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L-METHIONINE A VIABLE EXOGENOUS INTERVENTION FOR ENHANCED GROWTH, YIELD, ANTIOXIDANT AND IONIC ACTIVITIES IN HYBRID MAIZE

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Abstract

L-methionine (L-MET), an essential sulfur-containing amino acid belonging to the aspartate family, plays a critical role in agricultural crops by influencing their nutritional value. In this study, the objective was to evaluate the efficacy of exogenously administered L-MET in enhancing maize growth, yield, and essential metabolic processes. Two different concentrations of L-MET, namely 50 and 100 mg L-1, were compared to an untreated control to assess their impact on two maize hybrids, Dk-6317 and Dk-9108. The results demonstrated a significant positive effect on various parameters, including plant height, leaf characteristics, photosynthetic rate, chlorophyll content, and yield. The exogenous application of L-MET led to notable improvements in carotenoid levels, total soluble phenolics, proline, glycine betaine, malondialdehyde, hydrogen peroxide, and ion homeostasis in both maize hybrids, with statistical significance ($P \leq 0.05$) observed. Furthermore, a correlation analysis was conducted to examine the relationship between the investigated traits. The results indicated a progressive correlation between hundred grain weights (HGW) and the number of leaves (NL), cob length, and photosynthetic pigments. This suggests that photosynthesis traits significantly contribute to yield. Consequently, the findings strongly indicate that foliar application of L-MET has the potential to stimulate maize growth and enhance traits that contribute to increased yield by modulating various physiological and biochemical processes.

Keywords: Plant Growth Regulators (PGRs), L-Methionine (L-MET), Maize Hybrids, Foliar Application, S-Adenosyl-Methionine, Amino Acids, Essential Nutrients.

INTRODUCTION

The nutritional significance of legumes and grains decreased 50-75% by restricting vital amino acids in certain environmental stress conditions. Legumes and grains are vital sources of nutrition for humans, supplying a wide range of crucial nutrients such as proteins, carbohydrates, dietary fiber, minerals and vitamins. Legumes and grains are considered reliable sources of plant-based proteins because they contain a wide range

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of essential and non-essential amino acids. However, under certain stress conditions, such as drought, nutrient deficiency, or extreme heat, the synthesis and availability of certain necessary amino acids can be hampered. (Galili and Amir, 2013; Galili *et al.,* 2005). L-methionine, an indispensable amino acid classified within the aspartate family, plays a fundamental role in preserving crops' nutritional composition (Hesse and Hoefgen, 2003). Its significance lies in its indirect influence on diverse sub-cellular processes as a precursor to S-adenosyl methionine, the primary donor of methyl groups in biotic reactions. Methionine, characterized by a non-polar nature, a side chain containing sulfur, and a γ-carbon with an S-methyl (-CH3) thioether, plays a vital role in cellular mechanisms encompassing cell division, cell wall synthesis, chlorophyll biosynthesis, ripening, and senescence. These processes are intricately regulated by ethylene production and its precursor, S-adenosyl methionine (Galili et al., 2005).

Although plants serve as the key source of nutrition for organisms, certain essential nutrients, including amino acids like methionine and lysine, may be deficient in crucial crops (Bourgis et al., 1999; Amir, 2010). Global malnutrition affects more than 40 percent of the population, particularly micronutrients such as vitamin A, zinc, and iron. Furthermore, it extends to macronutrients such as methionine and lysine. These amino acids, which mammals cannot synthesize endogenously, must be acquired through dietary sources (Galili and Hofgen, 2002).

Exogenous application of growth regulators and hormones to crops, particularly through foliar spraying, represents a promising approach to enhancing cereal crops' nutritional significance. This technique promotes crop development and improves the harvest index by stimulating the synthesis of various endogenous hormonal substances (Zahir et al., 2005; Mustafa et al., 2016). Moreover, foliar spraying offers rapid leaf absorption, reducing the time lag between application and uptake by plants (Ahmad and Jabeen, 2005). Numerous studies have demonstrated the efficacy of foliar spraying with amino acids as a beneficial technique for crop improvement (Sh Sadak et al., 2014). Application of L-MET contributes to better absorption of sulfur and nitrogen, with its effectiveness being dependent on the concentration applied (Forde et al., 2014). Even at extremely low concentrations, foliar feeding exerts positive effects on plant physiological processes, ensuring optimal growth and resulting in higher yields and improved crop quality (Sher et al., 2017).

Zea mays L. (Maize) holds the distinction of being the world's third-largest and most vital grain crop, surpassed only by wheat and rice. Maize accounts for approximately 4.8% of the total cultivated land and contributes 3.5% to the overall agronomic production. It is anticipated to cover 1.0 million hectares and produce 3.3 million tons of grains each year (Chivasa et al., 2017). Because of the rise in poultry and cattle feed, maize consumption has been risen. Maize is a versatile crop known as Queen of Grains owing to its high genetic potential, and it is the principal source of food, feed, and industrial raw materials (Ramamurthy et al., 2020). Many types of maize are used in human food and animal feedstuff, particularly maize products (Nuss and Tanumihardjo, 2010).

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Building upon the introductory information, the present study formulated the hypothesis that the foliar application of L-methionine to maize hybrids would augment sulfur and nitrogen absorption. This enhanced nutrient uptake was expected to accelerate growth and lead to amplified yields in maize hybrids. Additionally, it was posited that foliar spraying of L-methionine would positively impact the traits contributing to yield, thereby resulting in improved grain production and overall yield outcomes.

