a decrease of 20.68%, 59.85% and 64.44% respectively, among which the decrease was significant under 7.5% and 10% PEG-6000 ($P < 0.05$). The GE of H24 decreased by 11.53%, 32.86% and 78.99% respectively compared with CK, and the GE under 10% PEG-6000 decreased significantly compared with CK ($P < 0.05$). The GE of H29 increased by 1.4% under 5% PEG-6000 treatment compared with CK, but the difference was not significant, while it decreased significantly by 42.80% and 67.50% under 7.5% and 10% PEG-6000 treatments respectively. The GE of H30 decreased significantly under three concentrations of PEG-6000 treatment compared with CK, with a decrease of 29.63%, 50.62% and 67.90% respectively. The GE of H47 decreased significantly under three concentrations of PEG-6000 treatment compared with CK, with a decrease of 34.19%, 35.90% and 70.94% respectively. The above results indicated that a certain degree of drought stress would inhibit the germination of wheat materials, and the inhibitory effect on germination intensified with the increase of stress degree.

**Effects of Drought Stress on Wheat Seed Germination Rate (GR).**As shown in Fig. 1 (b), the GR of the five wheat materials showed a downward trend with the increase of drought stress concentration simulated by PEG-6000. The GR of H9 under drought stress simulated by three concentrations of PEG-6000 decreased compared with CK, with a decrease of 16.70%, 38.80% and 54.0% respectively, among which the decreases were significant under 7.5% and 10% PEG-6000 ($P < 0.05$). The GR of H24 decreased by 5.42%, 10.53% and 74.44% respectively compared with CK, and the GR under 10% PEG-6000 decreased significantly compared with CK ($P < 0.05$). The GR of H29 decreased by 3.00%, 36.74% and 56.98% respectively compared with CK, among which the decrease was significant under 7.5% and 10% PEG-6000. The GR of H30 decreased significantly under three concentrations of PEG-6000 treatment compared with CK, with a decrease of 18.60%, 47.67% and 63.18% respectively. The GR of H47 decreased significantly under three concentrations of PEG-6000 treatment compared with CK, with a decrease of 20.88%, 47.81% and 52.86% respectively.

When the concentration of PEG-6000 was 5%, the order of GR of the five wheat materials was H29 > H24 > H9 > H47 > H30. Combined with the decrease data, compared with CK, H29 had the smallest decrease, followed by H24, and H47 had the largest decrease, indicating that H29 and H24 had relatively better GR under low-concentration (5% PEG-6000) drought stress, while H47 had relatively poor GR. When the concentration of PEG-6000 was 7.5%, the order of GR of the five wheat materials was H24 > H29 > H9 > H47 > H30. Combined with the decrease data, compared with CK, H24 had the smallest decrease, and H47 and H30 had a decrease of more than 47%, indicating that H24 had better GR under 7.5% PEG-6000 drought stress, while H47 and H30 had poor GR. When the concentration of PEG-6000 was 10%, the GR was in the order of H47 > H9 > H29 > H24 > H30. Combined with the decrease data, compared with CK, H47 had the smallest decrease and H24 had the largest, indicating that H47 was the least affected by stress under 10% PEG-6000.

**Effects of Drought Stress on Wheat Seed Germination Index (GI).** As shown in Fig. 2 (c), the GI of the five wheat materials showed a downward trend with the increase of drought stress concentration simulated by PEG-6000. The GI of H9 under drought stress simulated by three concentrations of PEG-6000 decreased compared with CK, with a decrease of 15.95%, 47.53% and 62.29% respectively, among which the decreases were significant under 7.5% and 10% PEG-6000 ($P < 0.05$). The GI of H24 decreased by 19.88%, 25.65% and 81.00% respectively compared with CK, and the GI under 7.5% and 10% PEG-6000 decreased significantly compared with CK ($P < 0.05$). The GI of H29 decreased by 9.83%, 39.82% and 67.66% respectively compared with CK, among which the decrease was significant under 7.5% and 10% PEG-6000. The GI of H30 decreased significantly under three concentrations of PEG-6000 treatment compared with CK, with a decrease of 36.76%, 59.29% and 73.81% respectively.The GI of H47 decreased significantly under three concentrations of PEG-6000 treatment compared with CK, with a decrease of 35.07%, 43.03% and 69.97% respectively. When the concentration of PEG-6000 was 5%, the GI of the five wheat materials was in the order of H9 > H47 > H29 > H24 > H30. Compared with CK, H29 had the smallest decrease, followed by H9, and H30 had the largest, indicating that H29 and H9 had strong germination ability under low-concentration (5% PEG-6000) drought stress, while H30 was relatively poor; when the PEG-6000 concentration was 7.5%, the GI was in the order of H47 > H24 > H9 > H29 > H30. Combined with the decrease data, compared with CK, H24 had the smallest decrease and H30 had the largest, indicating that H24 had strong germination ability under 7.5% PEG-6000 drought stress, while H30 was poor. When the PEG-6000 concentration was 10%, the GI was in the order of H9 > H47 > H29 > H30 > H24. Combined with the decrease data, compared with CK, H9 had the smallest decrease, followed by H47, and H24 had the largest, indicating that H9 and H47 had strong germination ability under 10% PEG-6000 drought stress, while H24 was poor.

