

Exogenous Applications of Naphthaleneacetic Acid and Indole-3-Acetic Acid on Selected Crops Under MgCl₂ Stress

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Abstract

*Salt stress in agricultural soils can induce significant stress detrimental to crops, the environment, and human health. This study evaluated the efficacy of topically administered phytohormones indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) in alleviating stress induced by magnesium chloride (MgCl₂) in *Abelmoschus esculentus* (A. esculentus) and *Zea mays* (Z. mays). In controlled laboratory (Petri plate) and field-pot environments, morphological, physiological, and biochemical responses were evaluated utilizing a Randomized Complete Block Design (RCBD) with three replications. Both low and high concentrations of MgCl₂ resulted in a significant reduction in biochemical and growth indicators, such as leaves number, area of leaf, and shoots and length of root. In A. esculentus and Z. mays, the combination of MgCl₂ with IAA and NAA significantly enhanced seedling growth. Biochemical investigations shows that the hormone-assisted (IAA and NAA) therapy revealed increased levels of carotenoids, proteins, carbohydrates, chlorophyll “a”, “b”, total chlorophyll and decline the proline contents in both crops compared to MgCl₂ stress. In conclusion, MgCl₂ resulted in significant adverse effects on both species; however, the foliar application of IAA and NAA mitigated stress-induced damage, whereas A. esculentus exhibited greater resistance compared to Z. mays.*

Keywords: Saline Stress, *A. esculentus*, *Z. mays*, Naphthaleneacetic Acid, Indole-3-Acetic Acid.

Introduction

Climate change poses a significant global threat to security of food and agricultural productivity. Rising global temperatures, altered precipitation patterns, increased evapotranspiration, and the rising frequency of extreme climatic events, including heatwaves, floods, and prolonged droughts, have intensified the effects of environmental stress on agricultural crops worldwide (Qadir et al., 2014). In Pakistan, increasing temperature (Asad et al., 2023) has significantly affected soil salinization

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and the sensitivity of agriculture, whereas it highly susceptible to climate change due to increasing temperatures, unpredictable monsoon patterns, glacial melt, recurrent droughts, and intense flooding. In low-income countries such as Pakistan reliant on environmentally friendly agricultural systems, climate-induced variations considerably threaten sustainable agriculture by altering agro-ecosystems, degrading soil quality, and obstructing plant growth.

Soil salinity represents a pervasive and detrimental environmental issue affecting agricultural systems worldwide, alongside various other abiotic stresses. Salinity occurs when soluble salts accumulate in the soil or irrigation water beyond the tolerance levels of plants. The accumulation arises from both anthropogenic activities, such as suboptimal irrigation practices, insufficient systems for drainage, excessive fertilizer use, and groundwater overexploitation, as well as natural processes including mineral weathering and the presence of saline base substances (Munns & Tester, 2008). In experimental contexts, salts such as magnesium chloride (MgCl₂) are commonly utilized to induce salinity stress, as they effectively establish osmotic stress and ionic imbalance, allowing for the systematic assessment of plant responses to salinity (Acosta-Motos et al., 2017). Salinity and heavy metal stress adversely affects plant development through various interconnected mechanisms, such as osmotic stress ion toxicity, nutritional deficiency, and oxidative damage. Elevated levels of magnesium and chlorine ions restrict water consumption, disrupt cellular ion balance, and inhibit enzymatic and metabolic functions (Fao, 2015). Salinity induces the generation of reactive oxygen species (ROS), resulting in the oxidative degradation of membranes in cells, proteins, and nucleic acids, which ultimately impairs growth, photosynthesis, and productivity (Wicke et al., 2012). Salinity affects nutritional homeostasis and induces excessive production of reactive oxygen species, resulting in oxidative stress.

The application of Plant Growth Regulators (PGRs) has proven to be an effective method for enhancing the tolerance of plants to abiotic stressors. Auxins, particularly indole-3-acetic acid (IAA) and its synthetic analog naphthaleneacetic acid (NAA), are crucial in regulating cell elongation, root development, nutrient uptake, and stress-related signaling pathways (Quamruzzaman et al., 2021). The external application of IAA, particularly through foliar spraying, facilitates rapid absorption and efficient regulation of biological and physiological processes in stress conditions (Khan et al., 2012). Previous studies indicate that the exogenous application of IAA and NAA can mitigate the adverse effects of salt by promoting root development, maintaining hormonal balance, enhancing photosynthetic efficiency, and strengthening antioxidant

defense mechanisms (Egamberdieva, 2009; Kaya et al., 2013). These reactions collectively enhance plant growth and improve stress tolerance under saline conditions. Therefore, this study aims to examine the impact of MgCl₂-induced salinity stress on the development performance of *Zea mays* (*Z. mays*) and *Abelmoschus esculentus* (*A. esculentus*), while also assessing the potential mitigating effects of foliar application of IAA and NAA.

Method and Materials

Selected Plant Species

Seeds of *Z. mays* and *A. esculentus* were collected at the KP research center, Pakistan. The obtained seeds were assessed for viability prior to the start of the experiment in the physiology laboratory at Bacha Khan University Charsadda, whereas the seed were sowing in in pots of control environment. Each pot was filled with same kind and quantity of soil. Subsequent to sowing, the seedlings were administered diverse concentrations of salt stress and hormones.

Laboratory Experiments

In the lab, the soil and 87 petri plates with filter sheets inside were autoclaved. Seeds were cleansed with 0.1% mercury chloride for 30 seconds, followed by two to three rinses with distilled water. Ten seeds were placed on each petri plate using Randomized Complete Block Design (RCBD). The Petri plates were thereafter treated with it every 24 hours (hrs) and kept at 28 °C. Each pot was allocated diverse concentrations of salt and hormones, whereas the specifications of these sets were mentioned in each table and graphs.

Percent Germination

$$\text{Percentage} = \frac{\text{No. of germinated seeds}}{\text{total seeds}} \times 100$$

Measurement of Agronomic Characteristics

The plant harvested after the treatments were completed, and agronomic traits such as shoots and length of roots, root and leaf counts, and dry and fresh biomass were recorded.

Biochemical Analysis

Soluble Sugar Analysis

The soluble sugar in the leaves was determine using the Mohammadkhani and Heidari (2008) technique. Ten milliliters (mL) of distilled water were mixed with a fresh leaf sample weighing 00.20 grams

(g) in a mortar. The material was then spun at 1000.00 rpm for about ten minutes (min). The residue was eliminated by transferring the supernatant to a new test tube. 5.0 mL of the anthrone reagent and 00.50 mL of extracts from plants were placed in a test tube. After that, the tubes were heated in a bath of water for around fifteen minutes. Subsequently, they were swiftly cooled with cold water. Green is the outcome of measuring at 620.00 nm against a blank reagent. The anthrone reagent was designed to function as the blank for the reagent. The carbohydrate content was quantified in micrograms per milligram of fresh weight after measurement in micrograms per mL.