MATERIAL METHODS

Experimental layout and site

To investigate the beneficial role of L-methionine (L-MET) on maize hybrid's, a pot trail was executed during the spring 2020 with foliar application of L-MET. The trails were performed in the lab and wire chamber of the Department of Botany, University of Agriculture, and Faisalabad. Monsanto Pakistan (Private) Limited provided kernels of spring hybrid maize Dekalb-6317 and 9108, which had an initial seed moisture content of 11.8 percent (on a fresh weight basis) and germination potential of 95 percent. The trails were laid out in a completely randomized design (CRD) in a factorial arrangement with four replications under natural environmental conditions. This experiment used large plastic pots of 12 inches in size filled with almost 8.0 kg of washed sand. Six seeds of each variety (Deklab-6317 and Deklab-9108) were sown in each of the plastic pots. Germination commenced on the third day following seed sowing, and it was completed on the seventh day. Plants were regularly irrigated with a sufficient amount of water to maintain the moisture content. Three L-methionine concentrations, i.e., 0, 50, and 100 mg L^{-1} were used. To maintain the nutritional content of sand media, Hoagland nutrient solution was used. Once the seedling was established, only three plants in each pot were kept till maturity. Overall weather conditions of experimental location during spring 2020 represented in the figure 3.1.

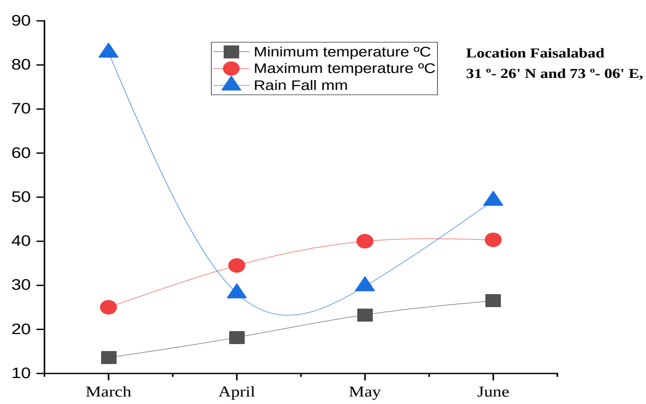

Figure 1: Maximum and minimum temperature and average rain fall of experimental location during spring 2020

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Growth and yield contributing attributes: Growth attributes were measured in relations to plant height and leaf area (Hunt, 2002) and number of leaves after 85th day of germination, while yield contributing traits were studied as cob length 100 grain weight and cob weight of two hybrid maize varieties Dk-6317 and Dk-9108 at the time of harvest.

Biochemical parameters

Chlorophyll determination essay: Arnon's (1949) technique was used to determine the contents of chlorophyll and carotenoids (mg/g f.w). 5ml of 80 percent acetone was used to homogenize the leaf samples (0.25 g) from 44-day-old plants. Kept the crude extract overnight, in the dark. Resulting extracts optical density stood measured for 663, 645, and 480nm, while 80 percent acetone was used as a reference for blank measurement. By applying the following formulae to the resulting optical density, chlorophyll and carotenoids contents were calculated.

$$\text{Chl.}\,a\ (\text{mg/g}) = \frac{12.7(\text{OD663}) - 2.69(\text{OD645})\ \text{x V}}{1000\ \text{x W}}$$

$$\text{Chl.}\,b\ (\text{mg/g}) = \frac{22.9(\text{OD645}) - 4.68(\text{OD663})\ \text{x V}}{1000\ \text{x W}}$$

$$\text{Total Chl.}\ (\text{mg/g}) = \frac{20.2\ (\text{OD645}) + 8.02\ (\text{OD663})\ \text{x V}}{1000\ \text{x W}}$$

Where V= Volume (ml)

W= Sample weight (g)

Estimation of total soluble phenolic: Phenolic contents were determined by the technique of Julkunen-Tiitto, 1985. In 1ml 80 percent acetone, the ground fresh material 0.5 g was combined. The mixture was left at 50°C for 1 hour and then centrifuged for 15 minutes at 12000rpm. In a microfuge tube, the supernatant was collected and kept at 20 °C until needed. In a test tube with a capacity of 10 ml, distilled water was used to dilute a 100 ml aliquot to 1 ml. 0.5 mL of folin-phenol Ciocalteu's reagent was added, followed by vortexes and 2.5 mL of 20% Na_2CO_3. The mixture was reduced in volume to 5mL and vortexes violently for 10 seconds. The absorbance at 750nm was assessed after twenty minutes using a spectrophotometer. The spectrophotometer was adjusted auto zero using 80 percent acetone.

Proline determination essay: Bates et al. (1973) technique used to determine proline content. 10ml 0f 3 percent aqueous sulphosalicylic acid was homogenized with 0.1g of fresh plant material and strained through Whatman no.2 filter paper. A mixture of 1ml of the filtrate, 2ml of acid ninhydrine, and 2ml of glacial acetic acid was added. The mixture was heated to 100°C for an hour in a water bath, and the reaction was then ceased in an ice bath. Sample solution was vortexed and treated with 4 ml of toluene. The free proline chromophore was poured into a test tube and absorbance was measured at 520 nm with a spectrophotometer (UV-1100). 2 ml of 3 percent aqueous sulphosalycylic acid was

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used for blanks. The standard calibration curve was formed by repeating the procedures with proline (10 to 50 g/2 ml).

The following formula was computed for the amount of free proline.