**Effects of Drought Stress on Wheat Seed Germination Drought Resistance Index (GDRI).** As shown in Fig. 2 (b), the GDRI of H9 under 5% PEG-6000 stress increased by 22.60% compared with CK, but the difference was not significant (*P* < 0.05), while it decreased by 37.70% and 53.80% under 7.5% and 10% PEG-6000 stress respectively, with significant differences (*P* < 0.05). The GDRI of H24 decreased by 19.90%, 25.67% and 81.03% respectively compared with CK, and the GDRI under 7.5% and 10% PEG-6000 decreased significantly compared with CK (*P* < 0.05). The GDRI of H29 under 5% PEG-6000 stress increased by 2.50% compared with CK, but the difference was not significant (*P* < 0.05), while it decreased by 31.57% and 63.27% under 7.5% and 10% PEG-6000 stress respectively, with a significant decrease under 10% PEG-6000 (*P* < 0.05).The GDRI of H30 decreased significantly under 3 concentrations of PEG-6000 treatment compared with CK, with a decrease of 28.57%, 54.00% and 70.47% respectively, among which the GDRI under 7.5% and 10% PEG-6000 decreased significantly compared with CK (*P* < 0.05). The GDRI of H47 decreased by 35.03%, 43.03% and 70.03% respectively under 3 concentrations of PEG-6000 treatment compared with CK.When the PEG-6000 concentration was 5%, the GDRI of the five wheat materials was in the order of H9 > H29 > H24 > H30 > H47. Compared with CK, H9 had a larger increase, followed by H9, and H47 had the largest decrease, indicating that H9 and H29 had strong germination drought resistance under low-concentration (5% PEG-6000) drought stress, while H47 was relatively poor; when the PEG-6000 concentration was 7.5%, the GDRI was in the order of H24 > H29 > H9 > H47 > H30. Combined with the decrease data, compared with CK, H24 had the smallest decrease and H30 had the largest, indicating that H24 had strong germination drought resistance under 7.5% PEG-6000 drought stress, while H30 was poor; when the PEG-6000 concentration was 10%, the GDRI was in the order of H9 > H29 > H47 > H30 > H24. Combined with the decrease data, compared with CK, H9 had the smallest decrease and H24 had the largest, indicating that H9 had strong germination drought resistance under 10% PEG-6000 drought stress, while H24 was poor. The germination drought resistance of the five wheat materials under different degrees of drought stress was basically consistent with GI.

**Effects of Drought Stress on Wheat Root Length (RL).** As shown in Fig. 3 (a), the RL of the five wheat materials showed a downward trend with the increase of drought stress concentration simulated by PEG-6000. Under low-concentration (5%) PEG-6000 stress, the RL of H47 decreased by 25.26% compared with the corresponding CK group, but the difference was not significant, and the decrease was the smallest compared with other materials. H24 was the next, with a decrease of 25.88%. The RL of H29 was the most inhibited, with a decrease of 45.16%, showing a significant difference ($P < 0.05$). Under medium-concentration (7.5%) PEG-6000 stress, H47 had the smallest RL decrease compared with the corresponding CK group, which was 31.65%. H30 had the largest RL inhibition, with a decrease of 85.77%. Under high-concentration (10%) PEG-6000 stress, the RL of all five wheat materials decreased significantly compared with the corresponding CK group, among which H47 had the smallest RL inhibition compared with the corresponding CK group, with a decrease of 42.21%. H30 had the largest RL inhibition, with a decrease of 79.85%. The above results indicated that the RL decrease of H47 under three different concentrations of PEG-6000 stress was lower than that of the other four materials, that is, the roots of H47 had strong ability to resist drought stress.

**Effects of Drought Stress on Wheat Seedling Length (SL).** As shown in Fig. 3 (b), the SL of the five wheat materials showed a significant downward trend compared with the CK group with the increase of drought stress concentration simulated by PEG-6000 ($P < 0.05$). Under low-concentration (5%) PEG-6000 stress, the inhibition degree of SL of the five wheat materials was in the order of H9 > H24 > H29 > H47 > H30. The SL of H9 decreased significantly by 12.79% compared with the corresponding CK group, with the smallest decrease compared with other materials; H24 was the next, with a decrease of 20.53%; the seedling growth of H30 was the most inhibited compared with CK, with a decrease of 62.09%. Under medium-concentration (7.5%) PEG-6000 stress, the inhibition degree of SL of the five wheat materials was in the order of H24 > H29 > H47 > H9 > H30. The SL of H24 had the smallest decrease compared with the corresponding CK group, which was 31.65%; the SL of H30 was the most inhibited, with a decrease of 85.77%. Under high-concentration (10%) PEG-6000 stress, the inhibition degree of SL of the five wheat materials was in the order of H47 > H9 > H24 > H29 > H30. The seedling growth of H47 was the least inhibited compared with the corresponding CK group, with a decrease of 79.20%; H30 was the most inhibited, with a decrease of 94.77%.

