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RESEARCH ARTICLE

Effect of Exogenous Application of Proline in Drought Stress Condition in Maize at Seedling Stage

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ABSTRACT

Maize (*Zea mays*) is a grass broadly cultivated for its seed, a cereal grain and it is a global staple food. However, water shortage poses a serious threat to its production globally. Drought stress negatively affects the growth and development of maize leading to, less germination, diminished grain yield and quality. The experiment was performed in a wire house of the department of Plant Breeding and Genetics, at The Islamia University of Bahawalpur. Six maize genotypes were screened for drought at various levels along with foliar application of proline during the seedling stage using complete randomize design (CRD). The major traits chlorophyll contents, shoot length, root length, fresh shoot weight, dry root weight, fresh root weight and dry root weight traits were studied. The best genotype G2 (P-1429) showed minimum reduction percentage in traits under the stress condition of drought as compared to normal condition. The best genotype G1 (P1-543) showed minimum reduction percentage in traits under the stress condition of drought along with Foliar Application of Proline. The results show that Foliar Application of Proline poses positive significant effect and reduces the negative effect of drought stress on the growth of maize seedling under drought stress. Keeping in view these genotype performances, we can increase the yield by using this Breeding material from above screening genotypes of maize under drought stress and overcome the challenge of drought in Agriculture sector of Pakistan.

Key words: Maize Drought Stress, Genotype Screening, Proline Foliar Application, Maize Growth Traits, Sustainable Agriculture

INTRODUCTION

Maize (*Zea mays* L.), is one of the most important cereal crops growing in the world. It is used as food for human consumption as well as food grain for animals (Moussa, 2001). Due to the rapid increase in the global population, there is a need for expansion in crop areas to meet food demand. Presently, maize production areas are around 165 million hectares, with production quantities of approximately 850 million tons and an average grain yield of 5200 kg ha-1. The area and production of maize is increasing day by day due to the high demand of human food, animal fodder, poultry feed, fish feed, starch formation, and oil supply (Alam *et al*., 2021). In 2021-2022 season, total area and production of maize was 1653 thousand hectares and 10.635 million tons, respectively in Pakistan (Economic Survey of Pakistan 2021-2022). Over the past 35 years, soil salinity has increased by approximately 26% in this region (Salehin *et al*., 2018; Clarke *et al*., 2015). This gradual increase has negatively impacted soil fertility and maize productivity (Sikder *et al*., 2016).

Maize is one of the most important cereal crops in the world (SHAH *et al*., 2023), with a global production of over 1 billion tonnes annually (Khalid, 2022). It is a staple food for many countries in Africa, Latin America, and Asia, providing a source of energy, protein, and other essential nutrients. In addition to its role in human nutrition, maize is also used for animal feed, bio-fuel production, and various industrial applications. The

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versatility and high productivity of maize make it a vital crop for global food security and economic development (BABAR *et al*., 2022).

Maize is a crucial crop in terms of global food security; however, its productivity is often constrained by a range of biotic and abiotic stresses (Khalid *et al*., 2021). Among these stresses, drought is considered one of the most severe limitations to maize production worldwide (Zafar et al., 2022). Drought stress affects maize at various growth stages, but it is particularly detrimental to the crop during the seedling stage, where even short periods of water deficit can have a significant impact on plant growth and development (Shahani *et al*., 2021; Zafar et al., 2021). Under drought stress, maize seedlings may exhibit reduced leaf area, chlorosis, wilting, and even death, leading to significant yield losses. The negative impacts of drought on maize productivity are exacerbated by the effects of climate change, which is expected to result in more frequent and severe drought events in many maize-growing regions worldwide (Khalid and Amjad, 2018). As such, there is a growing need to develop strategies to improve the drought tolerance of maize and enhance its resilience to water deficit conditions (Khalid *et al*., 2021).

Breeding for drought tolerance is one of the key strategies for improving maize productivity under drought stress. Over the past few decades, significant progress has been made in developing drought-tolerant maize varieties through conventional breeding and biotechnology approaches (Zafar et al., 2020). These varieties are characterized by their ability to maintain growth and yield under water-limited conditions and to recover quickly after water becomes available again. However, the adoption of these varieties by smallholder farmers in developing countries has been limited by several factors, including the high cost of seeds, limited availability of improved varieties, and lack of knowledge about their performance under different environmental conditions (Manan et al., 2022).

In addition to breeding for drought tolerance, several other approaches have been proposed for improving the drought resilience of maize, including agronomic practices, such as irrigation management and soil conservation, and the application of osmoprotectants, such as proline. Osmoprotectants are low molecular weight organic compounds that accumulate in plant tissues under stress conditions, where they act as protective agents against oxidative damage and help to stabilize cellular structures and membranes.