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

Glycinebetaine determination essay: Grieve and Grattan (1983) method was slightly modified for glycinebetaine. 0.25g leaf sample were extracted with 0.5 percent toluene. The extracts were stored at 4°C overnight. 1mL of supernatant was added to the extract, followed by 1mL of $2NH_2SO_4$. 0.5mL of the mixture was then mixed with 0.5mL of KI_3. After 90 minutes, the mixture was chilled by adding pre-chilled distil water and 1, 2-dichloroethane. Two layers were formed, remove the upper layer, and absorbance at 365nm was measured using a spectrophotometer.

MDA Determination essay: Heath and packer (1968) method was utilized to measure malondialdehyde (MDA). Fresh leaf sample (0.25g) was crushed in 3ml of TCA (1 percent w/v). It was then centrifuged for 15 minutes at 12000rpm.1 mL (0.5 percent v/v) Thiobutyric acid TBA produced in 20% TCA was added to 1 ml supernatant. Incubated at 95°C for 15 minutes before being chilled inside an ice bath. A spectrophotometer was used to measure the absorbance of the supernatant at 532 nm and 600 nm (UV-1100). The non-specific absorbance value was subtracted at 600 nm.

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

Determination of hydrogen peroxide: The amount of hydrogen peroxide present in leaf tissue was estimated by the technique of Velikova et al., (2000). An ice-cold mortar and pestle and were used to grind a fresh leaf sample (0.24g) in 2.5 ml of 0.1 percent (w/v) trichloroacetic acid (TCA). Follow the crushing; the mixture was centrifuged for 12 minutes at 10000rpm. A 0.5 mL aliquot of the supernatant was added to a fusion mixture comprising 0.5 mL $KHPO_4/KH_2PO_4$ buffer with pH 7.0 and 1 mL KI, vortexes, and absorbance measured at 390 nm using a UV-visible spectrophotometer with distilled water kept as a blank.

Analysis of ions: This experiment analyzed the ionic content in samples using digestion flasks. Initially, 0.5g samples were mixed with 5ml concentrated H2SO4 and left to incubate overnight. The conical flasks were placed on a hot plate at 350°C until fumes appeared. Once the fumes appeared, the flasks were removed from the hot plate, cooled, and gradually treated with 1 ml of H2O2. The flasks were then returned to the hot plate and left for 20 minutes, generating additional fumes. This process was repeated until the substance became clear and colorless. To increase the volume, distilled water was added to the extract, resulting in a final volume of 50 ml. The solutions were filtered and transferred to labeled bottles for sodium, potassium, and calcium ion content. A flame photometer was employed to measure Na, K, and Ca ions in maize plants' stems and roots. Standard curves were created using a graded series of standards containing Na, K, and Ca ions at concentrations of 10, 20, and 100 ppm. The readings obtained from the

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flame photometer for Na, K, and Ca ions were compared to the standard curve. This allowed the calculation of the actual amounts in milligrams per gram of dry weight.

Statistics: For the sand culture experiment, a completely randomized experimental design with four replications and three concentrations of L-MET (0, 50 and 100mgL^{-1} in foliar mode) were used. Statistics 8.1 and LSD test was used to analyze the data significance ($P \leq 0.05$) between variables using two-way analysis of variance (ANOVA), and origin was used to create all of the graphs.

RESULTS

Foliar application of L-methionine improved plant growth and yield components

The exogenous foliar spray of L-methionine (L-MET) exhibited a significant (P≤0.05) enhancement in the growth characteristics of hybrid maize varieties, as depicted in Figure 2. Notably, the influence of different maize hybrids was significantly distinct (P≤0.05), in addition to the impact of varying L-MET doses. Among the hybrids, Dk-6317 displayed a higher plant height and a higher leaf count compared to Dk-9108. Conversely, the absence of foliar application resulted in minimal plant height, while Dk-9108 exhibited a larger leaf area with the application of 100 mg L-1 L-MET (Figure 2).

Regarding yield characteristics, L-MET doses had a notable effect on cob length, cob weight, and hundred grain weight. Remarkably, hybrid Dk-6317 exhibited superior yield characteristics when subjected to a L-MET dose of 100 mg L-1. Furthermore, L-MET led to increased relative growth parameters such as leaf area index, number of leaves, and plant height. While L-methionine treatment demonstrated a non-significant association with leaf number and cob weight (Table 1), all other growth and yield-related characteristics exhibited a significant association with L-MET application.

Table 1: The least significant difference in data for various Growth, yield and chlorophyll contents of Maize (*Zea mays* L.) hybrids i.e Dk-6317 and Dk-9108 treated to varying doses of L-methionine (0, 50, and 100 mgL^{-1})

Treatments	Traits									
	PH	LA	No. of leaves	100 grain weight	Cob weight	Cob length	Chl *a*	Chl *b*	T Chl	Chl *a:b*
DK-6317	91^{a}	55^{b}	10.6a	12a	51a	8a	0.16a	0.18a	0.20a	1.8b
DK-9108	73^{b}	66^{a}	9.4b	10b	46b	7b	0.15b	0.15b	0.17b	2.9a
LSD	0.79	0.80	0.8	0.40	0.27	0.05	3.4e^{-03}	3.1e^{-03}	4.2e^{-03}	0.12
0mg L^{-1}	79^{c}	57^{b}	9.0c	12a	49a	6.9c	0.16a	0.18a	0.19a	1.8c
50mgL^{-1}	85^{a}	62^{a}	10.0b	11b	48b	7.7b	0.151b	0.17b	0.19b	2.3b
100mg L^{-1}	83^{b}	62^{a}	11a	10c	47c	8.3a	0.15c	0.15c	0.17c	2.9c
LSD	0.97	0.98	1.01	0.49	0.3	0.07	4.2e-^{03}	3.8e^{-03}	5.2e^{-03}	0.15
Interaction	*	**	Ns	*	n.s	**	n.s	n.s	*	**

