

## Article

# Exogenous Application of Biostimulants and Synthetic Growth Promoters Improved the Productivity and Grain Quality of Quinoa Linked with Enhanced Photosynthetic Pigments and Metabolomics

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**Abstract:** Modern agriculture is mainly concerned with maximum resource use efficiency linked with greater productivity to feed the growing global population. The exogenous application of biostimulants is considered a sustainable approach to improve the growth and productivity of field crops. The present study was carried out to explore the comparative impact of biostimulants and synthetic compounds on quinoa crop (cultivar UAF-Q7), as it has gained significant popularity among agricultural scientists and farmers throughout the world, due to its high nutritional profile. A two-year field experiment was carried out at the Research Area of Directorate of Farms, University of Agriculture, Faisalabad, Pakistan. Application of moringa leaf extract (MLE) produced the maximum total chlorophyll ( $5.11 \text{ mg g}^{-1}$ ) and carotenoids ( $1.2 \text{ mg g}^{-1}$ ), compared with the control. Antioxidants' activities and gas exchange attributes were also recorded as the highest following MLE application. Mineral elements in root and in shoot were found highest in response to MLE application. Similarly, application of MLE significantly improved the growth and yield attributes of quinoa. Mineral elements of grain (Mg, Zn and Fe) were also significantly enhanced. MLE was found to be more responsive in improving the growth and quality compared with synthetic compounds.

**Keywords:** antioxidant; gas exchange; growth; moringa leaf extract; proline; quinoa; yield

## 1. Introduction

*Chenopodium quinoa* (Willd.), a pseudocereal, originated from Andean regions and belongs to the family of *Amaranthaceae* [1]. The global production of quinoa is increasing significantly, being more than 161 thousand metric tons in 2019, and a major share of this production was from Peru and Bolivia [2]. Due to its high nutritional profile, quinoa is also known as “functional food” because it prevents various chronic disease [3]. It is a rich source of good quality protein, essential amino acids (particularly lysine (5.1–6.4%)

and methionine) [4,5], dietary fiber, nutrients (Ca, P, K, Fe, Zn, and Mg), and vitamins (A, B2, and E) [6]. Interestingly, quinoa grains contain small amounts of the important polyunsaturated fatty acids Omega-3 and Omega-6 [7] and are gluten-free. Additionally, quinoa seeds are used to lose weight and protect against coronary heart disease [8] as well as to lower blood sugar and insulin levels [9]. Quinoa has a high level of resilience to a variety of common environmental stresses, including drought, frost, soil salinity, disease, and pests [10,11]. Worldwide, demand for quinoa cultivation is increasing because it can survive under harsh environmental conditions as well as providing excellent nutritional content [1]. To improve quinoa yield, various strategies have been developed, one being the foliar spray of different natural and synthetic plant growth promoters at critical growth stages [12–14].

Currently, researchers are more interested in natural plant growth promoters to increase plant growth and development, because they are environmentally friendly. Natural or synthetic plant growth promoters are rich in phenolic compounds, antioxidants and nutrients which help enhance the fresh plant, dry biomass, and yield, by improving the nutrient uptake of roots [15–17]. Plant growth promoters (PGP) stimulate various physiochemical processes, which have a positive effect on economic yield and seed quality as well as increased tolerance against biotic and abiotic stresses [14,18]. Moreover, exogenous use of plant growth promoters improves seed germination [19–21] and plant growth [22,23], delays senescence [24], enhances root biomass [25,26] for mineral absorption, and hence increases seed quality [27,28] and quantity [29,30]. Hassanein et al. [31] reports that the addition of nano zinc fertilization at low concentrations combined with biostimulants, especially seaweed and moringa extracts, is an effective alternative to regular fertilization methods and contributes to the sustainable development of horticultural crops [32,33]. The foliar application of liquid seaweed extract [34] and organic compounds [35] was reported as being more applicable regarding nutrient availability for optimum development and growth, and an improved quality of field crops cultivated under unfavorable environments [36,37].

