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Original Article

Moringa leaf extract enhances the growth and yield characteristics of buckwheat genotypes by modulating the biochemical and physiological activities

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Abstract

Moringa leaf extract (MLE) as a biostimulant has demonstrated success in boosting the productivity of several agronomic crops, but its impact on Buckwheat crops remains unexplored. Buckwheat, recognized as an essential nutritional and functional food crop, often exhibits lower yields compared to major cereal crops grown in similar environments. Therefore, this research aimed to investigate the impact of different concentrations (1%, 2%, and 3%) of MLE on the agricultural performance of common buckwheat (CB) and tartary buckwheat (TB). A pot experiment was carried out according to completely randomized design with factorial arrangements having three replications. Results demonstrated significant improvements in growth parameters (branches, leaves, nodes, and internodes) for MLE-treated plants compared to the control group. Foliar treatment MLE 2% also increased chlorophyll content, improved membrane stability index (MSI) and relative water content (RWC), and enhanced biochemical composition (phenolic compounds, free amino acids, leaf proline, and soluble sugars) in both buckwheat genotypes followed by MLE 3% and MLE 1%. TB produced significantly higher grain yield (0.74 g) as compared to CB (0.43 g). The findings showed that a foliar treatment of MLE 2% led to increased grain yield in both TB (0.97 g) and CB (0.55 g) as compared to control group plants (0.37g TB and 0.22 g CB) respectively. This increase was associated with elevated activities of photosynthetic pigments, phenolic content, RWC, free amino acids, soluble sugars, and catalase in both buckwheat genotypes. In conclusion, MLE application at 2% significantly boosted the agriculture performance of buckwheat, and this study unlocked new insights into optimizing the productivity of the vital food crop.

Keywords: Biostimulant, Free amino acid, Phenolic, Buckwheat, Foliar application, Grain yield

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Introduction

Progressive future of human beings is totally depended upon the food security. It was reported that globally almost one hundred and fifty plant species are in farm practices, while only twelve among these are being used by humans for their survival (Fernie and Yan, 2019). Various crops were eradicated and genetically deteriorated due to the Green revolution (Chawla et al., 2023) Due to that currently not enough knowledge is available about the potential use of those neglected crops. To ensure food and nutritional security in scenario of climate changes, buckwheat is a component alternative to stable crop (Singh et al., 2020). These alternative crops have high nutrition, commercial value and a better diversification strategy for coping with the food insecurity (Ali and Bhattacharjee, 2023).

Buckwheat (*Fagopyrum esculentum*) forms the polygonaceae family and annual crop. It is widely growing crop while in Asiana and European countries it is included in genus Fagopyrum. It is used as a cereal crop having two species *F. esculentum* and *F. tataricum* are important (Sinkovič et al., 2020). It is a rich source of vitamin B, Zn, Cu, Mn, K, Fe, and Mg (Sofi et al., 2023). This cereal crop has distinct importance due to antibacterial, anticancer, antifatigue, and antibacterial properties (Pirzadah et al., 2020). In buckwheat, starch is the main ingredient which is more the 70 percent of the total dry weight (Chiang et al., 2023). Moist temperate, cool climate is favorable for buckwheat because it is short day plant while dry climate is also suitable for seed germination for this crop (Breslauer et al., 2023). Due to health promoting and rich nutritional profile, buckwheat has recognized as valuable functional food crop all around the world (Pirzadah et al., 2020). Cultivation of buckwheat in Pakistan is very limited while in the Indo-Himalayan region, it is considered a major crop and used as traditional food among human beings. Ye (1992) found that about fifteen species of buckwheat have reported, out of which only two species (common buckwheat and tatary buckwheat) are cultivated in Gilgit-Baltistan, the northern area of Pakistan (Ohnishi, 1994). Buckwheat's robust adaptability and minimal environmental demands make it suitable for cultivation in areas marked by arid and semi-arid conditions (Žvikas et al., 2016). Instead the increasing the growing demand of buckwheat as superfood crop, there is limited work has reported that provide the systematic information on improving buckwheat production (Babu et al., 2018).

There are various crop quality management strategies, while exogenous application is an innovative strategy for enhancing the crop quality, production and bioactive compounds specifically in arid and semi-arid regions. Furthermore, salicylates, silicone, ascorbic acid, moringa leaf extract (MLE) and vitamin B2 are the natural growth promoters which can increase the crop yield and quality (Riaz et al., 2022; Hafeez et al., 2021; Zahra et al., 2021).

MLE contained an abundance of vital macro and micronutrients, with a remarkable presence of antioxidants like soluble phenolic compounds, superoxide dismutase, amino acids, catalase, potassium, and total sugars (Aslam et al., 2020; Jahanzaib et al., 2022; Faisal et al., 2020). While for its growth promoting attributes zeatin, cytokinin considered. Foliar spray of MLE is also considered for regulating plant metabolic activities and, in abiotic stress conditions, it enhances the plant growth. While it boost about 25-30% of crop productivity (Zulfiqar et al., 2020). Buckwheat crop as functional food, there is limited number of works that provide some systematic information about the effects of MLE on the agriculture performance of buckwheat. Therefore, due to nutrient-rich composition of MLE, this study explored the potential of MLE as a bio-stimulant to enhance the agricultural performance of buckwheat by inducing physiological modifications

Material and Methods

Experimental site and plant material

Buckwheat crop seeds, i.e., common buckwheat and tartary buckwheat were acquired from a reputable farmer located in Skardu, within the Gilgit-Baltistan region of northern Pakistan. In 2022-23, the experiment was carried out in a wire house, under the proper optimal ambient light conditions, with a 10-hour photoperiod and a temperature range of 8/22°C (minimum/maximum) at the Botany Department, Ghazi University D. G. Khan-32200, Pakistan (30.05°North, 70.64°East, and 129 m above sea level).