Non-significant = ns, Significant * = p<0.05 and Significant ** = p<0.01

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Table 2: The least significant difference in data for various biochemical parameters and ionic analysis of Maize (*Zea mays* L.) hybrids i.e Dk-6317 and Dk-9108 treated to varying doses of L-methionine (0, 50, and 100 mgL^{-1})

Treatments	Traits											
	Carotenoids	Phenolic	Proline	GB	MDA	H_2O_2	Shoot Na^+	Root Na^+	Shoot K^+	Root K^+	Shoot Ca^{+2}	Root Ca^{+2}
DK-6317	0.29a	1.2a	1.5a	0.9a	0.5a	0.7a	23b	26b	50a	24a	18a	13a
DK-9108	0.20b	1.1b	1.1b	0.8b	0.3b	0.6b	31a	28a	34b	19b	15b	13a
LSD	$9.8e^{-03}$	0.01	0.06	0.03	$7.8e\text{-}^{03}$	0.02	0.9	0.72	0.79	1.17	0.8	0.78
0mg L^{-1}	0.22c	1.27a	1.4a	1	0.5a	0.7a	30a	30a	44a	23a	18a	14a
50mg L^{-1}	0.25b	1.24b	1.2b	0.9	0.4b	0.7b	27b	26b	42b	22b	17b	12b
100mg L^{-1}	0.27a	1.01c	1.2b	0.8	0.3c	0.5c	24c	25C	39c	20b	15c	11c
LSD	0.01	0.02	0.07	0.04	$9.6e^{-03}$	0.02	1.2	0.89	0.97	1.43	1.02	0.95
Interaction	**	**	*	n.s	**	n.s	*	*	n.s	n.s	n.s	n.s

Non-significant = ns, Significant * = p<0.05 and Significant ** = p<0.01

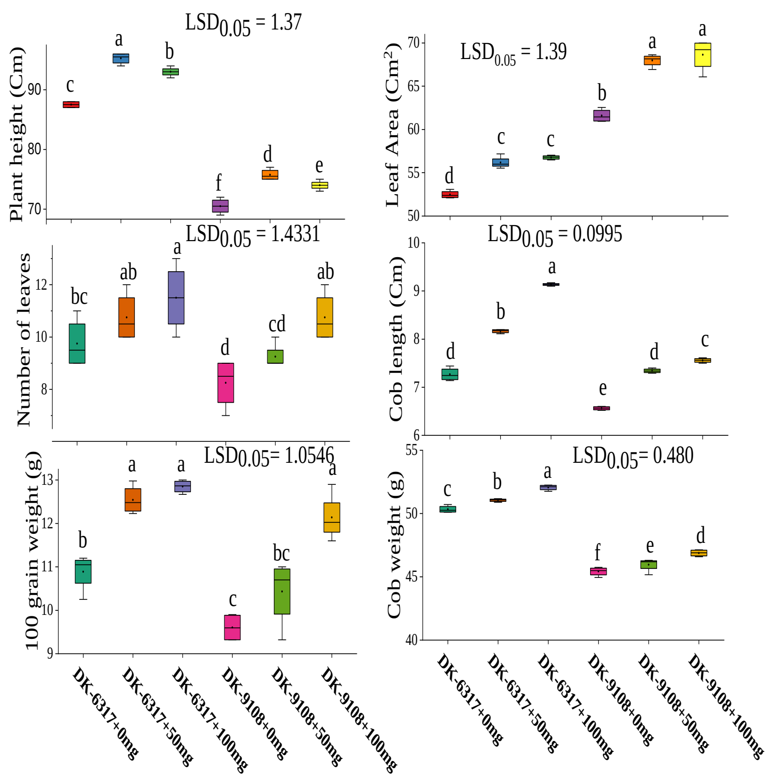

Figure 2: Effect of foliar applied L-methionine on (A) Plant height (B) leaf area (C) number of leaves (D) Cob length (E) 100 grain weight (F) cob weight of two maize hybrids i.e Dk-6317 and DK-9108.

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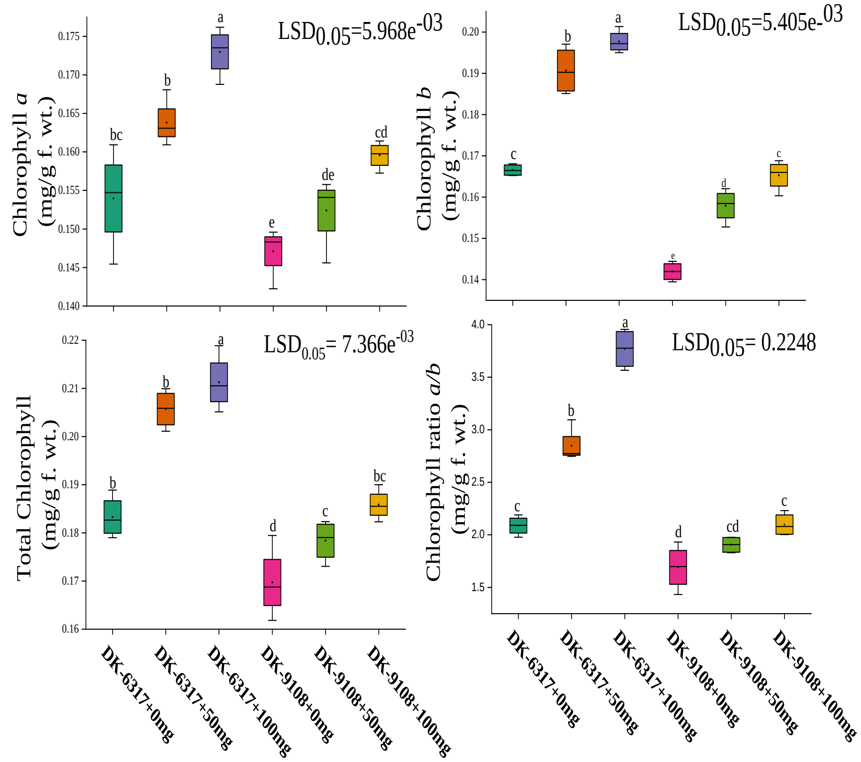