Proline is an amino acid that has been shown to increase the tolerance of plants to environmental stress. Many studies have investigated the effect of proline on seed germination and seedling growth in maize. One study by Aymen *et al*., (2014) found that proline priming significantly increased the seedling growth and root length of maize. The authors suggested that proline enhanced the synthesis of proteins and enzymes involved in cell division and elongation, leading to an increase in growth (Hassan *et al*., 2021).

Proline is one of the most common osmoprotectants in plants, and its accumulation in response to water deficit has been well documented in several species, including maize. Proline plays a crucial role in protecting plants from drought stress by regulating the osmotic potential of cells, stabilizing proteins and membranes, and scavenging free radicals (Farooq et al., 2021). Several studies have reported that exogenous application of proline can enhance the drought tolerance of maize seedlings by improving their water status, photosynthetic activity, and antioxidant defense mechanisms. Overall, the role of proline in mitigating drought stress and other stress conditions has been extensively studied in various crops, including maize. These studies provide valuable insights into the mechanisms underlying plant stress responses and offer potential strategies for improving crop productivity and resilience under challenging environmental conditions (AlKahtani et al., 2021).

Proline application significantly increased the relative water content, chlorophyll content, and biomass of maize plants, indicating a protective effect of proline on plant growth and development under drought stress. Proline application significantly improved the root length, shoot length, and dry weight of maize plants, indicating a positive effect of proline on plant growth and development under drought stress (Farooq *et al*., 2020).

MATERIALS AND METHODS

The focus of this research was to look at some morphological and physiological traits in maize under normal, drought conditions at seedling stage. The experiment was performed in a wire house (a glasses trial) of the department of Plant Breeding and Genetics, at The Islamia University of Bahawalpur. For this experiment 54 glasses were used for drought treatments, each treatment had three replications. Well sieved soil was arranged a day before the filling of glasses then the very next day; the glasses were filled with well-sieved soil. All glasses were arranged in planned layout (6 glasses in one replication from top to downward for 6 genotypes, each treatment had three replications (18 glasses).

The experiment comprised factor regarding drought with 3 treatments: normal watered (100%), (D1 50%) and water stress (D2 75% + Proline) with 6 maize genotypes, each treatment consists of 3 replications. The seeds of these genotypes were procured from Faculty of Agriculture, University of Agriculture Faisalabad, Pakistan. Sowing of maize was undertaken in soil having uniform soil moisture, and stress was imposed at 25 days after sowing. After stress imposition, soil moisture was determined on a daily basis and water losses were remunerated by adding water to achieve the described level of field capacity in respective treatments.

The study was laid out in a completely randomized design (CRD) with factorial arrangements, where each treatment had three replications. All maize genotypes having 2 seeds were sown on 12 October, at equal distance and a uniform stand was maintained by keeping eight seedlings per glass after completion of emergence. All other practices were kept uniform for each treatment of the experiment.

The glasses were watered every third day after the first irrigation date. The sowing date was noted as the first day of sowing and the germination date was recorded after 2-3 days of sowing. Some genotypes showed a delayed germination and some seeds took 5 to 7 days to germinate until March 7-8. However, two out of two seeds in some glasses germinated with a 100% germination rate. The germination rate was monitored daily by visiting the research area, even though some glasses showed a low germination rate. During this period, all glasses were watered normally with 40ml per glass.

Before the application of drought stress, 0.4% proline solution was made in the laboratory of the department of Plant Breeding and Genetics, IUB. To make the proline solution, measured out 2 grams of proline and dissolve it in 500 milliliters of distilled water. The solution was stirred until the proline has completely dissolved by using electromagnetic stirrer (Ali *et al*. 2013). After making solution, it was poured in foliar sprayer.

Drought stress was imposed 25 days (25th March) after sowing. The normal treatment received regular watering, while the D1 treatment received 50% less water (20ml per glass), and the D2 treatment received 75% less water (15ml per glass + Foliar spray of Proline solution). Proline is sprayed on the leaves of each glass when watered. After the initial application of stress, all glasses were watered and sprayed twice a week.