Pot experiment details

Buckwheat genotypes' seeds were planted in plastic pots measuring 45cm in height and 30cm in diameter, filled with sandy clay loam soil. The soil had a pH of 7.5 and an electrical conductivity (EC) of 1.32 dSm^{-1}. Five seeds were initially sown in each pot, but after

emergence, only three plants were retained per pot. The recommended NPK fertilizer doses (50:40:40 kg ha^{-1}) were applied at the time of sowing, utilizing urea, diammonium phosphate, and potash sulfate as the respective sources. Tap water was used for irrigation to fulfill the plants' water requirements. The CRD (complete randomized design) design was used with factorial arrangement primarily because of two factors: (1) buckwheat genotypes and (2) MLE foliar treatment, and it comprised three replications.

Foliar application of moringa leaf extract

Fresh, healthy, and mature leaves were taken from already well established moringa trees at Ghazi University's research farm. Before the extraction process, leaves were properly cleaned and stored in freezers for twenty-four hours. A local manufactured extracted machine was used for extraction of MLE. Furthermore, sieved extract was diluted with the distilled water and made the solution with 1, 2 and 3% ratio. (Basra et al., 2011). A one-liter capacity hand sprayer was used to apply the MLE foliar spray. The foliar spray of MLE treatments was applied weekly from emergence to initiation of flowering stage.

Growth parameters

Growth parameters like number of branches, leaf, node, internode, and plant height were recorded manually. Plant height was recorded with a meter rod at maturity

Determination of photosynthetic pigments

The determination of photosynthetic pigments, including chlorophyll content (a, b, and carotenoid) in fresh buckwheat leaves, followed the method by Arnon (1949). Young, fully expanded buckwheat leaves from each treatment were collected and preserved in a biochemical freezer at -30°C. Subsequently, these mentioned biochemical parameters were measures measured within one week according to established standard procedures. The absorbance of the leaf extracts was recorded at wavelengths of 663 nm, 645 nm, and 680 nm using the spectrophotometer.

Chlorophyll content a = (12.7 × OD 663 - 2.69 × OD 645) × V / 1000 × 1 / W

Chlorophyll content b = (12.7 × OD 645 - 4.68 × OD 663) × V / 1000 × 1 / W

Carotenoid = (OD 480 + 0.114 × OD 663) × V / 1000 × 1 / W

Here, OD (optical density) represents the measurement of light absorption, V stands for the volume of the MLE in milliliters, and W represents the weight of the fresh leaves in grams. The total chlorophyll content is calculated as the sum of chlorophyll content "*a*" and "*b*".

Biochemical analysis

Total free amino acid (FAA) was measured in leaf by Lee and Takahashi (1966) method**.** Leaf phenolic and free proline contents were determined according to the procedure outline by Bates et al. (1973), using the spectrophotometer (Uv400, Pfungstadt, Germany) while, soluble sugar in leaf tissues wea determined by following the protocol method by (Karkacier et al., 2003).

Catalase and hydrogen peroxide

Catalase activity (CAT) was assessed in accordance with the methodology protocol by Chance and Maehly (1955). In a cuvette, 100 µL of enzyme extract was mixed with 1.9 mL of phosphate buffer and 1 mL of a hydrogen peroxide (H_2O_2) solution at a concentration of 5.9 mM. Then, the absorbance at 240 nm was determined using a spectrophotometer (UV 4000; ORI, Germany). The hydrogen peroxide (H_2O_2) content was quantified following the procedure described by Velikova et al. (2000), using a spectrophotometer that measured the wavelength at 390 nm. For the preparation of the hydrogen peroxide (H2O2) solution, 0.1 g of fresh leaves were crushed in pre-chilled mortar and pestle in 5 mL of trichloroacetic acid (TCA) solution of 0.1% (w/v). Following this, the leaf samples were centrifuged for 15 minutes. After the centrifugation 0.5 mL supernatant was mixed with 05 mL of potassium phosphate buffer and I ml of potassium iodide solution at pH 7

Relative water content

Sample (0.5g) of fresh leaf were submerged in water till they reached a stable weight. The fully hydrated leaves were then weighed to obtain the saturated weight (Ws). Following this, the leaves were dried for 24 hours at 80 ℃ to ascertain the dry weight (Wd). The calculation of leaf relative water content (RWC) was performed using the formula established by Barrs and Weatherley (1962).

