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Comparative Analysis of Drought Tolerance in Xindan Maize Varieties at the Seedling Stage under PEG-Induced Stress

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Abstract: Drought is a major constraint on maize production, particularly in the Huang–Huai–Hai region of China. This study aimed to evaluate drought resistance of newly developed maize varieties at the seedling stage. A 20% polyethylene glycol (PEG) solution was used to simulate drought stress. Two maize varieties, Xindan 58 and Xindan 65, were analyzed for morphological and physiological traits, including plant height, primary root length, chlorophyll content, malondialdehyde (MDA), proline (PRO), and antioxidant enzyme activities (CAT, POD, and SOD). The results reveal that drought stress significantly reduced plant height (21.6%–45.1%) and primary root length (30.8%–40.1%). Chlorophyll content decreased by 20.5%–29.2%. In contrast, proline content increased markedly (358.1%–4438.7%), and antioxidant enzyme activities were enhanced. MDA content showed a decreasing trend in most varieties. The magnitude of these responses differed among varieties. Overall, both Xindan 58 and Xindan 65 exhibited drought resistance at the seedling stage, with Xindan 58 showing a stronger tolerance. These findings provide useful information for drought-resistant maize breeding and variety selection.

Keywords: Maize; Drought Stress; Seedling Stage; PEG; Physiological Response.

1. Introduction

Drought stress is one of the most important factors limiting crop production in northern China and represents the leading cause of yield loss among all abiotic stresses. In maize, drought can reduce annual yield by 20%–30%. Drought stress at the seedling stage primarily affects vegetative growth, which subsequently influences final yield [1]. It has been reported that drought inhibits plant height, leaf area, and dry matter accumulation [2]. In addition, drought induces excessive accumulation of reactive oxygen species (ROS) in leaves, leading to membrane lipid peroxidation [3–4]. It also reduces photosynthetic capacity by affecting chloroplast structure and chlorophyll content [5–6].

Drought tolerance varies among maize varieties. Therefore, evaluating drought resistance at the seedling stage is of great importance for genetic improvement and the selection of drought-tolerant germplasm. Previous studies have identified significant variation in drought tolerance among maize genotypes[7-10]. For example, Hengyu 321 has been reported to maintain high yield under both drought and well-watered conditions [7]. Comprehensive evaluations of multiple maize varieties have identified Lainong 14, Nongda 108, and Jinhai 5 as drought-tolerant, while Zhengdan 958, Zhongke 11, and Jundan 20 were classified as drought-sensitive [8]. Systematic studies integrating yield, drought resistance index, root traits, anthesis–silking interval (ASI), and physiological parameters have further identified stable drought-tolerant varieties [9]. At the germination stage, varieties such as Jingke 968, showed strong drought tolerance across different stress intensities [10]. In addition, PEG-induced water stress combined with cluster analysis has been used to classify maize germplasm into different drought tolerance levels and to identify drought-tolerant varieties [11].

The Huang–Huai–Hai region is located in the central maize production belt of China and is one of the major summer maize growing areas, with an annual planting area exceeding 13.33 million hectares and total production above 20 million tons. However, this region is characterized by uneven precipitation, distinct seasonal variation, high temperature, and strong evaporation, making it highly prone to drought [12–13]. Frequent drought events not only reduce crop yield but also threaten regional ecological stability. Therefore, the development and promotion of drought-tolerant maize varieties are essential for sustainable production in this region.

In recent years, the Xindan series maize varieties developed by Xinxiang Academy of Agricultural Sciences have been widely approved and cultivated in the Huang–Huai–Hai region due to their high yield and strong stress resistance. In this study, two maize varieties were used as experimental materials. A 20% PEG solution was applied to simulate drought stress at the seedling stage. Agronomic traits, chlorophyll content, membrane stability, osmotic adjustment substances, and antioxidant enzyme activities were analyzed to evaluate drought resistance. This study aims to provide a theoretical basis for genetic improvement of drought

tolerance and to support the selection of maize varieties with strong drought resistance and high yield potential.