RESULTS AND DISCUSSION

Chlorophyll Content

Results of three-way ANOVA for chlorophyll content revealed that there were significant effects of Genotype, Treatment, and Genotype-Treatment interaction on the response variable. The F-values for Genotype, Treatment, and G*Treat were 49.6, 220.66, and 11.31 respectively, with p-values less than 0.05, indicating that these factors have a significant impact on CC. The results also show that there was no significant effect of Replication on CC. The Grand Mean was 5.4898, indicating the average value of CC across all factors. The Coefficient of Variation (CV) was 6.27, indicating that the data had a low level of variation. Overall, these results suggest that Genotype, Treatment, and Genotype-Treatment interaction have a significant impact on CC, while Replication did not show any significant effect. All the genotypes showed significant differences in chlorophyll content. Furthermore, there was a significant effect of genotypic in to environmental interaction on chlorophyll content. Significant variation in chlorophyll content indicated t he presence of high genetic diversity among all the genotypes of maize (Table 1). The maximum chlorophyll content was shown by G1(8.44) in normal condition followed by G3(7.03). The minimum value was measured in G4(5.42) as represented in Fig. 1. In drought stress condition, maximum chlorophyll content was shown by G3(4.87) followed by G1(4.86). G3 being the maximum in chlorophyll content suggesting it is tolerant against drought. The minimum value was observed in G6(3.19). In drought stress with endogenously applied proline, maximum chlorophyll content was observed in G1(7.81) followed by G3(7.03). The minimum value was shown by G6(4.92) and G3(4.92). The given results concise with the results of El-Masry *et al*. (2020), El-Desoki *et al*. (2017) and Akram *et al*. (2021) which confirms that application of proline significantly increases the chlorophyll content in maize.

Table 1: Analysis of Variance for Chlorophyll Content

Source	DF	SS	MS	F
Repli	2	0.1237	0.0618	
G	5	29.4325	5.8865**	49.6
Treat	2	52.3725	26.1862	220.66
G*Treat	10	13.4205	1.342	11.31
Error	34	4.0348	0.1187	
Total	53	99.3839		
Grand Mean 5.4898	CV 6.27			

Table 2: Analysis of Variance for Shoot Length

Source	DF	SS	MS	F
Repli	2	7.6	3.802	
G	5	500.07	100.014	81.75
Treat	2	772.92	386.462	315.88
G*Treat	10	122.05	12.205	9.98
Error	34	41.6	1.223	
Total	53	1444.25		
Grand Mean 40.661	CV 2.72			

Shoot Length (cm)

Three-way ANOVA (analysis of variance) for shoot length showed that there were significant effects of Genotype, Treatment, and Genotype-Treatment interaction on the response variable. The F-values for Genotype, Treatment, and G*Treat were 81.75, 315.88, and 9.98 respectively, with p-values less than 0.05, indicating that these factors have a significant impact on shoot length. The Grand Mean was 40.661, indicating the average value of shoot length across all factors. The Coefficient of Variation (CV) was 2.72, indicating that the data had a low level of variation. These results suggest that Genotype, Treatment, and Genotype-Treatment interaction have a significant impact on shoot length. There was a significant effect of genotypic in to environmental interaction on shoot length. Significant variation in shoot length indicated the presence of high genetic diversity among all the genotypes of maize (Table 2). The maximum shoot length was shown by G4 (48.50) in normal condition followed by G2(47.60). The minimum value was measured in G5(40.33) (Fig. 2). In drought stress condition, maximum shoot length was

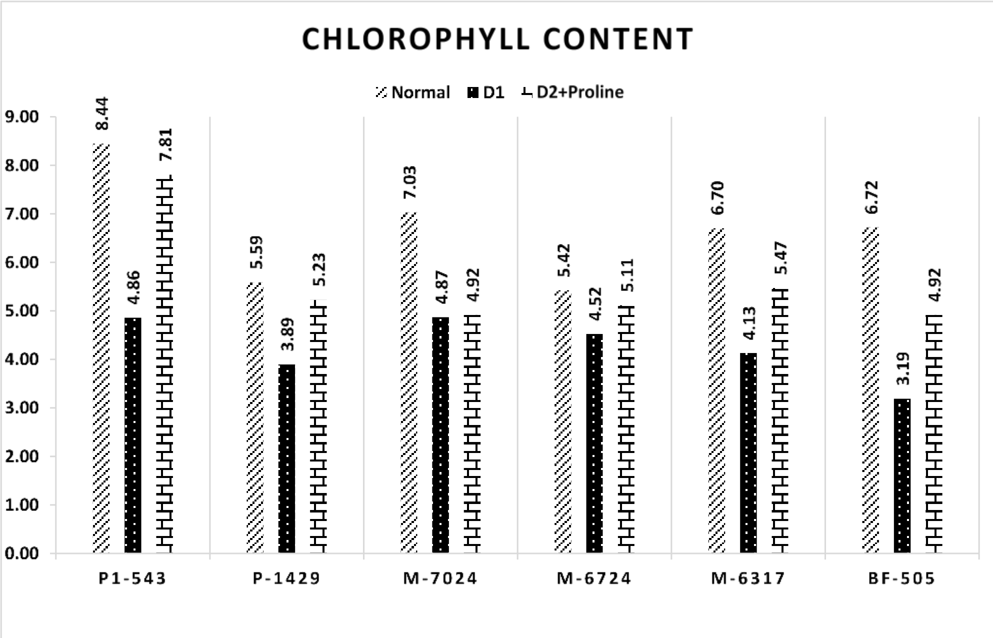

Fig. 1: LSD of Chlorophyll Content in Normal, Drought and Proline Applied Treatment