**Effects of Drought Stress on Wheat Leaf Chlorophyll (Chl) Content.** As shown in Fig. 4 (a), the Chl content in leaves of the five wheat materials showed a downward trend with the increase of drought stress concentration simulated by PEG-6000. The Chl content of H9 under drought stress simulated by 3 concentrations of PEG-6000 decreased compared with CK, with a decrease of 14.30%, 45.17% and 60.13% respectively, among which the decrease was significant under 7.5% and 10% PEG-6000 ($P < 0.05$). The Chl content of H24 decreased by 31.22%, 38.25% and 56.64% respectively compared with CK, among which the decrease was significant under 10% PEG-6000 ($P < 0.05$). The Chl content of H29 decreased by 5.22%, 67.41% and 82.52% respectively compared with CK, among which the decrease was significant under 7.5% and 10% PEG-6000 ($P < 0.05$). The Chl content of H30 decreased significantly under 3 concentrations of PEG-6000 treatment compared with CK, with a decrease of 20.71%, 36.67% and 64.23% respectively. The Chl content of H47 decreased by 3.07%, 14.40% and 25.82% respectively compared with CK, among which the decrease was significant under 10% PEG-6000 ($P < 0.05$). When the PEG-6000 concentration was 5%, the order of Chl content decrease of the five wheat materials was H47 > H29 > H9 > H30 > H24, indicating that under low-concentration (5% PEG-6000) drought stress, the Chl synthesis ability of H47 was less affected, while that of H24 was more affected. When the PEG-6000 concentration was 7.5%, the order of Chl content decrease was H47 > H30 > H24 > H9 > H29, indicating that under medium-concentration (7.5% PEG-6000) drought stress, the Chl synthesis ability of H47 was less affected, while that of H29 was more affected. When the PEG-6000 concentration was 10%, the order of Chl content decrease was H47 > H24 > H9 > H30 > H29, indicating that under high-concentration (10% PEG-6000) drought stress, the Chl synthesis ability of H47 was still less affected, while that of H29 was the most affected.

**Effects of Drought Stress on Wheat Leaf Malondialdehyde (MDA) Content.** As shown in Fig. 4 (b), the MDA content in leaves of the five wheat materials showed an upward trend with the increase of drought stress concentration simulated by PEG-6000. The MDA content of H9 under drought stress simulated by 3 concentrations of PEG-6000 increased compared with CK, with an increase of 13.53%, 63.63% and 167.71% respectively; the MDA content of H24 increased by 32.45%, 96.17% and 174.57% respectively compared with CK; the MDA content of H29 increased by 10.68%, 47.14% and 96.77% respectively compared with CK. These three materials showed significant differences compared with the corresponding CK under 7.5% and 10% PEG-6000 drought stress ($P < 0.05$). The MDA content of H30 increased significantly under 3 concentrations of PEG-6000 treatment compared with CK, with an increase of 58.90%, 115.04%, and 168.47% respectively; the MDA content of H47 increased by 34.09%, 73.71%, and 134.13% respectively compared with CK, with significant differences ($P < 0.05$). When the PEG-6000 concentration was 5%, the order of MDA content increase of the five wheat materials was H30 > H47 > H24 > H9 > H29, indicating that under low-concentration (5% PEG-6000) drought stress, H30 had the largest increase in MDA content, and H29 had the smallest; when the PEG-6000 concentration was 7.5%, the order of MDA content increase was H30 > H24 > H47 > H9 > H29, indicating that under medium-concentration (7.5% PEG-6000) drought stress, H30 had the largest increase in MDA content, and H29 had the smallest; when the PEG-6000 concentration was 10%, the order of MDA content increase was H24 > H30 > H9 > H47 > H29, indicating that under high-concentration (10% PEG-6000) drought stress, H24 had the largest increase in MDA content, and H29 had the smallest. The above results indicated that H29 could resist drought stress of the same degree with a relatively small increase in MDA content.

**Effects of Drought Stress on Wheat Leaf Proline (Pro) Content.** As shown in Fig. 4 (c), the Pro content in leaves of the five wheat materials showed an increasing trend with the increase of drought stress concentration simulated by PEG-6000, and all increased significantly compared with the corresponding CK group ($P < 0.05$). Under drought stress simulated by 3 different concentrations (5%, 7.5%, and 10%) of PEG-6000, the order of Pro content increase of the five wheat materials was H30 > H9 > H47 > H29 > H24, that is, H24 had the smallest increase (21.3%-93.6%), followed by H29 (27.4%-131.8%), and H30 had the largest (181.2%-762.1%). The above results indicated that H24 and H29 could resist different degrees of drought stress with a relatively small increase in Pro content.

**Correlation and Principal Component Analysis of Various Indices of Wheat under Different Treatments.** **Figure 10** shows that there were different degrees of correlation between different indices. GE was extremely significantly positively correlated with GR, GI, GDRI, RL, SL and Chl content, and extremely significantly negatively correlated with MDA content and Pro content.

As shown in Table 1, principal component analysis of 9 indices under drought stress obtained 2 principal components, with eigenvalues of 6.956 and 0.972, contribution rates of 77.294% and 10.800% respectively, and a cumulative contribution rate (88.094%) > 85%. The first 2 components could be extracted as comprehensive evaluation indices for wheat drought tolerance at the germination stage. Among them, the first principal component (PC1) had the highest contribution rate of 77.294%, which was a drought stress-sensitive principal component. GI had the highest eigenvector value of 0.970, followed by GE, GR, GDRI, SL, RL and Chl. Except for Chl, the eigenvector values of the other indices were greater than 0.8, indicating that PC1 was positively correlated with these 7 indices and could accurately reflect the changes of these 7 indices of wheat materials under salt stress. The second principal component (PC2) had a contribution rate of 10.800%, and its main eigenvectors were Chl, RL, and MDA.