RWC = (Wf − Wd/Ws − Wd) × 100

Membrane stability index

Fresh leaf tissue, 200 mg, was diced into small fragments and immersed in 10 mL of distilled water within boiling tubes. The samples were heated at

40°C for 30 minutes using a water bath, and the electrical conductivity of the resulting solution (EC1) was measured with a conductivity meter. Another sample subjected to the same treatment was heated at 100°C for 10 minutes, and the electrical conductivity of this solution (EC2) was likewise recorded. The leaf MSI% was then calculated using the equation formulated by Premachandra et al. (1990).

MSI = [1- EC1/EC2] x 100

Yield-related traits

Each pot's plants were individually labeled to calculate yield-related characteristics at maturity. The plants were manually harvested and allowed to air-dry in an open environment for two days. Following the drying period, oven dry weight of plants was determined after the drying the plant material in oven at 70°C for 48 hours. Grains were weighed using an electric balance and grain yield was recorded. The harvest index (HI) was also calculated by the following formula:

HI = Grain yield/Plant dry weight × 100

Statistical analysis

All data, including the growth, physiological parameters, and yield-related characteristics, were presented as mean values. Statistical analysis was conducted using analysis of variance (ANOVA) with SAS software, version 9.1 (Institute, Cary, NC, USA), followed by the LSD test at 5% probability level.

Results

A statistically significant difference (P≤0.05) was evident in various growth, biochemical, physiological membrane stability index, leaf relative water content), and yield-related traits among the foliar application of MLE treatments and buckwheat genotypes (Table 1). Except leaf phenolic content, soluble sugar levels, H_2O_2 concentration, and grain yield, non-significant interaction (Foliar Treatments* Genotypes) was observed for all the measured traits in this study (Table 1).

Growth related attributes

Growth data of buckwheat plants i.e. number of branches, number of leaves, number of nodes, and number of internodes presented in Table 2 and 3. A significant difference (p≤0.05) was recorded among foliar application of MLE treatments for growth related attributes (Table 1). Foliar treatment with MLE significantly enhanced the all the growth traits of buckwheat genotypes as compared to control (Table 2 and 3). Maximum value of growth traits was observed in foliar treatment with MLE 2% followed by MLE 3% and MLE 1%, while lower number of branches, number of leaves, number of nodes, and number of internodes was found in control (Table 2 and 3). Statistical difference (P≤0.05) was found between the buckwheat genotypes for number of branches and number of leaves. Tartary buckwheat (TB) produced the highest number of branches and number of leaves per plant than common buckwheat (CB) Table 2.

Table-1. Mean square values (P < 0.05) for yield-related traits, growth, and physiological character and yield traits of buckwheat genotypes grown under foliar treatments of MLE

Traits	Treatment (T)	Genotypes (G)	G*T
Degree of freedom	3	1	3
Growth and Agronomic traits			
Plant height (cm)	412.859 ***	412.859 ***	15.837$^{n.s}$
No of branches plant^{-1}	4.77778 **	4.16667 ***	0.50000$^{n.s}$
No of leaves plant^{-1}	32.3750 ***	22.0417 ***	2.3750$^{n.s}$
No of node plant^{-1}	6.48611***	2.04167$^{n.s}$	0.15278$^{n.s}$
No of internode plant^{-1}	5.81944**	3.37500$^{n.s}$	0.26389$^{n.s}$
No of seed plant^{-1}	260.042***	260.042***	10.153$^{n.s}$
Grain yield plant^{-1}(g)	0.25398***	0.58219***	0.03924*
Biological yield plant^{-1}(g)	1.16221***	1.93802***	0.02216$^{n.s}$
Harvesting Index(HI%)	203.376**	296.280 **	45.118$^{n.s}$
Physiological traits			
Chlorophyll *a* (mg/g fwt)	0.00541***	0.01005***	0.00034$^{n.s}$
Chlorophyll *b* (mg/g fwt)	0.00541***	0.01005***	0.00034$^{n.s}$
Carotenoid (mg/g fwt)	0.17962***	0.23187***	0.01088$^{n.s}$
Total chlorophyll	7.162***	0.00143***	1.370$^{n.s}$
Proline(µmol /g fwt)	6.104***	5.104***	8.486$^{n.s}$
Phenolics (µmol /g fwt)	0.03454***	0.02653***	0.00554**
Soluble sugar (µmol /g fwt)	0.54301***	1.57953***	0.03643 *
Free amino acid (mg/g fwt)	0.09541***	0.06827***	0.00054$^{n.s}$
Catalase (mg/g fwt)	4.4948 ***	2.47042***	0.08486$^{n.s}$
H_2O_2 (µmol /g fwt)	0.00789***	0.00060**	0.00100***
Membrane stability index	173.357***	125.456***	5.502$^{n.s}$
Relative Water Content	825.126***	133.043***	10.678$^{n.s}$

G = buckwheat genotypes, T= MLE treatments; * = significant (<0.05), ** = more significant (<0.01), *** = highly significant (<0.00), n.s. = non-significant

Table-2. Mean number of branches, number of leaves, and number of nodes plant of common buckwheat (CB) and tartary buckwheat (TB) grown under the foliar treatment of moringa leaf extract.

