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ASSESSMENT OF THE MACRO ELEMENTS IN MAIZE UNDER CADMIUM STRESS ALLEVIATION THROUGH EXOGENOUS APPLICATION OF PROLINE AND TREHALOSE

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Abstract

Cadmium toxicity is a significant environmental issue affecting crop growth and productivity globally. Maize, a rich source of nutrition, is an excellent model plant to study physiological changes responsible for reduced productivity under stressful conditions. This study assessed the effect of Cd concentrations on maize seedlings before and after exogenous application of proline and trehalose in 25 mM, 50 mM, and 75 mM concentrations. Trehalose has emerged as an important reducing sugar that can reduce the adverse impacts of Cd. Exogenous proline increased proline content and alleviated Cd-induced seedling growth inhibition. It also increased antioxidant enzyme activities and reduced reactive oxygen species accumulation. Compared with other concentrations, 75 mM of exogenous proline and trehalose was the most effective at mitigating Cd toxicity in maize. The results depicted that proline showed the better plant growth in Cd stressed maize plants due to better yield and cob level, lesser degradation of chlorophylls, and accumulation of essential mineral contents. Hence, the research concluded that exogenous application of proline and trehalose could be beneficial in reducing Cd toxicity in maize crop.

Key words: Antioxidant enzyme, Cd toxicity, Concentration, Proline, Trehalose, Macromolecule, Maize crop.

Introduction

Heavy metals interrupted the metabolic system which consequently effects the plant development and reduced its yield (Abid *et al*., 2017). It originates from industrial waste and is found in soil. It has become one of the key metal stresses which stance a serious risk to plants (Anjum *et al*., 2020). In crop plants, the noxiousness of cadmium decreases the uptake and transport of water and nutrients, escalates oxidative damage, upsets plant metabolic rate, and constrains plant functioning and morphology (Khan *et al*., 2016). Maize is the main cereal harvest, cultured under a wide range of soil types all over the world, though, human activities polluted the soils pose severe extortions to its productivity (Anjum *et al*., 2020). Toxic amounts of heavy metals alter or replace the structural elements of proteins, which result in the creation of links among heavy metals and sulfhydryl assemblies, which affect metabolic processes and normal plant function in a variety of ways (Chary *et al*., 2008).

Cadmium stress in plants results in the reduction of Fe, Mg, Mn, P, and S levels, decrease in growth and limits the leaf photosynthesis (Sun *et al*., 2004). These effects of cadmium toxicity also fluctuate with changing concentrations of cadmium stress from 0.1 to 5.0 (μM) (Farooq *et al*., 2016). Exogenous proline reduces the negative effects of Cd on immature date palms, decreasing oxidative damage, increasing hydration, photosynthetic-activity, and growth, while displaying higher antioxidant enzyme activity (Zouari *et al*., 2016). Trehalose is a non-reducing disaccharide extensively dispersed in environment (Avonce, 2005; Iordachescu & Imai, 2008), wherever it helps mainly as a protective agent against ecological pressures (Wiemken, 1990; Wingler, 2002).

Trehalose is the preliminary need for chitin production and chitin can decrease the stomatal opening by aggregate reactive oxygen species (ROS) components in plants (Paul *et al*., 2008, Zhou *et al*., 2012). Reactive oxygen species (ROS) such as hydrogen peroxide has a key role in modification of stomatal movements. Plants exposed to heavy metal stress have been demonstrated to benefit from proline foliar spraying. According to Nair *et al*., (2016), an increase in intracellular proline accumulation in Arabidopsis plants severely hindered the activity of other genes necessary for normal morphogenesis and amino acid synthesis. According to Rontein *et al*., (2002), proline is involved in cell osmoregulation, protein preservation during dehydration, and can serve as an enzymatic regular under pressure. It is a well-known organic molecule that mediates osmotic adjustment mediation during salinity stress in addition to sustaining subcellular structures that might be referred to as an energy bowl and a stress-related signal.

According to Ashraf & Foolad (2007), an excess of free proline has undesirable or adverse effects on cell development or protein activities; as a result, the intensity of the effect was associated with its concentration. The efficiency of foliar-sprayed proline is also dependent on the dosage employed, plant species, plant growth stage, and application period, according to the researchers.

Proline has a variety of defensive functions, including osmotic adjustment, stabilization of cell structures, and decrease in damage of photosynthetic machinery. A disaccharide called Trehalose which is non-reducing and is made up of 2 units of glucose. Tre may build up in a variety of plants as a result of abiotic stressors and work as a protein and membrane structural stabilizer, source of energy and have protection ability (Iordachescu & Imai, 2008).

In various plants, the use of Proline and Trehalose to increase environmental stress tolerance has been described. Exogenous Prohave protection mechanism against oxidative stress due to salt by lowering hydrogen peroxide and peroxidation of level of lipid and also improve defense of antioxidant and detoxifying system of methylglyoxal.

Presence of cadmium in the loam can have an impact on root absorption, as well as the following supply and transport of nutrients in plant life. There is connection between metal, and the usage, storing, and utilization of a variety of components (for example minerals as well as plant water absorption. Sugar beet roots with Cd-induced Fe deficiency were discovered (Khan *et al*., 2022).

After exposure to Cd, the absorption of B, K, P, S, Zn, Ca, Mn, and in pea was significantly reduced. Handling with cadmium at 1.0 M meaningfully condensed the level of B, K, Mg, Ca, Fe, Cu, Zn, and P in origins of (*Hordeum vulgare* L.), on the other hand the levels of the same essentials in sprouts were not exaggerated (Gan *et al*., 2017). The poisonousness of cadmium triggered a reduction in minerals absorption (*Atriplex halimus* L.). Cadmium poisoning reduces the amount of P, Mg, Ca, and N in alfalfa roots and shoots (Zhang *et al*., 2019).

Aims and objectives of study: To find out the most effective treatment of Proline and Trehalose under Cd stress.

To check the best concentration of foliar application for amelioration of Cd stress.

To find out the Cd tolerance levels of five varieties of maize.

Materials and Method

Five varieties of maize (MmR1-yellow 55MT, White Pearl-80 MT, Psc-yh-1898, Sargodha-2002, Super green maize) were selected for this experiment in order to check the toxic effect of Cd stress on different parameters (Physiological, morphological, Biochemical, yield, Physio-chemical) of maize as well as alleviation of Cd stress by foliar application of proline and trehalose. Similarly (Table 1) represented the treatment groups and (Table 2) is represented the different attributes of maize during experiment.

Table 1. Exogenous application of proline and trehalose.

T1	Control
T2	Cadmium stress
T3	Proline 75 mM
T4	Proline 50 mM
T5	Proline 25 mM
T6	Trehalose 75 mM
T7	Trehalose 50 mM
T8	Trehalose 25 mM

Plant bio-mass: After eight weeks of germination, two plants off each pot was then be removed, with the remaining 3 plants maintained for seed harvest. The height of the shoot and the length of the root was calculated using an iron meters scale, and the fresh weight of the shoot and root was quantified through the use of a weighing balance. The shoots and roots was then be dried in the oven for three days at 65ºC, and respective dried mass was estimated (Aimen *et al*., 2022).

Leaf surface area per plant (cm2).

Carleton & Foote's formula (1965) was used to figure out how much leaf area each plant had:

$$A\ (cm^2) = L \times W \times 0.65\ *$$

*The correction factor for a monocot plant

Biochemical variables Total soluble sugars: The method described by Yemm & Willis (1954) was used to calculate total soluble sugars. In brief, 10 ml of newly made anthrone reagent was pipetted into test tubes for sample, standard, and blank, and the test tubes was stored in ice-cold cooled water. In the test tube, 1.0 ml of sample extract and 0.2 to 1.0 ml of standard sugar solution (20-100 g) was mixed with 10 ml anthrone reagent to form the standard curve layer.

The contents were shaking after 3-5 minutes, while the test tubes remain immersed in ice-cold water. The contents of the test tube were heated very quickly for 10 minutes in a bath of boiling water, then quickly cooled in cold water. A blank (distilled water) was used to measure the absorbance at 625 nm. After making the standard curve and figuring out how much sugar was in the sample as a whole.

Total soluble proteins: Leaf samples (0.5 g) were mixed with 4% polyvinyl pyrrolidone (PVP) in a 10 mm potassium phosphate buffer (pH 7.0) containing 10 mM potassium phosphate. The mixture was then centrifuged at 12,000 g at 4 C for 15 minutes, and the supernatants were used right away to measure enzyme activity. The Bradford method was used to find out how much protein there was (Bradford, 1976). A 20 _L sample of the supernatant was mixed with 980 _L of the BioRad Bradford reagent. The absorbance was measured at 595 nm. The amount of protein was measured by comparing it to a standard curve made from bovine serum albumin.

Chlorophyll content: The Aron method was used to measure the amount of chlorophyll a and b (1949). Fresh leaves from each container were cut into 0.5cm pieces and smashed to get the chlorophyll out. This was done overnight at -100C with 80% acetone, and the absorbance of the mixture was measured between 645 nm, 663 nm, and 480 nm with a spectrometer. To figure out how much chlorophyll a and b there was, these formulas of Aron (1949) were followed.

Table. 2 Attributes of maize under experiment.

Morphological	Physiological	Biochemical	Yield	physiochemical
Plant height	Relative water content	Chlorophyll content	Kernel per cob	pH
No of leaves	Co2 Assimilation	Potassium	100 kernel weight	Electrical conductivity
Cob level	Transpiration rate	Calcium	Yield	Organic matter
Leaf area	Water use efficiency Stomatal conductance Sub-stomatalconductance	Phosphorus Sodium Total soluble sugar,protein, amino acids	Moisture	Available potassium Available phosphorus

Analytical procedure to determine minerals: After wet digestion these samples were diluted as required and subjected to mineral analysis by using (FPM) and (AAS), to evaluate the concentration of minerals. In (FPM) the Samples of sodium or potassium, was analyzed on Janway PFP-7 flame photometer then it was run a series of Na and K metal standards in flame atomic absorption spectroscope and drawn a calibration curve to find Na and K levels in given samples. In (AAS), all the experimental digested samples was analyzed for Ca by using Atomic absorption spectrophotometer, (AAS) (Anwar *et al*., 2004). The instrument was calibrated with respective standard before and after every analysis then concentrations of respective mineral in the samples was calculated and the sample values was compared with the respective reference values.

Soil analysis

The pH meter was used to measure the concentrations of the water samples, and the Conductivity Meter was used to calculate the electrical conductivity (EC). The pH of all soil samples was then be determined using a soil and water proportion of 1:2 (w/v). The combination was homogenized to produce a concentrated solution, and the samples was thoroughly mixed during measurement to ensure homogeneity. A pH meter was used to determine the pH of the soil. To determine electrical conductivity, create a suspension (1:2w/v) of 5g soil in 10 ml of distilled water. The soil to be used for the experiment was analyzed for the following physio-chemical parameters: pH, EC, N, P, K, Ca, Mg, Organic matter, Saturation percentage, and Texture (Rhue & Kidder, 1983).