Figure 3: Effect of foliar applied L-methionine on (A) Chlorophyll *a*, (B) Chlorophyll *b*, (C) Chlorophyll *a/b* ratio (D) Total chlorophyll contents of two maize hybrids i.e Dk-6317 and DK-9108

Application of L-methionine improves the photosynthetic characteristics

The exogenous spray of L-methionine (L-MET) exhibited a significant ($p \leq 0.05$) improvement in the chlorophyll content of hybrid maize varieties, as illustrated in Figure 3. The application of various L-MET concentrations resulted in enhanced levels of chlorophyll a, chlorophyll b, the chlorophyll a/b ratio, and total chlorophyll content. Notably, the hybrid Dk-6317 displayed higher chlorophyll content with the foliar spray of both 50 and 100 mg L-1 of L-MET. Conversely, the untreated control plants of the Dk-9108 variety exhibited the lowest chlorophyll content. These findings indicate that L-MET plays a crucial role as a photosynthetic regulator in maize, contributing to heightened photosynthetic activity and increased chlorophyll levels.

Application of L-methionine improved carotenoids, total soluble phenolic, proline, Glycinebetaine, malondialdehyde and Hydrogen peroxide, by enhancing plant defence mechanism

The exogenous application of L-methionine (L-MET) exerted a significant ($p \leq 0.05$) influence on the levels of carotenoids, total soluble phenolics, proline, glycinebetaine,

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malondialdehyde, and hydrogen peroxide in both hybrid maize varieties, Dk-6317 and Dk-9108, as indicated in Table 2. Notably, foliar treatment led to a significant increase (P≤0.05) in carotenoid content. Figure 4 illustrates that hybrid Dk-6317 exhibited a significant impact on defense compound activation. Moreover, the foliar application of L-MET resulted in a significant reduction (P≤0.05) in the levels of total soluble phenolics, proline, glycinebetaine, malondialdehyde, and hydrogen peroxide in both maize hybrids. It is noteworthy that hybrid Dk-6317 displayed a more pronounced effect on defense compound accumulation than Dk-9108.

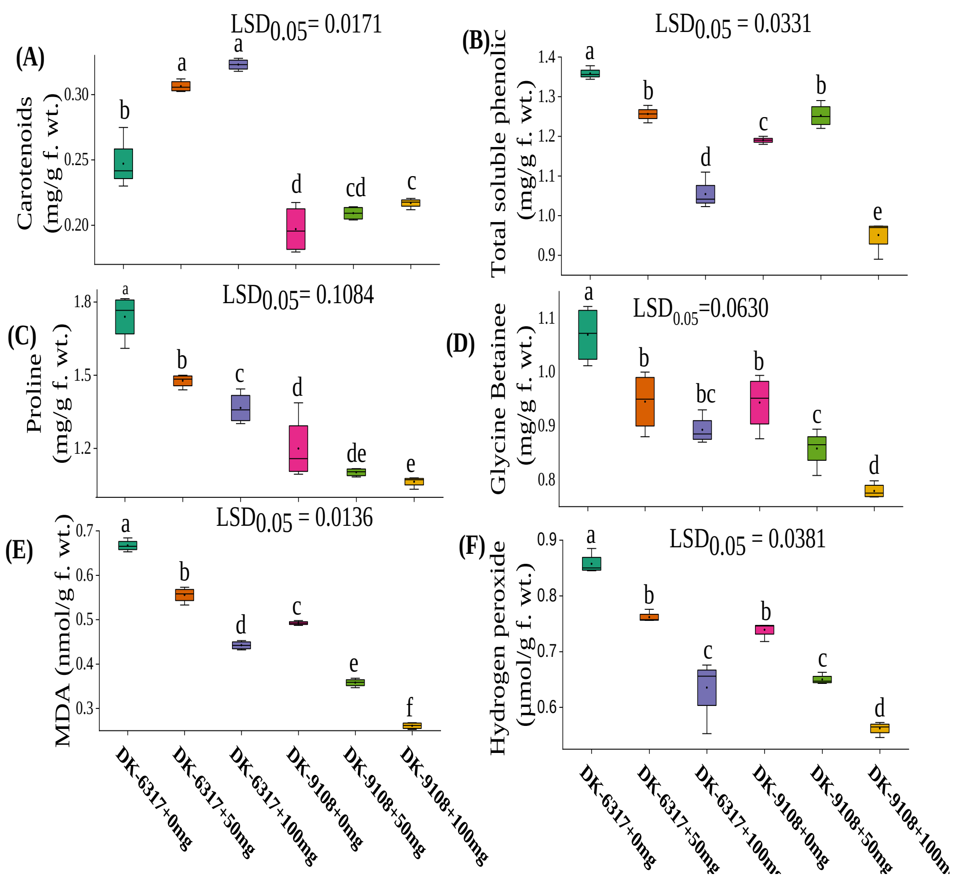

Figure 4: Effect of foliar applied L-methionine on (A) carotenoids (B) total soluble phenolic (C) proline (D) Glycinebetaine (E) lipid peroxidation (F) Hydrogen peroxide, by enhancing plant defence mechanism in two maize hybrids i.e Dk-6317 and DK-9108