2. Materials and Methods

2.1. Plant Materials

Two maize varieties, Xindan 58 and Xindan 65, were used as experimental materials. Both are nationally approved hybrids developed by Xinxiang Academy of Agricultural Sciences, with approval numbers Guoshenyu 20190238 and Guoshenyu 20190003, respectively. Two widely cultivated maize hybrids in the Huang–Huai–Hai region, Zhengdan 958 and Xianyu 335, were used as control varieties.

2.2. Experimental Design

Uniform and healthy maize seeds were surface-sterilized with 1% sodium hypochlorite for 10 min and rinsed 3–5 times with distilled water. The sterilized seeds were germinated in sterile sand at room temperature for 4 days.

Seedlings at the one-leaf stage were transferred to culture boxes (15 cm × 20 cm × 25 cm) and grown in Hoagland nutrient solution. The nutrient solution was aerated daily and renewed every 3 days. Growth conditions were maintained at 25/20°C (day/night), with a 14 h photoperiod, 65% relative humidity, and a light intensity of 400 μmol·m^{-2}·s^{-1}. Drought stress was applied at the three-leaf stage. Two treatments were established: CK (normal Hoagland nutrient solution) and PEG (Hoagland solution containing 20% polyethylene glycol). Each treatment included three pots with 10 plants per pot, and three biological replicates were conducted. Seedlings in the PEG treatment were exposed to drought stress for 8 days before sampling.

2.3. Measurements

Ten representative plants were selected from each replicate for analysis. The plant height was measured from the root base to the tip of the highest leaf and expressed in centimeters (cm). The primary root length and lateral root number were measured using an EPSON Expression™ 1680 root scanner and analyzed with WinRHIZO software. The Chlorophyll content: measured on the first fully expanded leaf below the youngest leaf using a SPAD-502 chlorophyll meter. The physiological parameters were determined as previously described. For example, Antioxidant enzyme activities were determined following standard protocols. Enzyme extracts were prepared according to Li et al. [14]; Superoxide dismutase (SOD) activity was measured following Dhindsa et al. [15]; Peroxidase (POD) activity was determined according to Tatiana et al. [16]; Catalase (CAT) activity was measured following Mishra et al. [17]; Malondialdehyde (MDA) content was determined according to Agarwal et al. [18], and proline (PRO) content was measured using the sulfosalicylic acid method [19].

2.4. Statistical Analysis

All data were processed and analyzed using Microsoft Excel 2011. Differences among treatments were evaluated based on standard statistical methods.

3. Results

3.1. Effects of Drought Stress on Plant Height and Root Traits at the Seedling Stage

To evaluate the effects of drought stress on seedling growth, plant height and root traits were measured under PEG treatment. The results showed PEG treatment significantly reduced both plant height and primary root length in all maize varieties compared with the control (CK) ($P < 0.01$). For plant height, the reduction was greatest in Xindan 58 (45.1%), followed by Xindan 65 (33.8%), while Xianyu 335 showed the smallest decrease (21.6%) (Fig. 1A). For primary root length, Xindan 65 exhibited the largest reduction (40.1%), whereas Xindan 58 showed the smallest decrease (30.9%) (Fig. 1B). No significant differences were observed in the number of lateral roots between PEG treatment and CK in all varieties (Fig. 1C). These results indicate that drought stress markedly inhibited both shoot and root growth at the seedling stage, with varying responses among different maize varieties.