Physiological parameter: Relative water content was recorded by the formula given by Pieczynski *et al*., (2013).

Principal components analysis (PCA): A multivariate approach called principal component analysis (PCA) examines a data table in which observations are characterized by a number of interrelated quantitative dependent variables. Its objective is to extract the crucial data from the table, represent it as a group of new, orthogonal variables known as principle components, and visualize the pattern of similarity between the observations and the variables as points on graph. The line that most accurately describes the geometry of the point swarm is the first principal component (PC1). It reflects the direction of the data's greatest variation. To obtain a coordinate value along the PC-line, each observation (yellow dot) may be projected onto this line. This number is referred to as a score.

Statistical Analysis

The obtained data will statistically be analyzed through analysis of variance (ANOVA). Correlation among studied parameters was also estimated. Each attribute's data was statistically analyzed using Mini tab 16. To find mean differences Tukey test and 3-way ANOVA were employed.

Results

Morphological parameters season 1

Plant height: Analysis of variance of morphological parameters for Plant height show that Season, Variety, Treatment, Season Variety, Season-Treatment, Variety-Treatment, and Season- Variety-Treatment all have a significant effect on Plant height. The height of the plant is between 161.325cm and 164.507cm. The tallest plants were found to be of Variety 1 Treatment 6 in the spring, and the shortest ones were of Variety 1 Treatment 6 in the fall (Fig. 1).

No. of leaves: The results of ANOVA in morphological parameters for number of Leaves depicted Significant effect in Season, and non- significant effect in Variety. Number of leaves in different season and variety treatments ranged from 9.25cm to 12.75cm. Highest values was found in Season 1, Variety 4, Treatment 7 and lowest was observed in Season 2, Variety 4 and treatment (Fig. 2).

Cob length: Analysis of variance of Cob length show significant effect in Season, Treatment, but not in Variety, Season Variety, Season Treatment, Season Variety Treatment, and Variety Treatment. Variety Treatment has the least significant effect. The amount of cob varies from 16.06 cm to 19.218cm. Season 1, Variety 2, and Treatment 6 all had the most cob level. Season 2's Variety 1 and Treatment 7 saw the least amount of activity (Fig. 3).

Leaf area: The results from ANOVA in leaf area parameters exhibited non-significant effect in Season. The mean concentration of leaf area fluctuated from 698.24 cm^2 to 691.83 cm^2. The highest value was present in Season and variety 1, treatment 3 and lowest was found in Season 2 Variety 1 and treatment 4 (Fig. 3).

Relative water content: The ANOVA of the physiological parameters of RWC showed that all had a significant effect, but Treatment, Season-Treatment, and Variety-Treatment did not. The average amount of RWC is between 70.45%. and 74.31% Season 1, Variety 1, Treatment 1 had the lowest value. Season 1, Variety 2, and Treatment 3 had the highest value (Fig. 4).

CO_2 Assimilation: The results of ANOVA in Carbon dioxide assimilation depicted significant effect non-significant effect. The concentration of CO_2 assimilation varies from to 63.11 mmol CO_2 m^{-2} s^{-1} - 67.22 mmol CO_2 m^{-2} s^{-1} . Maximum range was found in season, variety 1, treatment 7 and minimum was observed in season1, variety 1and treatment 2 (Fig. 5).

Transpiration rate: Analysis of variance in transpiration rate demonstrated significant effect in Season, and non-significant in Treatment, Season ×Treatment. The concentration of transpiration rate fluctuated from to 2.40 mmol/m^2/sec to 5.77 mmol/m^2/sec. the highest value was present in season 1 variety 2 treatment 4 and lowest was found in season, variety 2 and treatment 7 (Fig. 6).

Water use efficiency: The ANOVA for water use efficiency showed that Season, Variety, Season Variety, had a significant effect, while Treatment, Season, and Variety had no effect. The average water use efficiency was between 8.83 A/E and 12.37. Season 1 variety 1, treatment 2, and treatment 7 all had low and high levels of WUE (Fig. 7).

Stomatal concentration: In stomatal concentration the results of ANOVA depicted significant and least significant effect in season and treatment. Non-significant effect was in variety, Season× Variety, Season ×Treatment, Variety ×Treatment and Season ×Variety× Treatment. The concentration of stomata varies from 291 mmol/m^2/sec to 294.25 mmol/m^2/sec. The highest value was found in Season 1 variety 1, treatment 4 and lowest was observed in season 2 variety 5 and treatment 1 (Fig. 8).

Sub stomata CO_2 Concentration: The ANOVA for sub stomatal concentration demonstrated non-significant effect in variety, and Significant and least significant in season and treatment. The concentration of sub stomatal ranged from 251.75 mmol/m^2/sec to 254.5 mmol/m^2/sec. High level was observed in season 1 variety 3 treatment 6 while low level was found in season, variety 1 and treatment 2.

Biochemical parameters

Chlorophyll concentration: The analysis of variance in CC demonstrated significant effect in season, least significant effect in variety, Season × Variety, Variety × Treatment, Season × Variety × Treatment and non-significant effect in Treatment, Season × Treatment. Chlorophyll contents ranged from 10.91µg/ml to 14.12µg/m. Low level was found in Season 2 variety 1 treatment 7 and high level was observed in Season 1 variety 2 treatment 2 (Fig. 10).

Potassium: The results from ANOVA in Potassium showed in Season, variety, Season × Variety, Season × Variety × Treatment was significant effect. Non-significant and least significant was showed in treatment, Season ×Treatment and Variety× Treatment. The mean concentration of Potassium ranges from 26.39 mg/g to 22.82 mg/g. Maximum range was observed in season 1 variety 2 treatment 2 and minimum was found in season 2 variety 4 treatment 3 (Fig. 11).

Calcium: The ANOVA in calcium depicted non-significant effect in Season ×Variety× Treatment, Variety× Treatment, Season ×Treatment, treatment. Significant and least significant effect in season, Season ×Variety, variety. The level of Calcium in different season, variety and treatment varies from 5.54 mg/g to 1.52 mg/g. High Ca concentration was observed in season, variety 1 treatment 6 and low was found in season, variety 2 treatment 8 (Fig. 12).

Phosphorous: The results from Analysis of variance in phosphorous exhibited Significant and least significant effect in season, variety, Season × Variety and non-significant effect in Season × Variety × Treatment, Variety × Treatment, Season × Treatment, treatment. The trend of Phosphorous ranges from 5.28 mg/g to 1.62 mg/g. Maximum value was observed in season 1 variety 2 treatment 7 and minimum was found in season 2 variety 3 treatment 4 (Fig. 13).

Sodium: The ANOVA in sodium depicted significant effect in season while non-significant effect in Variety, Treatment, Season × Variety, Season × Treatment, Variety × Treatment and Season × Variety × Treatment. Sodium concentration ranged from 2.95 mg/g. to 8.32 mg/g. Highest value was present in season 1 variety 2 treatment 6 while lowest was observed in season 2 variety 1 treatment 7 (Fig. 14).

Total soluble sugar: Analysis of variance in total sol sugar showed significant and least significant effect in season, season × variety and variety while non-significant in treatment, Season × Treatment, Variety × Treatment and Season × Variety × Treatment. T0he trend of TSS ranged from 1.82%. to 5.07%. Season 1 variety 1 treatment 5 showed highest mean while lowest was observed in season 2, variety and treatment 5 respectively (Fig. 15).

Total soluble protein: The results from ANOVA in total Sol Protein exhibited significant and least significant effect in season, season × variety and variety but non-significant in treatment, Season × Treatment, Variety × Treatment and Season × Variety × Treatment. Level of TSP in different sources fluctuated from 7.11%. to 12.32% The highest value was observed in season 1 variety 1 treatment 6 and lowest was present in season 2 variety 3 treatment 3.

Total free amino acid: ANOVA of T F amino acid demonstrated non-significant effect in Season, Variety, Treatment, Season × Variety, Season × Treatment, Variety × Treatment and Season × Variety × Treatment. The mean of TFAA varies from 7226 mg/g to 8308mg/g. Extreme was present in season 2 variety 5 and treatment 5 and least was observed in season, variety 1 and treatment 8 (Fig. 16).

Yield parameters

Kernal Per Cob: The ANOVA results of yield parameters in KPC showed non-significant effect in Season, Variety, Treatment, Season × Treatment, Variety × Treatment and Season × Variety × Treatment and significant in Season × Variety. Different parameters in yield show variations from 162.25 to 258.75. The high value was present in season 2 variety 4 treatment 3 and low was found in season 1 variety 3 treatment 1 (Fig. 17).

Hundred kernal weight: In yield parameters the analysis of variance in HKW presented significant effect in season and non-significant variation in Variety, Treatment, Season × Variety, Season × Treatment, Variety × Treatment and Season × Variety × Treatment. The trend of HKW varies from24.25 gm to 27.25gm. Maximum was observed season 1 variety 3 treatment 1 and minimum was in season, variety, treatment 1 respectively (Fig. 18).

Yield: The ANOVA design of yield depicted significant and non-significant impact in season, treatment and Variety, Season × Variety, Season × Treatment, Variety × Treatment and Season × Variety × Treatment. The yield ranged from 80.5 gm/plant to 83.5 gm/plant. High and low content of yield in season, variety and treatment was found in 2, 4 8and 5 respectively (Fig. 19).

Moisture content: The ANOVA in moisture content influenced significant effect in Season, Variety, Season × Variety and Season × Variety × Treatment and non-significant in Treatment, Season × Treatment Variety × Treatment. Moisture contents ranged from 10.57% to 14.42%. Low level was found in Season 2 variety 2 treatment 7 and high level was observed in Season 1 variety 2 treatment 2 (Fig. 20).