Protein Analysis

Bradford (1976) assay reagent was used to extract and quantify soluble protein. Weigh 00.20g of leaves and then utilize cool pestle and mortar in 5.0 mL of K₃PO₄ buffer (00.10M, Ph = 07.00) to mix them together. Cheesecloth is utilized to creation of extracts filters, then makes it cooled and spun at 4000.00rpm for 10 min. Spin the refrigerator centrifuge at 12,000.00rpm for 20min. Supernatant was putted in a test tube, then add buffer until the extract volume is 5.00 mL. We add 04.80 mL of buffer to 00.20 mL of this extract to make it less concentrated.

Calculation

Fill a check tube with 00.10 mL of the extract that is likely to be impacted. We put 05.00 mL of Bradford essay in and shake. To generate the reagent blank, combine 00.10ml of buffer with 5mL of Bradford essay reagent. To find the opacity at 595.00 nm, use a spectrophotometer. The soluble protein was determined using a standard curve.

Determination of Photosynthetic

The Maclachlan and Zalik (1963) approach was used to determine the chlorophyll and carotenoid contents. After being reweighed, the leaves are macerated with a tiny amount of acid-treated sand in 3.00 mL of 80.0% acetone and centrifugation for 5 minutes at 1000 rpm. After that, the debris is cleaned three times using 1.00 cc of 80.00% acetone each time. A total volume of 07.00 mL is obtained by combining and diluting the supernatants with acetone. These results had visual densities of 663.00, 645.00, 480.00, and 510.00 nm.

$$Chl. a \left(\frac{mg}{g} \right) = \frac{12.30 D_{663} - 0.86 D_{645}}{d \times 1000 \times w}$$

$$Chl. b \left(\frac{mg}{g} \right) = \frac{19.30 D_{645} - 0.86 D_{663}}{d \times 1000 \times w}$$

$$Total\ Chl. = Chl. a + Chl. b$$

$$\text{Carotenoids} = \frac{(1000 A_{470} - 01.82 \text{ Chl } a - 85.02 \text{ Chl } b)}{198}$$

Proline Analysis of Leaves

The Bates et al. (1973) was employed to quantify proline levels in the leaves. The extract was added after 10.00 mL of 03.00% sulphosalicylic acids and 00.50g of frozen plant tissue were combined in a test tube and filtered through paper. The reaction was halted in an ice bath following an hour of heating the mixture to 100.00 °C, which included 02.00ml of acid ninhydrin and 02.00 mL of glacial acetic acid. Subsequently, 04.00 mL of toluene was incorporated, and the entire mixture was stirred vigorously for 14-25 seconds. Subsequently, the 02 stages were permitted to separate within the tubes. The chromophore containing toluene was transferred to a detached test tube. Absorbance was quantified at 520.00 nm. The quantity of proline with respect to fresh weight was calculated using a standard curve, with ranges of 20 to 100 mg/ml.

Results and Discussion

Shoot Growth of Selected Crops

Table 1 has provided the effect of various MgCl₂, IAA and NAA treatments on the field and laboratory-based parameters. In the field, the SL/AE/F values did not significantly (NS) differ between most treatments as compared to the control and this was an indication that salinity stress did not affect this parameter much in the field. Nevertheless, the mean values under combined treatments were relatively higher, with a trend of MgCl₂/IAA (18.7 ± 9.0) and MgCl₂/NAA (17.1 ± 4.5) indicating the positive effect of growth regulators in combination with MgCl₂. On the contrary, the SL/ZM/F parameter presented considerable differences in most treatments with a character of significance of the difference between the treatments. This gave the highest reading under MgCl₂ /IAA 75/100 ppm (25.5 ± 4.9) followed by MgCl₂ /IAA 75/50 ppm (22.5 ± 12.0) and MgCl₂ /NAA 25/50 ppm (22.5 ± 8.5) which indicated better performance at combined MgCl₂ and auxin applications. Control treatment had relatively lower values which indicate the ameliorative effect of exogenous growth regulators in the stress conditions.

A stronger treatment effect was found in laboratory data. In case of SL/AE/L, the majority of treatments differed significantly (with *) with the control. Maximum values were recorded at MgCl₂ /IAA 75/100 ppm as well as MgCl₂ /IAA/NAA, with the highest measure of 7.7 ± 2.1 and this is a good indication of controlled physiological responses. Some

treatments including MgCl₂ /IAA/NAA 75/50/50 ppm were found to be non-significant implying that there might be a concentration effect. On the same note, the applied treatments had a significant effect on the SL/ZM/L values. The highest value was found in MgCl₂ /IAA/NAA 100/100/100 ppm (8.7±.5) and at MgCl₂/IAA 50/50 ppm and MgCl₂/NAA 50/100 ppm and the lowest value was found in the control (4.5±.5). In general, in laboratory settings the differentiation of treatments was more apparent as compared to field settings.

Table 1: Effect of IAA and NAA on maize and *A. esculentus* under the induced stress of MgCl₂.

Treatments	Field Data		Lab Data	
	SL/AE/F	SL/ZM/F	SL/AE/L	SL/ZM/L
Control	15.7±06.6NS	08.4±01.3*	06.8±01.5*	04.5±10.5*
25-ppm	16.1±07.2NS	12.7±03.3*	06.8±01.8*	04.5±01.8*
50 -ppm	13.2±02.2NS	10.3±03.9*	09.6±00.2*	07.3±00.2NS
75 -ppm	15.0±05.8NS	12.8±06.7*	03.7±00.7*	05.±02.4*
100 -ppm	14.1±02.3NS	14.5±00.7*	04.20±01.4*	04.0±01.0*
MgCl ₂ /IAA25/50	18.3±07.0NS	12.3±00.9*	06.7±00.7*	04.8±02.3*
MgCl ₂ /IAA50/ 50	13.7±02.9*	14.6±06.3*	07.2±01.4*	07.4±00.4*
MgCl ₂ /IAA75/ 50	18.7±09.0*	22.5±12.0*	05.2±01.4*	05.2±02.8*
MgCl ₂ /IAA100/ 50	16.0±09.4*	11.0±00.7*	06.2±01.4*	04.0±00.7*
MgCl ₂ /IAA25/ 100	16.0±09.4*	16.0±02.8NS	06.2±01.4*	04.0±01.1*
MgCl ₂ /IAA50/ 100	10.4±01.3*	17.5±07.0*	06.2±02.8*	07.3±00.4NS
MgCl ₂ /IAA75/ 100	15.0±08.1*	25.5±04.9*	07.7±02.1*	05.4±02.9*
MgCl ₂ /IAA100/ 100	08.7±01.9NS	15.0±01.4*	06.2±01.4*	04.6±01.7*
MgCl ₂ /NAA25/ 50	15.5±08.7NS	22.5±08.5*	06.7±02.1*	04.7±02.1*
MgCl ₂ /NAA50/ 50	17.1±04.5NS	14.9±02.0*	05.7±00.7*	07.2±00.2NS
MgCl ₂ /NAA75/ 50	14.0±03.6NS	17.7±00.5NS	05.7±02.1*	04.4±01.4*
MgCl ₂ /NAA100/ 50	15.80±05.4NS	16.3±08.9NS	06.2±01.4*	04.0±00.8*
MgCl ₂ /NAA25/ 100	14.5±06.1NS	12.3±01.8NS	06.7±02.1*	04.9±02.3*
MgCl ₂ /NAA50/ 100	15.6±06.1NS	15.0±02.8*	05.7±00.7*	07.3±00.4*
MgCl ₂ /NAA75/ 100	17.0±06.6NS	13.9±01.5*	07.7±00.7*	05.6±03.0*
MgCl ₂ /NAA100/ 100	13.2±03.2NS	15.8±0.4*	06.7±00.7*	03.9±00.5*
MgCl ₂ /IAA/NAA25/50/50	12.6±03.9NS	16.3±0.4NS	06.2±01.4*	04.8±02.2*
MgCl ₂ /IAA/NAA50/50/50	16.2±12.1NS	17.0±02.8NS	06.7±00.7*	07.5±00.5NS
MgCl ₂ /IAA/NAA75/50/50	16.2±04.9NS	19.8±00.4NS	07.7±00.7NS	04.6±01.1*
MgCl ₂ /IAA/NAA100/50/50	17.0±06.0NS	19.9±04.9NS	07.2±01.4*	03.8±00.3*
MgCl ₂ /IAA/NAA25/100/100	11.7±04.4NS	15.3±07.4NS	06.7±00.7*	05.9±00.7*
MgCl ₂ /IAA/NAA50/100/100	12.0±03.8NS	16.4±05.1NS	07.7±00.7*	06.1±01.00*
MgCl ₂ /IAA/NAA75/100/100	15.5±04.6NS	16.5±00.0NS	07.2±01.4*	05.00±01.3*
MgCl ₂ /IAA/NAA100/100/100	11.6±01.5NS	11.7±01.4*	05.7±00.7*	08.7±00.5NS