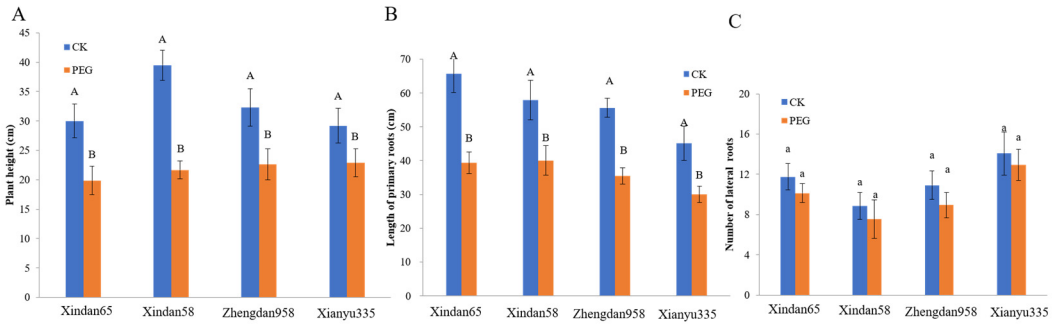


Fig 1. Effects of PEG treatment on plant height, primary root length, and lateral root number in four maize hybrids (Xindan 65, Xindan 58, Zhengdan 958, Xianyu 335).

(**A**) Plant height, (**B**) primary root length, (**C**) lateral root number. CK, control; PEG, polyethylene glycol treatment. CK, control; PEG, polyethylene glycol treatment. The differences between different capital and lowercase letters between the same variety treatments reached extremely significant (P<0.01) and significant (P<0.05) levels. Data are mean ± SD. The same notation applies to Figs. 2–5.

3.2. Effects of Drought Stress on Chlorophyll Content at the Seedling Stage

To assess the impact of drought stress on photosynthetic capacity, chlorophyll content was measured in maize seedlings. We found that PEG treatment significantly reduced chlorophyll content in the leaves of all maize varieties compared with CK ($P < 0.01$). Among the varieties, the reductions in chlorophyll content were 21.1% and 20.9% in

Xindan 65 and Xindan 58, respectively, which were comparable to that of Xianyu 335 (20.5%). In contrast, Zhengdan 958 showed the largest decrease (29.2%) (Fig. 2). These results suggest that the effects of drought stress on photosynthetic capacity were similar in Xindan 65 and Xindan 58, and both varieties exhibited relatively smaller reductions compared with Zhengdan 958.

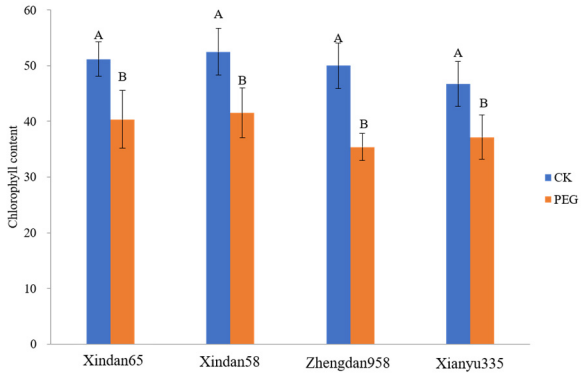


Fig 2. Effects of PEG treatment on chlorophyll content in four maize hybrids at seedling stage

Chlorophyll levels in Xindan 65, Xindan 58, Zhengdan 958, and Xianyu 335 under control (CK) and drought stress (PEG). Data represent mean ± SD.

3.3. Effects of Drought Stress on Membrane Lipid Peroxidation at the Seedling Stage

Malondialdehyde (MDA), a product of membrane lipid peroxidation, is commonly used as an indicator of membrane damage under stress conditions. As shown in Fig. 3, PEG treatment led to a decrease in MDA content in most maize varieties compared with CK, except for Xianyu 335. These reductions were statistically significant or highly significant. Among the varieties, Xindan 65 showed the largest decrease in MDA content (31.3%), whereas Xindan 58 exhibited a relatively smaller reduction (24.4%). These results suggest that Xindan 58 maintained relatively stable membrane integrity under drought stress.

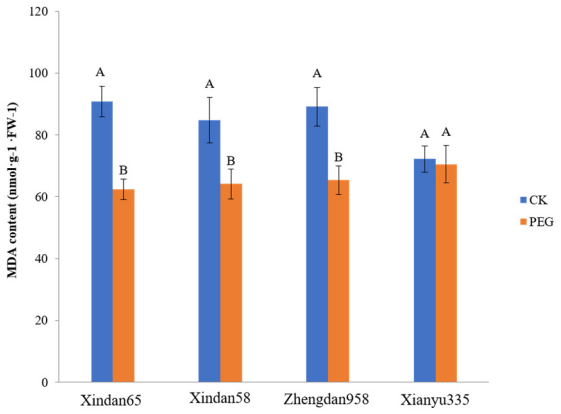


Fig 3. Effects of PEG treatment on MDA content in four maize hybrids at seedling stage.