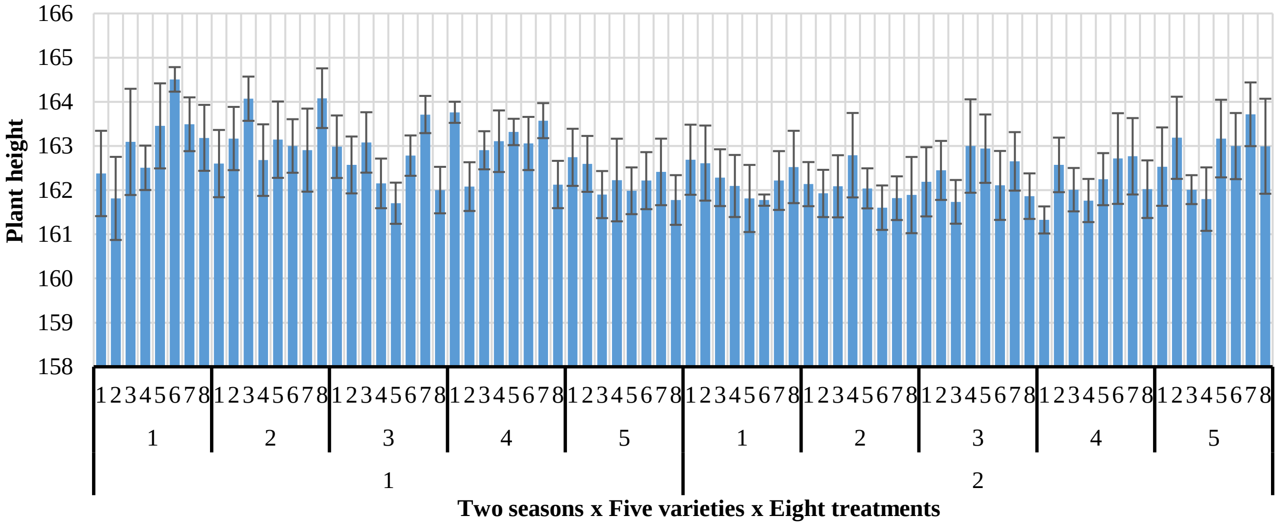

Fig. 1 . Bar graphs of three-way interaction (two seasons x five varieties x eighttreatments) of plant height (1,2* Season, 1, 2, 3, 4, 5 variety, 1, 2, 3, 4, 5, 6, 7, 8 *Treatment.

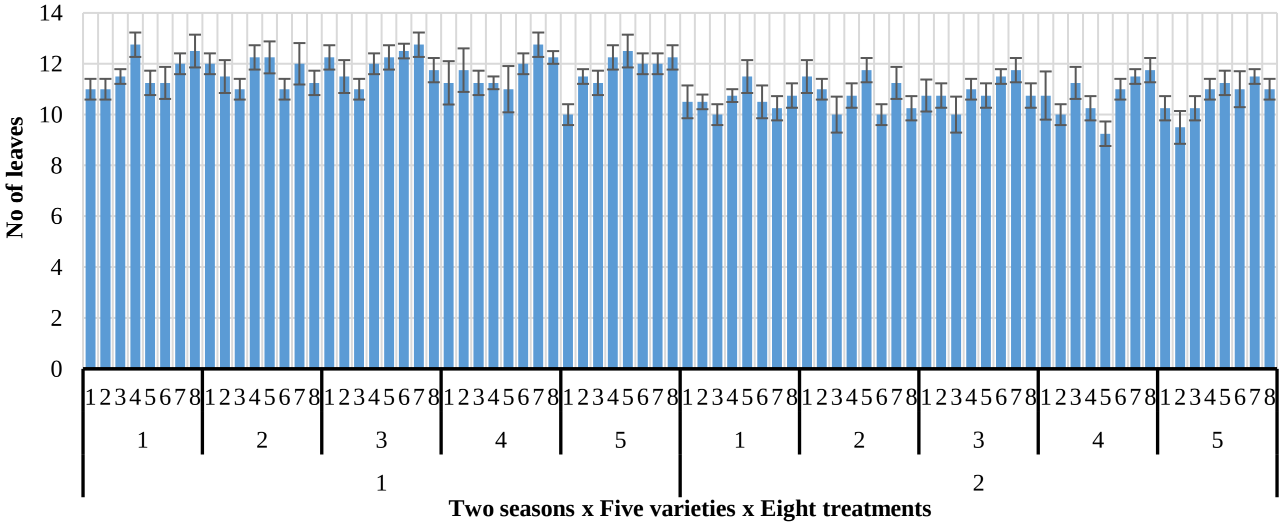

Fig. 2 . Bar graphs of three-way interaction (two seasons x five varieties x eighttreatments) of No of Leaves (1,2* Season, 1, 2, 3, 4, 5 variety, 1, 2, 3, 4, 5, 6, 7, 8*Treatment).

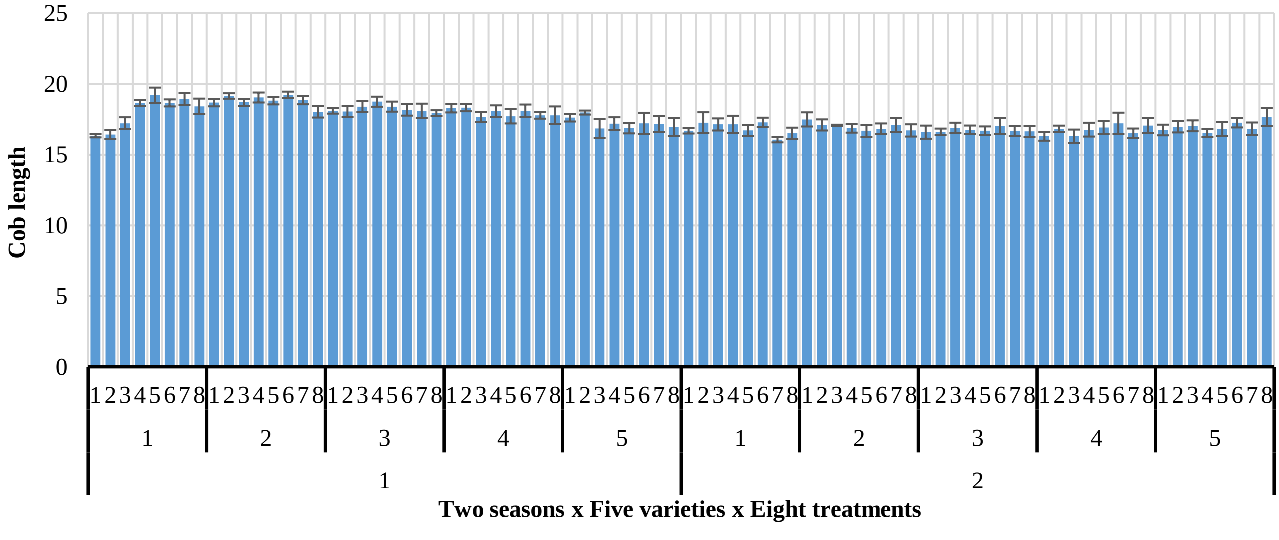

Fig. 3. Bar graphs of three-way interaction (two seasons x five varieties x eighttreatments) of Cob Length (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

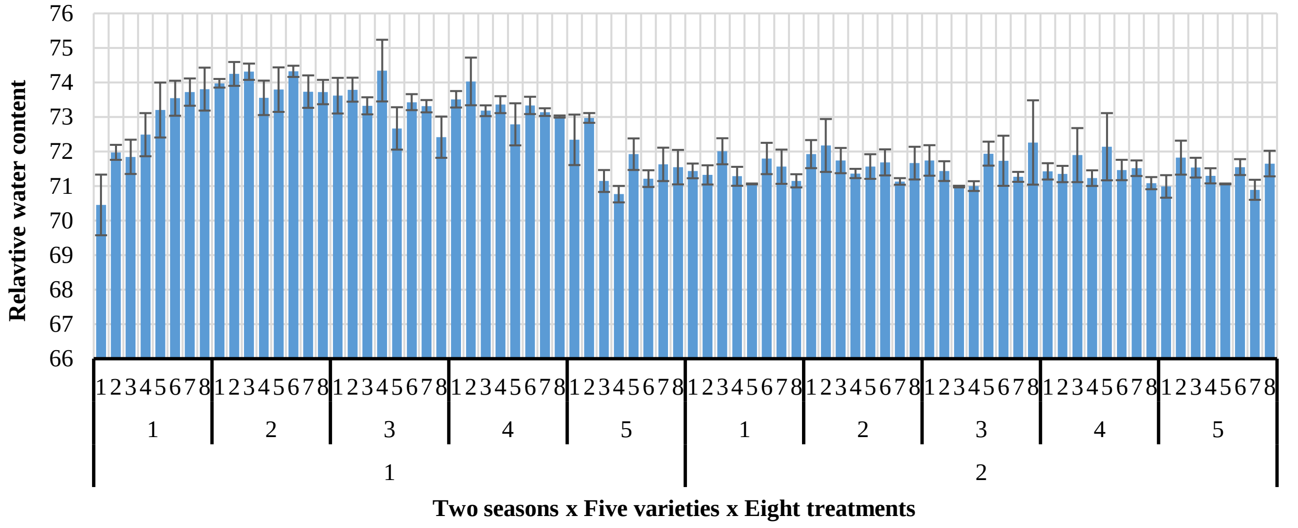

Fig. 4. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Relative Water content (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

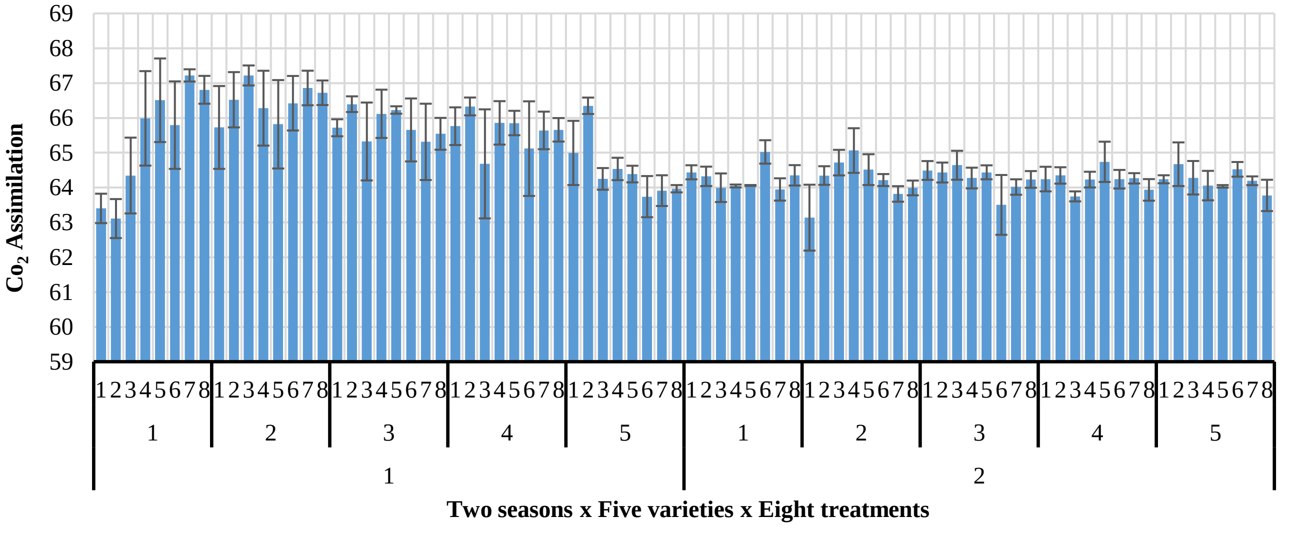

Fig. 5. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Co2 Assimilation (1,2*Season,1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

