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APPLICATION OF L-METHIONINE CONFERS SALINITY TOLERANCE IN MAIZE THROUGH MODULATION OF METABOLITES AND ANTIOXIDATIVE DEFENSE

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Abstract

The world's arid and semi-arid regions are frequently subjected to significant abiotic stresses, particularly salt stress, which threaten crop yield and production. In light of this, the current study aimed to assess the mitigating effects of salinity stress through different doses of L-methionine (0, 50, and 100 mg L-1), with the goal of promoting sustainable production of Dekalb maize hybrids (Dk-6317 and Dk-9108). The experiment was carried out during spring 2020 in a pot setup, where salt stress was induced by applying two levels of sodium chloride (0 and 120 mM). Exogenous L-methionine at 50 mg L-1 and 100 mg L-1 significantly improved maize growth, yield, photosynthetic pigments, and antioxidant defence characteristics. Salt stress significantly ($p \leq 0.05$) reduced plant height (17.89% and 21.40%), shoot fresh weight (17.13% and 17.42%), shoot dry weight (18.03% and 29.98%), root fresh weight (21.56% and 31.02%), root dry weight (33.37% and 30.51%), and leaf area (16.28% and 21.18%) of Dk-6317 and DK-9108, respectively, when compared to L-methionine treatments. Furthermore, correlation analysis revealed a significant ($p \leq 0.05$) correlation between various studied traits, including enzymatic and stress-related antioxidants, as well as yield attributes. As a result, antioxidant enzyme activity such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) promotes plant growth by positively correlating with photosynthetic pigments, resulting in higher yield attributes. It appears that L-methionine improves maize crop development when subjected to salt exposure, a novel aspect of L-methionine's beneficial role. A beneficial interaction between L-methionine and photosynthetic pigments suggests its potential to mitigate salt toxicity effects and enhance sustainable maize production.

Index terms__ L-Methionine, Dekalb Maize Hybrids, Bio-Stimulant, Amino Acid, Foliar Application, S-Adenosylmethionine (SAM).

INTRODUCTION

Soil salinity is among the major global concerns that affect crop production, threatening crop yield and growth. This would be a detrimental and widely dispersed factor for crop growth and development worldwide (Machado and Serralheiro, 2017). Each year, 45 million hectares of agricultural land are damaged, and 1.5 million hectares are lost to crop production due to salinity (Pereira et al. 2020). Despite moderate salinity levels, aggravated soil salinity has a significant negative impact on global maize production. The main reasons for its spread are low precipitation, excessive surface evaporation, poor drainage, repeated applications of fertilizers, various amendments, and low-quality irrigation practices (AbdElgawad et al. 2016). Salt-affected soils with high NaCl concentrations reduce plant growth by creating osmotic stress, nutritional instability, oxidative stress and metabolic disturbances, affecting plant biomass and yield production (Deinlein et al. 2014; Gharib et al. 2016). Higher soil salinity levels trigger osmotic stress, limiting nutrient absorption and reducing water absorption, which primes for physiological desiccation (Hafez et al. 2015; Hafez and Omara 2021). Now growing crops under saline conditions is a crucial element in fulfilling global food demand (Lynch 2019; Pereira et al. 2020; Ding et al. 2021).

Maize (*Zea mays* L.) is one of the major humanities crops after rice and wheat. In the past, this C4 crop prevented many food crises (Xie et al. 2018). Even at moderate salinity levels, soil salinization greatly impacts global maize production (Ali *et al.* 2017). In order to induce salinity tolerance in cereal grains including maize crops, soil amendments and foliar applications of organic/inorganic compounds will be advised (Kamran et al. 2019). Plant breeders develop salt-tolerant crop varieties. Crops are modified using genetic engineering techniques by introducing specific genes that improve salt tolerance (Gupta and Huang, 2014). Agronomic practices and soil amendments can reduce salt stress (Munns et al. 2006). Salt tolerance is a complex trait governed by several genes. It is difficult and time-consuming to identify and transfer all genes into commercial crop varieties. It is challenging to find suitable genetic resources with high salt tolerance. Crop wild relatives have higher salt tolerance, but transferring their traits to commercial cultivars while retaining desirable agronomic traits is quite difficult. Which would lead to the demise of salt tolerance strategies due to cost and time-consuming implementation. Improving salt tolerance in crops may jeopardize other key agronomic traits like yield potential or disease resistance (Munns and Gilliham 2015).

Bio-stimulants based on amino acids increase plant yields and production (Amin et al. 2011, Hassan et al. 2020). They act as precursors to natural growth regulators, which improve photosynthesis and plant development (Amin et al. 2011). L-methionine is a sulfur-retaining amino acid. It indirectly affects several cellular functions, such as being the precursor of S-adenosyl-methionine (SAM), which acts as a primary biotic methyl group donor. As an initiator of mRNA translation, it plays a prominent role in protein synthesis. This is necessary for a range of metabolic processes (Galili et al. 2005; Shekari and Javanmardi 2017; Trovato et al. 2021). Moreover, amino acid-based foliar treatments maintain a desirable pH value in cells because they retain both acidic and basic components (Aslam et al. 2001). L-methionine, which is an essential amino acid used for human and ruminant nutrition and is normally found in plants, is reduced in dietary value for humans and ruminants due to various environmental stresses (Galili et al. 2005). For this reason, scientists are focusing on the exogenous application of organic compounds, such as amino acids, to increase crops' nutritional value (Amira et al. 2005). Moreover, to improve methionine levels in crops, several breeding techniques, mutagenesis, and genetic engineering methods have been used (Wang et al. 2017).

Reportedly, a foliar spray of amino acids accelerates various physiological, chemical, and developmental responses in crops under stress situations. Exogenous L-methionine has been shown in recent studies to effectively alleviate stresses in a variety of crop species, including tomatoes, maize, bitter gourd, okra, and wheat. Most of the time, L-methionine regulates different defence mechanisms, particularly those connected to preserving ionic status, enhancement of chlorophyll contents and detoxification of free radicals (Alfosea et al. 2020; Shahid et al. 2021; Akram et al. 2020; Zulqadar et al. 2015; Ahmad et al. 2015). In plants, methionine acts as a precursor for the biosynthesis of a variety of stress-related compounds such as the synthesis of spermidine, putrescine, and spermine (polyamines), which are key to stress tolerance (Alcázar et al. 2010). It contributes to the synthesis of ethylene, a hormone that regulates plant growth and stress responses. Ethylene aids plants in dealing with stressors such as drought, salinity, and pathogen attack (Zulqadar et al. 2015). Methionine participates in the synthesis of glutathione, a crucial antioxidant in plants that aids in the scavenging of reactive oxygen species (ROS), which are produced during stress and can cause cellular damage. Methionine helps to reduce oxidative stress and improve plant tolerance to various stresses by maintaining an optimal glutathione level (Hossain et al. 2012). Moreover, as a sulphur-retaining amino acid it is essential for sulphur metabolism in plants. Sulphur is required for the synthesis of several significant molecules, including cysteine and phytochelatins. These molecules are involved in heavy metal detoxification, oxidative stress defence, and protection against environmental toxins (Chorianopoulou and Bouranis, 2022). Methionine is involved in stress signal transduction pathways in plants. It can function as a signaling molecule, activating or regulating stress-responsive genes. Plant stress signaling and defence mechanisms are aided by methionine-derived molecules, such as polyamines and jasmonic acid (Hendrix et al. 2017).