Table 1
Principal component analysis of each index of five wheat materials under drought stress

| Component | Principal component 1 | Principal component 2 |
|---|---|---|
| GE | 0.960 | -0.059 |
| GR | 0.934 | -0.084 |
| GI | 0.970 | -0.051 |
| GDRI | 0.915 | -0.259 |
| RL | 0.807 | 0.46 |
| SL | 0.869 | -0.275 |
| Chl | 0.631 | 0.737 |
| MDA | -0.906 | 0.243 |
| Pro | -0.871 | -0.05 |
| Eigenvalue | 6.956 | 0.972 |
| Contribution rate (%) | 77.294 | 10.800 |
| Cumulative contribution rate (%) (%) | 77.294 | 88.094 |

Comprehensive evaluation of wheat drought tolerance under different treatments by membership function value method. In this experiment, a total of 9 related indices including germination indices and physiological indices were used to analyze the comprehensive indices of the five wheat materials under different treatments by the membership function method, and the overall effects of different treatments on wheat drought tolerance were evaluated respectively. As shown in Table 2, according to the membership function values, under 5% PEG-6000 drought stress, the drought tolerance order of the five wheat materials was H47 > H9 > H24 > H29 > H30; under 7.5% PEG-6000 drought stress, the order was H47 > H24 > H9 > H29 > H30; under 10% PEG-6000 drought stress, the order was H47 > H9 > H29 > H30 > H24. According to the average membership function values of the five wheat materials under different concentrations of drought treatment, the comprehensive drought tolerance order was: H47 > H9 > H24 > H29 > H30.

Table 2
Comprehensive factor scores and rankings of drought tolerance of five wheat germplasm materials under different treatments

| Materials | PEG-6000 concentration | Subordinative function value | Ranking of drought tolerance |
|---|---|---|---|
| H9 | 0 | 0.875 | 2 |
| | 5% | 0.634 | 7 |
| | 7.5% | 0.389 | 13 |
| | 10% | 0.229 | 17 |
| H24 | 0 | 0.828 | 3 |
| | 5% | 0.627 | 8 |
| | 7.5% | 0.482 | 12 |
| | 10% | 0.088 | 20 |
| H29 | 0 | 0.742 | 5 |
| | 5% | 0.625 | 9 |
| | 7.5% | 0.356 | 14 |
| | 10% | 0.129 | 18 |
| H30 | 0 | 0.805 | 4 |
| | 5% | 0.620 | 10 |
| | 7.5% | 0.356 | 15 |
| | 10% | 0.127 | 19 |
| H47 | 0 | 0.935 | 1 |
| | 5% | 0.662 | 6 |
| | 7.5% | 0.537 | 11 |
| | 10% | 0.319 | 16 |

# DISCUSSION

Effects of Drought Stress on Germination and Growth Indices of Five Wheat Materials. The germination and seedling stage is the most sensitive period to drought stress and a key stage for early identification of crop drought resistance [6, 7]. In this study, five different wheat germplasm materials were selected to determine the changes of growth indices (GE, GR, GI, GDRI, RL and SL) and physiological indices (Chl content, MDA content, and Pro content) at the germination stage. Among the growth indices, GE and GI are indices to measure the germination speed and uniformity of seeds, GR can reflect the potential germination ability of seeds, GDRI can comprehensively evaluate the drought tolerance of different materials, and growth parameters such as RL and SL are external intuitive phenotypes of plant growth under stress, which can reflect stress resistance, that is, the greater the growth, the stronger the stress resistance, and vice versa. This study showed that drought stress significantly inhibited wheat seed germination and growth, and the inhibition degree was positively correlated with stress intensity, which was consistent with the research results of Wang et al. [40] and Mahpara et al. [41], that is, the germination indices of wheat materials were inversely proportional to the concentration of PEG-6000. The reason might be that drought stress limited seed water absorption, hindered the synthesis and transportation of growth hormones, affected the normal elongation of embryos and their smooth penetration through seed coats, and further affected the growth potential and growth potential of seeds. H47 performed outstandingly in RL and GR and GI under 10% PEG-6000 stress, which might be due to its root system's ability to maintain strong water absorption capacity, alleviating drought damage. This was consistent with the conclusion of Zhang et al. [42], who reviewed that wheat could enhance its absorption of soil moisture and adaptation to drought environment by regulating root development and changing root morphology (including root length, number of lateral roots and root hair density). That is, wheat varieties with developed root systems and strong osmotic adjustment ability could better maintain cell turgor under drought stress, ensuring normal seed germination and seedling growth. H29 and H9 performed well under 5% PEG-6000 stress, with small decreases in GR and GI, which might be because they could quickly activate their own defense mechanisms in mild drought environments, reduce membrane system damage during seed imbibition, or activate the activity of antioxidant enzymes in seeds, timely scavenge excessive reactive oxygen species produced by drought, and reduce membrane lipid peroxidation damage [43]. In contrast, H30 was extremely sensitive to drought. Under three different concentrations of PEG-6000 stress, the decrease in SL was among the highest. Under 10% PEG-6000 stress, its SL decreased by 94.77% compared with the control, far exceeding other materials. Wang et al. [27] also found that the two most affected indices of wheat at the germination stage were bud fresh weight and bud length, which decreased by 65.56% and 62.30% respectively compared with the control after drought stress. This indicated that H30 had poor cell membrane stability and low antioxidant system activity, making it difficult to resist oxidative damage caused by drought. In addition, it was found that the germination drought resistance of the five materials was highly consistent with GI. Under different concentrations of PEG-6000 stress, materials with higher GI had relatively stronger germination drought resistance, indicating that GI could be used as a reliable index for rapid evaluation of wheat drought resistance at the germination stage, providing an efficient evaluation basis for subsequent screening of drought-resistant wheat varieties.