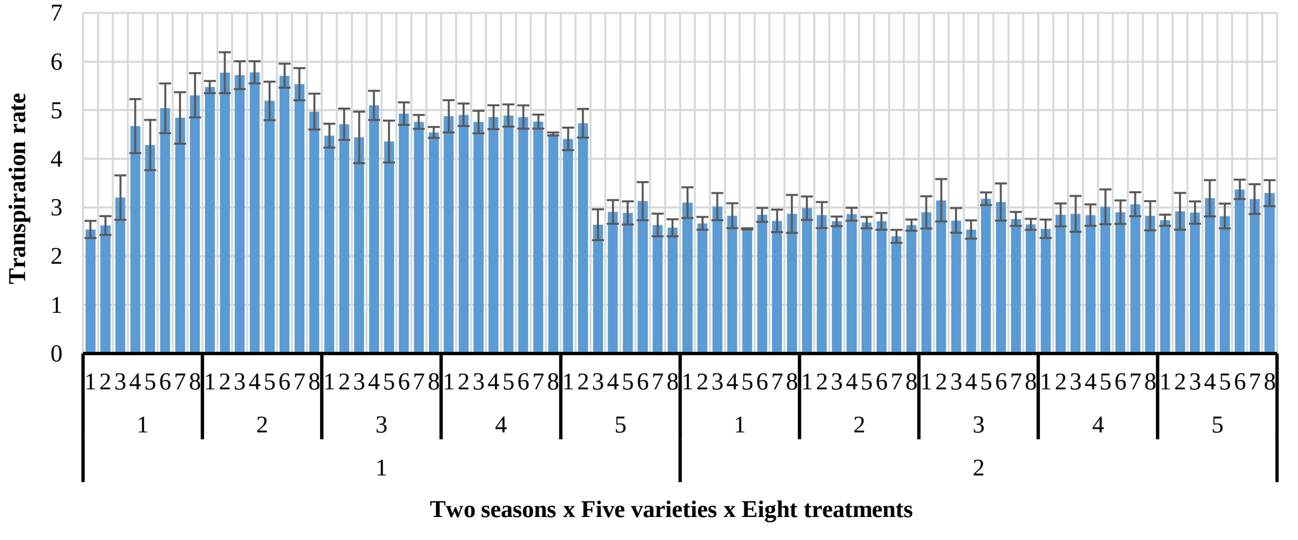

Fig. 6. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Transpiration (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

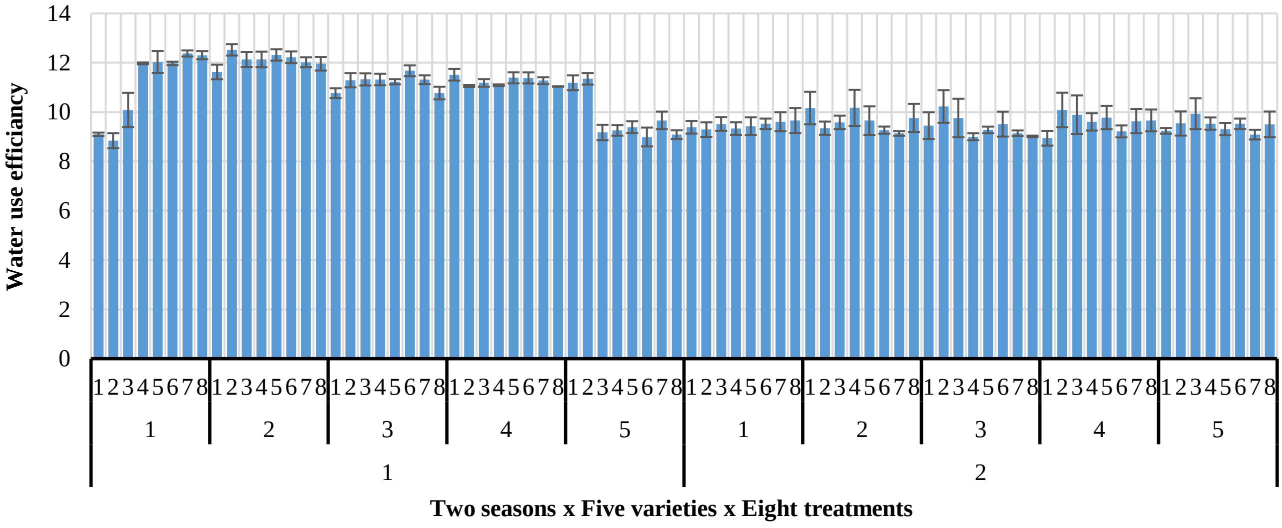

Fig. 7. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Water use Efficiency (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

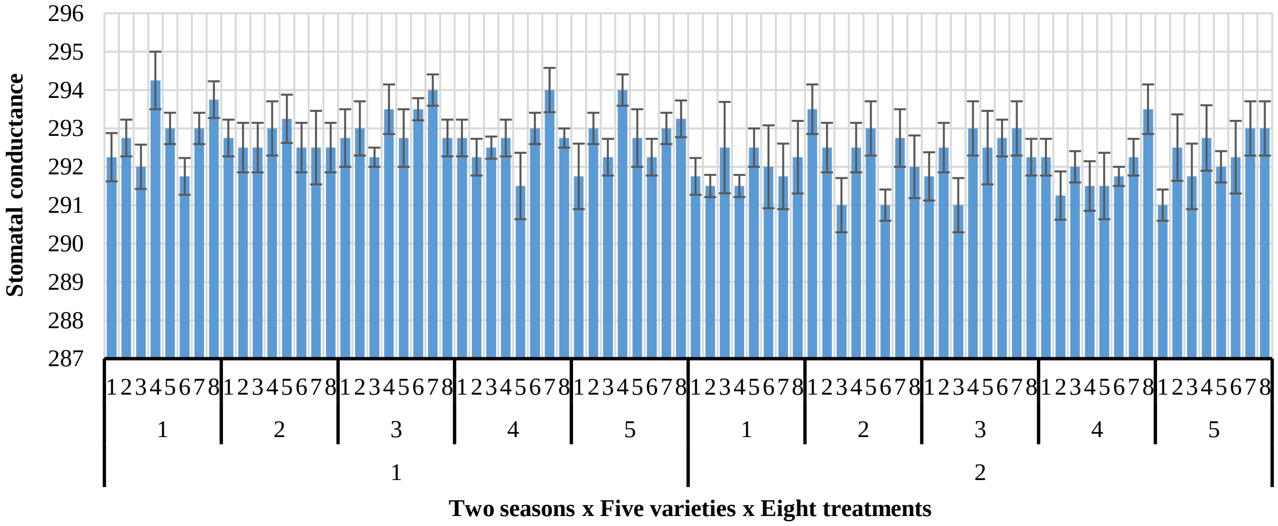

Fig. 8. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Stomatal Conductance (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

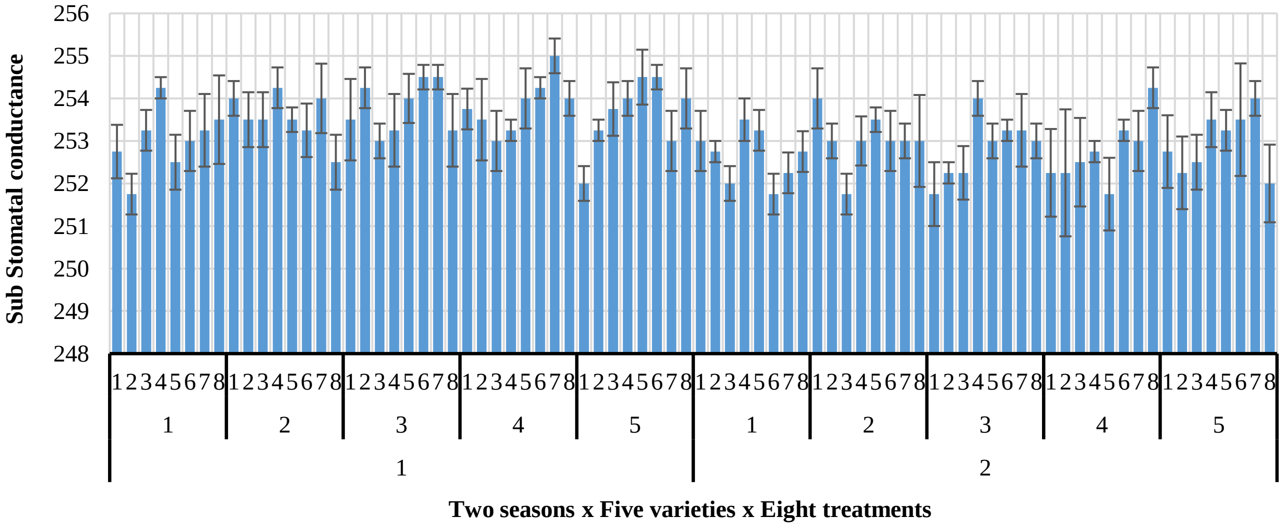

Fig. 9. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Sub-Stomatal Conductance (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

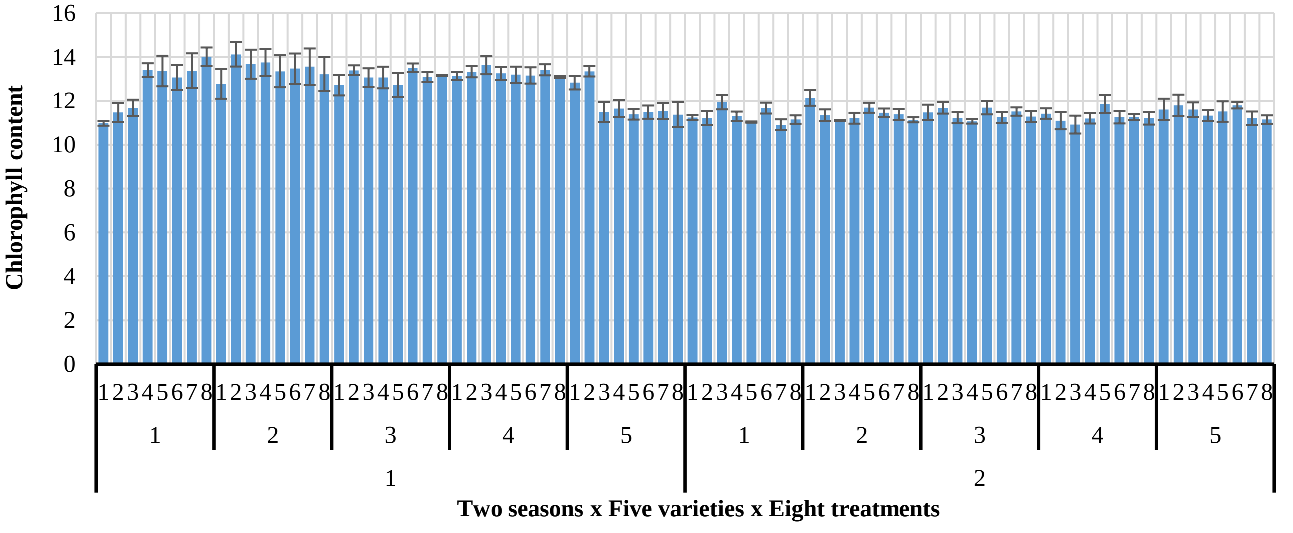

Fig. 10. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Chlorophyll content (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