Despite L-methionine's multiple roles, salt stress management processes remain elusive. L-methionine has also rarely been studied under saline conditions for its contribution to maize yield improvement, so further research is necessary to encourage its wider use in crop management. Like other cereal crops, maize is threatened by rising soil salinity, which poses serious challenges to its cultivation. Therefore, it is crucial for food security to develop technologies that address salt stress and maize productivity decline. Thus, this investigation explored the properties of these organic bio-stimulants in a salt stress environment, either alone or in combination, and examined the enzymatic antioxidant defense compounds linked to oxidative stress and plant growth attributes. Understanding how these bio-stimulants operate to enhance plant resistance to salt stress will result in novel approaches to harnessing bio-stimulants for global food demand. To address the aforementioned facts, it was hypothesized that: 1) a specific dose of bio-stimulants could improve maize growth and overcome NaCl toxic effects; and 2) applied bio-stimulants would boost maize plants' ability to tolerate salinity levels by altering their physiochemical and nutritional characteristics. A number of growth, photosynthetic efficiency, oxidative stress, antioxidant system, and yield parameters were evaluated with salt stress in maize hybrids treated with or without L-methionine foliar spray. The results of our findings would enable us to conclude that L-methionine plays an essential role in enhancing maize crop growth and productivity in stressful environments.

MATERIALS AND METHODS

Plant Material and Experimental Site

In spring 2020, pot experiments were carried out at the University of Agriculture Faisalabad Pakistan (31.2537 °N and 73.0438 °E). The city of Faisalabad lies in northeast Punjab, on flat, rolling terrain 186 meters (610 feet) above sea level. The experimental period was characterized by mild winters and moderately hot summers, ranging between 9 and 16°C (min) and 29.5 and 41.5°C (max). Monsanto Pakistan provided the seeds of hybrid spring maize Dekalb-6317 and Dekalb-9108, which had a moisture percentage of 11.8 and a germination capacity of 95 percent. This maize hybrid displayed an early maturity of 115 days, making it an excellent choice for mid- to late-season planting with high yield potential.

Treatment Application, Experimental Design and Sampling Time

The experiment was carried out in natural environment conditions with a completely randomized design (CRD) under factorial arrangements with four replications. To conduct the experiment, 12-inch pots were filled with 13.5 kg of thoroughly mixed sandy clay loam soil (sand 33%, silt 33% & clay 33%) having pH of 8.0, EC of 1.34 dsm -1, exchangeable K of 135.5 g kg -1, N of 0.40 g kg-1, P of 8.1 g kg-1, and organic matter of 2.01%. Six seeds of each hybrid Deklab (Dk-6317 and Dk-9108) were grown per pot. Pots were treated with 0 (control) and 120 mM NaCl after the 5th week of germination by applying 40mM in three steps to reach a final concentration of 120 mM, then exposed to 120 mM directly five times. For reduction of salt stress effects, foliar application of L-methionine was performed at seven-day intervals between early mornings with the first and last spray occurring at five and twelve weeks after planting (total 6 sprays). The combination of treatments was control, NaCl alone, and NaCl with L-methionine. The treatments included positive control (PC), L-MET 50mg L-1 (L1), L-MET 100mg L-1 (L2), negative control with NaCl 120 mM (NC), salinity + L-MET 50mg L-1 (S+L1), and salinity+ L-MET 100mg L-1 (S+L2). Sprinkler watering cans of 5L capacity were used every two days to apply fresh water to the plants. Once seedlings were established, only three plants were kept in each pot until maturity.

Meteorological Conditions

Weather data of subject duration of the year 2020 of experiment was collected from Agriculture Meteorology Cell University of Agriculture Faisalabad and presented in Figure 1.

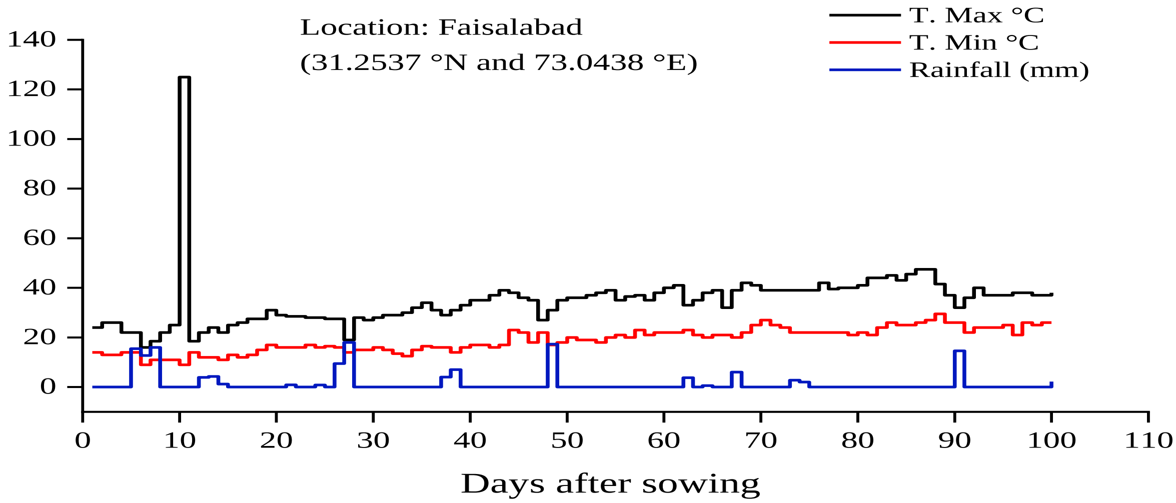


Figure 1: Temperature Variations and Rain Fall Pattern during the Whole Crop Season of spring 2020

Measurement of Plant Growth and Yield

As part of the plant growth measurement process, plant growth related attributes including plant height, shoot and root fresh and dry weights and leaf area were measured on the 85th day of planting (Hunt, 2002). Yield determination attributes such as cob length, cob weight, grains count per cob, and 100 grains weight were recorded after harvesting (115th day of planting).

Analysis of Chlorophyll Contents

For determination of chlorophyll contents leaf samples (0.25 g) from 85th-day-old plants were homogenized using 80% acetone. The centrifugation process was used to separate the insoluble cell debris from the soluble pigment extract. After that the extract's absorbance was measured at specific wavelengths 663, 645, and 480 nm (Arnon 1949).

Calculation of chlorophyll concentration:

$$\mathrm{Chl.}\,a\ (\mathrm{mg/g}) = \frac{12.7(\mathrm{OD}663) - 2.69(\mathrm{OD}645)\ \mathrm{x\ V}}{1000\ \mathrm{x\ W}}$$

$$\mathrm{Chl.}\,b\ (\mathrm{mg/g}) = \frac{22.9(\mathrm{OD}645) - 4.68(\mathrm{OD}663)\ \mathrm{x\ V}}{1000\ \mathrm{x\ W}}$$

$$\mathrm{Total\ Chl.}\ (\mathrm{mg/g}) = \frac{20.2\ (\mathrm{OD}645)\ +\ 8.02\ (\mathrm{OD}663)\ \mathrm{x\ V}}{1000\ \mathrm{x\ W}}$$

Phenolic Determination Essay

To determine the phenolic contents, a leaf sample was ground in 80% acetone. A 100 ml aliquot of the ground mixture was filtered, and the volume was brought to 1 ml with water from the filtered mixture. Afterwards use 0.5 ml of Folin-phenol Ciocalteu with the addition of 2.5 ml of 20% sodium carbonate, and the final volume was adjusted to 10 ml and the absorbance was recorded at 750nm (Julkunen-Tiitto 1985).

MDA Determination Essay

A leaf sample was homogenized with trichloroacetic acid (TCA). The leaf sample was centrifuged at 12,000 RPM for 15 minutes. The supernatant was then removed and 1 ml of thiobutyric acid was added, followed by incubation and cooling. Supernatant absorbance was determined at two wavelengths, 532 nm and 600 nm, using a spectrophotometer (UV-1100). By subtracting the non-specific absorbance from 600 nm, the absorbance value was adjusted (Heath and Packer 1968).

- MDA (mmol/ml) = A532-A600(155000)×106

Glycinebetaine Determination Essay

Leaf samples were extracted using distilled water. The resulting mixture was centrifuged and diluted 1:1 to 2N H2SO4. Afterward, 0.5 ml of the diluted mixture was taken out and treated with a cold IK-I2 (Periodide) solution (0.2 ml) and vortexed and cooled. After that 5 ml of 1-2 dichloroethane was added to the reaction mixture and the solution was vigorously vortexed. An absorbance measurement was made at 365 nm for the resulting solution (Grieve and Grattan 1983).