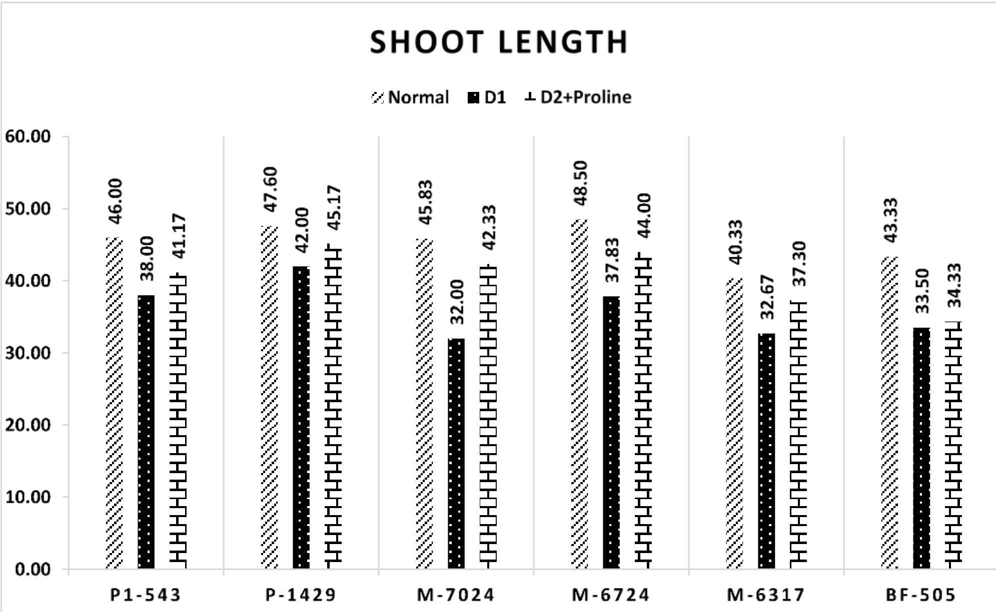

Fig. 2: LSD of Shoot Length in Normal, Drought and Proline Applied Treatment.

shown by G2(42.00) followed by G1 (38.00). G2 being the maximum in shoot length suggesting it is tolerant against drought. The minimum value was observed in G3 (32.00). In drought stress with endogenously applied proline, maximum shoot length was observed in G2 (45.17) followed by G4 (44.00). The minimum value was shown by G6 (34.33). The results suggest that the proline application in drought improves plant growth factors like shoot length. It concise with the results of Akram *et al*. (2021) that investigated the effect of proline application on the growth and yield of maize under water stress. The results showed that proline significantly increased the shoot length of maize seedlings, indicating improved growth and stress tolerance.

Root Length (cm)

Three-way ANOVA (analysis of variance) for root length indicated significant effects of genotype, treatment, and Genotype-Treatment interaction on the response variable. The F-values for genotype, treatment, and G*Treat were 34.89, 277.58, and 5.19 respectively, with p-values less than 0.05, indicating that these factors have a significant impact on root length. The Grand Mean was 38.991, indicating the average value of root length across all factors. The Coefficient of Variation (CV) was 3.40, indicating that the data had a moderate level of variation. These results suggest that Genotype, Treatment, and Genotype-Treatment interaction have a significant impact on root length. There was a significant effect of genotypic in to environmental