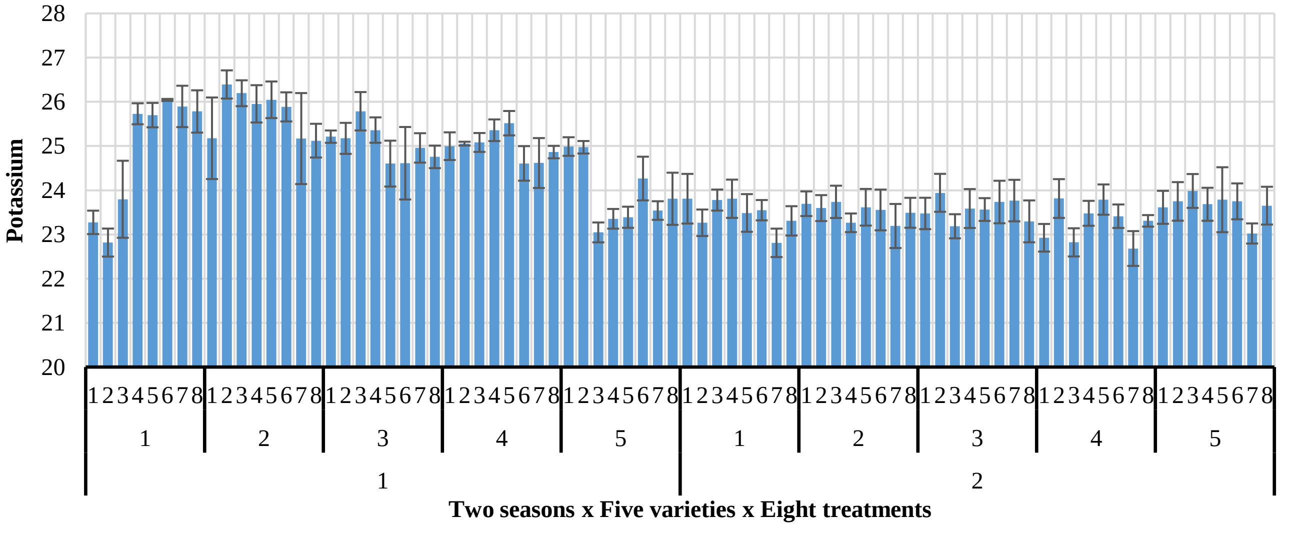

Fig. 11. Bar graphs of three-way interaction (two seasons x five varieties x eighttreatments) of Potassium (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

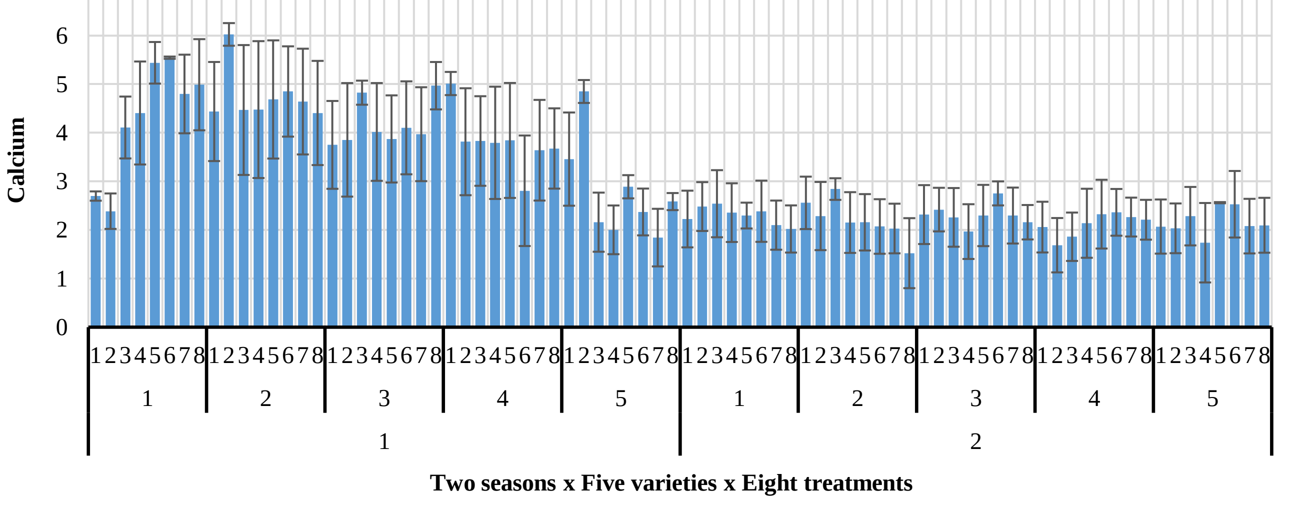

Fig. 12. Bar graphs of three-way interaction (two seasons x five varieties x eighttreatments) of Calcium (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

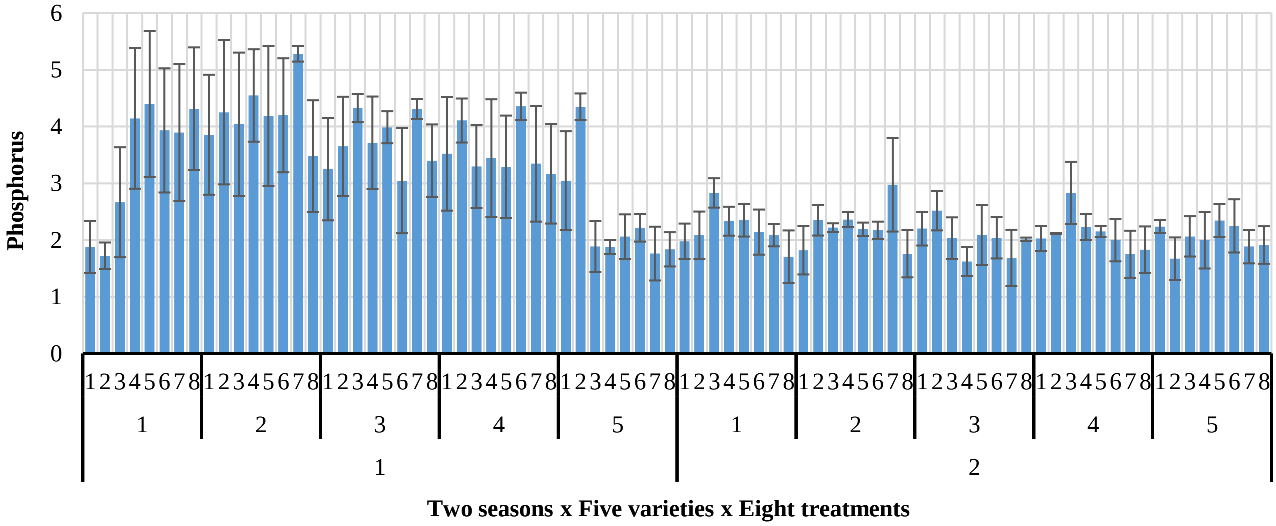

Fig. 13. Bar graphs of three-way interaction (two seasons x five varieties x eighttreatments) of Phosphorus (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

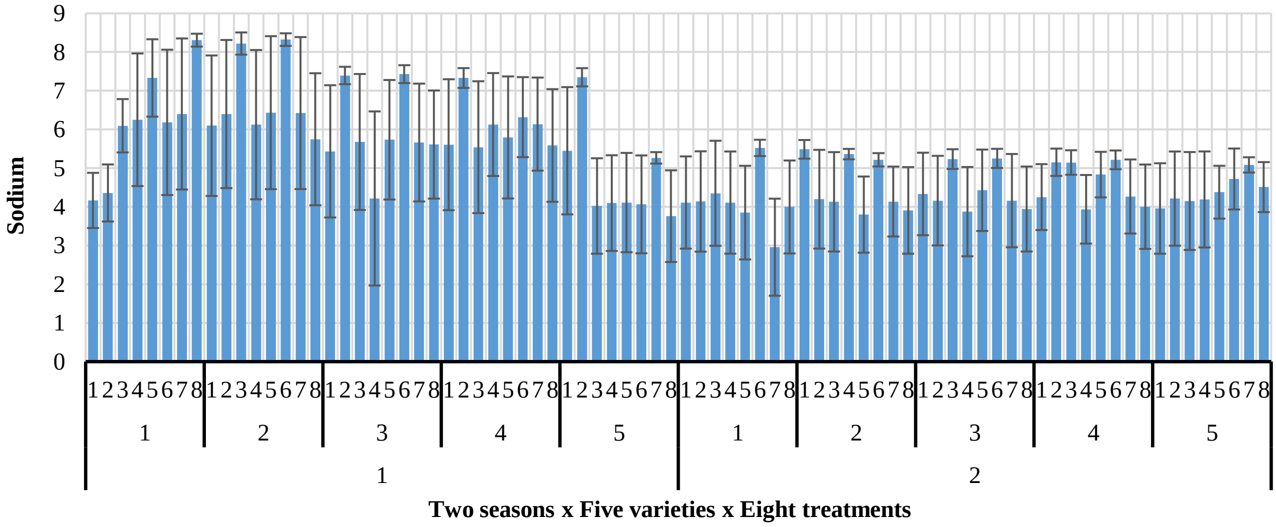

Fig. 14. Bar graphs of three-way interaction (two seasons x five varieties x eighttreatments) of Sodium (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

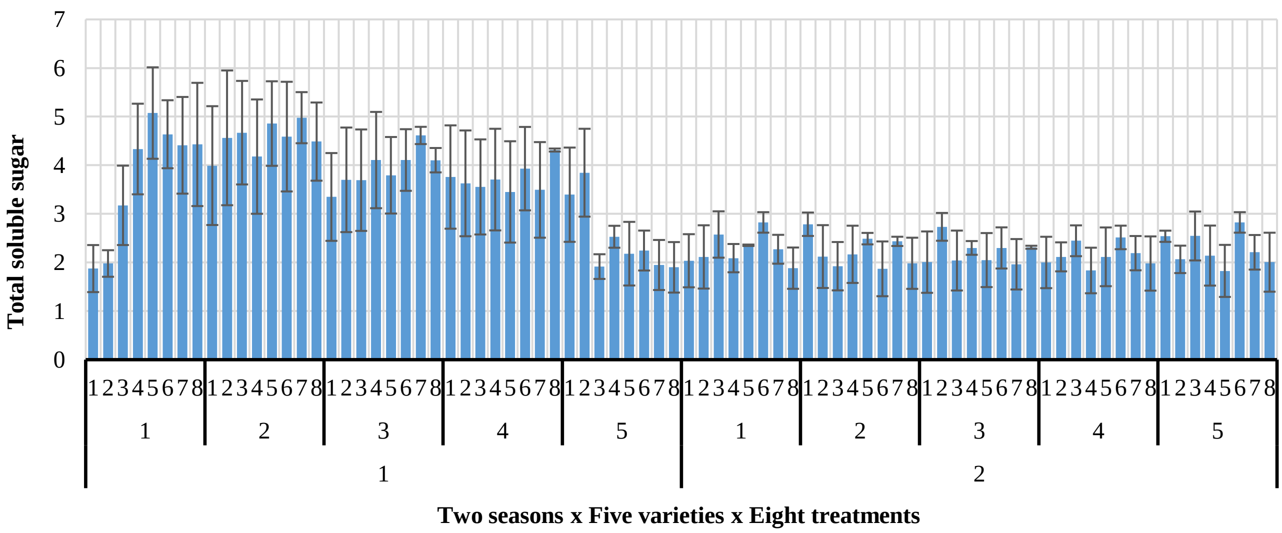

Fig. 15. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Total Soluble sugar (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