Proline Determination Essay

Fresh plant material (0.5g) was ground with three percent aqueous sulfosalicylic acid, and then heated for one hour at 100°C after adding acid ninhydrin and glacial acetic acid. An ice bath was used for the reaction, and toluene was used to extract the sample and measured at 520nm absorbance. In order to generate the standard curve, proline concentrations ranging from 10 g/2 ml to 50 g/2 ml were used. Using the appropriate formula, free proline was calculated. (Bates et al. 1973).

$$\text{µmoles proline/g fresh weight} \quad = \frac{\text{µg proline/ml} \times \text{ ml of toluene}}{(115.5\ \text{µg/mole})/\text{g sample}/5}$$

Free Amino Acid Determination Assay

For the amino acid assay, the Hamilton and Van Slyke (1943) technique was employed. 1.0 g leaf sample was extracted in phosphate buffer (pH of 7.2). The resulting mixture was centrifuged at 5000 rpm for 5 minutes, and the supernatant was used to measure the protein content. Next, 1 ml of the mixture was combined with 10% pyridine and 2% ninhydrin. The resulting solution was boiled for 30 minutes and the volume was increased to 50 ml. At 570 nm the extract's optical density was measured. To calculate free amino acid levels, a standard curve was established using lucine and the provided formula.

$$\text{Total amino acid (mg/g fresh weight)} = \frac{\text{Graph reading of sample x volume of sample x dilution factor}}{\text{Weight of the tissue x 1000}}$$

Total Soluble Protein Determination Essay

Protein contents were measured using the Bradford Method (1976). To extract the soluble proteins, 0.5 g of leaf tissue was mixed in 500 mM Tris-HCl buffer (pH 7.5). 100 mg of Comassie Brilliant Blue G-250 plus 50 ml (90%) of ethanol, and 100 ml (85%) of phosphoric acid were combined to formulate a reagent. The mixture was thoroughly combined and filtered. Total soluble protein was measured using this reagent.

Total Soluble Sugar Determination Essay

To determine the total soluble sugar content, Yoshida et al. (1976) method was utilized. Initially, the leaf sample was heated in 5 ml of distilled water, and the volume was adjusted to 50 ml. Following this, 5 ml of anthrone chemical was diluted filtrate. The resulting mixture was boiled at 90°C for 20 minutes and the absorbance was measured at 620 nm.

Hydrogen Peroxide Determination Essay

To determine the amount of hydrogen peroxide in leaf tissue, Velikova et al. (2000) technique was utilized. Initially, leaf samples were extracted in trichloroacetic acid, then centrifugation was done at 10000 rpm for 12 minutes. A solution of potassium phosphate buffer was added to the supernatant, vortexed, and the reading wavelength was measured at 390nm.

Antioxidant Determination Assay

Enzymatic antioxidants were determined using a leaf extract prepared for total soluble proteins. Peroxidase (POD) catalase (CAT) superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities were estimated according to detailed protocols developed by Chance and Maehly (1955), Giannopolitis and Ries (1977) and Nakano and Asada (1981) respectively. The peroxide solution mixture comprised of 100 µl of H_2O_2, 100 µl of guaiacol, and 100 µl of leaf extract. The absorbance changes were measured at 470 nm using a spectrophotometer. For CAT determination, the absorbance of the reaction mixture (0.1 ml of enzyme extract + 1 ml of 5.9 mM H_2O_2 + 1.9 ml of 50 mM phosphate buffer) was measured every 20 seconds at 240 nm. For SOD a reaction solution was prepared, comprising 50 mM NBT, 1.3 mM riboflavin, 13 mM methionine, 75 mM EDTA, 50 mM phosphate buffer (pH 7.8), and 50 mL enzyme extract. The mixture was then exposed to white fluorescent light for 20 minutes in reaction tubes. The reaction mixture absorbance was estimated at 560 nm. To investigate Apx activity, combined the 100 µl of enzyme extract, 100 µl of ascorbate (7.5 mM), 100 µl of H_2O_2, and 2.7 ml of potassium phosphate buffer with EDTA (pH 7.0) and measured absorbance at 290 nm.

Statistical Approach

Data were interpreted using Statistix 8.1 and the Origin Pro-2021 tool for graphical representation. At 5% probability, Fisher analysis of variance was used, and treatment means were compared using the LSD test. In addition, a two-tailed t-test (df-2) used for correlation analysis.

RESULTS

Growth and Development of Hybrids Maize

The morphological data of maize hybrids, DK-6317 and DK-9108, in normal (0 mM) and saline (120 mM) conditions with or without foliar L-methionine at different doses (0, 50, and 100 mg L-1) are presented in Table 1. Plant height (PH), shoot fresh weight (SFW), shoot dry weight (SDF), root fresh weight (RFW), root dry weight (RDF), and leaf area (LA) were significantly decreased ($p \leq 0.05$) by salt stress. However, maize hybrids treated with exogenously applied L-methionine alleviate salt stress significantly ($p \leq 0.05$). This application resulted in an increase in various morphological characteristics, including PH (14.52% and 8.9%), SFW (10.23% and 9.04%), SDW (16.84% and 33.5%), RFW (19.1% and 18.18%), RDW (16.84% and 15.35%), and LA (14% and 13.69%) of Dk-6317 and DK-9108 under 120 mM NaCl stress (S+L1) doses, respectively, compared to only negative control (NC) saline treatment.

Table 1: Plant Height, Shoot Fresh Weight, Root Fresh Weight, Shoot Dry Weight, Root Dry Weight and Leaf Area of Two Maize Hybrids (Dk-6317 & Dk-9108) Grown in Control, Nacl Alone and With Nacl With Growth Regulator L-Methionine for Different Foliar Applications

	Treatments	**PH (cm)**	**S.FW(g)**	**R.FW(g)**	**S.DW(g)**	**R.DW(g)**	**L.A**
DK-6317	**PC**	98.50±0.65c	51.76±0.28d	6.83±0.03c	6.99±0.07c	2.86±0.02c	76.97±1.04c
	L1	102.25±0.85b	54.79±0.20b	7.59±0.11b	7.45±0.04b	3.15±0.02b	80.42±0.48b
	L2	107.00 ±0.91a	57.02±0.10a	7.93±0.03a	7.73±0.07a	3.27±0.03a	83.11±0.05a
	NC	75.75 ± 1.31h	27.26±0.23i	3.76±0.07i	4.75±0.03i	1.45±0.02i	53.60±0.57i
	S+L1	86.75 ± 1.11f	30.05±0.15h	4.48±0.06g	5.55±0.03g	1.84±0.03g	61.12±0.28g
	S+L2	92.25 ± 0.85e	32.89±0.08g	4.79±0.09f	5.80±0.05f	2.17±0.03e	64.02±0.19f
DK-9108	**Treatments**	**PH(cm)**	**S.FW(g)**	**R.FW(g)**	**S.DW(g)**	**R.DW(g)**	**L.A**
	PC	82 ± 1.08g	46.00±0.06f	5.59±0.06d	6.12±0.10e	1.71±0.04h	68.40±0.15e
	L1	88.50 ± 0.65f	49.04±0.29e	4.73±0.09f	6.78±0.04d	2.07±0.03f	72.75±0.14d
	L2	95.50 ± 0.87d	52.90±0.04c	5.22±0.04e	7.06±0.06c	2.46±0.06d	76.32±0.16c
	NC	56.00 ± 1.29k	24.43±0.37k	2.74±0.06k	3.64±0.03j	1.05±0.03k	45.34±0.09k
	S+L1	61.00 ± 1.08j	26.64±0.18j	3.14±0.03j	4.86±0.02i	1.18±0.03j	51.55±0.14j
	S+L2	71.25 ± 1.11i	29.58±0.19h	3.97±0.01h	5.20±0.02h	1.51±0.04i	57.52±0.13h

L-methionine for different foliar applications (i.e. positive control (PC), L-MET 50mg (L1), L-MET 100mg (L2), Negative control with NaCl 120mM (NC), Salinity+L-MET 50mg (S+L1) and Salinity+L-MET 100mg (S+L2) for Plant height(PH), shoot fresh weight(SFW), root fresh weight(RFW), shoot dry weight(SDW), root dry weight(RDW) and leaf area(LA) of two maize hybrids (Dk-6317 & Dk-9108). Alphabetic letters represent statistically significant differences at $p \leq 0.05$

Yield Attributes of Hybrid Maize

Salt stress significantly ($p \leq 0.05$) decreased yield attributes. Results showed that exogenously applied 50 mg L-1 L-methionine to maize hybrids DK-6317 and DK-9108 had higher cob weights (33.28% and 34.37%), longer cobs (20.12% and 17.06%), higher 100 grains weights (5.27% and 2.48%), and greater grains count per cob under 120 mM NaCl stress (S+L1) (Figure 2 a, b, c and d). Furthermore, addition of L-methionine at 100 mg L-1 along with salt treatment (S+L2) significantly ($p \leq 0.05$) improved cob weight

(62.92% and 74.02%), cob length (36.44% and 29.51%), 100 grains weight (47.37% and 28.76%), and grains count per cob (17.17% and 3.64%), as compared to NC saline treatment alone.