Exogenous L-methionine regulated the mineral homeostasis in maize hybrids

The foliar application of L-methionine (L-MET) had a significant impact on the accumulation of Na+ ions, as well as the build-up of K+ and Ca2+ ions in the stems and roots of maize hybrids, as depicted in Figure 5. Mineral ion homeostasis in relation to maize hybrids exhibited a significant (p ≤0.05) differentiation from the significant (P≤0.05)

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impact of L-MET doses (Figure 5). The concentration of 100 mg L-1 L-MET demonstrated a more favorable ionic balance, whereas the absence of foliar application of L-MET in the dk-9108 hybrid resulted in the lowest level of ionic balance (Table 2). Notably, in hybrid Dk-6317, the foliar spray of L-MET significantly (p ≤0.05) reduced the accumulation of Na+ ions, while concurrently promoting a substantial increase in K+ and Ca2+ ions in both stem and root tissues. Conversely, in hybrid Dk-9108, L-MET effects on ion balance were lower.

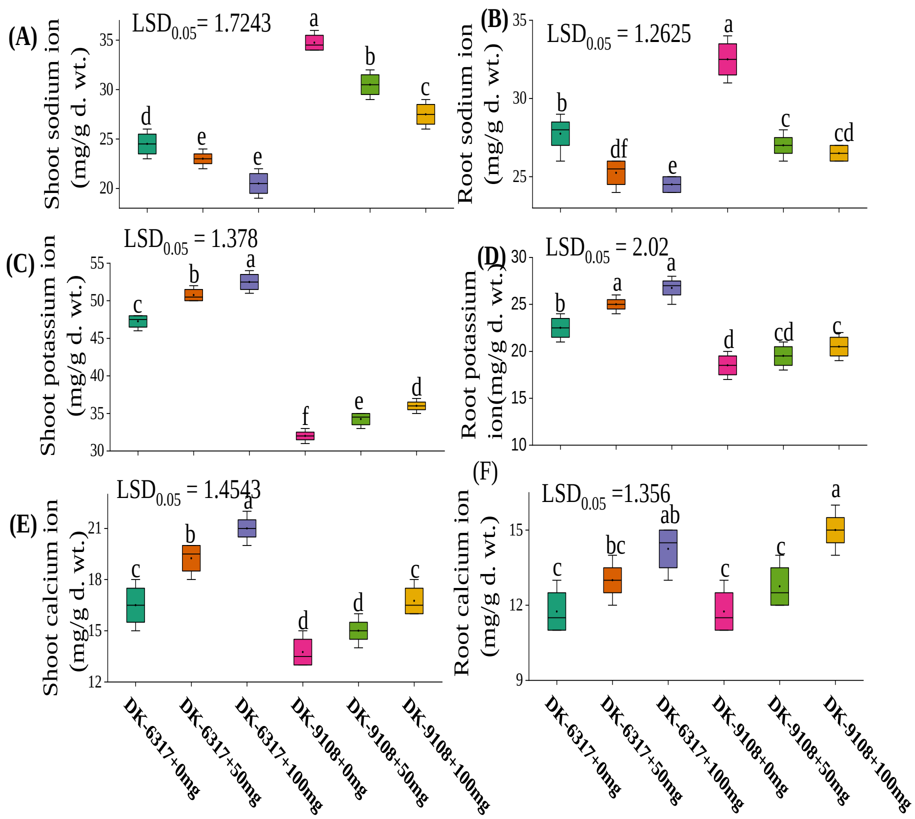

Figure 5: Effect of foliar applied L-methionine on (A) shoot sodium ion (B) root sodium ion (C) shoot potassium ion (D) Root potassium ion (E) Shoot calcium ion (F) root calcium ion of two maize hybrids i.e Dk-6317 and DK-9108

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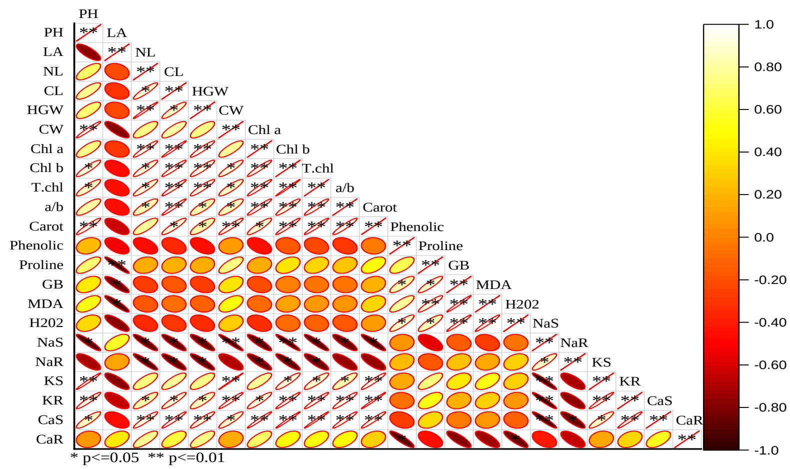

Figure 6: Pearson correlation among growth, yield, physiochemical and ionic traits i.e. plant height (PH), leaf area (LA), number of leaves (NL), cob length (CL), 100 grain weight (HGW), cob weight (CW), chlorophylls, (Chl *a*), (Chl *b*), (Chl *a/b*), (T.Chl), carotenoids (Carot), phenolic, proline, glycinebetain (GB), malondialdehyde (MDA), hydrogen peroxide(H2O2), sodium ion shoot (NaS), sodium ion root (Nar), potassium ion shoot (KS), potassium ion root (KR), calcium ion Shoot (CaS) and calcium ion root (CaR).