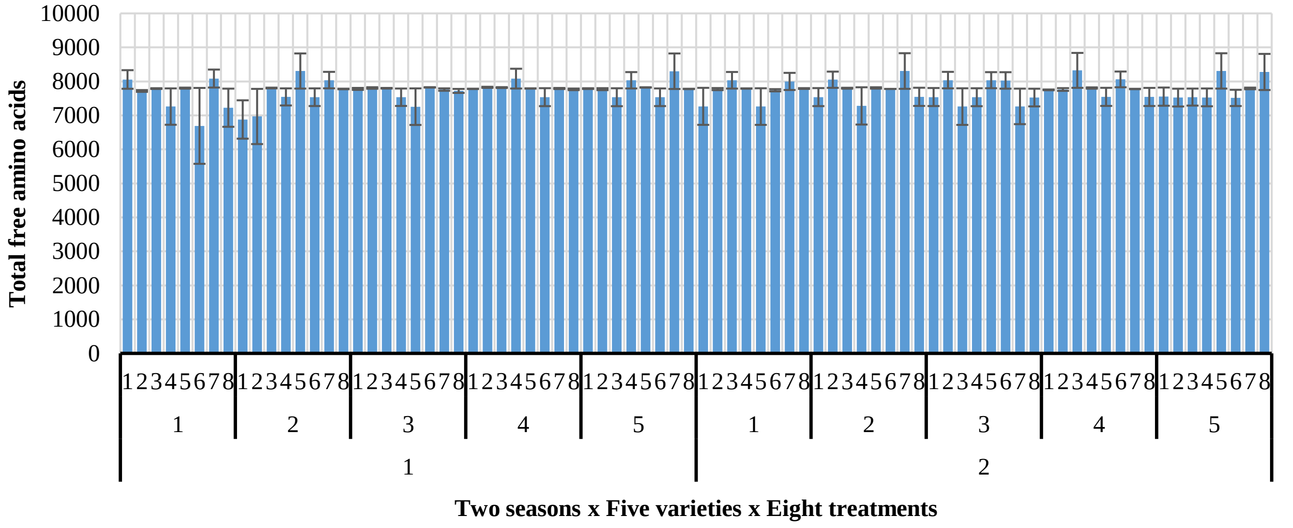

Fig. 16. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Total free Amino acids (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

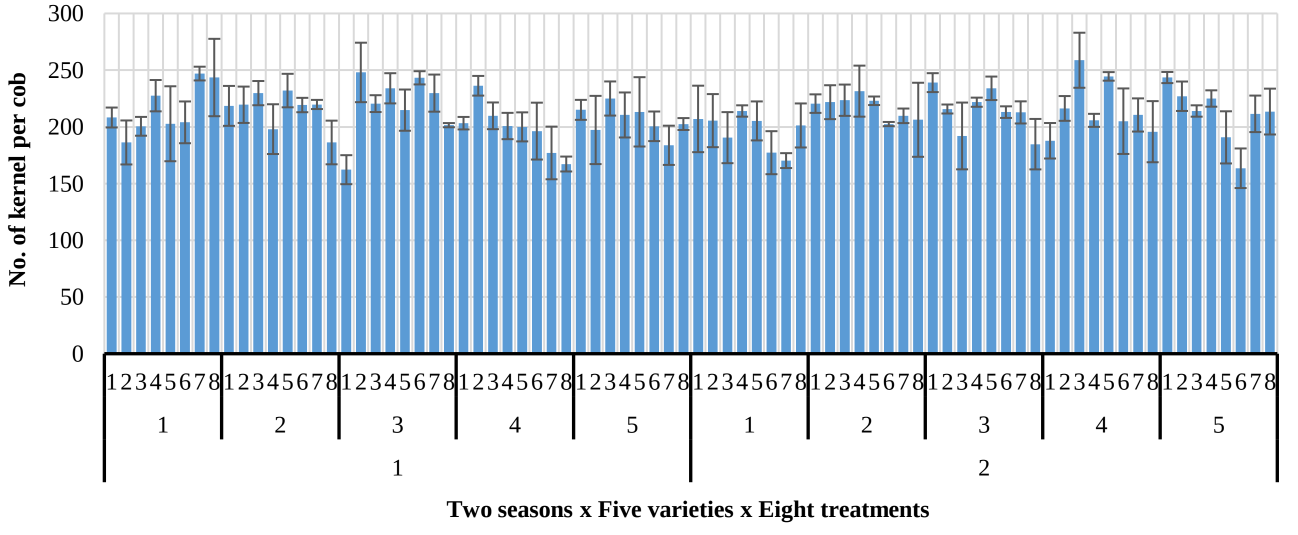

Fig. 17. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of No of Kernal per Cob(1,2* Season, 1,2,3,4,5 variety,1,2,3,4,5,6,7,8*Treatment).

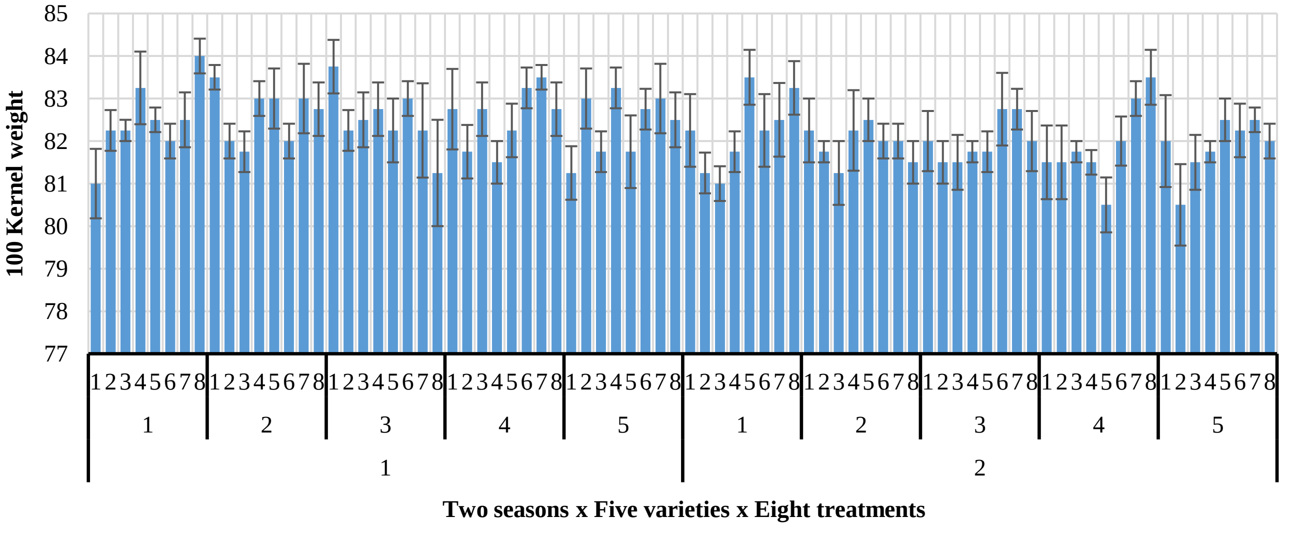

Fig. 1 8 . Bar graphs of three-way interaction (two seasons x five varieties x eighttreatments) of 100 Kernal weight (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

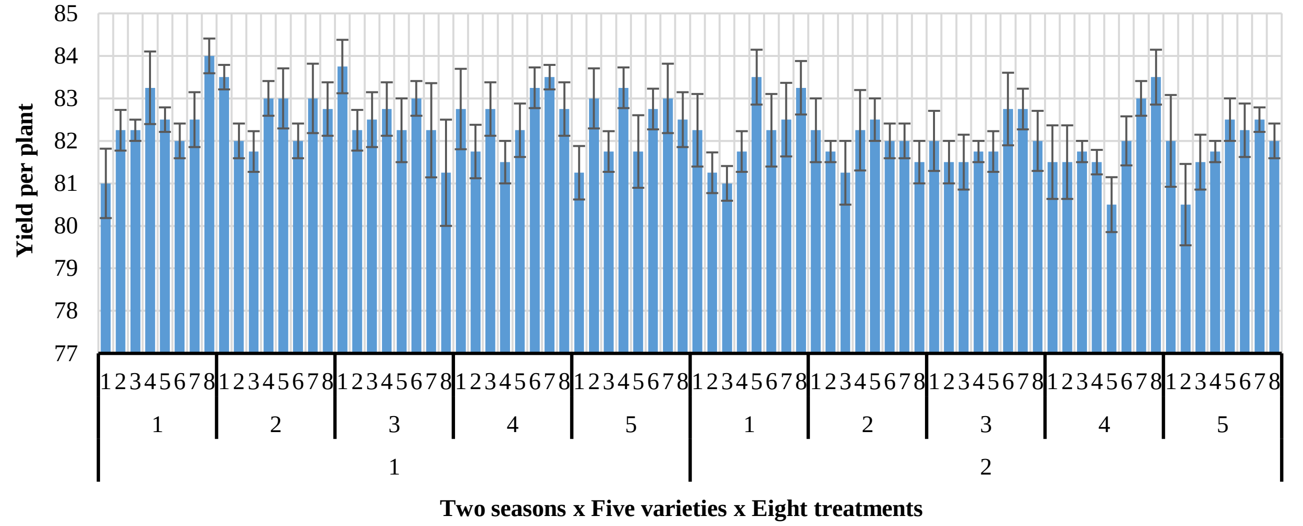

Fig. 19. Bar graphs of three-way interaction (two seasons x five varieties x eighttreatments) of Yield (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