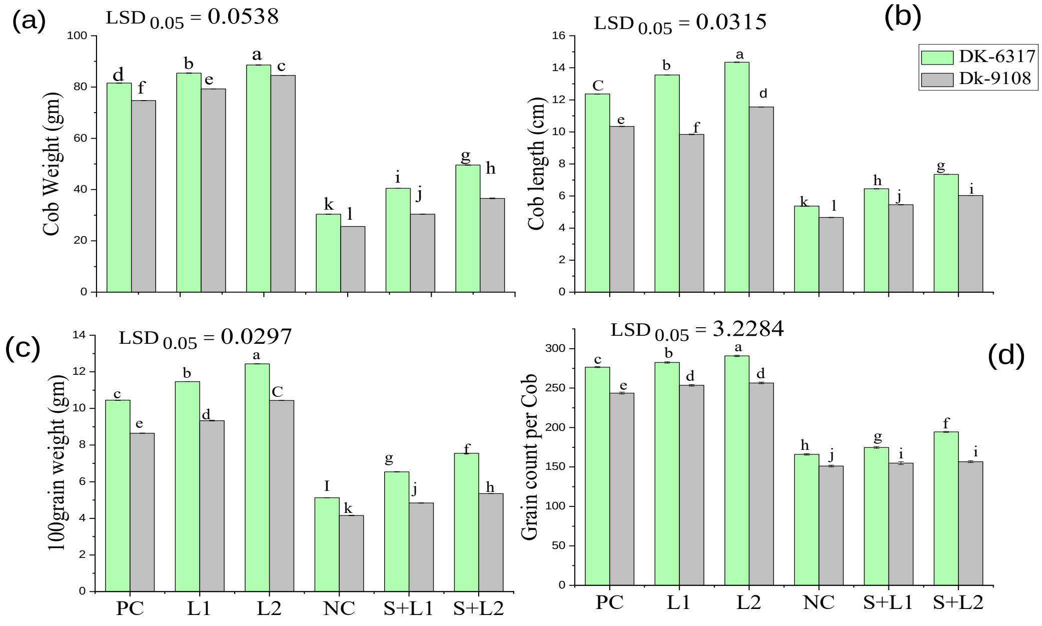


Figure 2: Cob Weight, Cob Length, 100 Grains S Weight And Grains S Count Per Cob of Two Maize Hybrids Dk-6317 and Dk-9108 Grown In Control, Nacl Alone, and Nacl with Growth Regulator L-Methionine Different Foliar Applications (I.E., Positive Control (Pc), L-Met 50mg (L1), L-Met 100mg (L2), Negative Control with Nacl 120mm (Nc), Salinity+ L-Met. Alphabetic Letters on the Bar Graph Represent the Statistically Significant Differences at *p* ≤ 0.05

Physiological Characteristics of Maize Hybrid

Salt stress significantly (*p* ≤0.05) reduced the properties of Chl a, Chl b, and total Chl a+b in DK-6317 (66.50%, 45.30%, and 42.57%) and in DK-9108 (54.84%, 56.42%, and 55.63%) (Figure 3 a, b, and c). The combination treatments (S+L1) and (S+L2) significantly (p≤0.05) mitigate the negative effects of saline treatment by improving photosynthesis up to (28, 21.99 and 33.56%) in DK-6317 and (9.54, 17.33 and 12.99%) in DK-9108, respectively. Similarly, carotenoids contents showed significant (*p*≤0.05) reduction under salinity salt stress and showed 9.8% and 7.22% decrease in Dk-6317 and DK-9108 as well (Figure 2 d). However, maximum carotenoid contents of 6.19% and 3.82% in DK-6317 and DK-9108 were detected with no salinity stress (Figure 2 d).

Biochemical Traits

NaCl toxicity significantly (p≤0.05) altered the activities of various biochemical traits in fresh leaf samples of maize hybrids, and the subsequent foliar application of L-methionine had a substantial (p≤0.05) impact on these traits. Specifically, under saline conditions, there was a marked rise in phenolic and free amino acid contents in DK-6317 (30.73%, 25.2%) and in DK-9108 (36.34%, 21.88%), respectively (Figure 4 a & b). However, L-methionine treatment significantly (p≤0.05) mitigated salt stress effects by reducing total phenolic and total free amino acid contents. Additionally, salt treatment (120 mM NaCl) substantially decreased soluble sugars and total soluble protein content (Figure 3 c & d), but these levels were significantly improved by L-methionine (S+L1) and (S+L2). Notably, (S+L2) resulted in the highest improvement in DK-6317 (28.51% and 22.87%) and in DK-9108 (27.65% and 28.64%) compared to saline treatment (NC), while the maximum decrease was observed in DK-6317 (49.14% and 33.13%) and in DK-9108 (69.30% and 40.42%) under control conditions (PC) (Figure 4 c & d).The results revealed an increase in proline content among the tested maize varieties significantly (p≤0.05), DK-9108 exhibited the highest proline accumulation (40.55%) under salt stress, while its proline content was the lowest (6%) under non-stressed conditions. The maximum proline content was observed in DK-6317 and DK-9108 in response to NaCl treatment, followed by application of 50mg L-1 (S+L1) and 100mg L-1 (S+L2) L-methionine, which slightly mitigated the excessive production of this osmolyte. In comparison, the lowest proline content was recorded in DK-6317 and DK-9108 under control conditions (6.43 and 8.95%, respectively).

