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# The effects of induced osmotic stress on specific physiological responses in various maize cultivars

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**Abstract**
Drought is one of the main consequences of climate change and negatively affects plant growth and development, resulting in reduced yield. The objective of this study was to investigate the effects of the physiological responses to drought in some maize corn hybrids. The tested maize hybrids were physiologically and biochemically evaluated and the following parameters were monitored: germination stage, chlorophyll content, and proline content in leaves. These analyses highlight the hybrid stress response. The lowest average germination percentage (26.8056%) was observed in the medium supplemented with 190 g/l PEG. Osmotic stress had a very significant negative influence on chlorophyll accumulation in the tested hybrids. The highest proline content was recorded in the Kashmir and P9398 hybrids, indicating their enhanced osmotic adjustment capacity under stress conditions. The superior performance of certain hybrids (Kashmir for proline, P9911 for chlorophyll and germination) could be leveraged in breeding programs aimed at improving stress resistance.

**Keywords:** drought, corn, proline, chlorophyll

## Introduction

Maize is a highly versatile and economically significant crop, utilized extensively in the food, feed, fodder, and biofuel sectors. Additionally, it is widely processed in dry and wet milling industries to produce a range of products, including flour, cooking oil, pharmaceuticals, glue, artificial sweetener, alcoholic beverages, and starch [20]. Furthermore, maize is used in the production of ethanol fuel. Like all other crops, maize plants grown under natural conditions are exposed to various abiotic stresses throughout their life cycle [14,15,17]. Water deficit stress is considered as one of the most important environmental factors that adversely affect maize production [3, 17, 18]. Prolonged periods of water deficit will result in smaller leaves, premature flowering and a longer anthesis–silking interval, ultimately decreasing yield potentials [4, 6]. Maize seedlings growing under water stress conditions exhibit several important physiological responses, including decreased cell turgor [7, 9], leaf rolling [12], inhibited CO2 exchange and decreased photosynthetic efficiency and chlorophyll contents [5,14, 15]. The photosynthetic and gas exchange responses are the most sensitive to water deficits [25], and maintaining relatively high photosynthetic activity levels may enhance plant drought tolerance. Water stress is a significant environmental factor that severely impacts maize growth and development, inducing irreversible changes at physiological, biochemical, and molecular levels. The severity of these effects depends on the intensity, duration, cultivar, and stage of plant development under stress conditions. Research has demonstrated the substantial effects of water stress on physio morphological, phonological, biochemical, and molecular traits in maize [19,22,23,24,27]. Studies suggest that maize grain yield could be reduced by 10-27% due to decreased irrigation frequencies [13]. Furthermore, a projected decrease of 15-20% in global maize yield and production per year is anticipated due to heat and drought stress conditions, which pose a significant threat to maize production [8].

## Material and Method

The biological material used consisted of maize hybrids from different FAO groups:(P9398- FAO 330; P9911- Group FAO 410; P9757- Group FAO 370; Kashmir- FAO 370).

Determination of germination capacity. The germination capacity of plants can be assessed in a laboratory setting by inducing water stress using a hypertonic solution containing PEG 6000. This method allows for the evaluation of drought tolerance in plants from the seed germination phase. To establish the germination capacity, 100 seeds were used in three repetitions for each medium variant. The seeds were sterilized with 30% ethanol for 3 minutes, then rinsed with sterile distilled water to remove ethanol residues. (Srivastava et al. 2010). The seeds were tested under normal conditions (V0-water) and under stress conditions (V1-143g/lH2O, V2-190g/lH2O). The water stress conditions were created using a PEG 6000 solution. For this purpose, different concentrations of PEG 6000 dissolved in distilled water were used. The seeds were placed in Petri dishes on filter paper, and 5 ml of the osmotic solution was added to each dish along with distilled water for control. The boxes were kept in the growth chamber under day/night photoperiod conditions of 10/14 hours. The temperature during the study period was 20°C, and the humidity was 55%. Water was added to all dishes with osmotic solutions and was added every 3 days. Seed germination was recorded at 7, 14, and 21 days.