Effects of Drought Stress on Chlorophyll (Chl) Content in Leaves of Five Wheat Materials. Chl plays a role in absorbing, transferring, and converting light energy, and is the main functional pigment for plant photosynthesis. Changes in its content directly reflect the response ability of the photosynthetic system to stress, which is positively correlated with the degree of stress damage to plants [5, 29,41]. In this study, the Chl content in leaves of the five wheat materials decreased with the increase of PEG-6000 concentration, which was consistent with the research results of Chen et al. [44], but the degree of influence varied significantly among different materials. Under three different concentrations of PEG-6000 stress, H47 had the smallest decrease in Chl content, indicating that its Chl synthesis system had strong tolerance to drought, which might be because H47 could maintain high δ-aminolevulinic acid (ALA) synthetase activity in drought environments, ensuring a stable supply of Chl precursor substances [45]; at the same time, its antioxidant system could effectively scavenge reactive oxygen species, reduce chloroplast membrane lipid peroxidation, maintain the integrity of thylakoid membrane structure, and thus ensure the normal progress of Chl synthesis and metabolism. This was consistent with the conclusion of Mahpara et al. [41] that drought-tolerant wheat varieties could maintain Chl content by stabilizing chloroplast structure under drought. In contrast, H29 had the largest decrease in Chl content under high-concentration stress, suggesting that its photosynthetic system had poor adaptability to drought. It might be that H29 had a significant decrease in stomatal conductance under high-concentration stress, leading to insufficient $CO_2$ supply and blocked carbon assimilation, indirectly inhibiting Chl synthesis [26]; at the same time, drought-induced oxidative stress might damage the expression regulation of Chl synthesis-related genes, accelerating Chl degradation [31]. H24 had a large decrease in Chl under low-concentration stress, which might be related to its early sensitivity to drought, and its photosynthetic system might need a longer time to activate effective defense mechanisms.

Effects of Drought Stress on MDA Content in Leaves of Five Wheat Materials. MDA, as the end product of membrane lipid peroxidation, is produced in large quantities during plant growth under stress, and can form insoluble compounds with other macromolecular substances, interfering with normal cell functions [46]. The change in its content is positively correlated with the degree of stress, reflecting the antioxidant capacity and stress response of seeds, so MDA content can be used to evaluate stress resistance [47]. In this study, with the increase of PEG-6000 concentration, the MDA content in leaves of the five wheat materials increased significantly, indicating that drought stress destroyed the integrity and structural stability of the plasma membrane system in plant leaves, led to metabolic disorders in plants, caused an imbalance between the production and scavenging of reactive oxygen species, and further aggravated membrane lipid peroxidation, resulting in a large amount of MDA. This was basically consistent with the changes in MDA content under drought stress in rice [15,16], soybean [19], barley [21], and other plants. However, there were significant differences in the increase among different materials. H29 had the smallest increase in MDA content under low, medium, and high-concentration stress, indicating that its cell membrane structure had strong stability in drought environments, which might be because H29 had an efficient antioxidant defense system, which could quickly activate antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD), timely scavenge excessive reactive oxygen species (ROS) induced by drought, thereby reducing membrane lipid peroxidation, and maintaining cell membrane integrity, which was consistent with the conclusion of Li et al. [12] that wheat with high antioxidant enzyme activity had low MDA accumulation.In contrast, H30 had the largest increase in MDA content under low and medium-concentration stress, indicating that its cell membrane was extremely sensitive to drought. The possible reason was that H30 had low basic activity of antioxidant enzymes, which could not timely scavenge ROS, leading to intensified membrane lipid peroxidation and massive accumulation of MDA. H24 had the largest increase in MDA content under high-concentration stress, which might indicate that its antioxidant system was "overloaded" under severe drought, unable to continuously resist oxidative damage, resulting in uncontrolled membrane lipid peroxidation.

Effects of Drought Stress on Pro Content in Leaves of Five Wheat Materials. Pro is a water-soluble amino acid, a key osmotic adjustment substance for plants to cope with stress, which can act as an antioxidant to scavenge reactive oxygen species and protect cell membrane stability. Its accumulation is closely related to plant drought resistance and can be used as an important physiological index to identify plant drought resistance [43,46]. In this study, the Pro content of the five wheat germplasm materials increased significantly with the increase of PEG-6000 concentration, reflecting the stress mechanism of plants to reduce cell water potential and maintain osmotic balance by accumulating Pro. This change trend of Pro content was basically consistent with that in rice [15,16], soybean [19], barley [21], and other plants under drought stress. By actively accumulating the osmotic adjustment substance Pro to increase cell osmotic potential, maintain cell water absorption and retention capacity, avoid excessive salt entering cells, alleviate salt stress damage to cells, and thus resist the harm of stress to plants.However, there were significant differences in the increase of Pro among different materials. H24 and H29 could effectively resist drought with a small increase, indicating that they might have more efficient osmotic adjustment strategies. H30 had the largest increase in Pro content but weak drought resistance, which was inconsistent with the results of Li et al. [12] and Yang et al. [29]. The possible reason was that its antioxidant capacity was insufficient (excessive accumulation of MDA), resulting in serious cell damage, leading to the overactivation of the Pro synthesis metabolic pathway but failing to effectively play a regulatory role.