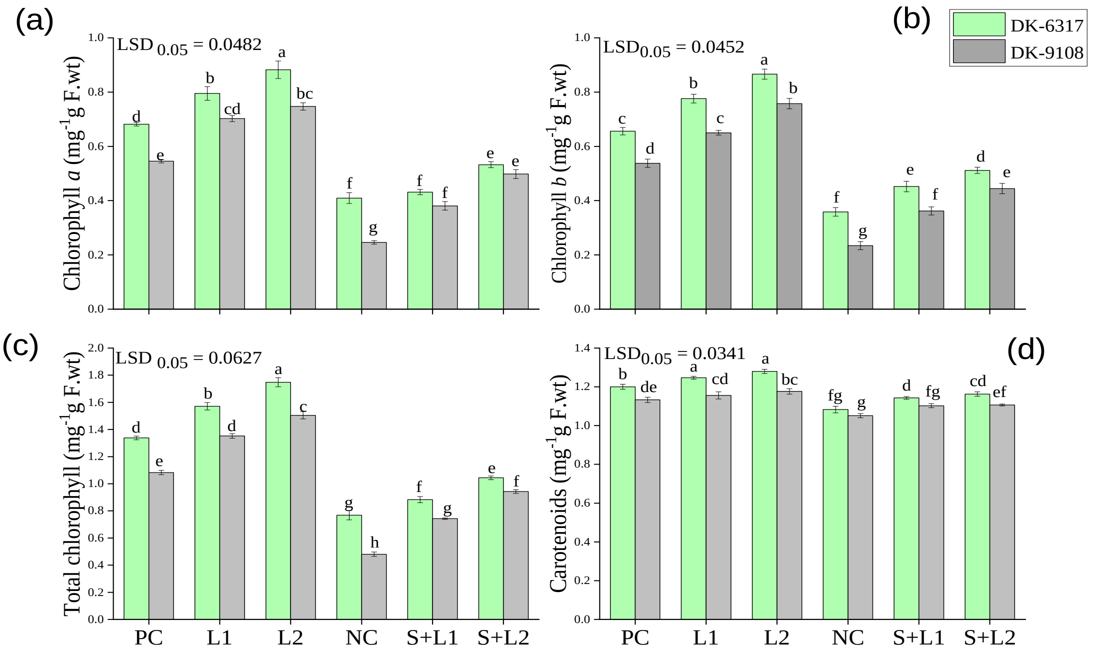


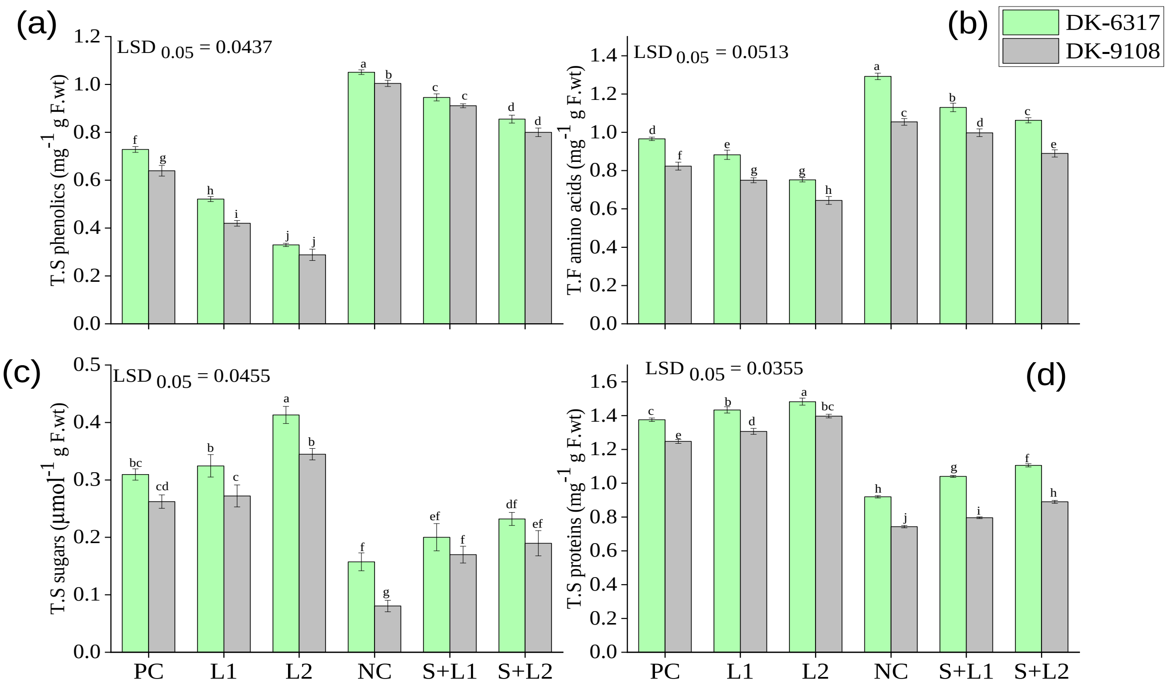
Figure 3: Chlorophyll *a, b* total, and carotenoid content of two maize hybrids Dk-6317 and Dk-9108 grown in control, NaCl alone, and NaCl with growth regulator L-methionine different foliar applications (i.e., positive control (PC), L-MET 50mg (L1), L-MET 100mg (L2), negative control with NaCl 120mM (NC), salinity+ L-MET. Alphabetic letters on the bar graph represent the statistically Significant differences at *p* ≤ 0.05

Oxidative Stress Markers

The toxicity of salt stress and ionic imbalance had a significant rise (*p*≤0.05) in malondialdehyde and hydrogen peroxide levels of both hybrid maize i.e. Dk-6317 and Dk-9108 respectively, as compared to control treatment without L-methionine application (Figure 4 f, h). However, results revealed that the application of 100 mg L-1 (S+L2) reduced the levels of malondialdehyde (by 36.40% and 40%) and hydrogen peroxide (by 25.63% and 16.04%) in leaves of both maize hybrids. In contrast, the lowest malondialdehyde and hydrogen peroxide levels were observed without salt stress in the (L1) and (L2) treatments. This resulted in a reduction percentage of (15 and 23%) and (38.32 and 33%) in DK-6317 and DK-9108, respectively.

Glycinebetaine

Under saline conditions, both maize hybrids DK-6317 and DK-9108 exhibited a significant rise (*p* ≤0.05) in glycinebetaine content compared to non-saline conditions. However, L-methionine reduced glycinebetaine overproduction. DK-9108 had the highest GB value under saline conditions, while DK-6317 had a relatively low GB value. The addition of L-methionine promoted and controlled excessive glycinebetaine production in both hybrids, irrespective of salt treatment. The highest GB values were observed with 100mg L-1 (S+L2) (26.77 and 35.11) in DK-6317 and DK-9108, respectively, followed by 50mg L-1 (S+L1) (29.63 and 43.73) under saline conditions (Figure 4 g).



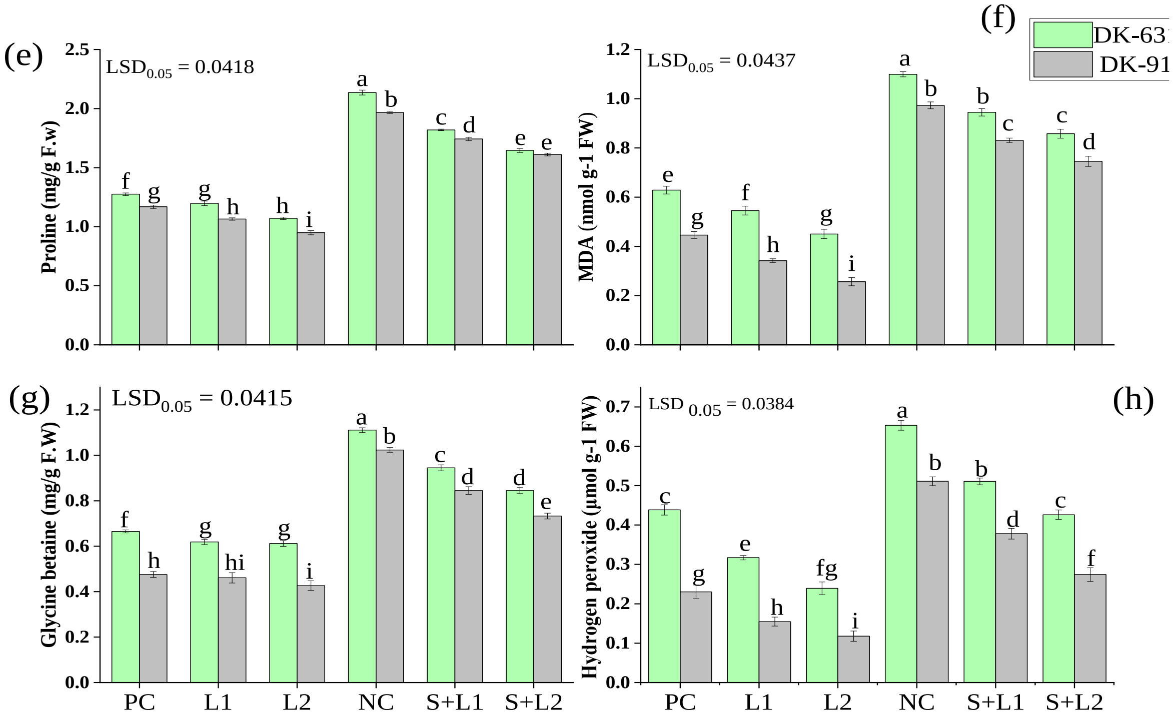


Figure 4: Total soluble phenolic, TFA, soluble sugars, total TSP, Proline, glycine betaine, hydrogen peroxide, and malondialdehyde contents of two maize hybrids, Dk-6317 and Dk-9108, grown in control, NaCl alone, and with NaCl with growth regulator L-methionine's different foliar applications (i.e., positive control (PC), L-MET 50mg (L1), L-MET 100mg (L2), and negative control with NaCl 120 mM (NC). The alphabetic letters on the bar graph represent the statistically Significant differences at *p* ≤ 0.05.