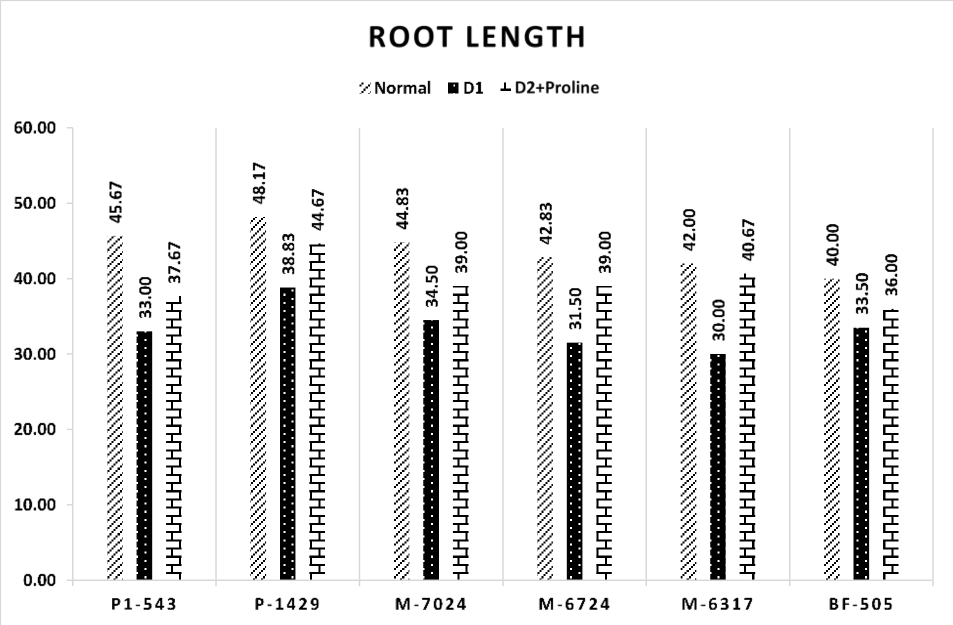

Fig. 3: LSD of Root Length in Normal, Drought and Proline Applied Treatment.

Table 3: Analysis of Variance for Root Length

Source	DF	SS	MS	F
Repli	2	3.73	1.866	
G	5	305.8	61.16	34.89
Treat	2	973.18	486.588	277.58
G*Treat	10	90.94	9.094	5.19
Error	34	59.6	1.753	
Total	53	1433.25		
Grand Mean 38.991		CV 3.40		

interaction on root length. Significant variation in root length indicated the presence of high genetic diversity among all the genotypes of maize (Table 3). The maximum root length was shown by G2(48.17) in normal condition followed by G1(45.67). The minimum value was measured in G6(40.00) (Fig. 3). In drought stress condition, maximum root length was shown by G2(38.82) followed by G3 (34.50). G3 being the maximum in root length suggesting it is tolerant against drought. The minimum value was observed in G5(30.00). In drought stress with endogenously applied proline, maximum root length was observed in G2(44.67) followed by G5(40.67). The minimum value was shown by G6 (36.00). The results suggests that the proline application in drought improves plant growth factors like shoot length. It concise with the results of Aymen *et al*., (2014) found that proline significantly increased the seedling growth and root length of maize.

Fresh Shoot Weight (g)

According to Three-way ANOVA (analysis of variance) for fresh shoot weight, the response variable is significantly affected by genotype, treatment, and genotype-treatment interaction. The statistical analysis shows that the F-values for these factors are higher than the critical value, indicating that they have a strong impact on fresh shoot weight. The Grand Mean of fresh shoot weight is calculated as 4.3056, which represents the average value of FSW across all factors. The Coefficient of Variation (CV) is relatively low, indicating that the data has a low level of variability. These results imply that genotype, treatment, and their interaction have a significant effect on fresh shoot weight, and can help in identifying the best genotype and treatment conditions to optimize fresh shoot weight in the given experimental setting (Table 4).

The maximum fresh shoot weight was shown by G5(4.98) in normal condition followed by G1(4.91). The minimum value was measured in G2(4.39) (Fig. 4). In drought stress condition, maximum fresh shoot weight was shown by G5(4.33) followed by G4 (4.21). G5 being the maximum in fresh shoot weight suggesting it is tolerant against drought. The minimum value was observed in G1 (3.23). In drought stress with endogenously applied proline, maximum fresh shoot weight was observed in G6(4.65) followed by G5(4.55). The minimum value was shown by G1 (4.20). The given results concise with the results of Sadeghi *et al*. (2010) investigated the effect of proline application on maize growth and yield under water stress conditions which proline application significantly increased shoot weight and yield compared to the control treatment under both normal and water stress conditions.

Fresh Root Weight (g)

According to the ANOVA table for fresh root weight, there are significant differences in the response variable due to the factors of genotype, treatment and the Genotype-Treatment interaction. The F-values for these factors are higher than the critical value, indicating that they have a significant impact on fresh root weight. The Grand Mean of fresh root weight is calculated as 3.9774, representing the average value of fresh root weight across all factors. The Coefficient of Variation (CV) is relatively high, indicating that the data has a moderate