Determination of proline content. Bates method was used for measure of proline [2]. To quantify the amount of proline in plants, they were subjected to water stress using polyethylene glycol (PEG) solutions applied at two concentrations, with water serving as a control. The plants were cultivated under controlled conditions, with a 14/10 day/night photoperiod and a temperature of 24/18 degrees Celsius. Proline was extracted from the plants at various time intervals following stress induction, specifically: - after three days from stress induction with a PEG 6000 solution at 143g/l H2O, - after 21 days from stress induction with a PEG 6000 solution at 190g/l H2O. This method relies on the principle that proline reacts with ninhydrin or ninhydrin acid to form a yellowish-reddish compound, which can be extracted into toluene. Finally, the upper phase containing red complex was used to measure the proline at a wavelength of 520 nm by a spectrophotometer (Shimadzu UV-160A Model, Japan).

Measurement of chlorophyll contents. To determine the drought-induced changes in chlorophyll content were assessed using a SPAD-502 portable chlorophyll meter (Konica Minolta Inc., Tokyo, Japan). The third fully expanded leaf (from the top) of each seedling was analyzed after the 3-day drought, 21-day drought. Each leaf was analyzed three times at different sites. The chlorophyll content of each leaf was based on the average of three readings.

To determine the significance of the differences between the varieties, the processing of the experimental data was done by variance analysis and the t-test. The meanings were expressed based on symbols [8].

## Results and Discussion

From the analysis of variance (table 1) it is observed that seedling age and interaction with the environment have a distinctly significant effect on germination.

The results indicate that seedling age significantly influences germination percentage in maize. Compared to 7-day-old plantlets, those aged 14 and 21 days showed significantly higher germination means ($p < 0.01$ and $*p < 0.001$, respectively). However, the difference between 14 and 21 days was not statistically significant, suggesting a potential saturation point in the effect of age on germination beyond 14 days.

The influence of environmental conditions on maize germination was assessed by comparing three variants (V0, V1, and V2). Although variant V0 showed the highest germination rate (85.30%) and V2 the lowest (26.80%), the differences between the treatments (V1–V0: –34.77%, V2–V0: –58.50%, V2–V1: –23.72%) were not statistically significant, as they did not exceed the minimum LSD value at the 5% level (2.23%). These results suggest that, under the tested conditions, environmental variations had no significant effect on seed germination.

**Table 1. Analise of variance**

| Cause of variability | SP | GL | S2 | F test |
|---|---|---|---|---|
| Total | 78104.10 | 107 | | |
| Large plots | 5301.52 | 8 | | |
| Repetitions | 441.91 | 2 | | |
| A | 4468.46 | 2 | 2234.23 | 22.85 |
| Error A | 391.15 | 4 | 97.79 | |

| | | | | |
|---|---|---|---|---|
| Medium plots | 65992.83 | 18 | | |
| B | 62333.85 | 2 | 31166.93 | 1554.39** |
| A x B | 3418.37 | 4 | 854.59 | 42.62* |
| Error B | 240.61 | 12 | 20.05 | |
| Small plots | 6809.75 | 81 | | |
| C | 3608.25 | 3 | 1202.75 | 33.05 |
| A x C | 142.72 | 6 | 23.79 | 0.65 |
| B x C | 854.44 | 6 | 142.41 | 3.91 |
| A x B x C | 239.33 | 12 | 19.94 | 0.55 |
| Error C | 1965.00 | 54 | 36.388 | |

**Table 2. Results on the effect of seedling age on germination**

| Seedling age | Average (%) | | Relative value % | Difference | Signification. |
|---|---|---|---|---|---|
| 14 days -7 days | 56.30 | 45.50 | 123.75 | 10.80 | ** |
| 21 days -7 days | 60.83 | 45.50 | 133.70 | 15.33 | *** |
| 21 days -14days | 60.83 | 56.30 | 108.04 | 4.52 | |
| | | | LSD 5% 5.71 | LSD 1% 8.64 | LSD0.1%13.89 |

**Table 3. Results on the effect of the environment on germination in corn**

| Variants | Average (%) | | Relative value (%) | Difference | Signification |
|---|---|---|---|---|---|
| V1-V0 | 50.52 | 85.30 | 59.23 | -34.77 | - |
| V2-V0 | 26.80 | 85.30 | 31.42 | -58.50 | - |
| V2-V1 | 26.80 | 50.52 | 53.05 | -23.72 | - |
| | | | LSD5%2.23 | LSD1%3.08 | LSD0.1%4.24 |

The results showed that the hybrid P9398 had the highest germination rate (63.77%), with significantly higher values compared to P9911, P9757, and Kashmir ($p < 0.001$). Among the remaining hybrids, P9757 and Kashmir performed better than P9911, with differences of 4.66% ($p < 0.01$) and 3.40% ($p < 0.05$), respectively. No significant difference was found between Kashmir and P9757, suggesting similar germination potential under the tested conditions.