Antioxidant Activity

As shown in (Figure 5, a, b, c & d), the study examined the effects of NaCl toxicity and subsequent L-methionine supplementation on antioxidant enzyme activity in maize hybrid plants. As a result of salt stress, superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT) were significantly altered. The maximum increase in enzyme activity was detected in response to 120 mM NaCl stress. This was observed with DK-6317 plants showing 54.72%, 66.76%, 56.38%, and 62.25% increases. In addition, DK-9108 plants reported 74.78%, 71.67%, 53.79%, and 71.37% increases in SOD, POD, APX, and CAT activities, respectively. The study found that exogenous L-methionine application (S+L2) on salt-stressed plants improved SOD activities and decreased the values of antioxidant enzymes significantly ($p \leq 0.05$), by up to 40.29% and 33.36% for POD, 48.78% and 60.45% for APX, and 33.82% and 27.23% for CAT in seedlings of Dk-6317 and Dk-9108, respectively, compared to salt stress treatments (S2).

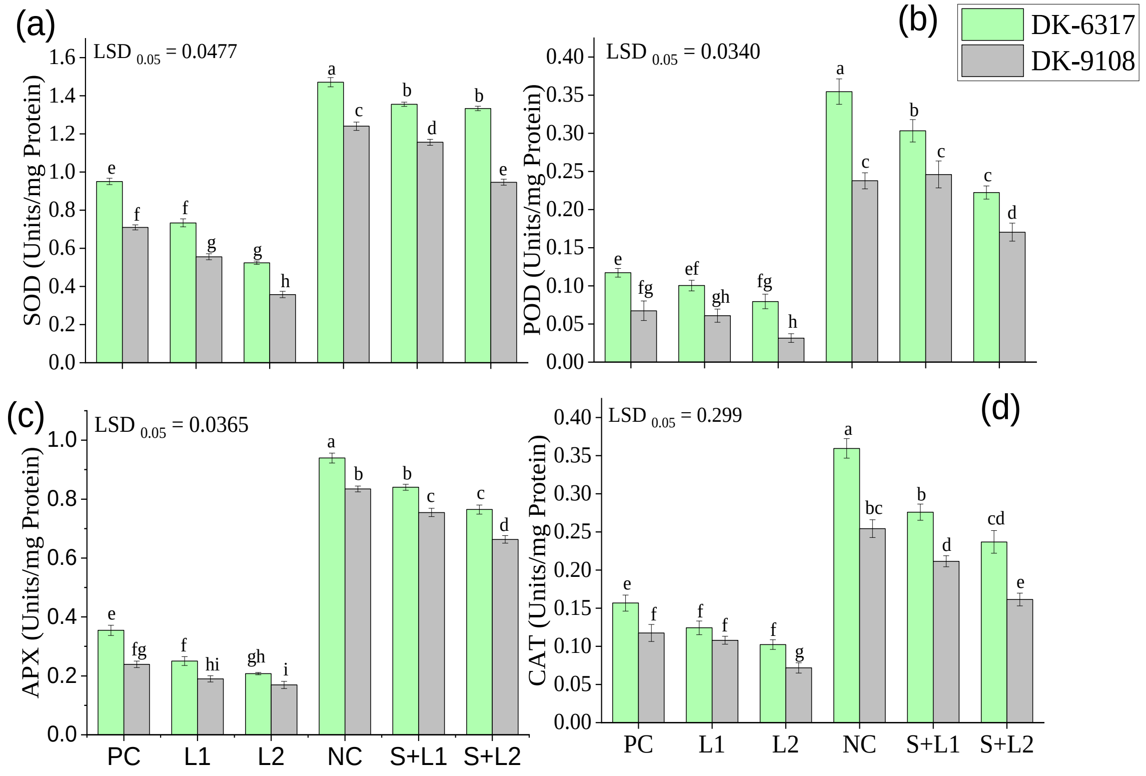


Figure 5: SOD, POD, APX, and CAT content of two maize hybrids Dk-6317 and Dk-9108 grown in control, NaCl alone, and NaCl with biostimulant L-methionine different foliar applications (i.e., positive control (PC), L-Met 50mg (L1), L-Met 100mg (L2), negative control with NaCl 120mM (NC), salinity+ L-Met 50mgAlphabetic letters on the bar graph represent the statistically significant differences at *p* ≤ 0.05

Correlation Analysis

The correlation study was done to measure the relationship between the investigated traits (Figure 6). All growth-related traits showed a significant relationship with plant height (PH), including fresh and dry weight of shoot and root, as well as leaf area (LA) which showed a positive correlation with photosynthetic pigments. There are positive correlations between total chlorophyll and growth-related traits, which results in higher crop yields. Enzymatic stress indicators including glycinebetaine (GB), phenolic content, and proline concentration are negatively associated with photosynthesis activity. Glycinebetaine (GB) and proline were strongly correlated with malondialdehyde (MDA), suggesting that hybrids with higher MDA concentrations also had greater GB and proline accumulation. Additionally, it was demonstrated that this enzymatic activity promotes plant growth effectively by correlating photosynthetic pigments through the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). A negative correlation was exhibited between amino acid (AA) content and plant height (PH), root fresh weight (RFW), leaf area (LA) and carotenoids. Hydrogen peroxide (H2O2) levels in the roots showed a negative correlation with fresh and dry root weight and plant height. A rise in root H2O2 levels results in a decline in biomass production.