Key words: * stands for significant ($p < 0.05$), NS (non-significant), SL (shoot length), ZM (*Z. mays*), and AE (*A. esculentus*), L (lab analysis) and F for field analysis.

All the findings are that a combination of MgCl₂ with IAA and / or NAA is more efficient than the single ones in controlling the impact of stress, especially in laboratories. Out of all the treatments, MgCl₂ / IAA at 75/50 and 75/100 ppm were the most effective and could suggest that they assist in stimulating stress tolerance processes. Maize, a major crop, is considered to be more vulnerable to salinity compared to cotton or

barley (Junghans et al., 2006). Significant alterations in the osmolality of shoot sap during salt stress have been demonstrated to sustain shoot turgor and growth in salt-resistant hybrids).

Table 2: MgCl₂ impact on root elongation of the targeted crops under different IAA and NAA treatments.

Methods	Field analysis		Laboratory Analysis	
	RL/AE/F	RL/ZM/F	RL/AE/L	RL/ZM/L
Control treatment	05.2±03.6*	06.0±01.4*	03.3±01.1*	02.5±00.4*
25 -ppm	07.7±04.1*	09.4±03.7NS	03.2±01.1*	02.6±00.8*
50 -ppm	05.1±01.6*	09.4±03.4NS	04.3±00.1*	04.3±00.2*
75 -ppm	06.3±02.2*	09.8±06.7NS	03.4±01.3*	03.1±01.3*
100 -ppm	06.5±02.1*	11.5±01.4NS	03.0±00.8*	02.4±00.2*
MgCl ₂ /IAA25/50	09.6±05.2NS	09.3±01.8NS	03.3±01.3*	02.4±00.2*
MgCl ₂ /IAA50/50	06.5±04.1*	10.5±00.7NS	04.5±00.2*	02.9±01.1*
MgCl ₂ /IAA75/50	07.7±03.8*	11.6±06.9NS	03.4±01.3*	04.2±00.2*
MgCl ₂ /IAA100/50	09.6±10.8NS	19.8±11.7*	02.7±00.3*	03.2±01.7*
MgCl ₂ /IAA25/100	04.6±00.5*	08.4±00.6NS	03.20±01.2*	02.7±00.5*
MgCl ₂ /IAA50/100	06.5±6.3*	12.3±03.2NS	04.5±00.1*	02.1±00.0*
MgCl ₂ /IAA75/100	09.3±4.0*	13.8±06.7*	03.5±01.6*	04.2±00.3*
MgCl ₂ /IAA100/100	08.2±10.3NS	21.5±05.0NS	03.4±01.3*	03.2±01.4*
MgCl ₂ /NAA25/50	07.3±04.7*	11.0±04.1*	03.3±01.2*	02.5±00.4*
MgCl ₂ /NAA50/50	04.9±01.5NS	14.0±01.4*	04.4±00.1*	02.7±00.8*
MgCl ₂ /NAA75/50	08.4±03.5NS	11.3±01.8*	03.6±01.6*	04.2±00.5
MgCl ₂ /NAA100/50	07.2±04.6*	14.0±00.7*	02.6±00.1*	02.1±00.2*
MgCl ₂ /NAA25/100	06.6±03.5*	13.3±08.9NS	03.3±01.2*	02.8±00.8*
MgCl ₂ /NAA50/100	07.1±03.8NS	09.3±00.4*	04.3±00.0*	02.9±01.1*
MgCl ₂ /NAA75/100	05.3±01.5*	11.5±02.2*	03.7±01.6*	04.3±00.4*
MgCl ₂ /NAA100/100	04.5±01.3*	10.10±1.6*	02.7±00.3*	03.3±01.5*
MgCl ₂ /IAA/NAA 25/50/50	07.7±06.9NS	13.00±00.7*	03.3±01.3*	02.5±00.4*
MgCl ₂ /IAA/NAA 50/50/50	07.4±02.5*	13.0±00.7NS	04.6±00.3*	02.8±00.9*
MgCl ₂ /IAA/NAA 75/50/50	07.0±02.9NS	13.5±03.5NS	02.3±00.2*	04.2±00.2*
MgCl ₂ /IAA/NAA 100/50/50	05.9±03.0*	16.0±00.0NS	02.7±00.2*	03.4±01.4*
MgCl ₂ /IAA/NAA 25/100/100	03.8±02.0*	16.8±03.9NS	03.1±10.00*	02.4±00.5*
MgCl ₂ /IAA/NAA 50/100/100	07.0±03.8NS	09.3±01.1NS	04.4±00.5*	04.2±00.2*
MgCl ₂ /IAA/NAA 75/100/100	04.0±00.2*	13.3±05.3NS	03.6±01.5*	03.1±01.6*
MgCl ₂ /IAA/NAA 100/100/100	05.8±02.2*	10.2±02.8NS	02.7±00.3*	03.3±10.0*

Keywords: * stands for significant ($p < 0.05$), NS (non-significant), RL (Root length), ZM (*Z. mays*), and AE (*A. esculentus*), L (lab analysis) and F for field analysis.