**Table 4. Results regarding the effect of the hybrid on germination in corn**

| Hybrids | Average (%) | | Relative value % | Difference | Signification. |
|---|---|---|---|---|---|
| P9911-P9398 | 48.33 | 63.77 | 75.78 | -15.44 | ooo |
| P9757-P9398 | 53.00 | 63.77 | 83.10 | -10.77 | ooo |
| Kashmir-P9398 | 51.74 | 63.77 | 81.13 | -12.03 | ooo |
| P9757- P9911 | 53.00 | 48.33 | 109.66 | 4.66 | ** |
| Kashmir- P9911 | 51.74 | 48.33 | 107.05 | 3.40 | * |
| Kashmir-P9757 | 51.74 | 53.00 | 97.62 | -1.25 | - |
| | | | LSD 5% 3.38 | LSD 1% 4.58 | LSD 0.1% 6.12 |

For proline content, the analysis of variance (table 5) shows that the differences between the analyzed hybrids were real and were significantly influenced by the heterogeneity of the experimental conditions.

**Table 5. Analise of variance**

| Cause of variability | SP | GL | S2 | F test |
|---|---|---|---|---|
| Total | 1.22 | 107 | | |
| Large plots | 0.32 | 8 | | |
| Repetitions | 0.06 | 2 | | |
| A | 0.22 | 2 | 0.11 | 9.95* |
| Error A | 0.04 | 4 | 0.01 | |

| | | | | |
|---|---|---|---|---|
| Medium plots | 0.65 | 18 | | |
| B | 0.33 | 2 | 0.17 | 8.25* |
| A x B | 0.07 | 4 | 0.02 | 0.93 |
| Error B | 0.24 | 12 | 0.02 | |
| Small plots | 0.25 | 81 | | |
| C | 0.02 | 3 | 0.01 | 4.26 |
| A x C | 0.05 | 6 | 0.01 | 4.13 |
| B x C | 0.02 | 6 | 0.00 | 2.03 |
| A x B x C | 0.05 | 12 | 0.00 | 2.16 |
| Error C | 0.10 | 54 | 0.001 | |

Proline content in maize seedlings increased with age, reaching 0.81 mg/g at 21 days compared to 0.70 mg/g at 7 days. The difference between 21-day-old and 7-day-old seedlings was statistically significant ($p < 0.01$), while differences between 14 and 7 days, and between 21 and 14 days, were not significant. These results suggest that proline accumulation is enhanced as seedlings mature, particularly after two weeks of growth.

**Table 6. Results on the effect of seedling age on proline content in corn**

| Seeding age | Average (mg/g) | | Relative value % | Difference | Signification |
|---|---|---|---|---|---|
| 14 days -7 days | 0.75 | 0.70 | 108.17 | 0.05 | |
| 21 days -7 days | 0.81 | 0.70 | 115.80 | 0.11 | ** |
| 21 days -14days | 0.81 | 0.75 | 107.05 | 0.05 | |
| | | | LSD 5%0.06 | LSD1%0.09 | LSD 0.1%0.14 |

The proline content in maize seedlings increased in response to PEG 6000-induced osmotic stress. At the highest concentration (V2), proline levels reached 0.827 mg/g, significantly higher than the control (V0, 0.691 mg/g) ($p < 0.05$). Although proline content also increased at the intermediate level (V1), the difference from the control was not statistically significant. These results suggest that a threshold level of stress is required to trigger a significant increase in proline synthesis. Our results align with previous work showing PEG-induced proline accumulation in maize seedlings [21]. Specifically, only the highest PEG concentration (V2) led to a significant proline increase of +0.136 mg/g ($p < 0.05$), representing ~20% rise consistent with documented proline increases ranging from 25–183% under osmotic stress. This threshold response is well documented, where only beyond moderate stress levels does proline biosynthesis ramp up. Notably, studies involving acetic acid or sorbitol co-treatment report even larger proline accumulation, suggesting that biochemical priming can amplify this osmo protective mechanism, Collectively, these findings underscore proline's role as both a marker and mediator of drought stress, and point to priming strategies as potential enhancers of stress tolerance.