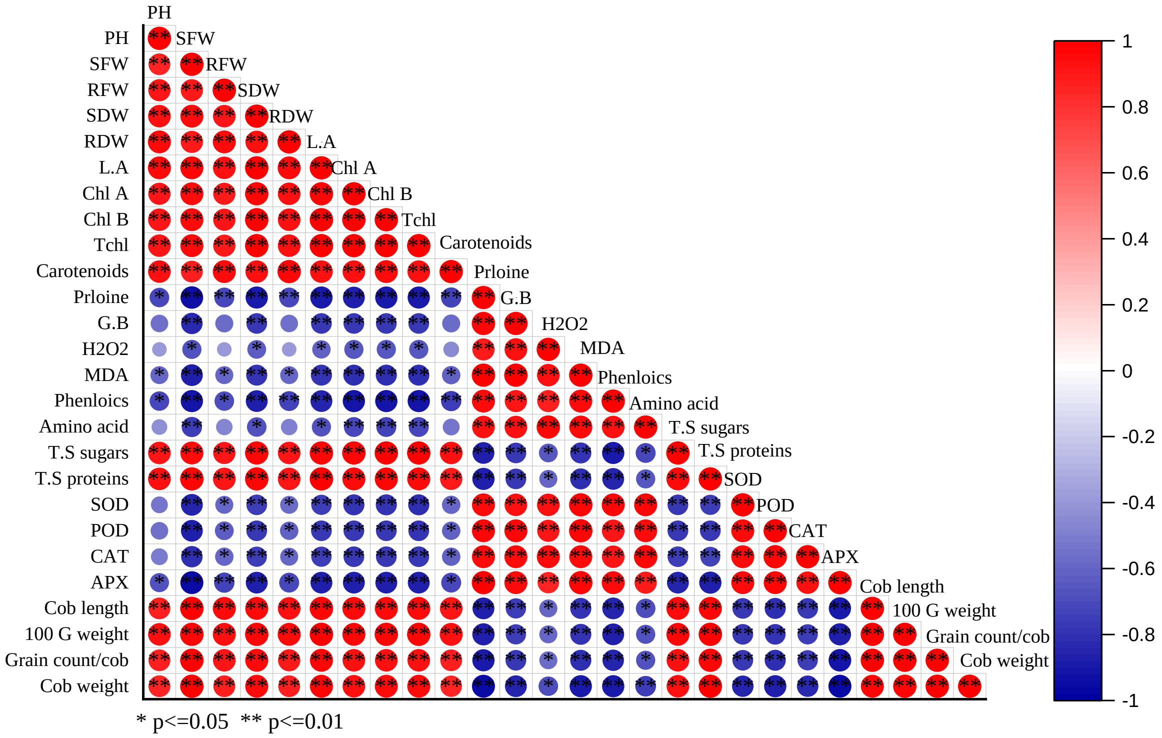


Figure 6: Pearson correlation among growth, chlorophyll contents, biochemical, enzymatic and non-enzymatic antioxidants traits i.e. plant height (PH), shoot fresh weight (SFW), Root fresh weight (RFW), shoot dry weight (SDW), Root dry weight (RDW), Leaf area (LA), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (T.Chl), carotenoids, proline, glycinebetain (GB), hydrogen peroxide(H_2O_2), malondialdehyde (MDA), phenolics Amino acid (AA), total souluble sugars (TSS), Total soluble proteins (TSP), Superoxide (SOD), Peroxidase (POD) Catalyase (CAT) and ascorbate (APX), cob length, 100 grain weight (100 G weight), grain count per cob and cob weight

DISCUSSION

Salinity hinders maize plants' normal development which adversely affects their physicochemical and biochemical processes, resulting in lower yield characteristics (Pirasteh et al. 2022). In field conditions irrigation water and salt affected soils with exceeded limits of sodium chloride (NaCl) can cause osmotic effect-induced loss of leaf turgor, Na+ and Cl- toxicity in various plant tissues, and potential nutritional imbalances (Hussain et al. 2018). Deklab maize hybrids' tolerance to saline soils can vary depending on the specific variety and salt stress severity. Despite the fact that some dekalb hybrids may be more resistant to salt stress than others, there is no detailed information available about exactly how these hybrids handle salt stress in saline soils. This study, however, might contribute to salt tolerance mechanisms of deklab varieties. The genotypes Dk-6317 and Dk-9108 have been specifically bred or selected to tolerate high soil salt levels. As a result, they can maintain normal growth and development even at elevated salt concentrations. This can lead to higher yields, which can be particularly helpful in areas where soil salinity is a limiting factor in crop productivity. Further, L-methionine has been demonstrated to effectively alleviate abiotic stress in various crops, such as tomatoes, maize, bitter gourds, okra, and wheat. Previous studies have used a concentration of L-methionine between 100 and 300 mg L-1, which is effective (Alfosea et al. 2020; Shahid et al. 2021; Akram et al. 2020; Zulqadar et al. 2015; Ahmad et al. 2015).

In the current study, L-methionine at a dosage of 50 and 100 mg L-1 has been shown to significantly ($p \leq 0.05$) enhance growth and yield characteristics compared to both control and salt stress treatments. Based on these results, our initial hypothesis becomes true that applying a specific dose of bio-stimulants would boost maize growth and overcome NaCl's toxic effects. By altering maize plants' physiochemical and nutritional characteristics, bio-stimulants would enhance their ability to tolerate salinity levels. The present study revealed the impact of low exogenous L-methionine doses on cultivated plants' growth parameters (Table 1). Based on the results, L-methionine may interact with nutrients and phytohormones to positively influence metabolism and growth. Regarding plant height, both hybrids exhibited satisfactory growth under pot conditions. However, the hybrid Dk-6317 displayed superior height performance (12.04% increase) compared to Dk-9108 when 100 mg L-1 foliar spray was applied. Furthermore, this increase in plant height was associated with enhanced growth-related characteristics in Dk-6317, including shoot and root fresh and dry weight (7.78%, 51.97%, 9.50% and 32.69%) respectively as well as leaf area (8.89%). Methionine's effect may be responsible for this, as it contributes to the synthesis of a wide range of importnat compounds, such as polyamines, that play a crucial role in cell division, differentiation, and stress response (Pál et al. 2015). Exhibited results coincide with several past studies investigating the mechanisms by which salinity reduces plant growth, including osmotic stress, ionic toxicity, decreased photosynthesis, decreased cell division, and impaired nutrient absorption (Perveen and Hussain 2021; Khan et al. 2019; Saqib et al. 2019). An earlier study by Mehak et al. (2021) found that methionine ameliorates drought stress in sunflower plants by improving growth, metabolites, and oxidative defenses.

Methionine serves as a precursor for the biosynthesis of various phytohormones, including ethylene and polyamines. Ethylene plays a crucial role in regulating various physiological processes in plants, such as fruit ripening and yield related traits. The application of methionine may stimulate the synthesis of these phytohormones, influencing plant growth, development, and yield (Iqbal et al. 2013). Under normal conditions, L-methionine exhibited a positive effect on several yield-related characteristics compared to the negative control (NC). Specifically, treatments labeled as L1 and L2 enhanced cob weight, cob length, 100 grains weight, and grains count per plant with the exogenous application of L-methionine at concentrations of 50 and 100 mg L^{-1} (56% and 61%, 52% and 44%, 42% and 48%, 38% and 38%) and similarly L2 (44% and 56%,47% and 48%,48% and 47%,33% and 38%) displayed a superior response concerning the discussed yield-related traits respectively in dk-6317 and dk-9108 (Figure

2). This could be due to the role of methionine in nitrogen metabolism, which is an essential nutrient for plant growth. It acts as a precursor for various compounds, such as S-adenosylmethionine (SAM), which is a methyl donor involved in nitrogen assimilation. By supplying methionine via foliar application, plants can potentially improve nitrogen utilization efficiency, leading to increased yields (Capaldi et al. 2015). Methionine supplementation may enhance the uptake and utilization of essential nutrients such as iron and zinc. These nutrients are crucial for plant growth and yield formation. By improving nutrient uptake and efficiency, methionine can positively impact yield-related traits (Mousavi et al. 2021). These findings are consistent with earlier research on sunflowers and wheat (Mehak et al. 2021; Maqsood et al. 2022) which suggests that L-methionine could help mitigate salt-induced yield loss in economically significant crops like maize.

Chlorophyll, the primary colored substances involved in plant photosynthesis and play a vital role in numerous physiological processes (Kamble et al. 2015). The chlorophyll content of hybrid maize improved significantly when exogenous L-methionine was applied and photosynthetic characteristics improved significantly. Both hybrids performed well, however DK-6317 outperformed in terms of chlorophyll and carotenoid content. Chauhan et al. (2018), found that elevated levels of salt were the main reason for the formation of proteolytic enzymes that cause chlorophyll degradation and decrease photosynthesis in salty environments (Hu et al. 2021). Sulfur-retaining amino acids, particularly methionine and cysteine act as a viable source of sulfur which benefits protein and chlorophyll production (Amir 2010). Acquisition and assimilation of sulfur are integral to plant metabolism, and a deficiency in sulfur results in diminished chlorophyll content, reduced efficiency of pigment system II (PS II), and lower levels of ribulose 1,5-bisphosphate carboxylase (Rubisco) content (Iqbal, et al. 2013). Hence, the chlorophyll content of the studied hybrid maize plants improved significantly when exogenous L-methionine was applied which appears to be a viable source of green photosynthetic pigments, resulting in stronger antioxidant and metabolic defences, especially under normal conditions (L1 and L2). Similarly, the present findings coincide with Almas et al. (2021), that L-methionine was found to enhance tomato chlorophyll content under salt stress conditions suggesting that L-methionine could be a promising approach. Previous studies have also reported a reduction in green chlorophyll content in maize when exposed to salt regimes (Qu et al. 2012; El-Esawi et al. 2018).