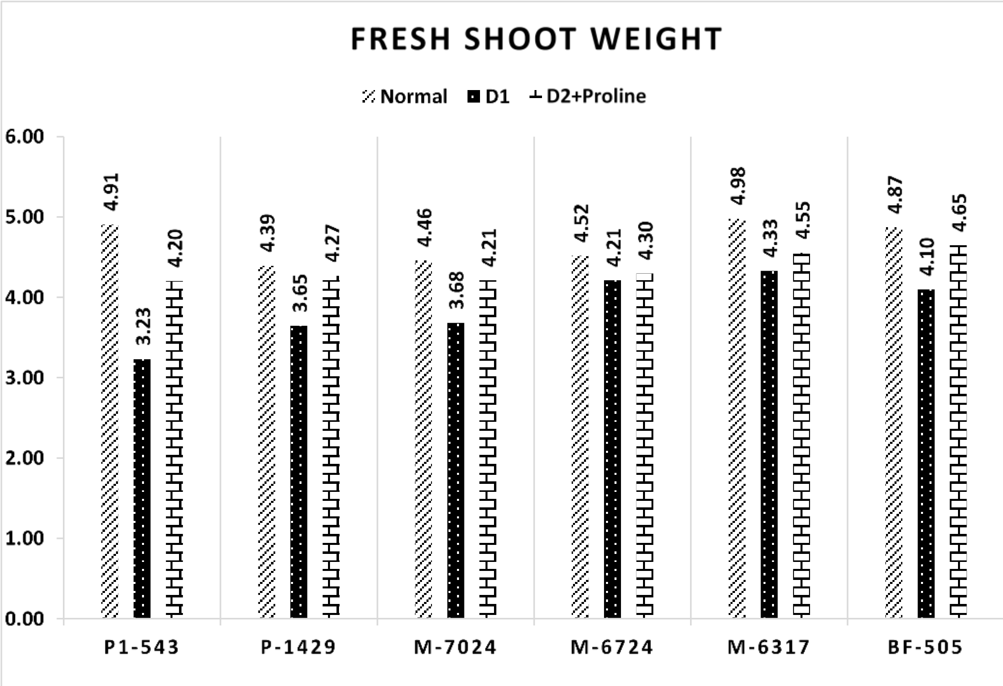

Fig. 4: LSD of Fresh Shoot Weight in Normal, Drought and Proline Applied Treatment

Table 4: Analysis of Variance for Fresh Shoot Weight

Source	DF	SS	MS	F
Repli	2	0.0724	0.03621	
G	5	2.418	0.48361	47.16
Treat	2	6.1581	3.07907	300.26
G*Treat	10	1.7527	0.17527	17.09
Error	34	0.3487	0.01025	
Total	53	10.7499		
Grand Mean 4.3056		CV 2.35		

Table 5: Analysis of Variance for Fresh Root Weight

Source	DF	SS	MS	F
Repli	2	0.065	0.03251	
Treat	2	10.7759	5.38795	187.1
G	5	2.2089	0.44178	15.34
Treat*G	10	2.0281	0.20281	7.04
Error	34	0.9791	0.0288	
Total	53	16.057		
Grand Mean 3.9774		CV 8.12		

level of variability. These results suggest that the choice of genotype, treatment and their interaction can significantly affect fresh root weight (Table 5).

The maximum fresh root weight was shown by G5(4.91) in normal condition followed by G2(4.72). The minimum value was measured in G4(4.15) as represented in Fig. 5. In drought stress condition, maximum fresh root weight was shown by G2(3.91) followed by G3(3.74). G2 being the maximum in fresh root weight suggesting it is tolerant against drought. The minimum value was observed in G6(3.10). In drought stress with endogenously applied proline, maximum fresh root weight was observed in G1(4.43) followed by G2(4.14). The minimum value was shown by G6 (3.63). The given results concise with the results of Aymen *et al*. (2014) investigated the effect of proline priming on maize growth and yield under water stress conditions. The results showed that proline priming significantly increased Root weight and yield compared to the control treatment under both normal and water stress conditions.

Dry Shoot Weight (g)

The analysis of variance (ANOVA) was conducted for dry shoot weight, which showed significant ($P < 0.01$) variation among genotypes and treatments. The results indicated that both genotypes and treatments significantly affected dry shoot weight. The interaction between genotypes and treatments also showed a significant effect on dry shoot weight. This implies that the performance of genotypes varied significantly under different treatments. The overall results suggest that there is substantial genetic variation among the tested genotypes for dry shoot weight, and that appropriate treatments can significantly affect the yield of maize. The coefficient of variation (CV) was found to be 10.55%, indicating moderate variation among the experimental units (Table 6).

The maximum dry shoot weight was shown by G1(1.49) in normal condition followed by G2(1.06). The minimum value was measured in G5(0.87) (Fig. 6). In drought stress condition, maximum dry shoot weight was shown by G1 (0.62) followed by G5 (0.58). G3 being the maximum in dry shoot weight suggesting it is tolerant against drought. The minimum value was observed in G6 (0.34). In drought stress with endogenously applied proline, maximum dry shoot weight was observed in G1(0.73) followed by G5 (0.64). The minimum value was shown by G2 (0.45).