Among different natural PGPs, it has been reported that 3% water extract of moringa leaf extract (MLE) and sorghum (sorgaab) are very effective for plant growth and development under both control and environmentally stressful conditions [38]. Moringa leaf juice is used as plant growth regulator because it improves the seed germination rate, plant development and yield by about 25–30% [39]. Toscano et al. [40] reported that the biostimulant impact of MLE varies from species to species, and even cultivar to cultivar. MLE is rich in antioxidants, protein, minerals and beta-carotene, which are normally deficient among the food of under-developed countries [41]. Moreover, MLE is an excellent source of zeatin (phytohormone) which helps to stimulate crop growth, development and grain yield, not only under normal conditions but even under harsh weather conditions [42,43]. External use of MLE was also found very effective in improving the growth and biochemical attributes of rocket plants [44], wheat [13] and quinoa crop [38]. At low concentration, sorghum water extract (sorgaab) is used on crops as a growth promoter because it is a good source of phenolics and other growth improving elements [45]. It has been reported that exogenous application of sorgaab on wheat [46] and rice [47] decreased weed density and dry weight, and increased crop yield. Application of mineral elements either alone or in combination with plant growth promoters improved the growth attributes of agronomic and horticultural crops [48,49].

There are also many synthetic plant growth promoters such as ascorbic acid and hydrogen peroxide, which play a crucial role in plant developmental processes [38]. Ascorbic acid (AsA) is ubiquitously present in plants and is involved in cell division, cell expansion, photosynthesis and in scavenging of reactive oxygen species [50]. It is also important as a cofactor for a large number of photosynthetic enzymes in plants [51]. At low concentrations, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) acts as a signaling molecule and increases plant tolerance against adverse environmental conditions by improving the antioxidant defense system [52]. It also enhances seedling growth, photosynthetic rate, and stomatal conduc-

tance of plants [53,54]. As previously reported, at low concentrations, exogenous use of  $H_2O_2$  has improved the physiological process of wheat [55], as well as cotton yield, by regulating enzymatic antioxidants' activities [56]. Keeping in view the above rationale, the aim of the current study was to investigate the comparative impact of natural (moringa leaf extract and sorghum water extract) and synthetic (ascorbic acid and hydrogen peroxide) growth enhancing substances on the growth, gas exchange attributes, physiological, biochemical and yield parameters of quinoa crop.

## 2. Materials and Methods

### 2.1. Experimental Particulars

The field experiment was conducted at the research area of the Directorate of Farms, University of Agriculture, Faisalabad, Pakistan ( $31.4180^\circ$  N and  $73.0790^\circ$  E) during the crop cultivation season of 2016–2017. The same experiment was repeated during the next year (2017–2018) to validate the results obtained in the first experiment. UAFQ-7 is a new and first variety of quinoa approved by the Federal Seed Certification and Registration Department, Islamabad, Pakistan [57]. Five seeds per hill of cultivar UAF-Q7 were sown in the soil manually with a spacing of 30 and 15 cm from row to row, and hill to hill, respectively. At the two-leaf stage, thinning was performed to maintain one seedling per hill. There were five rows (10 plants in each row) with 50 plants in each experimental unit of  $150\text{ cm} \times 150\text{ cm}$  area. Furthermore, N, P, and K was supplied at a rate of  $75\text{ Kg ha}^{-1}$ ,  $60\text{ Kg ha}^{-1}$  and  $60\text{ Kg ha}^{-1}$ , respectively. To fulfill the requirements of primary nutrients, muriate of potash (60%  $K_2O$ ), diammonium phosphate (46%  $P_2O_5$  and 18% N) and urea (46% N) were used. The complete quantity of P and K together with one third of the quantity of N were used at the time of sowing, and the remaining N was split equally and applied at the first and second irrigation. Four irrigations were supplied throughout the course of experimentation. Weeds were controlled manually. The design of the experiment was randomized complete block design (RCBD) with four replicates per treatment. The treatment plan consisted of control (no spray), moringa leaf extract (MLE) at 3%, sorghum water extract (sorgaab) at 3%,  $H_2O_2$  at  $100\ \mu\text{M}$ , and ascorbic acid (AsA) at  $500\ \mu\text{M}$  to study the above-discussed objectives. The weather data, including maximum temperature, minimum temperature, average temperature, relative humidity and rainfall, are presented in Figure 1.