Growth of Root

MgCl₂ stress and exogenous auxins (IAA and NAA) had a significant effect on Root Length (RL) in the presence of field (F) and laboratory (L) conditions (Table 2 above). It produced effects in RL alone, but when used together with the plant development regulators, root growth was altered significantly, suggesting that the salt-induced stress effects were alleviated. The control plants in field conditions were regarded to have a relatively lower root length of (5.2 -3.6), whereas low stress of MgCl₂ (25 ppm) slightly increased RL (7.7 ± 4.1). Nevertheless, a further addition of MgCl₂ higher than 50 ppm concentration did not yield a consistent enhancement and varied responses. Some of the treatments did

not vanish into insignificance and showed sensitivity of root growth with increasing salt level (Several treatments were statistically significant). The use of MgCl₂ and IAA significantly enhanced root length in the field, with the highest values of RL of 9.6 5.2 and 9.6 10.8 at MgCl₂/IAA 25/ 50 and 100/ 50, respectively, and non-significant (NS) differences with the control. Equally, MgCl₂/IAA 75/100 yielded greater RL (9.3 ± 94.0), which implies that medium-to-high levels of auxin concentrations were productive in fostering root growth in saline stress.

Conversely, the NAA treatments that involved MgCl₂ were a mixed response. There was moderate improvement of the RL at 75/50 (MgCl₂/NAA) (8.4 ± 3.5) and 25/50 (7.3 ± 4.7) and no additional improvement was attained in higher concentrations indicating a narrow concentration range in NAA-mediated root growth under MgCl₂ stress. The addition of MgCl₂ to IAA and NAA also had an additional effect on RL. MgCl₂ /IAA/NAA 25/50/50 and 50/50/50 had similar or higher values of RL than the control, which revealed the synergistic effect of auxins at balanced concentrations. Nonetheless, the combined doses (e.g. 100/100/100) did not produce a significant effect on RL, which is associated with the significance of hormonal equilibrium.

Root length became higher than field observations under laboratory conditions (RA/M/F), which pointed to less variability in the environment. The maximum RL was observed in MgCl₂/ IAA 100 / 50 (19.8 ± 11.7) and MgCl₂ / IAA 100 /100 (21.5 ± 5.0) which had significant or non-significant responses compared to control, respectively, indicating a strong stimulatory effect of IAA on root elongation under controlled conditions. The RL was also significantly enhanced by MgCl₂ NA treatment, especially at 50/50, 75/50 and 100/50 which validates the efficacy of NAA in the laboratories. Root length values in the treatments of laboratory AE and M (RL/AE/L and RL/M/L) were comparatively lower and steadier between treatments. The majority of MgCl₂-only treatments led to the attainment of significantly low RL in comparison with control, whereas the additions of IAA and/or NAA led to a consistent increase in root length. It is worthy to note that, MgCl₂/IAA 75/50, MgCl₂/NAA 75/100 and MgCl₂/IAA/NAA 75/100/100 showed a relatively higher RL value, which validated the use of auxins in protecting the root growth inhibition caused by MgCl₂. Maize exhibits a positive response to both forms of auxins, whereas lady finger responds exclusively to IAA. Salt stress inhibits primary root elongation by diminishing root meristem activity (West et al., 2004). Elevated salinity affects plant growth and development by reducing water potential, altering nutrient uptake, and facilitating the accumulation of detrimental ions (Zhang & Shi, 2013).

Aux/IAA proteins are transient and localized in the nucleus, playing a crucial role in regulating root development and initiating auxin signaling.

Leaf Number and Area

The application of MgCl₂ in conjunction with IAA yielded variable outcomes. The highest leaf number (LN) in LN/AE/F was observed in MgCl₂/ IAA 25/50 (12.00 ± 9.6) and 50 /100 (11.33 ± 8.8), showing no significant differences from the control. This indicates that IAA partially alleviates the effects of MgCl₂ stress. In LN/M/F, MgCl₂/IAA (11.50 ± 2.2) and 50/100 (10.00 ± 7.1) resulted in comparatively improved LN, indicating the beneficial impact of elevated IAA doses on leaf development in saline conditions (Table 3).

Table 3: Auxin (IAA and NAA) and MgCl₂ effect on leaf numbering of selected crops.

Treatments	Field Data	
	LN/AE/F	LN/MZ/F
Control	06.33±02.1NS	06.50±05.00*
25-ppm	06.66±05.1*	04.50±00.8NS
50-ppm	06.66±03.1*	06.00±01.5*
75-ppm	08.33±03.22NS	11.00±08.5NS
100-ppm	09.33±05.6*	07.00±01.5*
MgCl ₂ /IAA25/50	12.00±09.6NS	05.00±01.4*
MgCl ₂ /IAA50/50	05.00±02.7*	07.50±00.8*
MgCl ₂ /IAA75/50	04.66±00.6*	05.50±02.2*
MgCl ₂ /IAA100/50	08.00±02.7*	07.50±03.6*
MgCl ₂ /IAA25/100	04.00±03.0*	06.0±01.5*
MgCl ₂ /IAA50/100	11.33±08.8NS	10.00±07.1*
MgCl ₂ /IAA75/100	07.33±04.2*	11.50±02.2*
MgCl ₂ /IAA100/100	07.33±03.1*	08.00±01.5*
MgCl ₂ /NAA25/50	05.00±01.8*	06.00±01.5*
MgCl ₂ /NAA50/50	10.66±02.1NS	09.50±00.8*
MgCl ₂ /NAA75/50	06.66±01.6NS	07.00± 02.9NS
MgCl ₂ /NAA100/50	04.66±01.6*	05.50±02.2*
MgCl ₂ /NAA25/100	08.66±05.1*	06.50±02.2*
MgCl ₂ /NAA50/100	09.00±03.7*	06.00±01.5*
MgCl ₂ /NAA75/100	07.33±01.6*	07.00±01.5*
MgCl ₂ /NAA100/100	06.66±03.1*	05.00±00.0*
MgCl ₂ /IAA/NAA25/50/50	06.00±02.0*	05.50±00.8*
MgCl ₂ /IAA/NAA50/50/50	08.66±00.6*	06.50±00.8*
MgCl ₂ /IAA/NAA75/50/50	08.00±02.7*	06.50±00.8*
MgCl ₂ /IAA/NAA100/50/50	04.66±00.6*	07.00±00.1*
MgCl ₂ /IAA/NAA25/100/100	06.00±04.6*	06.00±00.1*
MgCl ₂ /IAA/NAA50/100/100	06.66±03.5*	06.00±02.9NS
MgCl ₂ /IAA/NAA75/100/100	06.33±02.1NS	05.00±01.5NS
MgCl ₂ /IAA/NAA100/100/100	06.00± 03.7NS	08.00±00.2NS

Keywords: * stands for significant ($p < 0.05$), NS (non-significant), LN (Leaf number), ZM (*Z. mays*), and AE (*A. esculentus*), and F for field analysis.

LN was found to be influenced by the application of MgCl₂ in conjunction with NAA. A moderate increase in leaf number was observed

at MgCl₂/NAA ratios of 50/50 (10.66 ± 2.1) and 25/100 (8.66 ± 5.1). Consequently, higher MgCl₂ levels appeared to decrease leaf number in LN/AE/F, suggesting a concentration effect. No significant difference in LN was observed between the NAA treatments at LN/M/F, with the highest LN recorded in the MgCl₂/NAA 50/50 treatment (9.50 ± 0.8). MgCl₂ combined with IAA and NAA exhibited relatively stable LN values under both field conditions. In LN/AE/F, leaf development was regulated by a hormonal balance in most combined treatments, maintaining LN close to the control level.