Correlation analysis

Pearson correlation study was exhibited to estimate the association among the studied traits (Fig. 6). Hundred grain weight (HGW) presented a positive correlation with the number of leaves (NL) and cob length, thus showing that maize hybrids exhibiting the highest number of leaves and cob length had the maximum grain weight. However, chlorophyll *a*, *b*, total and a/b ratio of chlorophyll were strongly positively correlated with the number of leaves (NL), cob length (CL), hundred grain weight (HGW), and cob weight (CW), inferring that chlorophyll related traits are significantly yield-contributing traits. Antioxidant traits, including glycinebetain (GB), exhibit a negative correlation with phenolic and proline content. While malondialdehyde (MDA) also demonstrated a negative correlation between proline and glycinebeatin (GB), indicating that hybrids with higher MDA concentrations also had higher GB and proline accumulation. Moreover, sodium shoot (NaS) and sodium root (NaR) were negatively correlated with the number of leaves (NL), cob length (CL), hundred grain weight (HGW), cob weight (CW), chlorophyll *a, b,* total, *a/b* ratio and carotenoid. Ionic contents comprising potassium in shoot (KS), potassium in root (KR) and calcium in shoot (CaS) were found to have a positive correlation with growth, yield and physiological traits whereas calcium in root (CaR) showed an insignificant correlation with growth, yield and physiological attributes.

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However, calcium in root (CaR) correlated negatively with phenolic and hydrogen peroxide (H2O2), indicating that the higher the calcium concentration in root, the lower the phenolic and hydrogen peroxide content.

DISCUSSION

The utilization of amino acid combinations and their derivatives to enhance the quality and nutritional value of legume and cereal crops is a widely adopted practice worldwide (Colla et al., 2015). In our study, we specifically focused on investigating the effects of a single amino acid, L-methionine (L-MET), on regulating the nutritional composition, growth parameters, and harvest potential of maize (*Zea mays* L.) in two maize hybrids, namely DK-6317 and DK-9108. Multiple aspects, including growth characteristics, yield parameters, physicochemical properties, and biochemical attributes, were examined following the foliar application of different doses of L-MET. The significance of L-MET lies in its status as an essential amino acid, as its deficiency in plants can reduce the nutritional value of protein-rich crops for both humans and animals (Amir and Hacham, 2002).

The findings of the current investigation elucidated the positive impact of L-methionine (L-MET) foliar application on maize hybrid growth and yield characteristics. Notably, the foliar spray treatment engendered notable improvements in biomass production. This was evidenced by significant enhancements in plant height, leaf number, and leaf area. Furthermore, crucial yield-related attributes, including cob length, cob weight, and 100-grain weight, exhibited significant improvements as a result of L-MET foliar application. The observed growth response of plants to L-MET foliar spray highlights the efficacy of even low doses of amino acids, underscoring their role not only as nutrient sources but also as signal transducers. Consequently, these molecules can act as signaling agents, mediating various advantageous physiological processes in plants (Teixeira et al., 2017; Shekari and Javanmardi, 2017).

The exogenous spray of L-methionine (L-MET) at dosage of 50 and 100 mg L-1 exerted a reinforcing effect on biomass production, thereby enhancing the growth and yield characteristics of both maize hybrids. These observations align with previous studies, including Perveen and Hussain (2021), Zulqadar et al. (2015), and Hasegawa et al. (2013), which reported similar growth-promoting effects of exogenous L-MET in maize, okra, and mustard plants. One key physiological metric crucial for plant growth is chlorophyll levels maintenance in plant leaves. This directly impacts plants' ability to trap and utilize carbon through photosynthesis (Flood et al., 2011). Even at minute concentrations, L-MET elicited a significant increase in various physiological parameters, ultimately enhancing photosynthetic activity. This can be attributed to methionine's role as a precursor to other amino acids involved in nitrogen metabolism. As nitrogen is an essential component of chlorophyll, the pigment responsible for capturing light energy during photosynthesis, augmenting nitrogen availability through methionine supplementation may positively influence photosynthetic activity.

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Plant carotenoids play a vital role in the photosynthetic antennas and reaction centers, thereby influencing light energy absorption, dissipation, and transmission throughout photosynthesis (Stange and Flores, 2012). The present study demonstrated that L-methionine (L-MET) significantly enhanced carotenoids levels in maize plants, contributing to improved photosynthetic activity. Notably, the hybrid dk-6317 exhibited higher carotenoid production than dk-9108. This enhancement can be attributed to methionine's role as a precursor in several metabolic pathways, including carotenoid biosynthesis. When provided with increased methionine, plants can produce higher carotenoids amounts. These findings align with similar studies conducted by Zulqadar et al. (2015) in Okra, where foliar L-MET treatments resulted in increased carotenoid levels. The consistency of the current results with those obtained from sweet cherry rootstocks reported by Sarropoulou et al. (2013) further strengthens the evidence supporting L-MET's positive influence on carotenoid production in various plant species.