**Table 7. Results regarding the effect of PEG 6000 concentration on proline content in corn**

| Variants | Average (mg/g) | | Relative value (%) | Difference | Signification |
|---|---|---|---|---|---|
| V1-V0 | 0.755 | 0.691 | 109.34 | 0.064 | |
| V2-V0 | 0.827 | 0.691 | 119.68 | 0.136 | * |
| V2-V1 | 0.827 | 0.755 | 109.45 | 0.071 | |
| | | | LSD 5%0.07 | LSD1%0.09 | LSD0.1%0.13 |

From table 8, the results demonstrate that proline content varies among maize hybrids, with Kashmir showing the highest average value (0.773 mg/g) and P9757 the lowest (0.735 mg/g). A statistically significant difference was observed between Kashmir and P9757 ($p < 0.01$), suggesting that Kashmir may have superior osmotic regulation capabilities under stress conditions. These findings are consistent with previous studies indicating genotypic variability in proline accumulation, often linked to drought tolerance [16, 21]. The lack of significant differences between Kashmir, P9911, and P9398 may indicate that these hybrids share comparable stress-adaptive responses in terms of osmolyte accumulation.

**Table 8. Results regarding the effect of the hybrid on on proline content in corn**

| Hybrids | Average (mg/g) | | Relative value (%) | Difference | Signification |
|---|---|---|---|---|---|
| P9911-P9398 | 0.755 | 0.769 | 98.22 | -0.014 | |
| P9757-P9398 | 0.735 | 0.769 | 95.59 | -0.034 | o |
| Kashmir-P9398 | 0.773 | 0.769 | 100.63 | 0.005 | |
| P9757- P9911 | 0.735 | 0.755 | 97.31 | -0.020 | |
| Kashmir- P9911 | 0.773 | 0.755 | 102.45 | 0.018 | |
| Kashmir-P9757 | 0.773 | 0.735 | 105.28 | 0.039 | oo |
| | | | LSD5%0.02 | LSD1%0.03 | LSD0.1%0.04 |

Proline as the most stable amino acid is common in many plants and naturally accumulates in large amounts in response to the environmental stresses. In addition to its role as osmolyte, proline plays a role in the stabilization of micro-cellular structures such as membranes, proteins and destroys free radicals in stress situation. In terms of stress, plants may be compatible with the production of metabolites such as amino acid, antioxidant and hormones to counteract the effects of stress and continue growing. In fact, proline as a chemical chaperone, stabilize proteins natural form and inhibits disturbing the folding of enzymes [26]. In some reports, a correlation is shown between accumulation of proline and plant resistance to environmental stresses [1]. Research indicates that by increasing the concentration of drought stress, proline increases. In stress conditions, glutamate that is the precursor for the synthesis of chlorophyll and proline goes into proline production.

Analysis of variance (Table 9) reveals that the age of seedlings and the interaction with water deficit have a distinct significant effect on chlorophyll accumulation.

The chlorophyll content of corn seedlings, measured by SPAD values, showed no significant change between 7 and 14 days. However, a significant increase was observed at 21 days compared to both 7 and 14 days, indicating continued chlorophyll accumulation with seedling maturation. The rise of 2.62 to 3.05 SPAD units ($p < 0.001$) reflects enhanced photosynthetic capacity as the seedlings develop, consistent with established growth patterns in maize and other cereals.

Increasing PEG 6000 concentration adversely affected chlorophyll content in corn seedlings. While the lower PEG concentration (V1) caused no significant change relative to the control (V0), the higher PEG level (V2) significantly reduced chlorophyll content by approximately 3.7 to 4.3 SPAD units ($p < 0.001$). This decline likely reflects osmotic stress-induced damage or inhibition of chlorophyll biosynthesis, consistent with reports in maize and other crops under drought or osmotic stress conditions [11].

The chlorophyll content differed significantly among the corn hybrids tested. P9911 exhibited the highest SPAD values, significantly exceeding P9398 and P9757, which had the lowest chlorophyll content. Kashmir showed intermediate values, significantly higher than P9757 but not significantly different from P9398 or P9911. This suggests that P9911 may have enhanced photosynthetic potential, which could contribute to improved growth and stress tolerance. These findings are consistent with previous reports highlighting genotypic variation in photosynthetic pigment content in maize [11].