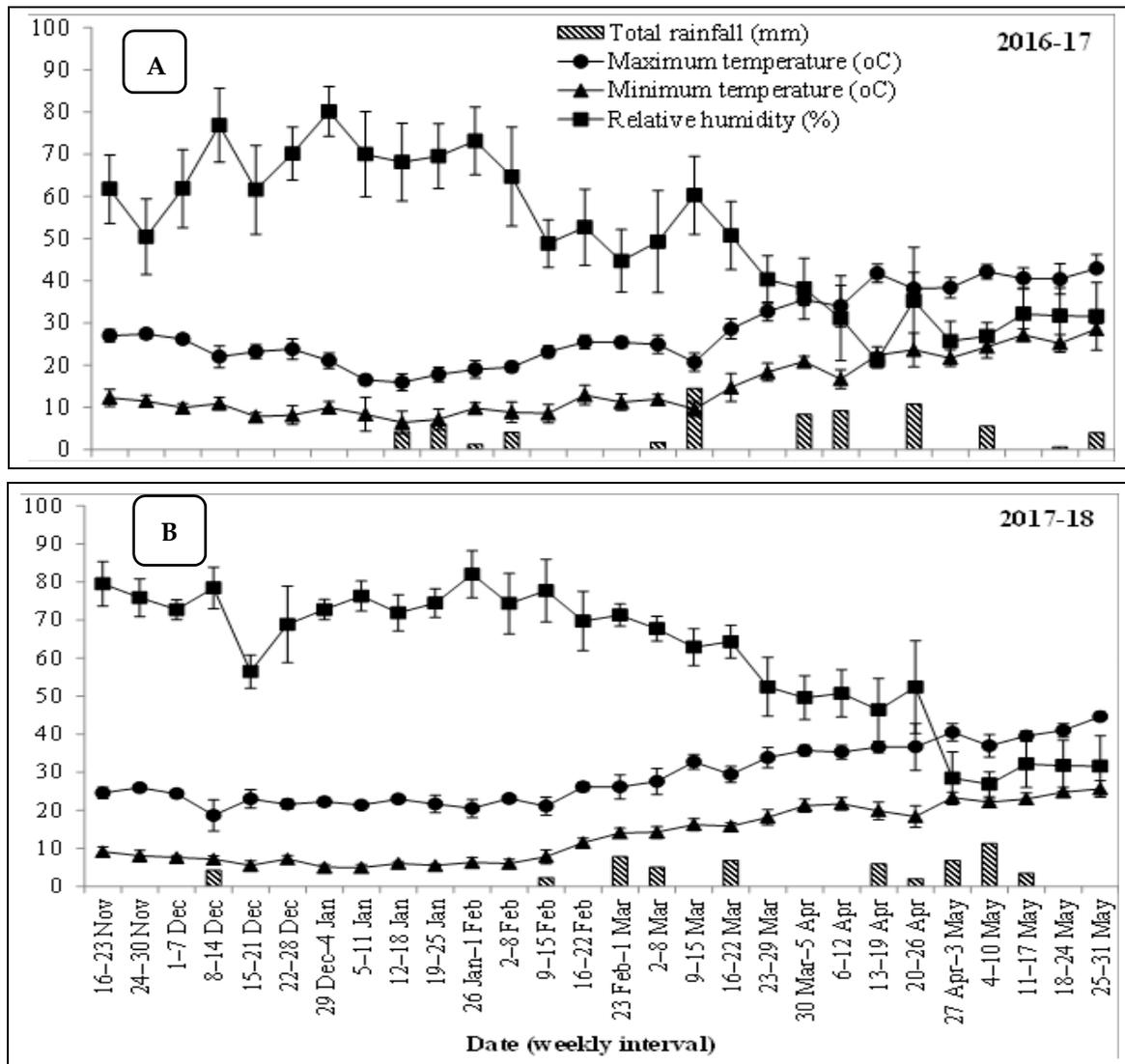
### 2.2. Treatment Plan and Implementation