Methionine is a precursor to secondary metabolites such as phenolic compounds, which play significant roles in plant defense, pigmentation, and signaling. It is involved in the formation of diverse chemical classes, including phenolic compounds, alkaloids, flavonoids, glucosinolates, and terpenoids. In the present study phenolic compounds increased with salt imposition and elevated levels were seen at 120 mM NaCl stress without exogenous L-methionnine Dk-6317 produced more phenolic compounds than dk-9108, however foliar application didn’t show any significant improvement in maintaining elevated levels. Methionine donates methyl groups to various acceptor molecules such as phenolic compounds and alkaloids, through enzymes called methyltransferases. This methylation process alters secondary metabolites' properties and activities, including their stability, solubility, and interaction with other molecules. Its involvement as a precursor, methyl group donor, and signaling molecule contributes to the diversity and functionality of these metabolites (Alseekh et al. 2020). Similarly, total free amino acids (fig. 3, c & d) were significantly elevated under salt stress conditions compared to control plants. These elevated levels of total free amino acids showed that plants tend to produce more compatible solutes to overcome salt stress effects. However, both hybrids significantly varied in the accumulation of free amino acids dk-6317 excel as compared to dk-9108. Previous research has also demonstrated that amino acids can alleviate salinity stress by augmenting soluble sugar levels and ultimately enhancing plant growth (Reddy et al. 2016). It was found that L-methionine can influence the production of various enzymes used in amino acid bio-synthesis and redox-homeostasis, leading to increased amino acid content in plants and enhanced resistance to saline conditions (Zhang et al. 2017b).

Total soluble sugars and total soluble proteins significantly decreased with salt stress. A maximum decline occurred at 120 mM salt stress without foliar L-methionine treatment in DK-9108. However, exogenous L-methionine application at 100 mg L-1 increased total sugar and soluble protein contents. This could be due to methionine's crucial role in protein synthesis. It is the initiator amino acid for most protein chains, which is essential for protein synthesis (Sauter et al. 2013). Bahmani et al. (2015) have also demonstrated that exogenous sulfur-retaining amino acids, including methionine and cysteine, can enhance salt stress tolerance. Methionine-rich proteins, which protect against various stresses, including oxidative stress. These proteins may contribute to maize's antioxidant defense by acting as antioxidants or by participating in signaling pathways that regulate antioxidant responses. Methionine sulfoxide reductases (MSRs) repair proteins damaged by oxidation. As a result, they reduce methionine sulfoxide residues back to methionine, restoring protein functionality. Maize MSRs are crucial for maintaining cellular redox balance and protecting proteins from oxidative damage (Zhang and Weissbach, 2008).

Proline and glycinebetaine act as a compatible osmolyte and offer several protective benefits to plants in stress alleviation by enhancing membrane stability, and inducing stress-responsive genes (Sadak et al. 2020). Studies have shown that L-methionine upregulates P5CS genes, leading to increased proline synthesis and accumulation under stressful conditions (Filippou et al. 2013). In this study, glycine-betaine and proline levels increased in maize leaf tissues as a consequence of salt stress, but decreased with foliar application, which contradicted L-methionine's effect and low values were observed in control conditions with 50 and 100 mgL-1 L-methionine foliar application (Fig. 3, e & g). Glycinebetaine is synthesized via a two-step pathway from choline, a common constituent of plant cell membranes, and L-methionine, which acts as a cofactor which helps glycine-betaine accumulate in abiotic stresses (Yu et al. 2020). In addition, previous studies have demonstrated that exogenously applied various other amino acids, like arginine, tryptophan and proline, can reduce stress severity in different plant species under salt stress (Hojagan et al. 2017; Paradisone et al. 2015).

Salinity increases lipid peroxidation malondialdehyde (MDA) membrane damage and hydrogen peroxide (H_2O_2) contents in maize plants. Present study showed an elevation of these contents under salt stress of 120 mM NaCl in both hybrid maize plants, however Dk-6317 showed more elevated levels of MDA and H_2O_2.Similarly, in stressful environments, plants activate biochemical markers such as reactive oxygen species (ROS), hydroxyl radical (OH), peroxide (O_2), and hydrogen peroxide (H_2O_2), which enhance the defense system in crop plants (Pitzschke et al. 2006). As ROS damage plant tissues, methionine increases the antioxidant defence system, resulting in membrane damage mitigation (El-Esawi et al. 2019). Methionine acts as a precursor for glutathione synthesis. Glutathione is a potent antioxidant that neutralizes ROS and protects against oxidative damage by scavenging ROS such as MDA and H2O2 and preventing their harmful effects (Meister and Anderson, 1983). Methionine donates a methyl group through transmethylation. This methyl group is utilized in various biochemical reactions, including the synthesis of significant molecules like DNA, RNA, proteins, and phospholipids. By facilitating these reactions, methionine indirectly contributes to cellular integrity and function, reducing ROS production (Gerke et al. 2012).

Salt stress significantly influences antioxidant enzyme activity, as shown in Figure 4 (a, b, c, and d). Studies conducted on different crops such as tomato, wheat, and maize have reported that exogenous L-methionine applications protect against oxidative damage (Shahid et al. 2021). Superoxide dismutase (SOD) activity increased at 120 mm NaCl salt stress. Similarly, exogenous L-methionine via foliar spray promoted SOD activity, though these increases were not statistically significant at doses of 50 and 100 mg L-1. The peroxidase (POD) content increased under saline conditions and varied significantly with L-methionine. The hybrid maize variety Dk-6317, in particular, demonstrated more pronounced variations in POD activity when 50 and 100 mg L-1 L-methionine doses were applied. Under salt stress, ascorbate peroxidase (APX) activity rose significantly, indicating a potential measure of salt stress toxicity. Exogenous L-methionine application, on the other hand, had no discernible effect on either hybrid maize variety. However, catalase (CAT) activity showed significant variations under salt stress when supplemented with exogenous L-methionine. The concentration of 100 mg L-1, in particular, significantly reduced catalase content, and this effect was more pronounced in the dk-9108 variety. Catalase activity did not change statistically significantly under control conditions. Additionally, L-methionine plays a crucial role in regulating ROS scavengers in plant systems (Carillo 2018). In specific crops, such as tomatoes, foliar spraying controls SOD and APX activity (Almas et al., 2021). Similarly, Nasibi et al. (2016) suggested that cysteine reduces salt stress in wheat by producing antioxidant enzymes like SOD, CAT, APX, and POD play a crucial role in scavenging ROS produced during salt stress by converting superoxide to O_2 and H_2O_2 and decomposing H_2O_2 into oxygen and water (Qamer et al. 2021; Elkelish et al. 2021; Jaha et al. 2022). It is imperative to note that methionine's specific effects on antioxidant enzymes in maize plants may vary depending on a variety of factors. These factors include the plant's developmental stage, the treatment concentration and duration, and environmental conditions. Interactions with other nutrients and signaling molecules within the plant may also influence these effects. Overall, while methionine has shown promise in increasing antioxidant enzyme activity in maize plants, more research is needed to fully understand the mechanisms involved and optimize its application in agricultural practices.

CONCLUSIONS

The study found that using exogenous L-methionine concentrations of 50 mg and 100 mg L-1 improved morphological and physiological parameters under salt-stressed maize hybrids. As a result, L-methionine supplementation can be considered a beneficial practice for improving salt tolerance in maize crops. In comparison of DK-6317 and DK-9108 hybrids, it was discovered that DK-6317 had higher salt tolerance and produced significantly more biomass and yield under salt stress conditions. The study suggests that using L-methionine and selecting salt-tolerant hybrids can be implemented as long-term agricultural measures to assure yield and quality in areas where maize cultivation is threatened by rising salinity stress. Conclusively, it is imperative to note that methionine foliar application effectiveness can vary depending on plant species, application method, concentration, and environmental conditions. Additionally, methionine response may also depend on plant nutrient status and other factors. In addition, such trials should be used to modify and refine methionine application to other crops. This is for potential effects of methionine foliar application.

Conflict of Interest

The researchers state that they conducted the study without any existing financial or commercial associations that could be interpreted as a possible source of conflict.

Author’s Contribution

Each of the authors listed has provided significant, hands-on, and intellectual contributions to the research and approved its publication.

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