**Table 9. Analysis of variance for chlorophyll in corn hybrids**

| Cause of variability | SP | GL | S2 | F test |
|---|---|---|---|---|
| Total | 2950.73 | 107 | | |
| Large plots | 534.86 | 8 | | |
| Repetitions | 326.75 | 2 | | |
| A | 196.87 | 2 | 98.43 | 35.01* |
| Error A | 11.25 | 4 | 2.81 | |
| Medium plots | 694.85 | 18 | | |
| B | 384.60 | 2 | 192.30 | 20.71* |
| A x B | 198.84 | 4 | 49.71 | 5.35 |
| Error B | 111.41 | 12 | 9.28 | |
| Small plots | 1721.02 | 81 | | |
| C | 349.20 | 3 | 116.40 | 6.87 |
| A x C | 249.49 | 6 | 41.58 | 2.45 |

| | | | | |
|---|---|---|---|---|
| B x C | 29.99 | 6 | 5.00 | 0.29 |
| A x B x C | 177.30 | 12 | 14.77 | 0.87 |
| Error C | 915.05 | 54 | 16.945 | |

**Table 10. Results on the effect of corn seedling age on chlorophyll content**

| Seedling age | Average (SPAD) | | Relative value % | Difference | Signification |
|---|---|---|---|---|---|
| 14 days -7 days | 31.96 | 32.39 | 98.69 | -0.42 | |
| 21 days -7 days | 35.02 | 32.39 | 108.12 | 2.62 | *** |
| 21 days -14days | 35.02 | 31.96 | 109.55 | 3.05 | *** |
| | | | LSD5%0.96 | LSD 1%1.46 | LSD 0.1%2.35 |

**Table 11. Results on the effect of PEG (6000) concentration on chlorophyll in corn**

| Variants | Average (SPAD) | | Relative value % | Difference | Signification |
|---|---|---|---|---|---|
| V1-V0 | 34.72 | 34.17 | 101.62 | 0.55 | |
| V2-V0 | 30.47 | 34.17 | 89.18 | -3.69 | ooo |
| V2-V1 | 30.47 | 34.72 | 87.76 | -4.25 | ooo |
| | | | LSD 5% | LSD1% | DL 0.1% |
| | | | 1.523 | 2.097 | 2.887 |

**Table 12. Results on the effect of the hybrid**

| Hybrids | Average (SPAD) | | Relative value % | Difference | Signification |
|---|---|---|---|---|---|
| P9911-P9398 | 35.71 | 32.45 | 110.03 | 3.25 | *** |
| P9757-P9398 | 30.76 | 32.45 | 94.77 | -1.69 | - |
| Kashmir-P9398 | 33.57 | 32.45 | 103.44 | 1.11 | - |
| P9757- P9911 | 30.76 | 35.71 | 86.13 | -4.95 | ooo |
| Kashmir- P9911 | 33.57 | 35.71 | 94.01 | -2.14 | - |
| Kashmir-P9757 | 33.57 | 30.76 | 109.14 | 2.81 | * |
| | | | LSD 5%2.30 | LSD 1%3.12 | LSD 0.1%4.17 |

## Conclusions

Corn germination significantly improves with increasing seedling age, being highest at 21 days compared to 7 and 14 days. Proline content increases with seedling age, showing a significant difference between 21 and 7 days, indicating greater proline accumulation as the plant develops, which may be related to enhanced stress adaptation. Higher PEG concentrations, simulating osmotic stress, cause a significant increase in proline content, especially at the highest treatment level. Chlorophyll content significantly decreases at higher PEG concentrations, suggesting impaired photosynthetic capacity under severe osmotic stress conditions. The Kashmir hybrid showed the highest proline content, significantly higher than P9757, suggesting better osmotic stress tolerance. P9911 exhibited the highest chlorophyll content and germination rates, significantly outperforming P9398 and P9757, indicating superior photosynthetic potential and seedling vigor. Significant differences among hybrids in biochemical and physiological parameters highlight the importance of selecting hybrids adapted to specific environmental and stress conditions. Chlorophyll content increased significantly at 21 days compared to 7 and 14 days, reflecting ongoing accumulation of photosynthetic pigments as seedlings mature. The superior performance of certain hybrids (Kashmir for proline, P9911 for chlorophyll and germination) could be leveraged in breeding programs aimed at improving stress resistance.

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