Phenolic compounds, as highly potent non-enzymatic secondary metabolites in crop plants, serve as effective antioxidants that scavenge reactive oxygen species during specific stress conditions (Pearse et al., 2005). Our findings indicate that untreated plants exhibit optimal levels of total soluble phenolics. However, the addition of L-methionine (L-MET) at dosage of 50 and 100 mg L-1 resulted in a reduction in phenolic content, thereby enhancing plant defense responses. Proline and glycinebetaine are well-known organic osmolytes that accumulate in plant tissues in response to various abiotic stressors (Sohag et al., 2020). In line with previous studies conducted by Riffat and Ahmed (2018) and Cai and Gao (2020), our study demonstrated that L-MET foliar application led to decreased concentrations of proline and glycinebetaine. Glycinebetaine, in particular, plays multiple defensive roles, including the stability of enzyme and protein structures, the reduction of reactive oxygen species (ROS), and the maintenance of membrane integrity under stressful situations (Paradisone et al., 2015). Additionally, the exogenous application of glycinebetaine has been shown to enhance antioxidant defenses and nutrient balance in safflower seedlings (Alasvandyari et al., 2017).

Too much generation of reactive oxygen species (ROS), such as hydrogen peroxide, can impede growth under certain environmental conditions (Bonnefont et al., 2011). In our study, the exogenous treatment of L-methionine (L-MET) at a concentration of 100 mg L-1 ensued in a reduction in hydrogen peroxide levels in maize plants. This effect can be attributed to L-MET's regulatory role in maintaining appropriate hydrogen peroxide concentrations in plants (Most and Papenbrock, 2015). L-MET contains sulfur, a critical component of various antioxidants that scavenge free radicals generated by ROS. Therefore, administering L-MET helped maintain malondialdehyde concentrations in maize plants (Most and Papenbrock, 2015). Our findings are consistent with those of Miller (2010) and Quan et al. (2008), who reported that stressors significantly increase hydrogen peroxide and malondialdehyde concentrations in plants. Gong et al. (2016) also demonstrated that overexpression of S-adenosyl-methionine synthetase resulted in reduced levels of O2, H2O2, and MDA in tomatoes, further supporting our findings with previous studies.

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Stabilizing ion homeostasis through efficient ion absorption and compartmentalization is crucial for normal plant growth and development. Imbalances in ion concentrations can lead to ionic toxicity and disrupt nutritional equilibrium in plants (Rouphael et al., 2018). Our findings demonstrate that L-methionine (L-MET) treatment resulted in elevated levels of K+ and Ca2+ ions, while reducing Na+ ion content in both maize and hybrid plants. Specifically, L-MET at 50 mg and 100 mg L-1 concentrations significantly decreased Na+ content. Furthermore, a foliar spray of L-methionine substantially increased maize K+ content. K+ ions play a crucial role in managing osmotic stress in plants and regulating stomatal movement (Hasanuzzaman et al., 2018). Additionally, L-MET treatment improved the reduction of harmful ions such as Na+ in both stems and roots; resulting in a substantial enhancement of K+ and Ca2+ concentrations in both stem and root tissues of hybrid maize.

The current findings exhibit consistent outcomes with a prior investigation conducted by de Freitas et al. (2018), which examined the impact of exogenous proline application on maize. Similarly, Riffat and Ahmad (2018) reported comparable results in maize subjected to salt stress. Exogenous sulfur application led to improvements in K^+ and Ca^{+2} ions, along with a significant reduction in Na^+ toxicity. The data recorded in relation to yield metrics for both treated and untreated plants indicated that foliar application of L-MET provided protection against yield-related characteristics. Notably, exogenous L-MET resulted in a twofold increase in 100-grain weight compared to when not applied. These findings further suggest that L-MET can be utilized as a natural plant growth enhancer or plant growth regulator to enhance maize physiological performance.

The observed results revealed a significant increase in yield-related attributes, such as the cobs per plant, cob length and weight, and 100-grain weight, in both hybrid maize varieties, namely Dkl-6317 and Dkl-9108. These findings are consistent with Rady et al., (2019), who also demonstrated the beneficial impact of salicylic acid on maize yield production. Furthermore, Shahid et al. (2021) reported similar outcomes, emphasizing the improvement in yield-related characteristics through the exogenous application of L-MET. The application of L-MET to maize hybrids exhibited a positive association with key yield-contributing traits, such as 100-grain weight, cob length, and weight, which can be attributed to enhanced photosynthetic attributes. Moreover, a positive correlation was observed between ionic elements, particularly calcium and potassium. Ultimately, this facilitates yield-related traits growth and development.

CONCLUSION

The aforementioned discourse elucidates that the application of exogenous foliar L-methionine (L-MET) resulted in superior performance of maize hybrids compared to control or untreated plants. It is a reasonable inference to conclude that L-methionine augments plant photosynthetic pigments and contributes to energy conservation, enhancing plant yield. The observed augmentation in growth and yield can be attributed to heightened photosynthetic activity facilitated by increased levels of chlorophyll and

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carotenoids. Notably, the application of L-methionine at a dosage of 100 mg/L exhibited the most profound influence on maize plant growth and incited a significant increase in physiological factors within maize leaves. Hence, it can be deduced that L-methionine can increase crop yield, serving as an alternative to conventional fertilizers. Additionally, a comparative analysis between two maize hybrids, dk-6317 and dk-9108, revealed that dk-6317 manifested superior yield and growth when subjected to exogenous L-MET application compared to hybrid dk-9108. To further advance our understanding of how amino acids influence various parameters such as hormonal equilibrium, substance transfer, and antioxidant metabolic activity, future investigations should prioritize unraveling the impact of amino acids on genetic transcription. This comprehensive analysis would elucidate the functioning of amino acids as bio-stimulants in maize plants.

Contributions from the authors: The research study and methodology were created by Muhammad Arfan and Hassan Munir. Reema Arshad carried out the garden experiment, lab analysis, data collection, and initial draught writing. Farooq Ahmad reviewing, and editing the project.

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