Evaluation Indices of Drought Resistance of Five Wheat Materials. Crop drought resistance is a quantitative trait controlled by multiple genes [24, 25]. A single drought resistance index is difficult to comprehensively and accurately evaluate drought resistance. Principal component analysis and membership function method are effective tools for systematic evaluation of wheat drought resistance. The cumulative contribution rate of the two principal components extracted in this study reached 88.094%, which concentrated the key information of 9 indices. Among them, the first principal component (PC1) had a contribution rate of 77.294%, mainly including 7 indices such as GI and GR, reflecting the core response of wheat germination and early growth under drought stress, which was consistent with the conclusion that germination indices were key evaluation bases for drought tolerance in previous studies [16, 22, 26]. GI had the highest eigenvector value (0.970), indicating that it was the most sensitive to drought stress and could be used as an important index for rapid screening of drought-tolerant varieties. The second principal component (PC2) focused on Chl content, RL and MDA content, revealing the synergistic effect of photosynthesis, root development, and cell membrane stability on wheat drought resistance.The membership function method comprehensively quantified multi-index data and clarified the drought tolerance order of the five wheat germplasm materials. H47 ranked first under different concentration stresses, which was consistent with its excellent performance in indices such as GR, RL and Chl content, verifying the reliability of the comprehensive evaluation method. In contrast, H30 had the worst drought tolerance, which was comprehensively reflected in its high MDA accumulation, low Chl stability, and weak germination ability in the evaluation. It is worth noting that the rankings of H24 and H29 fluctuated under different stress concentrations, which might be due to differences in their trade-off strategies between osmotic adjustment (less Pro accumulation) and antioxidant capacity (small increase in MDA).

In conclusion, this study conducted PEG-6000 simulated drought stress with different concentrations on five wheat germplasm materials, and found that with the intensification of stress, the inhibitory effect on the six growth indices (GE, GR, GI, GDRI, RL and SL) enhanced; Chl content decreased with the increase of stress concentration; MDA content and Pro content generally showed an upward trend with the increase of PEG-6000 concentration, and the higher the PEG-6000 concentration, the more significant the difference. From the perspective of principal component analysis, six indices in principal component 1, including GE, GR, GI, GDRI, RL and BL (with eigenvector values all > 0.8), could be used as the main evaluation indices for drought tolerance of wheat materials. Combined with the membership function method, the comprehensive evaluation of drought tolerance of the five wheat materials showed that the comprehensive ranking was H47 > H9 > H24 > H29 > H30, that is, H47 had the highest comprehensive evaluation value of drought resistance and the strongest drought tolerance. In conclusion, this study can provide excellent germplasm resources and basic research materials for wheat drought-resistant variety breeding and drought-resistant gene mining.

## Declarations

# COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies with human participants performed by any of the authors.

### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

# Author Contribution

AUTHOR CONTRIBUTIONSXiaofei Yang: conception and design, acquisition of data, analysis and interpretation of data, drafting the article; Wenting Zhang: manuscript drafting and critical revision of important intellectual content; Wenjing Dang: data interpretation and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

# Data Availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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# Figures