Treatments	Number of branches per plant			Number of leaves per plant			Number of nodes per plant		
	CB	TB	Mean (T)	CB	TB	Mean (T)	CB	TB	Mean (T)
Control	2.3	3.0	2.66 c	5.0	6.7	5.83 c	3.7	3.3	3.50 c
MLE 1%	3.7	4.0	3.83 b	8.3	9.0	8.66 b	5.7	4.7	5.16 ab
MLE 2%	4.0	5.7	4.83 a	10.3	12.0	11.2 a	6.3	5.7	6.00 a
MLE 3%	3.7	4.3	4.0 b	8.3	12.0	10.2 ab	5.0	4.7	4.83 b
Mean (G)	3.4 b	4.3 a		8.0 b	9.9 a		5.2 a	4.6 a	
CVCs	G=0.57, T=0.81, G×T=ns			G=1.15, T=1.62, G×T=ns			G=0.77, T=1.09, G×T=ns		

CVC = Critical value for comparison; G = Genotypes, CB = Common buckwheat, TB = Tartary buckwheat T = Treatments.

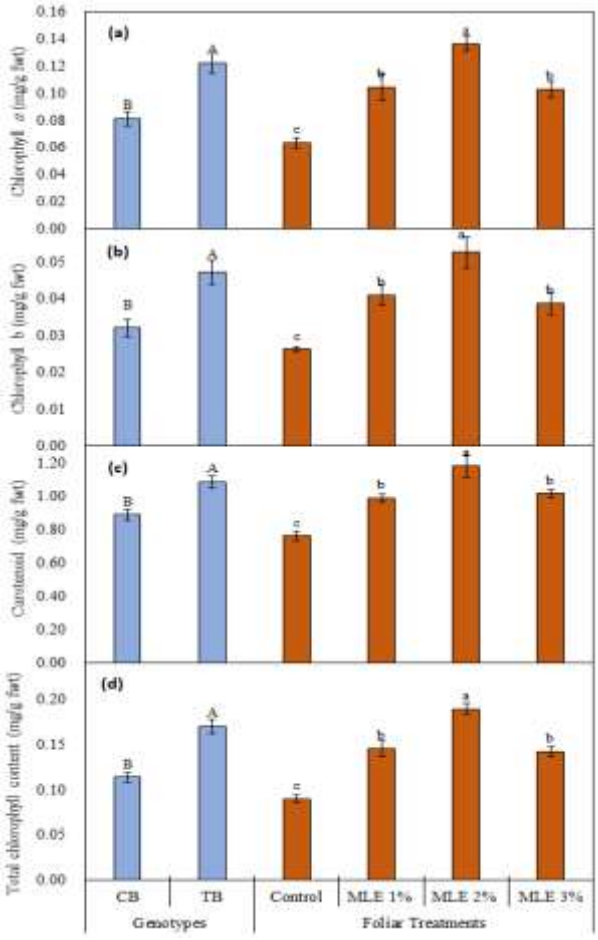

Figure-1. Effect of foliar treatments with moringa leaf extract (MLE) on biosynthesis of photosynthetic pigments i.e. (a) chlorophyll *a*, (b) chlorophyll *b*, (c) carotenoid, and (d) total chlorophyll content in leaves of common buckwheat (CB) and tartary buckwheat (TB). Same letter on bars mean they did not differ significantly at *p* = 0.05.

Biosynthesis of photosynthetic pigments

The biosynthesis of photosynthetic pigments i.e. chlorophyll content *a*, chlorophyll content *b*, carotenoid and total chlorophyll content were recorded at the peak in buckwheat genotypes (Figure 1). Significant variation ($P \leq 0.05$) was observed among MLE application treatments and buckwheat genotypes for biosynthesis photosynthetic pigments. Foliar application of MLE significantly stimulated the biosynthesis of chlorophyll content *a*, *b* and carotenoid in both buckwheat genotypes as compared to control (Figure 1). Maximum value of chlorophyll contents was observed in MLE 2% treated plants followed by 3% and 1% (Figure 1). While among buckwheat genotypes TB showed maximum value of chlorophyll content *a*, *b* and carotenoid than common CB (Figure 1).

Leaf phenolic, soluble sugar and hydrogen peroxide

The impact of foliar application of MLE treatments on biochemical analysis i.e. leaf phenolic content, leaf soluble sugar and hydrogen peroxide are shown in Figure 2. Remarkable impact of foliar treatment of MLE on H_2O_2 activities in buckwheat genotypes (Figure 2a). Lowest value of H_2O_2 concentration was observed in MLE 2% treated plants of TB and CB genotypes respectively followed by MLE 3% and MLE 1% (Figure 2a). Maximum H_2O_2 activities were found in non-treated plants (Figure 2a). Foliar treatment of MLE significantly enhanced the activities of leaf phenolic contents and soluble sugar content. Maximum value of leaf phenolic (Figure 2b) contents and soluble sugar (Figure 2c) was found in MLE 2% treated plants of TB and CB respectively. While the minimum value of leaf phenolic contents and soluble sugar was recorded in control group plants (Figure 2b-c)