The given results concise with the results of Muhlich *et al.* (2008) found that seed application with proline significantly increased the dry shoot weight of maize under both normal and drought conditions.

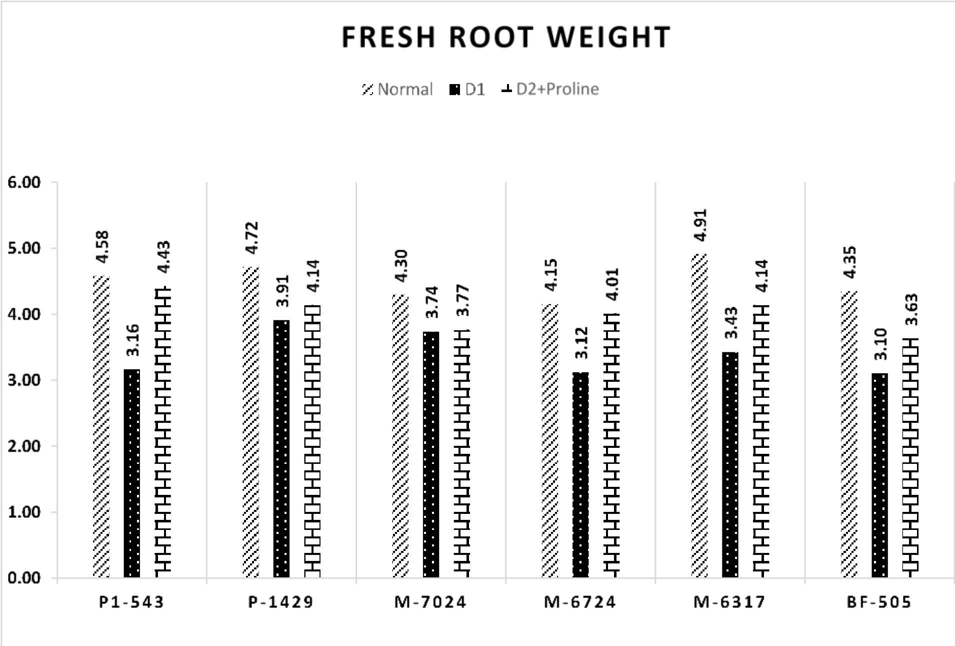

Fig. 5: LSD of Fresh Root Weight in Normal, Drought and Proline Applied Treatment.

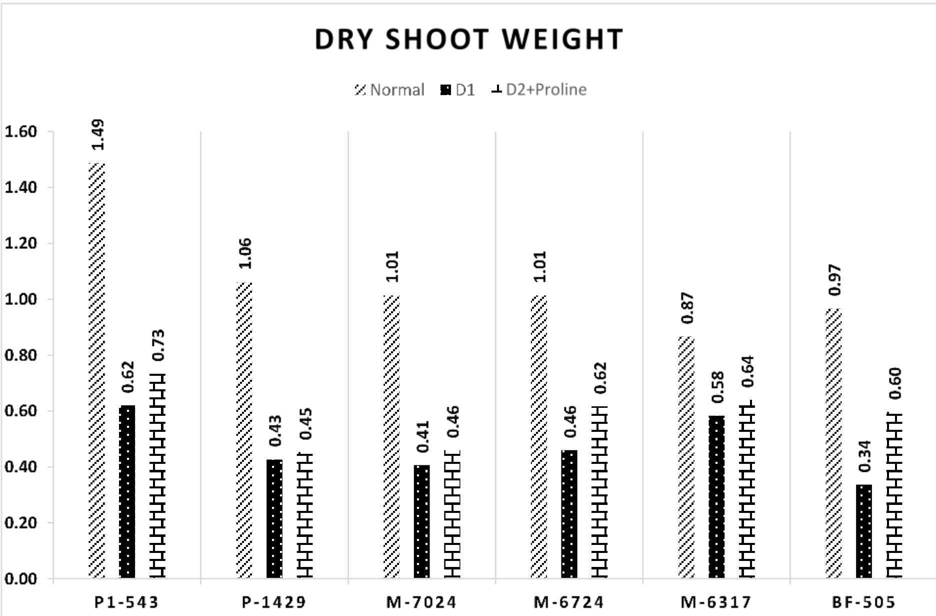

Fig. 6: LSD of Dry Shoot Weight in Normal, Drought and Proline Applied Treatment.