Table 4: Auxin (IAA and NAA) and MgCl₂ effect on leaf area of selected crops.

Treatments	Field Data	
	LA/EA/F	LA/ZM/F
Control	$02.34 \pm 00.1^*$	$01.6 \pm 01.91\text{NS}$
25 -ppm	$03.65 \pm 01.2^*$	$01.4 \pm 00.7\text{NS}$
50 -ppm	$02.65 \pm 03.1^*$	$00.9 \pm 00.5\text{NS}$
75 -ppm	$02.32 \pm 03.2^*$	$01.0 \pm 00.5\text{NS}$
100 -ppm	$04.32 \pm 01.6^*$	$01.9 \pm 00.3\text{NS}$
MgCl ₂ /IAA 25/50	$03.19 \pm 00.5\text{Ns}$	$01.0 \pm 00.2\text{NS}$
MgCl ₂ /IAA 50/50	$02.67 \pm 00.7\text{Ns}$	$01.5 \pm 00.3\text{Ns}$
MgCl ₂ /IAA 75/50	$02.13 \pm 00.4\text{Ns}$	$00.5 \pm 00.8^*$
MgCl ₂ /IAA 100/50	$02.92 \pm 00.7^*$	$10.00 \pm 00.5^*$
MgCl ₂ /IAA 25/100	$02.82 \pm 00.8^*$	$01.8 \pm 01.2\text{Ns}$
MgCl ₂ /IAA 50/100	$03.53 \pm 00.8\text{Ns}$	$01.2 \pm 00.2\text{Ns}$
MgCl ₂ /IAA 75/100	$03.41 \pm 00.6\text{Ns}$	$01.4 \pm 00.2\text{Ns}$
MgCl ₂ /IAA 100/100	$02.64 \pm 00.7^*$	$01.2 \pm 00.4\text{Ns}$
MgCl ₂ /NAA 25/50	$03.24 \pm 00.6\text{Ns}$	$01.1 \pm 00.2\text{Ns}$
MgCl ₂ /NAA 50/50	$03.39 \pm 00.2\text{Ns}$	$01.3 \pm 00.2\text{Ns}$
MgCl ₂ /NAA 75/50	$02.69 \pm 00.5^*$	$00.8 \pm 00.1\text{Ns}$
MgCl ₂ /NAA 100/50	$03.06 \pm 00.4\text{Ns}$	$00.8 \pm 00.6\text{Ns}$
MgCl ₂ /NAA 25/100	$02.85 \pm 00.7^*$	$01.1 \pm 00.2^*$
MgCl ₂ /NAA 50/100	$04.19 \pm 00.4\text{Ns}$	$01.2 \pm 00.3\text{Ns}$
MgCl ₂ /NAA 75/100	$03.69 \pm 00.7\text{Ns}$	$01.5 \pm 00.3\text{Ns}$
MgCl ₂ /NAA 100/100	$03.13 \pm 00.4\text{Ns}$	$00.8 \pm 00.8^*$
MgCl ₂ /IAA/NAA 25/50/50	$02.92 \pm 00.6^*$	$10.0 \pm 00.5^*$
MgCl ₂ /IAA/NAA 50/50/50	$02.82 \pm 00.8^*$	$01.8 \pm 01.2\text{Ns}$
MgCl ₂ /IAA/NAA 75/50/50	$03.52 \pm 00.8\text{Ns}$	$01.2 \pm 00.2\text{Ns}$
MgCl ₂ /IAA/NAA 100/50/50	$03.42 \pm 00.6\text{Ns}$	$01.2 \pm 00.3\text{Ns}$
MgCl ₂ /IAA/NAA 25/100/100	$02.64 \pm 00.7^*$	$01.3 \pm 00.30\text{Ns}$
MgCl ₂ /IAA/NAA 50/100/100	$02.67 \pm 03.6^*$	$01.1 \pm 00.2\text{Ns}$
MgCl ₂ /IAA/NAA 75/100/100	$03.34 \pm 02.1\text{NS}$	$01.3 \pm 00.2\text{Ns}$
MgCl ₂ /IAA/NAA 100/100/100	$02.04 \pm 00.7^*$	$01.3 \pm 00.0\text{NS}$

Keywords: * stands for significant ($p < 0.05$), NS (non-significant), LA (Leaf area), ZM (*Z. mays*), and AE (*A. esculentus*), and F for field analysis.

The application of treatments such as MgCl₂/IAA/NAA at concentrations of 100/100/100 (8.00 ± 0.2) in LN/M/F demonstrated improved LN compared to the control, suggesting a potential synergistic effect from the dual application of auxins under MgCl₂ stress conditions. Field condition Leaf area (LA) was found to react differently to MgCl₂ stress and the application of auxin (Table 4 above). MgCl₂ used alone in

LA/EA/F found that there was a concentration dependent variation in leaf area where higher concentrations were observed at 25 and 100 ppm as compared to the control and that intermediate concentrations were lower or equivalent. The use of IAA did not change or significantly improve LA, but showed a relatively higher value at 50/100 and 75/100, and no significant and striking change in LA/M/F was observed except at MgCl₂/IAA 100/50, which showed a strong stimulatory effect. NAA usage achieved a moderate significantly improved LA/EA/F (to 50/100, 75/100) although there was minimal effect on LA/M/F, with most treatments being insignificant. The joint use of IAA and NAA strongly stabilized LA/EA/F to control levels with a slight increase of 75/50/50, and a large increase of LA/M/F was once more observed at 25/50/50 indicating that there may be a synergistic effect of auxin at certain concentrations. Development is believed to be affected by various physiological systems, and the decline in growth following salt therapy has been thoroughly examined in numerous studies (Munns, 2002). The crop's size, leaves size, root counts, leaf width, number of spikes, and quantity of cones were all impacted by hormones (Karimi & Maleki, 2015).

Photosynthetic Pigments

Chl. a, b, and total chl. concentrations are vital pigments used for photosynthesis in plants that are critical to the production of glucose and other substances. The maximum measured Chlo. "a" concentration was 00.757 ± 00.00 under the S/IAA 100/100 treatment for *A. esculentus*, whereas maize revealed a value of 02.06 ± 00.43 under the S/NAA 50/100 treatment. The greatest chlorophyll "b" concentration was 05.31 ± 01.11 for *A. esculentus*, attained with a 25 ppm magnesium chloride treatment, while maize reached its peak with the S/IAA 100/100 treatment at 01.57 ± 00.41 (Figure 1).