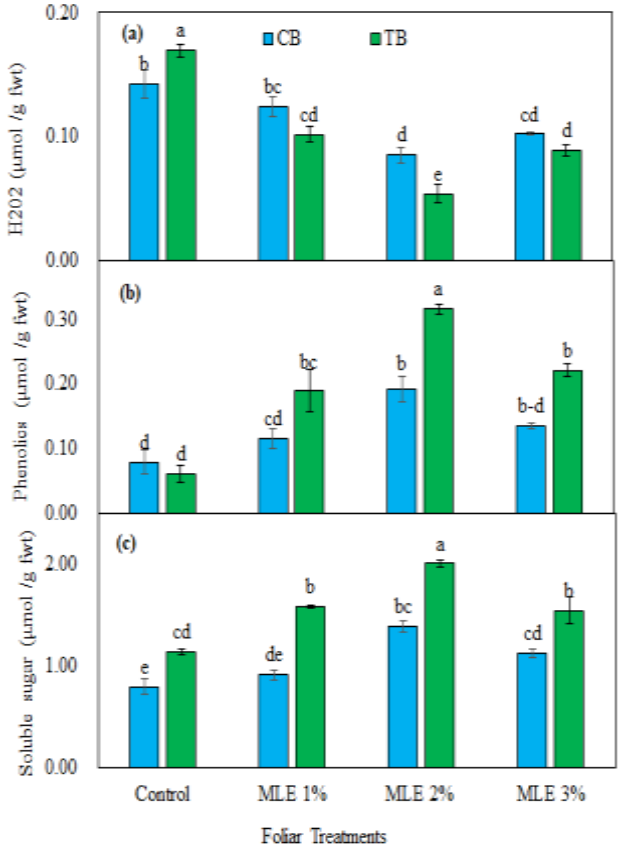

Figure-2. Effect of foliar treatments with moringa leaf extract (MLE) on (a) hydrogen peroxide (H_2O_2), (b) leaf phenolic content, and (c) leaf soluble sugar of common buckwheat (CB) and tartary buckwheat (TB). Same letter on bars mean they did not differ significantly at p = 0.05.

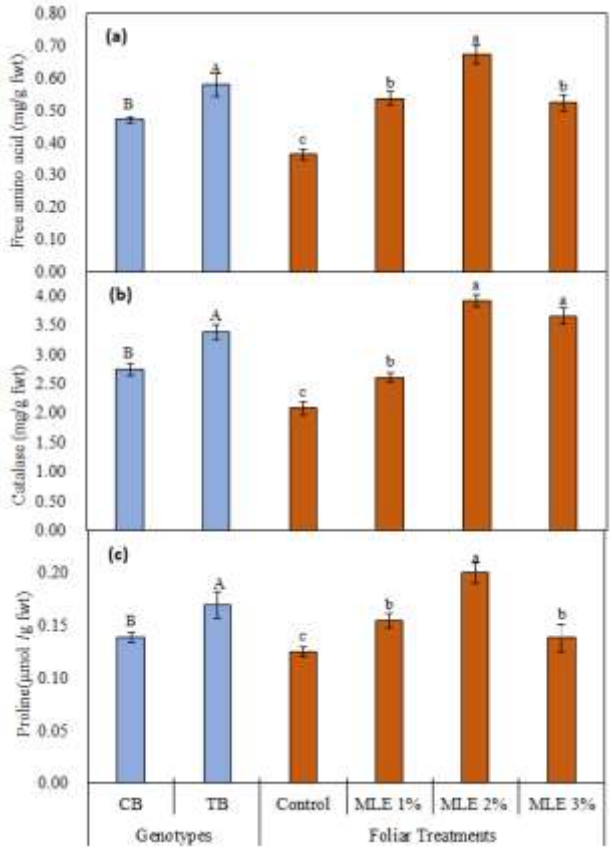

Figure-3. Effect of foliar treatments with moringa leaf extract (MLE) on (a) free amino acid (FAA) in leaf, (b) catalase (CAT) in leaf, and (c) leaf proline in common buckwheat (CB) and tartary buckwheat (TB). Same letter on bars mean they did not differ significantly at p = 0.05.

Free amino acid and catalase activities

MLE significantly stimulated the free amino acid (FAA), catalase (CAT) and leaf proline activates in both buckwheat genotypes (Figure 3a-c). FAA and CAT and praline activity was most remarkable in MLE foliar treated plants compared to control. The highest activity of FAA, CAT and leaf proline was recorded in MLE 2% treated plants followed by MLE 3% and MLE 1% (Figure 3a-c). Minimum value FAA and CAT and proline concentration was found in control group plants. Among the genotypes, the lowest activity of FAA, CAT and leaf proline was recorded in TB compared to CB genotypes (Figure 3a-c)

Membrane stability index (MSI) and leaf relative water content (RWC)

Significant variation ($p \leq 0.05$) was observed among MLE foliar treatments and buckwheat genotypes for MSI and leaf RWC. Foliar application of MLE significantly improved the leaf RWC and MSI in both buckwheat genotypes as compared to control (Figure 4a-b). Maximum MSI and leaf RWC was found in MLE 2% treated plants followed by MLE 3% and MLE 1% (Figure 4a-b). Among buckwheat genotypes, TB significantly improved the MSI and leaf RWC traits compared to CB (Figure 4).