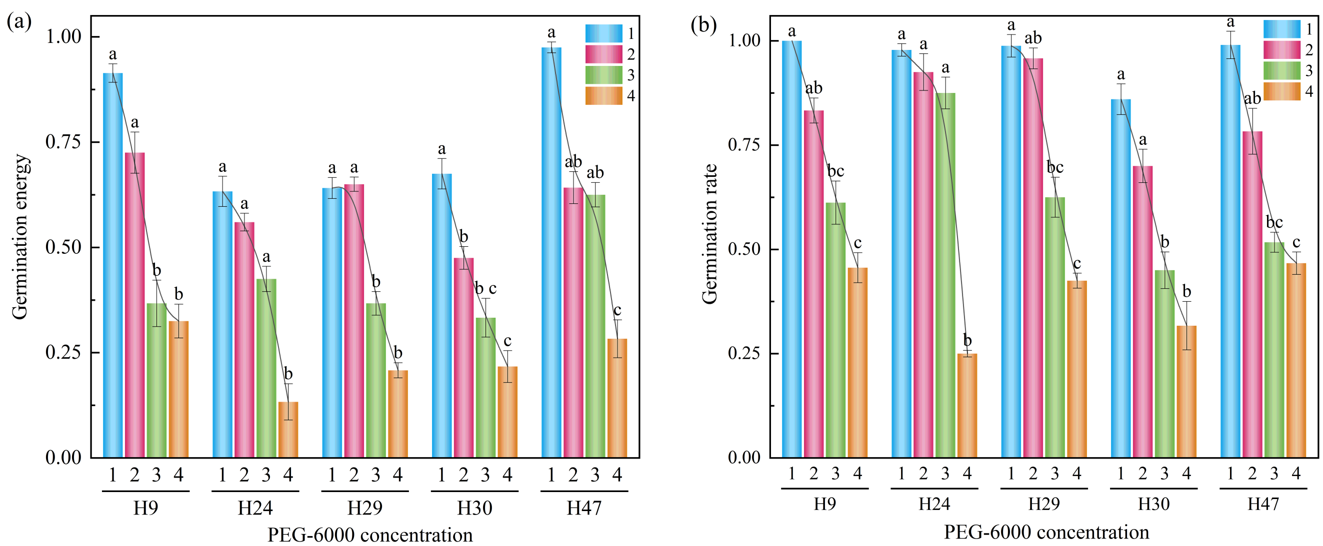
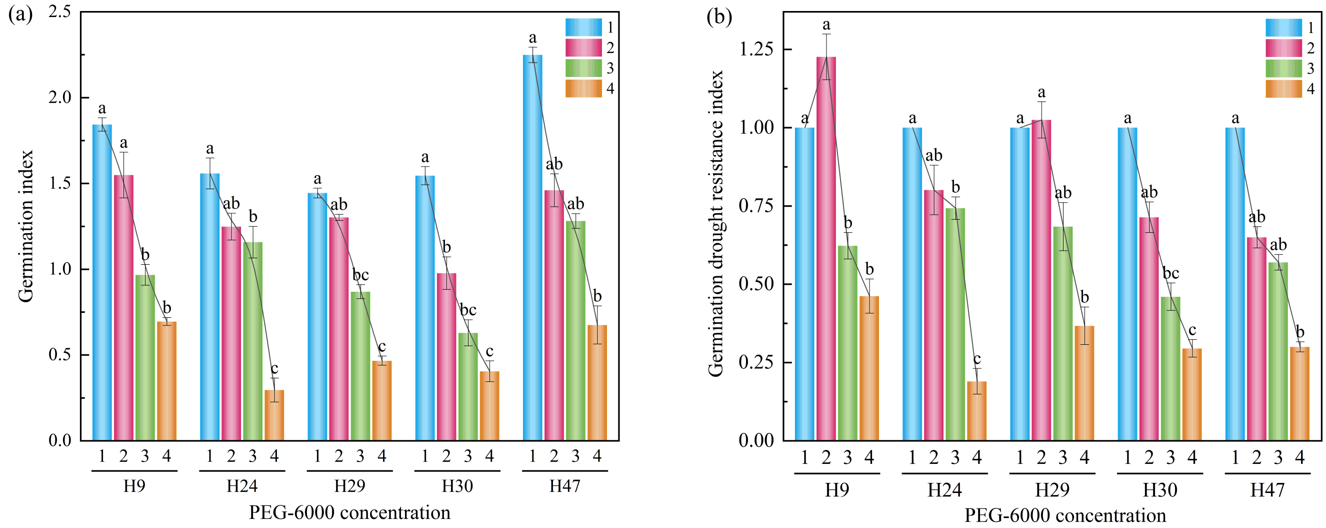



**Figure 1**

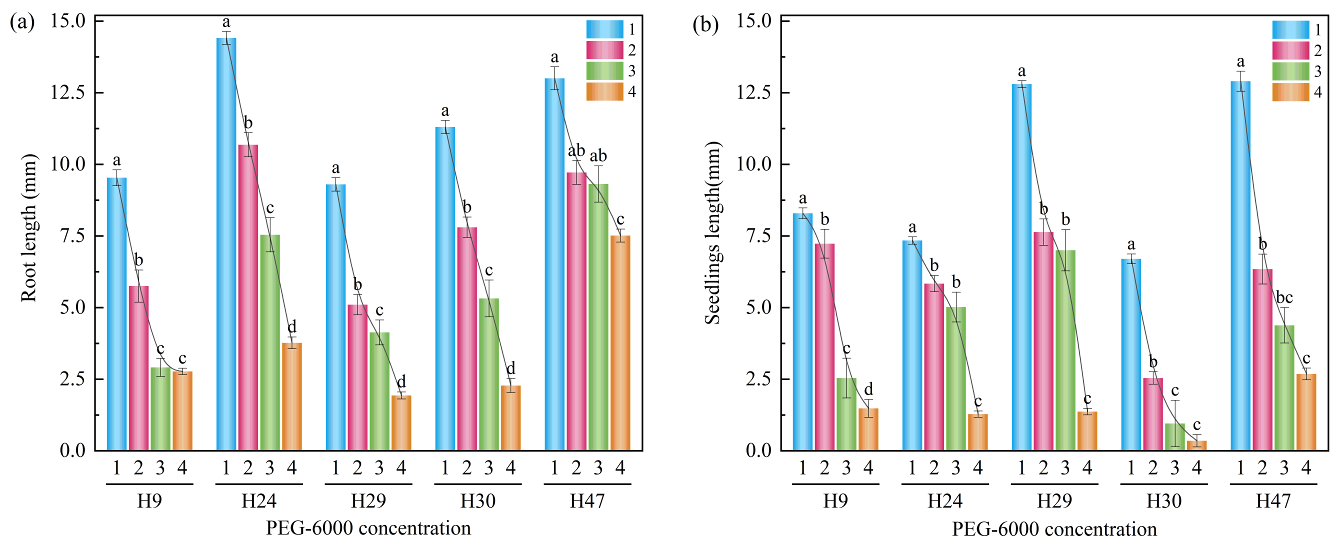
The changes in germination energy (a) and germination rate (b) of five wheat materials under PEG-6000 drought stress at different concentrations

**Note:** Abscissa 1-4 represent the concentrations of drought treatments in seed treatment section. Different letters indicate significant differences between treatments (*P* < 0.05). The same as below.