Research indicates that the regulation of photosynthetic pigments is influenced by chemicals and hormones. Chlo. 'a' and 'b', the primary energy-conversion pigments, exhibit variation in *A. esculentus* and maize, highlighting species-specific strategies for light absorption. The S/NAA 50/100 treatment in maize yielded the highest Chlo. "a" concentration (Figure 2), suggesting that naphthalene acetic acid effectively activates primary reaction centers. Maize, as a C₄ plant with a structured photosynthetic system, likely benefited from NAA by enhancing thylakoid membrane integrity or postponing leaf senescence, thereby elevating chlorophyll 'a' concentration per unit area. Synthetic maize IAA enhance the essential components of light-dependent processes. *A. esculentus* demonstrated peak chlorophyll 'a' concentration under a specific hormonal treatment (S/IAA 100/100), suggesting a greater reliance on Indole-3-

acetic acid for pigment synthesis in *A. esculentus*. Conversely, *A. esculentus* Chlo. 'b' levels exhibited the most significant physiological alteration. Following treatment with 25ppm $MgCl_2$, the quantity increased to 5.37 ± 1.11 , suggesting the presence of a direct metabolic bottleneck. The two species employ distinct strategies regarding pigment asset. Maize exhibits higher levels of chlorophyll "a" for the primary electron transport in photosystems. Administration of mineral supplements to lady finger resulted in unexpected alterations in the levels of its accessory pigment, chlorophyll 'b'. The variation in the development of C3 and C4 mechanisms may account for the observed discrepancy. *A. esculentus* exhibits a preference for larger antennae to enhance photon absorption, while maize favors elevated "a" concentration for optimal carbon fixation. Similarly, rhizospheric fungi also mitigates the adverse effect of abiotic stress (Ali et al., 2025), as comparable to IAA NAA.

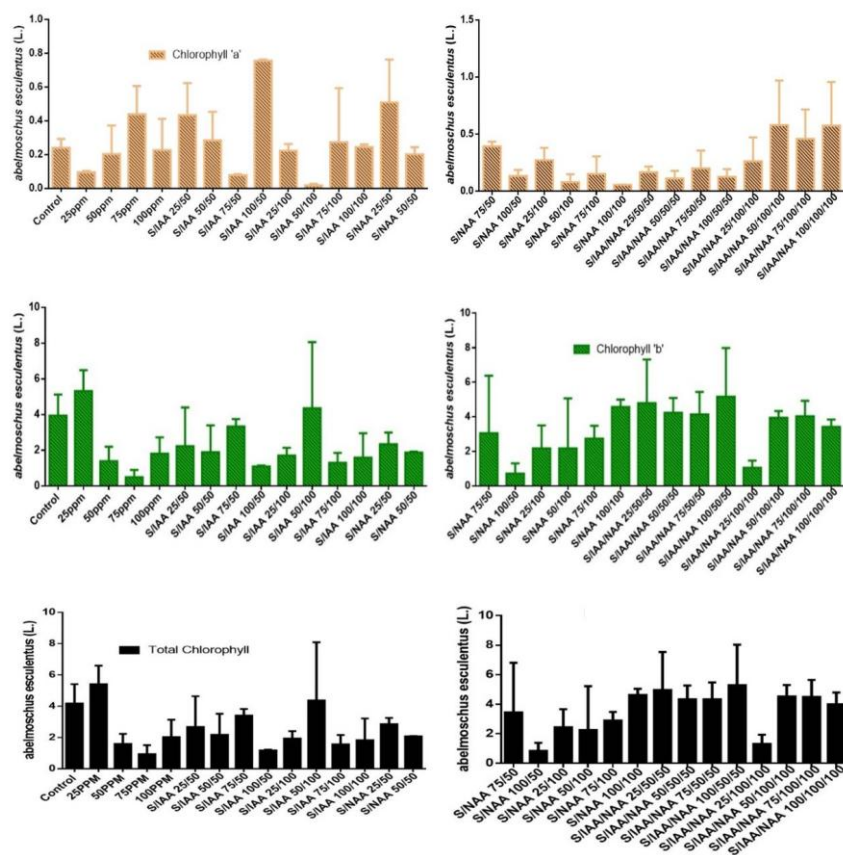


Figure 1: Effects of hormonal (IAA and NAA) and $MgCl_2$ on photosynthetic pigments of *A. esculentus*.

Additionally, increased levels of IAA in lettuce leaf discs led to accelerated chlorophyll degradation. Carotenoids, known for their role as scavengers of oxygen radicals, are prevalent in plant tissues (Lavelli et al., 2009). Prior studies have shown a decline in total chlorophyll levels, attributed to either its degradation or a reduction in its synthesis rate (Doganlar et al., 2010).

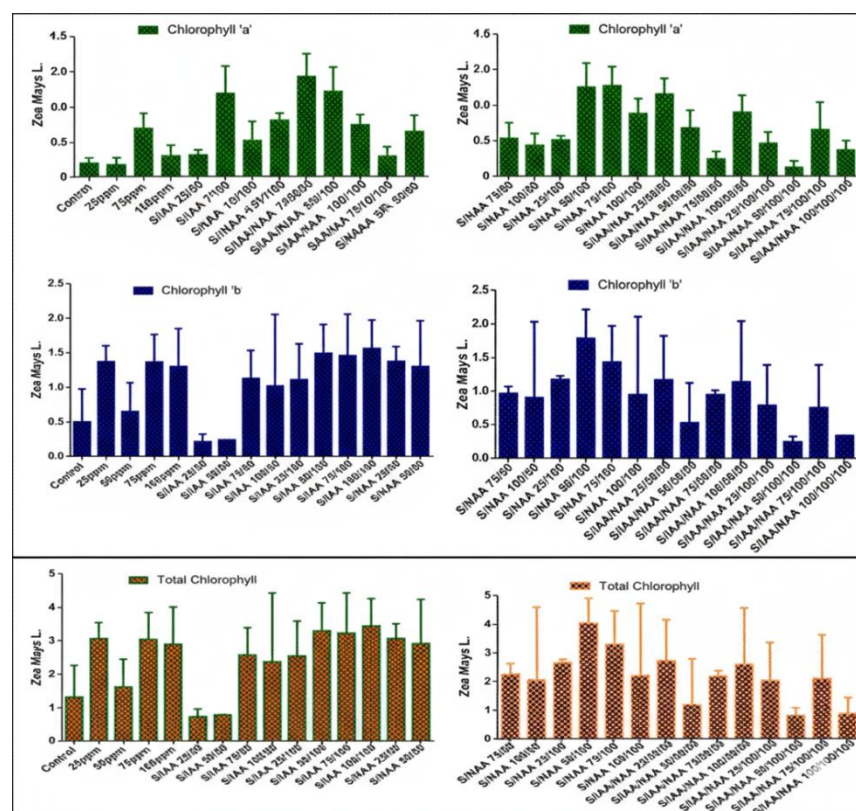


Figure 2: Effect of $MgCl_2$ and hormonal (IAA and NAA) on photosynthetic contents of *Z. mays*.

Carotenoids Contents

Increased concentrations of $MgCl_2$ led to a reduction in carotenoid levels; conversely, the introduction of hormones resulted in elevated carotenoid levels. The highest carotenoid concentrations in *A. esculentus* were observed at S/IAA 50/50, with a maximum value of 02.46 ± 00.56 and a minimum of 00.25 ± 00.29 at S/NAA 25/100. The maximum concentration in maize was 01.05 ± 01.27 at S/NAA 100/100, while the minimum was 00.42 ± 00.01 at S/IAA 50/50 (Figure 3). Hormonal imbalance, osmotic stress, and additional factors are believed to

significantly influence salinity-induced variations in plant productivity (Albacete et al., 2008).