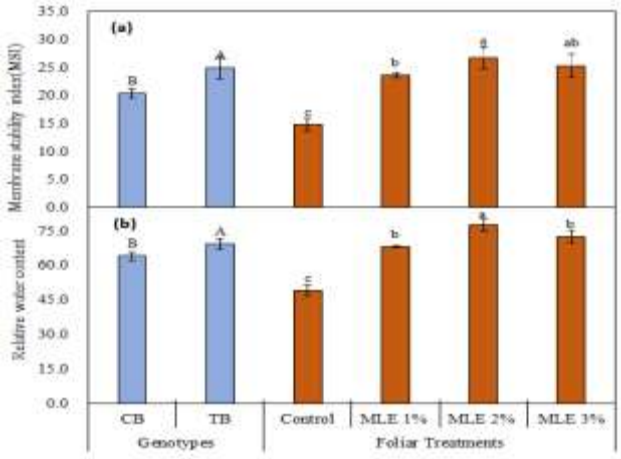

Figure-4. Effect of foliar treatments with moringa leaf extract on (a) membrane stability index (MSI%), (b) leaf relative water content (RWC%) in leaves of common buckwheat (CB) and tartary buckwheat (TB). Same letter on bars mean they did not differ significantly at p = 0.05.

Agronomic and yield attributes

Significant variation was observed among the genotypes and moringa treatments for yield related attributes such as plant height, number of seed per plants, grains yield per plant, biological yield per plant and harvest index. Foliar treatment with MLE significantly enhanced all agronomic and yield characteristics of buckwheat genotypes as compared to control (Table 3 and 4). Maximum value of yield traits was observed in foliar treatment with MLE 2% followed MLE 3% and MLE 1%, while lower value of number of seed per plants, grains yield per plant, biological yield per plant and harvest index internodes was found in control (Table 3 and 4). Moreover, TB has higher number of seed and grain yield per plant than CB (Table 3 and 4).

Table-3. Mean number of internodes, seeds per plant, and plant height of common buckwheat (CB) and tartary buckwheat (TB) grown under the foliar treatments of moringa leaf extract.

Treatments	Number of internodes per plant			Number of seeds per plant			Plant height (cm)		
	CB	TB	Mean (T)	CB	TB	Mean (T)	CB	TB	Mean (T)
Control	3.0	2.3	2.66 c	6.0	10.3	8.17 c	19.3	30.3	24.8 c
MLE 1%	5.0	3.7	4.33 ab	11.0	19.7	15.3 ab	33.7	40.5	37.1 b
MLE 2%	5.3	4.7	5.00 a	13.0	22.0	17.5 a	42.3	47.7	45.0 a
MLE 3%	4.0	3.7	3.83 b	10.7	15.0	12.8 b	34.0	37.3	35.7 b
Mean (G)	4.3 a	3.6 a		10.2 b	16.7 a		32.3 b	38.9 a	
CVCs	G=0.82, T=1.16, G×T=ns			G = 1.82, T =2.57, G×T=ns			G = 3.09, T =4.34, G×T=ns		

CVC = Critical value for comparison; G = Genotypes, CB = Common buckwheat, TB = Tartary buckwheat T = Treatments.

Table-4. Mean grain yield, biological yield per plant, and harvesting of common buckwheat (CB) and tartary buckwheat (TB) grown under the foliar treatments of moringa leaf extract.

Treatments	Grain yield per plant (g)			Biological yield per plant (g)			Harvesting Index (HI%)		
	CB	TB	Mean (T)	CB	TB	Mean (T)	CB	TB	Mean (T)
Control	0.22	0.37	0.29 c	1.5	2.1	1.81 b	15.7	18.2	16.9 b
MLE 1%	0.47	0.67	0.56 b	2.2	2.8	2.47 a	21.8	24.1	22.9 b
MLE 2%	0.55	0.97	0.75 a	2.2	2.6	2.41 a	24.6	37.7	31.2 a
MLE 3%	0.47	0.88	0.70 a	2.6	3.2	2.88 a	18.4	28.7	23.6 b
Mean (G)	0.43 b	0.74 a		2.1 b	2.7 a		20.2 b	27.1 a	
CVCs	G=0.09, T=0.13, G×T=00000			G=0.34, T=0.49, G×T=ns			G = 4.83, T= 9.66, G×T=ns		

CVC = Critical value for comparison; G = Genotypes, CB = Common buckwheat, TB = Tartary buckwheat T = Treatments.