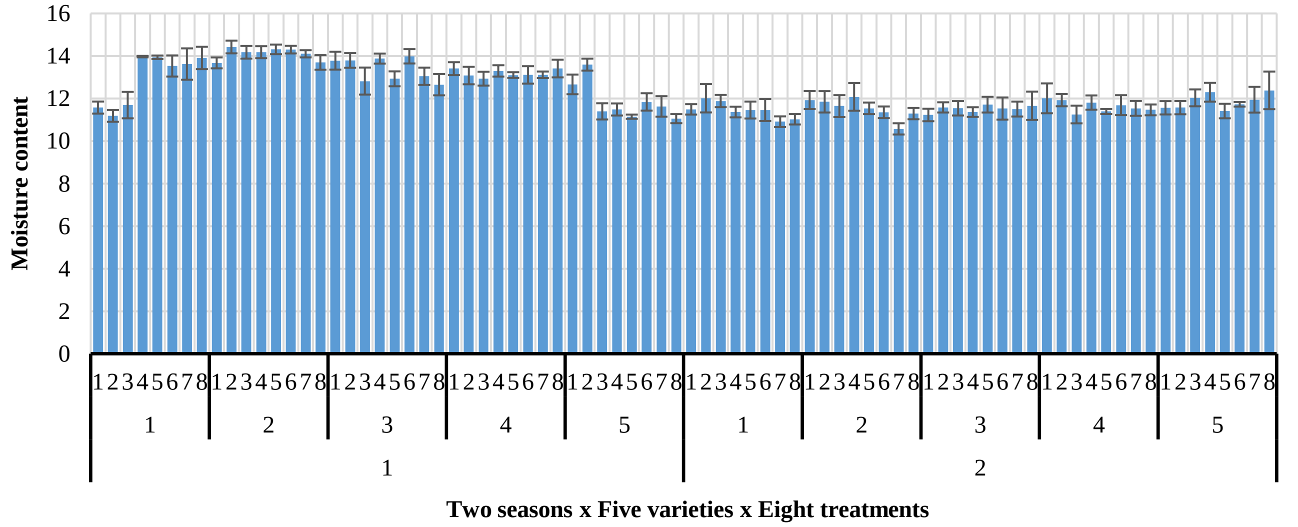

Fig. 20. Bar graphs of three-way interaction (two seasons x five varieties x eighttreatments) of Moisture. (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

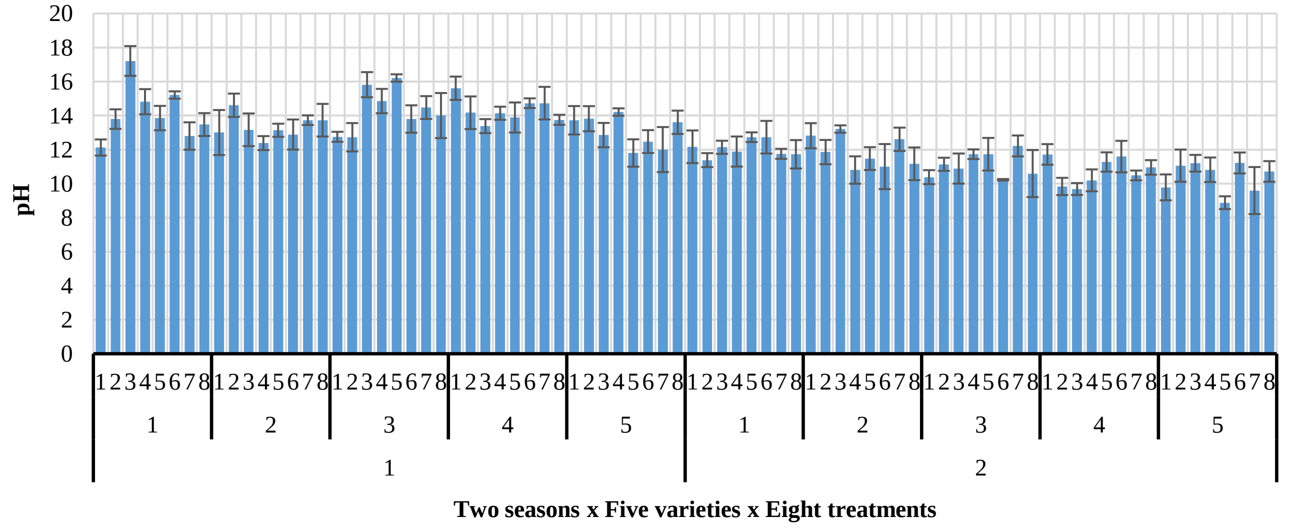

Fig. 21. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Ph (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

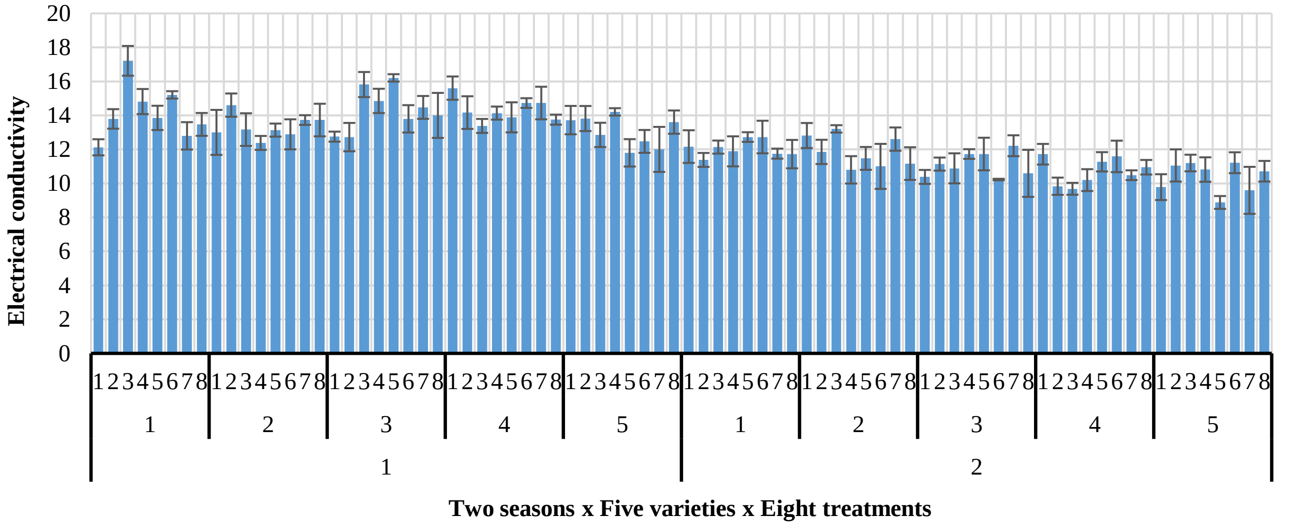

Fig. 22. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Electrical Conductivity (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

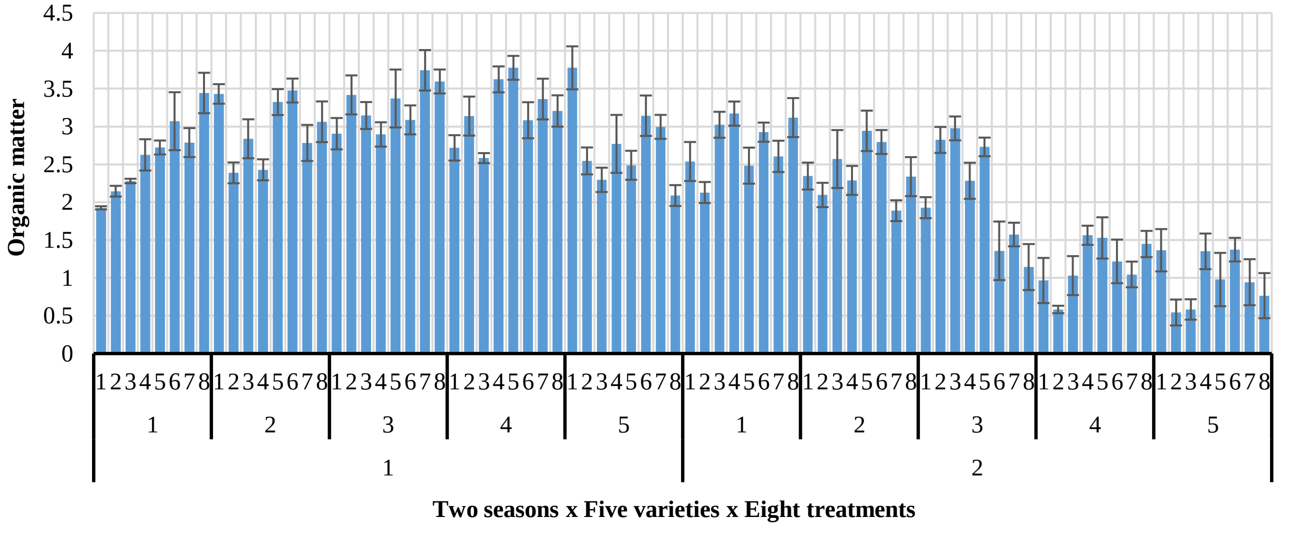

Fig. 23. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Organic Matter (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

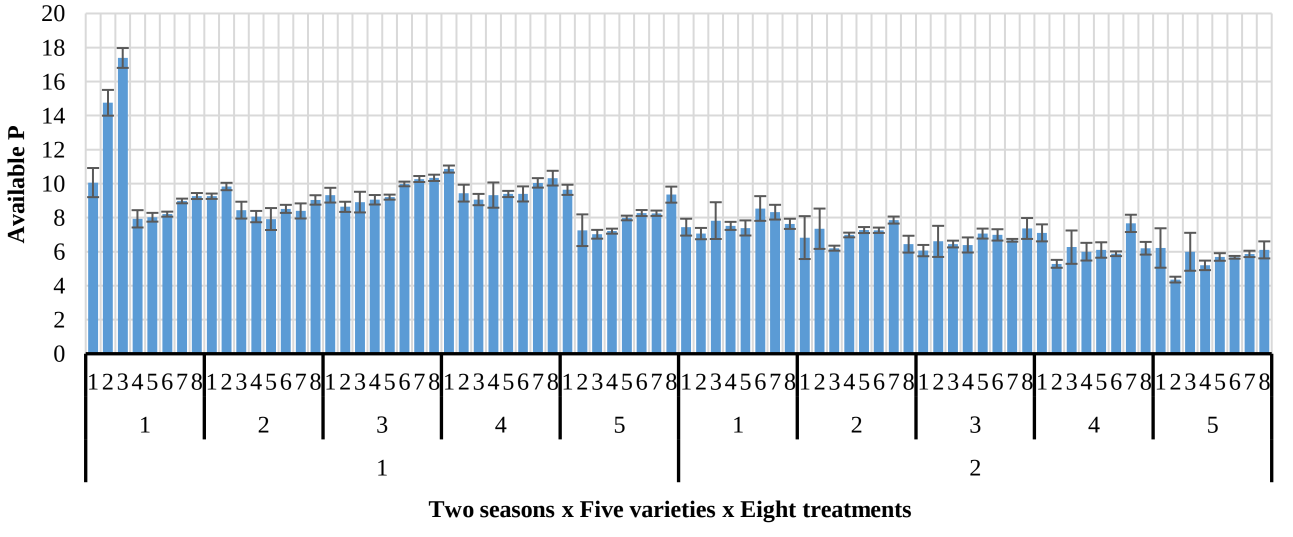

Fig. 24. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Available Phosphorus (1,2* Season, 1,2,3,4,5 variety,1,2,3,4,5,6,7,8*Treatment).