Table 6: Analysis of Variance for Dry Shoot Weight

Source	DF	SS	MS	F
Repli	2	0.02154	0.01077	
G	5	0.65853	0.13171	23.63
Treat	2	3.61031	1.80516	323.84
G*Treat	10	0.39242	0.03924	7.04
Error	34	0.18952	0.00557	
Total	53	4.87233		
Grand Mean 0.7078		CV 10.55		

Dry Root Weight (g)

Three-way ANOVA (analysis of variance) significant effects of genotype, treatment, and genotype-treatment interaction on dry root weight. Specifically, the F-values for Genotype, Treatment, and G*Treat were 17.82, 80.5, and 2.27 respectively, with p-values less than 0.05, indicating that these factors have a significant impact on DRW. The Grand Mean was 0.6594, indicating the average value of dry root weight across all factors. The Coefficient of Variation (CV) was 15.77, indicating that the data had a moderate level of variation. Overall, these results suggest that Genotype, Treatment, and Genotype-Treatment interaction have a significant impact on dry root weight. Significant variation in fresh root weight indicated the presence of high genetic diversity among all the genotypes of maize (Table 7).

The maximum dry root weight was shown by G2(1.08) in normal condition followed by G1(1.02). The minimum value was measured in G6(0.60). In drought stress condition, maximum dry root weight was shown

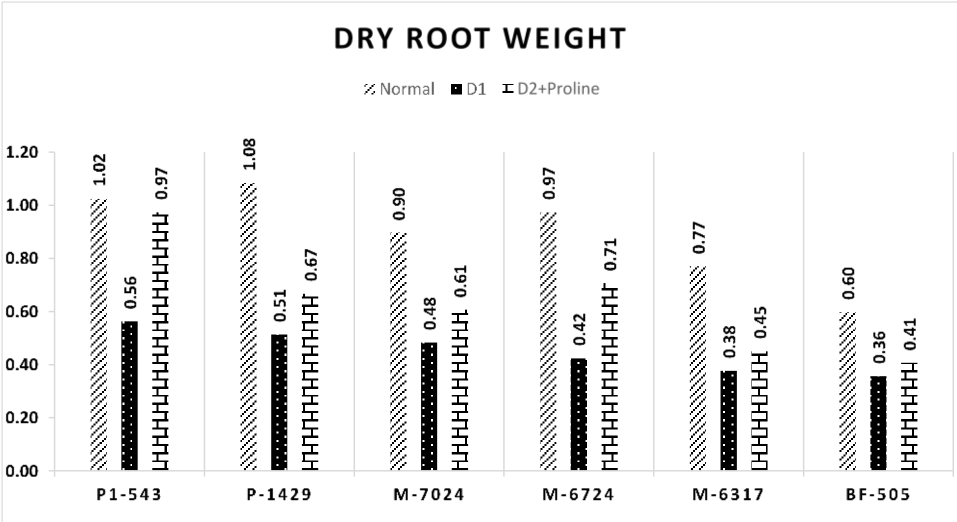

Fig. 7: LSD of Dry Root Weight in Normal, Drought and Proline Applied Treatment.

Table 7: Analysis of Variance for Dry Root Weight

Source	DF	SS	MS	F
Repli	2	0.07501	0.03751	
G	5	0.96326	0.19265	17.82
Treat	2	1.74098	0.87049	80.5
G*Treat	10	0.24598	0.0246	2.27
Error	34	0.36766	0.01081	
Total	53	3.39288		
Grand Mean 0.6594		CV 15.77		

by G1(0.56) followed by G2 (0.51). G1 being the maximum in dry root weight suggesting it is tolerant against drought. The minimum value was observed in G6 (0.36). In drought stress with endogenously applied proline, maximum dry root weight was observed in G1(0.97) followed by G3(0.71). The minimum value was shown by G6 (0.41) as represented in Fig. 7. The given results concise with the results of Jianchao *et al*. (2010) found that proline seed application significantly increased the dry root weight of maize compared to Normal condition.

Conclusion

The maximum chlorophyll content was shown by G1(8.44) in normal condition and G1(7.81) in foliar applied proline application at 75% drought. The maximum shoot length was shown by G4 (48.50) in normal condition and (45.17) in foliar applied proline application at 75% drought. The maximum root length was shown by G2(48.17) in normal condition and G2(44.67) in foliar applied proline application at 75% drought. The maximum fresh shoot weight was shown by G5(4.98) in normal condition and G6(4.65) in foliar applied proline application at 75% drought. The maximum fresh root weight was shown by G5(4.91) in normal condition and G1(4.43) in foliar applied proline application at 75% drought. The maximum dry shoot weight was shown by G1(1.49) in normal condition and G1(0.73) in foliar applied proline application at 75% drought. The maximum dry root weight was shown by G2(1.08) in normal condition and G1(0.97) in foliar applied proline application at 75% drought. Foliar application of proline on plants decreased the drought effect on plants and increase the plant tolerance to drought stress.

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