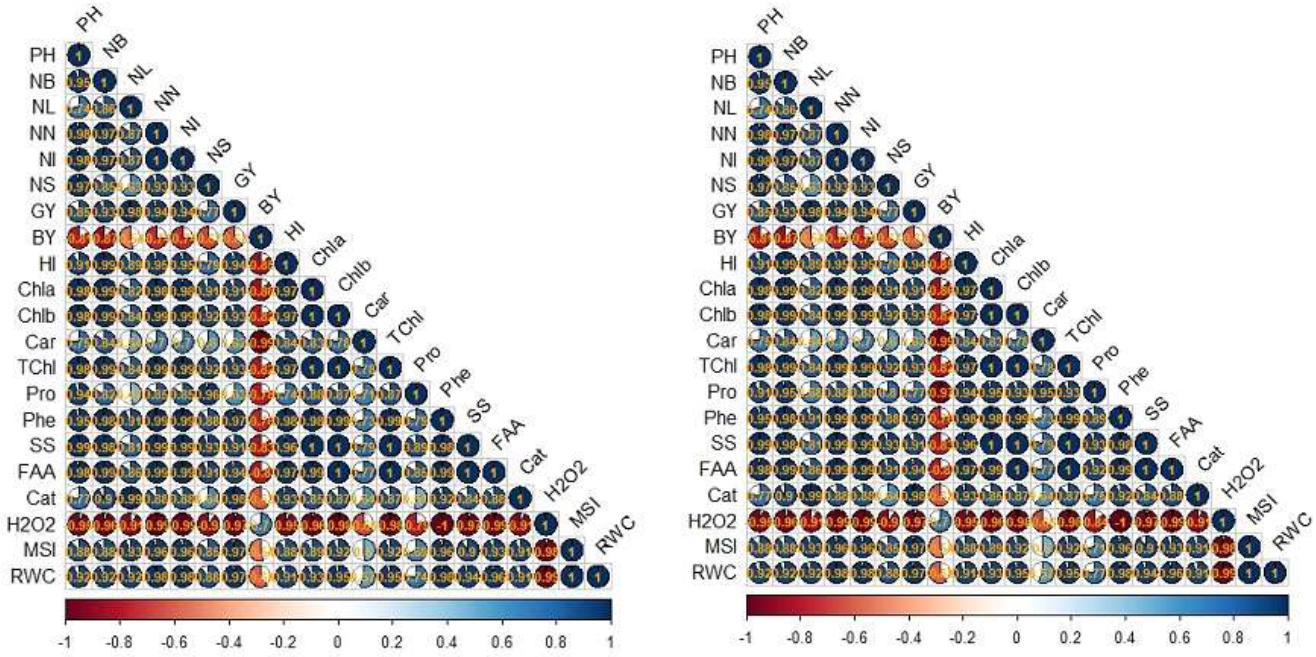

Figure-5. Pearson's correlation among growth, yield, quality, biochemical attributes of common buckwheat (A) and tartary buckwheat (B) cultivated. Number of branches (NB), number of leaves (NL), number of node (NN) and number of internode (NIN), plant height (PH), number of seed (NS), grain yield (GY), biological yield (BY), chlorophyll a, (Chla a) chlorophyll b (Cha b), carotenoid (Car), leaf proline content, (Prol) leaf phenolic (Phenol), leaf soluble sugar (SS), free amino acid (FAA), catalase (CAT), hydrogen peroxide (H2O2), membrane stability index (MSI%), leaf relative water content (RWC%).

Correlation

The correlation among growth, yield, quality, and biochemical attributes of common buckwheat (A) and tartary buckwheat (B) are presented in Figure 5. A positive and strong correlation was observed among all the traits except H_2O_2. Hydrogen peroxide (H_2O_2) had a negative correlation with the all-reaming traits in both CB and TB genotypes (Figure 5A-B).

Discussion

This research explored the effect of MLE (moringa leaf extract) on agriculture performance of buckwheat crop. Results of the experiment confirmed that foliar applied with 2% MLE treatment significantly improved the growth traits (Table 2 and 3), agronomic and yield characteristic (Table 4), which was strongly correlated with the physiological and biochemical traits (Figure 5). MLE contained many secondary metabolites and growth regulators such as cytokines, that may have enhanced the plant growth. MLE contains cytokinin hormone which is responsible for increasing the number of leaf and area because this hormone actually increase the leaf bud formation and cell division (Elzaawely et al., 2017). Latif and Mohamed (2016) reported that foliar application of MLE had a remarkable effect on growth of common beans by controlling stem elongation, improved the GA metabolism and signaling activities. Due to the presence of nitrogen, phosphate, and potassium in MLE, it dramatically increases the number of leaf also in wheat and okra plants (Meireles et al., 2020). Our result is supported by many studies, in tomatoes it boosts the number of nodes due to the presence of gibberellins, cytokinins, and auxins in MLE (Khan et al., 2017a; Khan et al., 2020; Zahra et al., 2022; Ali et al., 2018)

Statistically significant difference was observed among the MLE foliar treatments for physiological traits (Table 1). Foliar-applied MLE treated buckwheat plants significantly enhances their ability for biosynthesis of chlorophyll pigments i.e.,

chlorophyll content "a" and chlorophyll content "b", carotenoid, and total chlorophyll content than control group plants (Figure 1a-d). This effect of MLE may be attributed to the enhance the concentration of phytohormones such as chlorophyll and carotenoids in plant (Ali et al., 2018). The utilization of MLE has shown promising potential in increasing the concentration and activity of chlorophyll pigments in wheat and quinoa (Khan et al., 2020; Rashid et al., 2021). In addition, Moringa plant leaves have rich source of macronutrients that increase the chlorophyll concentration. MLE promotes the early production of cytokinins, which helps prevent premature leaf aging. This, in turn, leads to an expansion in leaf surface area and an enhancement in the synthesis of photosynthetic pigments (Rashid et al., 2021). Iron and magnesium are very important components for chlorophyll synthesis and these are also helpful for the proporphyrin transformation into chlorophyllide while, MLE is the excellent source of iron and magnesium (Biel et al., 2017). Irshad et al. (2022) found that the MLE applied treatment led to elevated chlorophyll levels in chickpea plants. Bakhtavar et al. (2015) also found that in early sowing of maize results maximum content of chlorophyll due to the MLE application. These results have similarity with the results of this research study. Foliar application of MLE under the favorable growing condition significantly improved the photosynthetic pigments in wheat (Khan et al., 2017a). Foliar applied MLE at the tillering and topping stages of wheat plants, has been shown to increase the concentrations of both chlorophylls *a* and *b* (Nawaz et al., 2013). The observed growth enhancement in plants following MLE foliar application may be attributed to the presence of various metabolites, including zeatin and phenolic compounds (Foidl et al., 2001).