MDA levels in Xindan 65, Xindan 58, Zhengdan 958, and Xianyu 335 under control (CK) and drought stress (PEG). Data represent mean ± SD.

3.4. Effects of Drought Stress on Osmotic Adjustment (Proline Content) at the Seedling Stage

Proline (PRO) is a major osmotic adjustment substance in maize seedlings. As shown in Fig. 4, PEG treatmen significantly increased proline content in all maize varieties compared with CK ($P < 0.01$). Among the varieties, Xindan 58 exhibited the greatest increase (4438.7%), followed by Xindan 65 (3514.3%). These results indicate that proline accumulation was markedly enhanced under drought stress, with higher levels observed in Xindan 58 and Xindan 65.

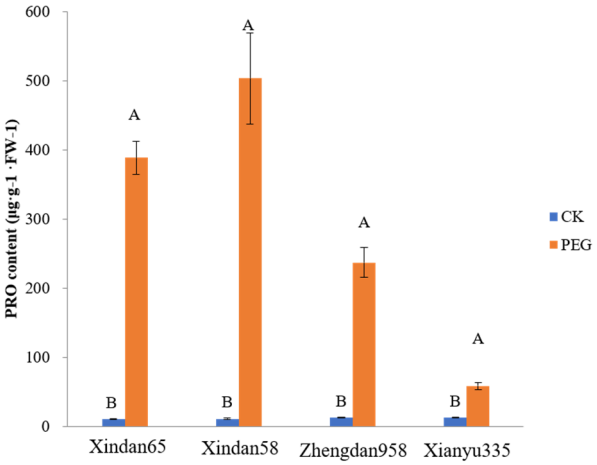


Fig 4. Effects of drought stress on proline (PRO) content in four maize hybrids at seedling stage

Seedlings of Xindan 65, Xindan 58, Zhengdan 958, and Xianyu 335 were grown under control condition (CK) or treated with PEG (polyethylene glycol) to impose drought stress. Proline (PRO) content was measured in leaf tissues. Data are presented as mean ± SD (or SE) from at least three biological replicates.

3.5. Effects of Drought Stress on Antioxidant Enzyme Activities at the Seedling Stage

To explore the effects of drought Stress on antioxidant enzyme activities, catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in all maize varieties were determined. The results revealed that the PEG treatment increased the activities of CAT, POD, and SOD in all maize varieties compared with CK (Fig. 5A), although the magnitude of increase varied among varieties. Except for SOD activity in Xindan 65, which showed no significant change, the activities of all three enzymes in the other varieties increased significantly ($P < 0.01$) (Fig. 5A-C). Among the varieties, CAT activity showed the greatest increase in Xindan 58 (88.6%) and the smallest increase in Xindan 65 (46.4%). The order of increase in CAT activity was: Xindan 58 > Zhengdan 958 > Xianyu 335 > Xindan 65 (Fig. 5B). For POD activity, the increase followed the order: Xindan 58 > Zhengdan 958 > Xindan 65 > Xianyu 335 (Fig. 5C). For SOD activity, the increase followed the order: Xindan 58 > Zhengdan 958 > Xianyu 335 > Xindan 65 (Fig. 5A). These results suggest that Xindan 58 has strong drought tolerance and a greater ability to alleviate oxidative damage caused by drought stress.

Seedlings of Xindan 65, Xindan 58, Zhengdan 958, and Xianyu 335 were subjected to PEG (polyethylene glycol) treatment to simulate drought stress. Activities of three

antioxidant enzymes were measured: (**A**) superoxide dismutase (SOD), (**B**) catalase (CAT), and (**C**) peroxidase (POD). Data are presented as mean ± SD (or SE) from at least three independent replicates.