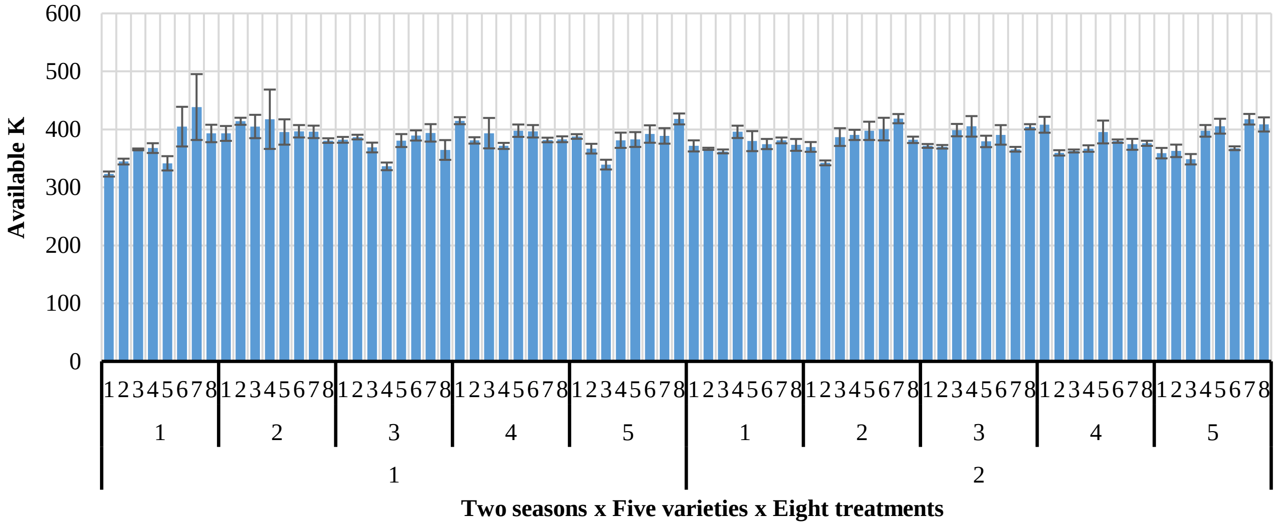

Fig. 25. Bar graphs of three-way interaction (two seasons x five varieties x eight treatments) of Available Potassium (1,2* Season, 1,2,3,4,5 variety, 1,2,3,4,5,6,7,8*Treatment).

Physico-biochemical parameters of soil: In physicochemical parameters the results from analysis of variance in pH showed significant and least significant in season, variety, Season × Variety and Season × Treatment, while non-significant variation in treatment, Variety × Treatment and Season × Variety × Treatment. (Fig. 23). The pH varies from 7.92 to 11.97. Maximum was observed in season 2 variety 1 treatment 3 and minimum was in season 2 variety 5 treatment 5. Results obtained from ANOVA of electrical conductivity showed significant and non-significant effect in Season, Variety, Season× Variety, Variety × Treatment and Treatment, Season × Treatment, Season × Variety × Treatment (Fig. 23). The trend of EC showed variations from 17.21 ms/cmto 9.68 ms/cm. Highest EC was observed in season 1 variety 1 treatment 3 and lowest concentration was present in season 2, variety 4 treatment 3 (Fig. 24). The results of ANOVA in osmoregulation showed significant and least significant variation in Season, Variety, Treatment, Season × Variety, Season ×Treatment, Variety × Treatment and Season × Variety × Treatment. The mean concentration of OM fluctuated from 0.54% to 3.77%. High content of OM was found in season1, variety5, treatment 1 and low content was present in season2, variety5, treatment 2 (Fig. 23).

Available phosphorous: The analysis of variance of A P showed significant effect in Season, Variety, Treatment, Season× Variety, Season × Treatment, Variety × Treatment and Season × Variety × Treatment. The Available phosphorous concentration ranges from 4.35 mg/kg to 17.38 mg/kg. Maximum value was present in season 1 variety 1 treatment 3 and minimum concentration was observed in season, 2 variety 5, treatment 2 (Fig. 24).

Available potassium: Analysis off variance for available potassium demonstrated non- significant variation in season, Season × Variety, Season × Treatment and significant in Variety, Treatment, Variety × Treatment while least significant in Season × Variety × Treatment. The trend of AP varies from 323 mg/kg to 438.5mg/kg. Highest concentration was observed in season 1, variety 1, treatment 7 and lowest was present in season 1, variety 1, treatment 1 (Fig. 25).

Discussion

This study examined the effects of proline and trehalose on maize growth and development in order to reduce cadmium stress. The results indicated that Plant height increase from 161.325cm to 164.507cm when subjected to Trehalose 75 mM which was compared to previous studies, as Alharby *et al*., (2021).

In current research leaf area fluctuated from 691.83 cm to 698.24cm. The highest value was present under Proline 75 mM treatment and lowest was found under Proline 50 mM treatment. Cadmium stress is a major environmental stressor that slows growth, cuts flower production, and shrinks leaf area (Farooq *et al*., 2016;). The Available phosphorous concentration ranges from 4.35% to 17.38%. Maximum value was present in Proline 75 mM treatment and minimum concentration was observed at cadmium stress. The percentage of total P in soil that is easily available for absorption by plant roots is referred to as soil available P (Ziadi *et al*., 2013).

According to Ashraf & Foolad (2007), exogenous Pro administration might enhance the K concentration in guard cell membranes, which is critical for keeping stomata open, enhancing stomatal conductance, and boosting chlorophyll content. Proline provides an extra benefit in soil salinity since it may reduce Na absorption while increasing K uptake, shielding plants after the negative belongings of Na, as highlighted by Dong *et al*., (2006).

The trend of EC showed variations from 9.68 ms/ cm to 17.21ms/cm. Highest EC was observed in Proline 75 mM treatment and lowest concentration was present in Proline 50 mM treatment. Soil electrical conductivity may be used to monitor the mineralization of organic materials in the soil and can be used to quantify soluble nutrients. To determine the impacts of salt, the electrical conductivity of all obtained soil samples was tested.

The pH varies from 7.92 to 11.97. Maximum Basic pH was observed at Proline 50 mM treatment 4 and minimum was in Trehalose 75 mM treatment. According to (Walecka-Hutchison & Walworth, 2006), soil pH is a degree of the alkalinity or else acidity of soil, and the current pH value was greater than Dong *et al*., (2006), and the standard allowed limits from WHO for pH values in the soil are required to vary between 6 and 8.5, as provided by Ziadi *et al*., (2013).

Moisture contents ranged from 10.57% to 14.42%. Low level was found in Trehalose 50 mM treatment and high level was observed at cadmium stress. Barnwal *et al*., (2012) discovered that maize moisture content (12.8-29.0 percent), which was greater than our observed value, was higher under cadmium.

The yield ranged from 80.5 gm /Plant to 83.5 gm/Plant. High concentration was found at Trehalose 50 mM treatment and low content of yield was found at cadmium stress. Under normal circumstances, greater Cd concentrations caused serious harm to maize plant development and output. Cd is a hazardous metal that has no physiological role in plants, as is well known. In plants growing in Cd-stressed soil, slowed growth is a typical sign. It might be because poisonous Cd is involved in plant metabolism, or because large concentrations of antagonistic harmful metals limit the absorption of other vital nutrients as result was compared with Chang *et al*., (2013).

Different parameters in yield show variations from 162.25 to 258.75. High value was present in Proline 75 mM treatment and low was found at control site. while trend of hundred kernel weight varies from 27.25 gm to 24.25 gm. Maximum was observed at Trehalose 50 mM treatment and minimum was in Proline 25 mM treatment.

Kernel per cob estimates were compared to those provided by Chang *et al*., (2013), who reported Kernel per cob (29.47 percent, 28.88 percent) with substantial phenotypic and genotypic coefficients of variation which was lower than current reported value. In current reported work Level of TSP in maize fluctuated from 7.11% dry wt to 12.32% dry wt. The highest value was observed in treatment Trehalose 75 mM and lowest was present in treatment Proline 75 mM. Stress on the protein and oil content of seeds can be lessened by applying trehalose on the leaves of plants instead of or in addition to compost. The fact that trehalose treatments made maize more nutritious was in line with what Wingler (2002) found.

The trend of total soluble sugar ranged from 1.82% dry wt. to 5.07% dry wt. treatment Trehalose 75 mM showed highest mean while lowest was observed Proline 75 mM treatment. Sodium concentration ranged from 2.95 mg/g dry wt. to 8.32 mg/g dry wt. Highest value was present in Trehalose 75 mM treatment while lowest was observed Trehalose 50 mM.

Zouari *et al*., (2016) found comparable results for corn, while Chang *et al*., (2013) reported similar results for maize and broad bean plants. Trehalose treatment reduced ion leakage and raised the ratio of $Na+$ ions in maize seedling leaves, suggesting that it may reduce stress by stabilizing plasma membranes (Zeid, 2009).

The Stomatal Concentration varies from 291 to 294. The highest value was found Proline 50 mM treatment and lowest was observed in control group. Stomata are helpful in gas exchange in vascular plants (Zhang & Reynold, 2019). According to Farooq *et al*., (2016), the concentration of sub stomatal ranged from 251.75% to 254.5%. High level was observed in Trehalose 75 mM while low level was found in Cadmium stress. In current research transpiration rate fluctuated from 5.77% to 2.40%. The Highest value was present under Proline 50 mM treatment and lowest was found under Trehalose 50 mM treatment.

Recommendations and Conclusion

Regular monitoring of heavy metals, particularly cadmium, is crucial to prevent excessive accumulation in the food chain. Clean wastewater before irrigation and avoid consuming polluted crops to lower health risks. Improved agricultural techniques and research on metal transfer from the environment to humans and animals are necessary. Exogenous application of proline and trehalose can reduce cadmium toxicity in maize in Sargodha, with a recommended concentration of 75 mM.

Cadmium stress significantly reduces maize growth and yield, leading to a decrease in photosynthetic pigments and increased reactive oxygen species (ROS). However, Trehalose 75 mM supplementation can increase maize production by reducing Cd uptake, enhancing photosynthetic pigments, TSP, and FAA, and scavenging ROS through antioxidant enzymes. Exogenous proline (75 mM and trehalose 75 mM, 50 mM) also improves morphological features by protecting photosynthetic pigments and reducing cadmium intake, thus alleviating cadmium growth inhibition in maize.

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