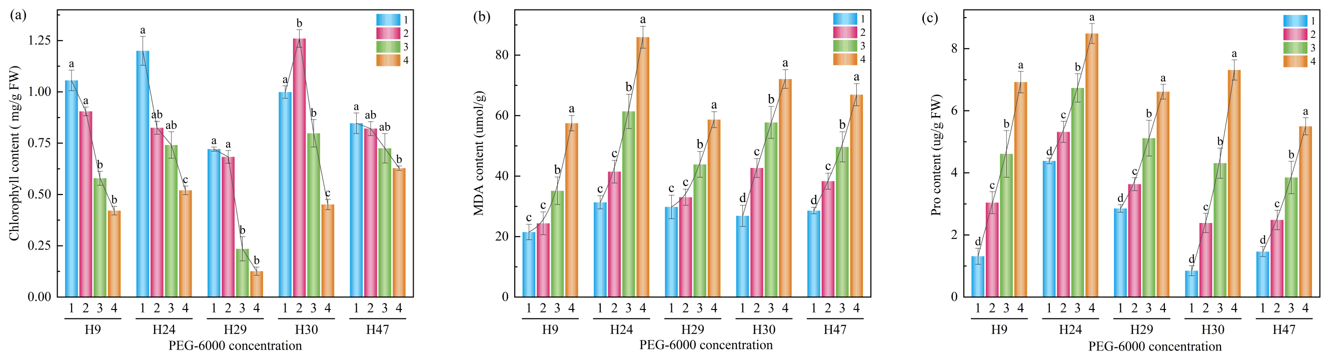

## Figure 2

The changes in germination index (a) and ermination drought resistance index (b) of five wheat materials under PEG-6000 drought stress at different concentrations



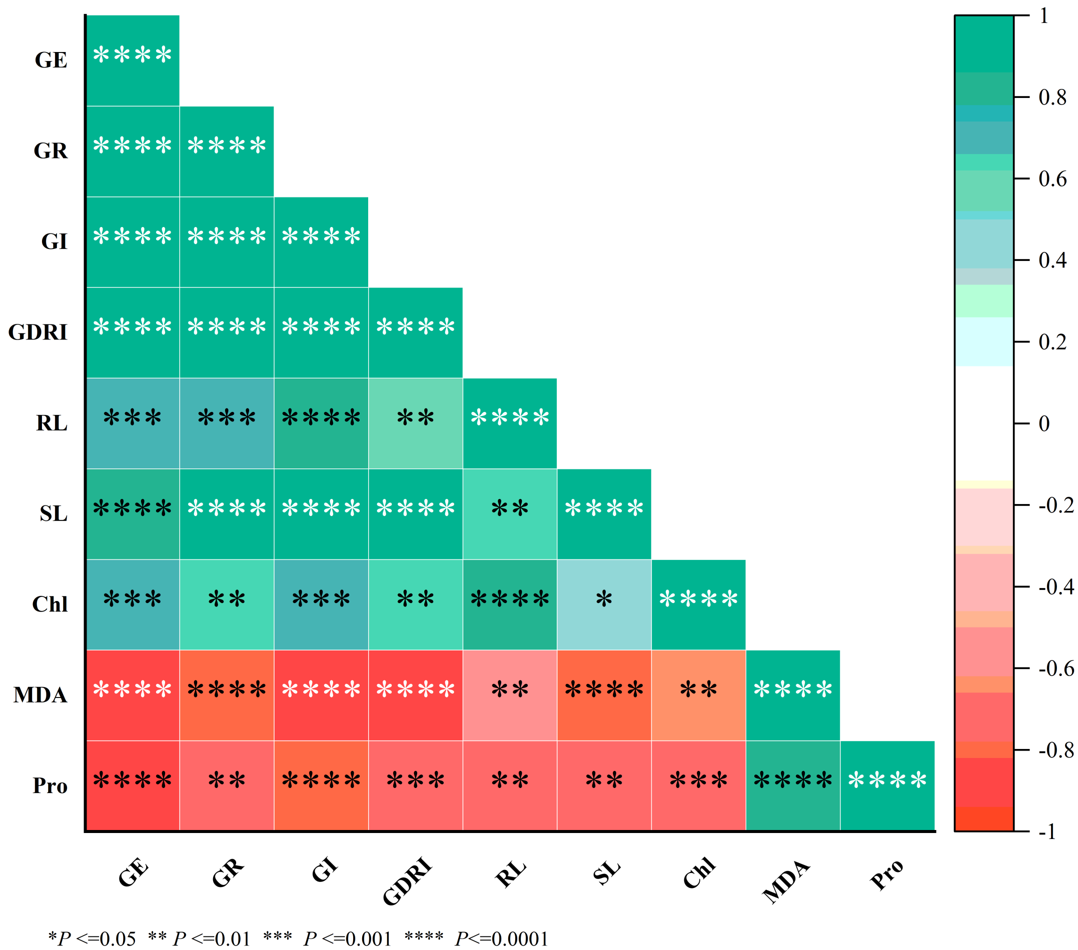

## Figure 3

The changes in root length (a) and seedling length (b) of five wheat materials under PEG-6000 drought stress at different concentrations




## Figure 4

The changes in Chl (a), MDA (b) and Pro (c) content of five wheat materials under PEG-6000 drought stress at different concentrations




## Figure 5

Correlation analysis between various indexes under different treatments

**Note:** * indicates a significant correlation, **or *** indicates an extremely significant correlation. The green square indicates a positive correlation, the red square indicates a negative correlation.