Among the treated plants with MLE, improves the leaf phenolic contents, soluble sugar content (Figure 2b-c), free amino acid, CAT and leaf proline according to the results (Figure 3a-c). The scientists hypothesized that the increase was due to the presence of osmoprotectants like soluble sugar, free amino acid, free proline and phenolic compound in the Moringa leaf extract. These osmoprotectants may translocated from MLE solution to active parts of plants and increased their endogenous concentration increase RWC and MSI which contribute to health and turgidity of cell (Abd El-Mageed et al., 2017). Moringa leaf extract as biostimulant that contributed to maintaining the membrane integrity, as observed in present study (Figure 4a-b). It is also reported that proline and sugar are compatible solute contributing the osmotic adjustment (Abd El-Mageed et al., 2017). Culver et al. (2012) found the similar result of MLF on the soluble sugar content of rape plants.

The heightened concentrations of phytohormones, flavonoids, and phenolics in moringa leaves, as observed by Mashamaite et al. (2022), may contribute to the increased levels of these compounds in MLE-treated plants. Additionally, the optimal presence of carotenoids, vitamins, and minerals in moringa could influence metabolic processes, potentially elevating phenolic concentrations in buckwheat (Figure 2b). This dual action positions MLE as a growth promoter and natural antioxidant. Verifying our findings, Nasir et al. (2016) reported that MLE application during critical growth periods increased total phenolic content. Remarkably, our results (Figure 2a) indicate a significant reduction in H_2O_2 content in plants treated with MLE. Antioxidants such as alpha-tocopherol and catalase, when taken up from the leaf surface and translocated into cells, play a crucial role in enhancing plant tolerance by improving their ability to effectively scavenge reactive oxygen species (Abd El-Mageed et al., 2017). The reduced levels of H2O2 can be attributed to the antioxidants present in Moringa leaf extract, including polyphenols and flavonoids (Zahra et al., 2021). Foliar application of MLE enhanced the agricultural performance of buckwheat plants, leading to improvements in number and weight of grain, and overall yield (refer to Table 3 and 4). The grain yield was strongly linked with the growth traits i.e. number of branches and number of leaves, and including the physiological traits i.e. RWC, MSI, proline content and levels of photosynthetic pigments a and b (Figure 5). These findings align with previous research, such as the studies conducted by Khan et al. (2017a) and Khan et al. (2017b). Basra et al. (2011) and Yasmeen et al. (2016) also reported the positive effects of MLE on crop growth and yield of field crops due to the presence of ascorbate, essential phyto-components, zeatin, vitamin, carotenoid and antioxidants. Numerous research has been reported that the application of MLE can significantly enhance crop yields by approximately 20-35% under both normal and stressful conditions (Elzaawely et al., 2017). Additionally, research has indicated that the exogenous application of MLE can elevate endogenous hormone levels, thereby promoting plant growth (Khan et al., 2017b; Irshad et al., 2022).

These results are in harmony with Phiri and Mbewe (2010), they reported that grain formation and increase in wheat production is due to the presence of micronutrients in MLE. Further, Afzal and Iqbal (2015) also reported that foliar application of MLE during the crucial growth stage of wheat increases the 1000-grain weight, quality, and biological yield. MLE is also responsible for increasing the fresh and dry weight, shoot and root length and net assimilation rate of wheat. Yasmeen et al. (2012) reported that the fresh and stored MLE, used separately and in combination with plant growth boosters, enhanced the number biomass, 1000 grains weight, vegetative production and the economic yield of wheat.

Conclusion

Moringa, well-known for its rich mineral nutrients and antioxidants, emerges as a potent agent in boosting the productivity of buckwheat genotypes. MLE-treated buckwheat plants exhibit a remarkable maintenance of essential factors, including higher RWC, MSI, and levels of photosynthetic pigments (a and b). The application of 2% MLE via foliar spray emerges as a effective strategy, resulting in a substantial improvement in buckwheat agriculture performance. The findings of this study not only shed light on the positive correlation between MLE application and buckwheat yield but also opens avenues for further exploration and application of MLE in optimizing the buckwheat productivity

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Sabir IA & Alam P: Funding acquisition and literature review
Leghari SK: Conceptualization of the study and developed research methodology
Saddiq MS: Conceptualization of the study and writing of original draft of manuscript
Khan S: Writing of original draft, critical review and editing of the manuscript
Fatima EM & Raza MH: Statistical analysis and data interpretation
Sajid M & Iqbal R: Critical review and editing of the manuscript
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