Several studies indicate that the growth promoters increase the concentrations of carotenoids and chlorophyll (Figure 4). For example, Iqbal et al. (2006) demonstrated that phytohormone treatments frequently increased pigment levels in maize, wheat, cotton, broad bean, and parsley, with notable effects observed in cotton and parsley. This may arise from phytohormones promoting protochlorophyll (ide) synthesis or suppressing pigment degradation. Phytohormones play a crucial role in influencing the impact of salinity and heavy metals on photosynthetic characteristics and membrane integrity (Pazuki et al., 2013).

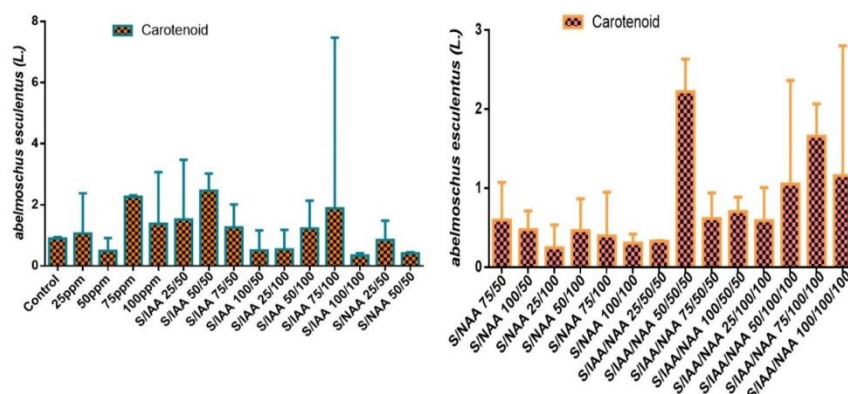


Figure 3: Effect of hormonal (IAA and NAA) and MgCl₂ on carotenoid levels A. esculentus.

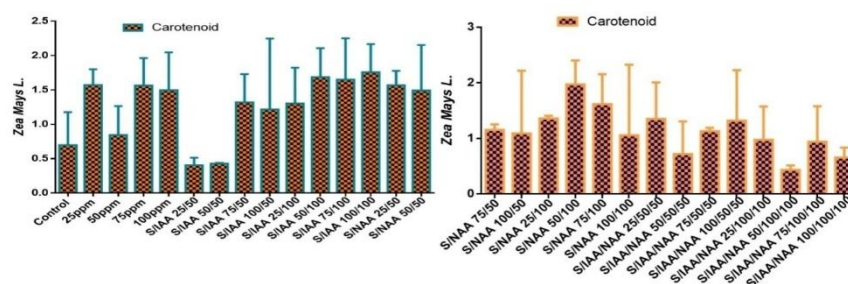


Figure 4: MgCl₂ and hormonal effects on carotenoid contents of Z. mays.

Carotenoids possess the potential to detoxify plants from the effects of reactive oxygen species due to their antioxidant properties (Verma & Mishra, 2005). Salinity resulted in a decrease in carotenoid levels in both genotypes. Carotenoids are known to quench oxygen and triplet chlorophyll while absorbing light energy for photosynthesis. The

durability of chloroplast membranes may be influenced by the splitting into light-harvesting complexes (LHCs) and the lipid stage of thylakoid membranes. The membranes exhibit reduced fluidity and decreased susceptibility to lipid oxidation (Demmig-Adams & Adams III, 2002). They eliminate excess energy through the utilization of the xanthophyll cycle. The reduction in carotenoid levels indicates that plants do not predominantly rely on carotenoid protection to manage salt stress.

Sugar and Protein Contents

Protein and carbohydrates dropped at both low and high magnesium chloride levels. Field testing for both crops demonstrated that a combination of foliar sprays, including auxin type IAA and NAA, significantly ($p \leq 0.05$) mitigated the toxicity of magnesium chloride salinity (Figures 5 and 6). The control group exhibited the highest sugar content, while the S/NAA 75/50 group demonstrated the greatest protein content. The *A. esculentus* plant in the S/IAA/NAA 50/100/100 group exhibited the lowest levels of sugar and protein. Figures 5 and 6 indicate that maize exhibited maximum sugar concentrations at S/NAA 50/100, while protein levels were highest in the control condition and lowest at S/IAA/NAA 50/100/100 with regard to sugars and proteins.

Salinity typically induces the accumulation of osmolytes such as proline, which is decreasing sugars, and polysaccharides (Fedina et al., 2002). In saline conditions, plants produce unique proteins that enhance their development and growth. The elevated protein levels in salt and heavy metals tolerant cultivars likely result from their enhanced osmotic regulation processes (Ahmad et al., 2024), which mitigate the cytotoxic effects of sodium relative to susceptible cultivars, thereby averting protein degradation during salt stress. Under salt stress, an accumulation of soluble amino acids was considerably improved in the leaves of the examined maize, with the most pronounced growth (113%) observed in a salt-tolerant variety. All tested genotypes, with the exclusion of the saline resistance maize genotype BR5033, exhibited soluble carbohydrate levels in leaves and roots that were similar to or lower than those observed in saline environments (Azevedo Neto & Tabosa, 2000). In contrast, Hussein et al. (2007) found that maize exhibited increased proline levels and decreased concentrations of arginine, lysine, serine, and glutamic acid under salt stress. The concentrations of glycine exhibited no variation. Many PGRs and antioxidants are often administered topically to lessen the negative effects of salt stress on plants. Exogenous plant hormones and osmoprotectants that lessen the negative effects of salt stress in maize include IAA, GA₃, cytokinins, ABA, polyamines, and salicylic acid. They promote osmotic adjustment to maintain turgor, enhance nutrient

absorption, increase antioxidant levels, and detoxify reactive oxygen species, thus ensuring membrane and enzyme stability (Kaya et al., 2010).

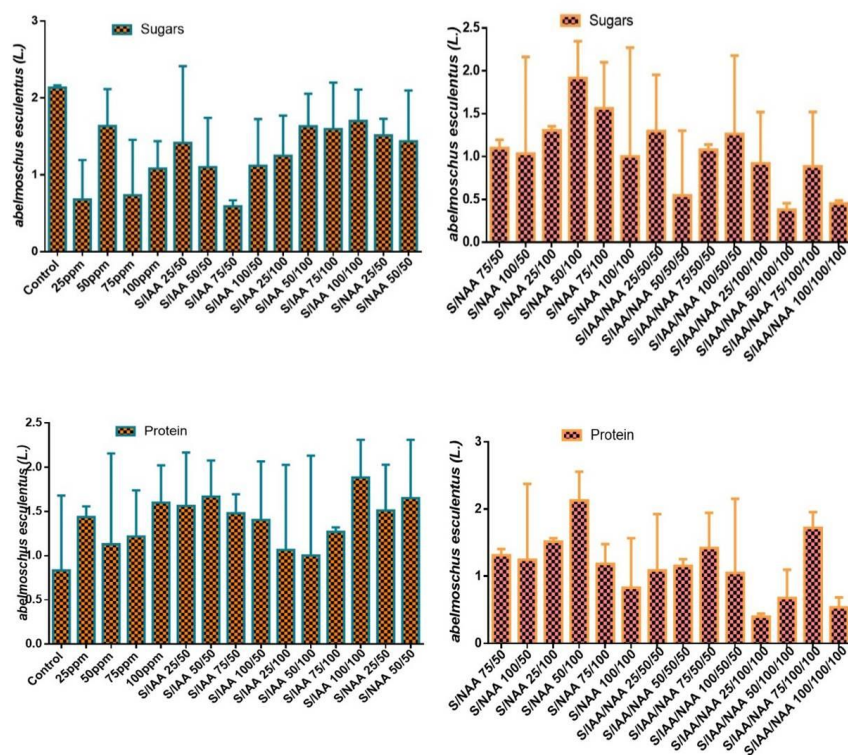


Figure 5: MgCl₂ and hormonal effects on total sugar and protein concentrations of *A. esculentus*.