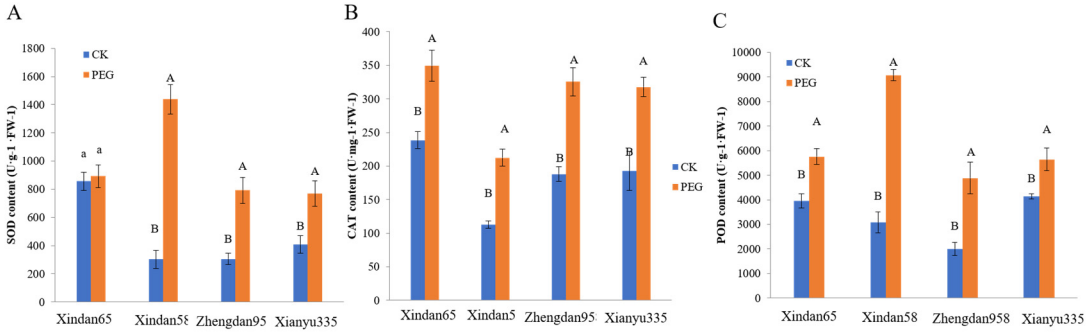


Fig 5. Effects of PEG treatment on protective enzyme activities in four maize hybrids

4. Discussion

Previous studies have shown that drought stress inhibits plant growth by restricting leaf and stem elongation, resulting in reduced leaf area and overall biomass. At the physiological level, osmotic adjustment, osmotic protection, and antioxidant defense systems are considered key mechanisms underlying drought tolerance [25]. In the present study, drought stress significantly reduced plant height, primary root length, and chlorophyll content in all maize varieties, indicating strong inhibition of vegetative growth and photosynthetic capacity. Changes in MDA content further reflected the effects of drought on membrane stability. Although MDA content is generally reported to increase under stress, it may decrease under prolonged stress or during recovery phases [22]. In this study, MDA content decreased in most varieties under drought treatment, suggesting differences in membrane lipid peroxidation responses among genotypes.

Proline accumulation is a common response to drought stress and plays an important role in osmotic adjustment. The significant increase in proline content observed in all varieties indicates enhanced osmotic regulation capacity under drought conditions. In addition, the activities of antioxidant enzymes (CAT, POD, and SOD) increased to varying degrees, demonstrating activation of the antioxidant defense system to mitigate oxidative damage. These results are consistent with previous findings [23–25].

Notably, the magnitude of response varied among varieties, indicating genotypic differences in drought tolerance. Xindan 58 showed relatively smaller changes in MDA content, greater accumulation of proline, and higher increases in antioxidant enzyme activities, suggesting better membrane stability, stronger osmotic adjustment capacity, and enhanced oxidative stress tolerance. Xindan 65 also exhibited relatively strong responses, although to a lesser extent. Overall, the coordinated regulation of osmotic adjustment and antioxidant defense appears to play a critical role in drought tolerance at the seedling stage, with Xindan 58 showing a more effective adaptive response to drought stress.

5. Conclusion

This study evaluated two newly developed maize varieties, Xindan 58 and Xindan 65, from the Huang-Huai-Hai region. Under drought stress, plant height, primary root length, and chlorophyll content decreased significantly; MDA content also decreased significantly or highly significantly, while proline content and the activities of CAT, POD, and SOD increased. Comprehensive analysis shows that both varieties possess seedling-stage drought resistance, with Xindan 58 being stronger than Xindan 65. These findings highlight the value of integrated physiological evaluation and support the use of Xindan 58 and Xindan 65 in drought-prone areas of the Huang-Huai-Hai region.

Author Contributions

X.Z. designed the project. L.T., X.W., F.W., and D.S. conducted experiments. L.T., X.W., Z.H., S.L., Y.H., and Z.Z. analyzed the data. L.T., X.W., and X.Z. wrote the manuscript. X. Z. supervised the project. All authors read and approved the manuscript.

Competing Interests

The authors declare no competing interests.

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