Proline Contents

Both low and high concentrations of magnesium chloride elevated proline levels in both crops; however, the combination of IAA and NAA reduced proline contents, while no effect was observed from the hormones at high MgCl₂ concentration (Figures 7 and 8). The highest proline content of 01.95 ± 00.23 was observed in treatment S/NAA 75/50, whereas the lowest value was 00.310 ± 00.11 in S/IAA 25/50. Maize demonstrated the greatest increase in proline levels at S/NAA 25/50 and S/IAA/NAA 25/50/50, whereas proline le Salt stress is a critical global issue that affects various molecular, biochemical, and physiological processes in plants, leading to a marked decline in agricultural productivity.

The application of exogenous proline enhanced photosynthetic pigments, primarily increasing CO₂ diffusion and photosynthesis rates

(Ali et al., 2007). The introduction of proline enhanced water relations in saline conditions, likely due to improved water absorption. Understanding the role of proline in plant water status is essential for examining its beneficial effects in saline environments, as the osmotic action of salts initially limits plant development following salt accumulation (Munns & Tester, 2008). The ability of a plant to retain water under saline conditions may enhance its salt tolerance by reducing excessive ion concentration through the dilution effect (Romero-Aranda et al., 2006) decreased at the S/IAA/NAA 50/100/100 concentration.

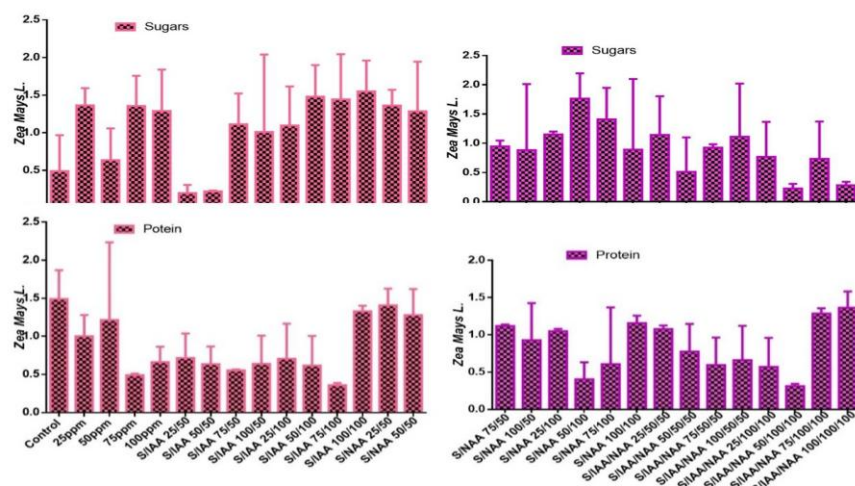


Figure 6: Effect of $MgCl_2$ and plant hormones on total sugar and protein levels of *Z. mays*.

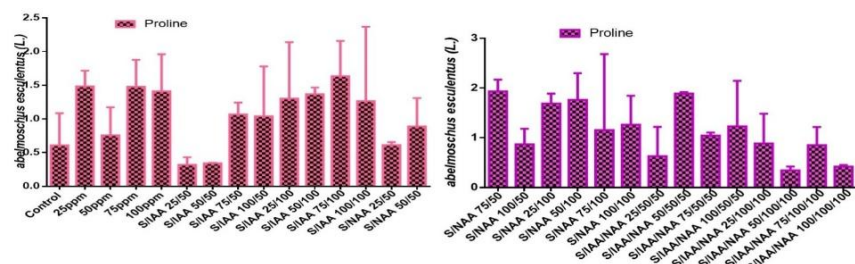


Figure 7: Hormonal and $MgCl_2$ effects on Proline contents of *A. esculentus*.

In our trials, foliar Proline demonstrated the greatest capacity for water retention under saline conditions. The *P. maritime* flora demonstrated similar results (Khedr et al., 2003). The application of Proline as a foliar spray enhanced the salinity tolerance of rice, attributed

in part to an increase in relative water content. Cherry tomatoes exhibit elevated phenol levels in response to salt stress (Hassan et al., 2015). Phenol concentration increased in a number of rice genotypes subjected to different degrees of salt stress (Hussain et al., 2013). Similarly, Gómez-Caravaca et al. (2011) reported that the phenolic content of *Chenopodium quinoa* increased in response to salt stress, aligning with our observations.

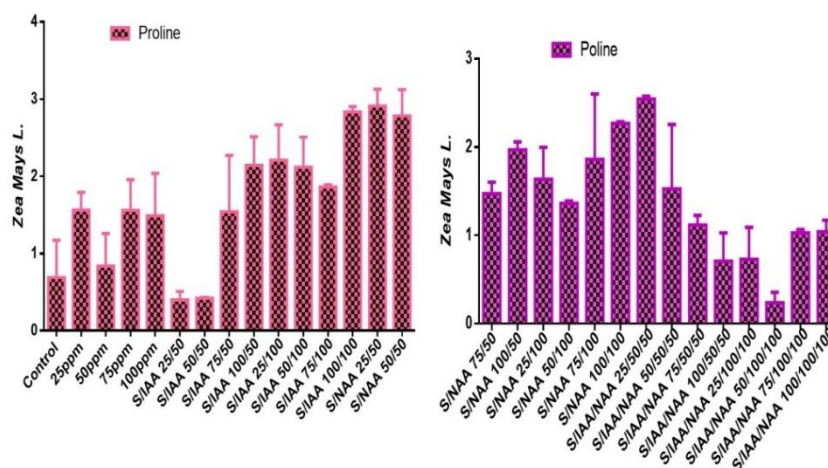


Figure 8: $MgCl_2$ and hormonal effects on Proline contents of *Z. mays*.

Conclusion

The present research demonstrates that $MgCl_2$ serves as a harmful abiotic stressor, adversely affecting the morphological parameters of the selected species, *Z. mays* and *A. esculentus*. Foliar applications of IAA and NAA significantly mitigated the adverse impacts on shoot length, root quantity, leaves numbers and area. The biochemical responses of *Z. mays* and *A. esculentus* to $MgCl_2$ stress led to reductions in the levels of chlorophyll “a” “b,” total chlorophyll, carotenoid, protein, and sugar compared to control, IAA, and NAA treatments. Proline concentrations were highest following $MgCl_2$ treatment in both *A. esculentus* and *Z. mays* species. Trace quantities of heavy metals, while essential to the ecosystem, require specific attention. Therefore, it is crucial to develop more sensitive detection techniques and integrate diverse methodologies.

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