Fresh leaves were collected from fully grown moringa trees located at the research farm of the Department of Agronomy, University of Agriculture, Faisalabad. Moringa leaf extract was prepared according to the methodology described by Khan et al. [58]. Before extraction, healthy and disease-free leaves were rinsed with distilled water and kept in a freezer overnight. The extract was filtered using Whatman filter paper and further diluted with distilled water to make a 3% solution. For preparing sorgaab, sorghum leaves were collected, chopped into pieces and dried under shade. The chopped material was soaked for 24 h in distilled water in 1:10 (*w/v*) ratio [59]. Soaked material was filtered, and the filtrate was diluted to make a 3% concentration. The optimum concentration of MLE (3%), sorgaab (3%), hydrogen peroxide ( $100\ \mu\text{M}$ ) and ascorbic acid ( $500\ \mu\text{M}$ ) used here, have already been established in different studies as optimum growth enhancers [60,61]. All treatments were applied twice: first at stem elongation and then at anthesis stage.

### 2.3. Estimation of Leaf Physiological and Biochemical Attributes

In accordance with the methodology of Arnon [62], the total chlorophyll contents were determined, although carotenoids were measured by the Davies method [63]. A 0.1 g sample of fresh leaves was ground in 80% acetone solution and made to a volume of 10 mL. Absorbance was taken at 480, 645 and 663 nm by use of a spectrophotometer. All measurements were taken ten days after the second round of foliar treatment application using fully extended leaves. Fully extended leaf was used to determine gas exchange

attributes on sunny days. Stomatal conductance ( $g_s$ ) ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) and substomatal  $\text{CO}_2$  concentration ( $C_i$ ) were recorded by infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Ascorbic acid was estimated using the procedure of Mukherjee and Choudhuri [64]. The concentration of malondialdehyde (MDA) was determined using the procedure of Heath and Packer [65]. Total free amino acids were determined by following the method of Hamilton and Van Slyke [66]. Total soluble protein of leaf was measured using the Coomassie brilliant blue (CBB) dye-binding procedure of Bradford [67]. Anthocyanins and free proline were measured by following the procedures of Stark and Wray [68], and Bates et al. [69], respectively.



**Figure 1.** Weather data including maximum temperature, minimum temperature, relative humidity and rainfall of experimental station during crop growing seasons of 2016–2017 (A), and 2017–2018 (B).

#### 2.4. Estimation of Seed Quality Attributes and Root Shoot Minerals

Phosphate (P) content was determined using the procedure of Yoshida et al. [70], where 0.5 g dried plant material was heated in 5 mL distilled water for one hour. The volume of filtrate was made up to 50 mL with  $\text{d.H}_2\text{O}$ , whereupon 1 mL was extracted into a test tube to which 2 mL nitric acid (2 N) and 1 mL molybdate-vanadate reagent were added, and the volume made up to 10 mL. The volume was vortexed, and after 20 min the absorbance was taken by spectrophotometer at 420 nm, with  $\text{d.H}_2\text{O}$  run as blank. Sulfur (S) content was estimated using the method of Tendon et al. [71]. An amount of 10 mL of plant

extract was taken in a volumetric flask, to which was added 1 mL 6 N HCl, 1 mL solution of 0.5% acacia gum, and 0.5 g barium chloride crystal, and the flask was held still for 60 s. Flasks were then whirled until the crystals dissolved, and the absorbance of samples were noted at 440 nm using the spectrophotometer. The presence of ions (Mg, Zn and Fe) in the seed were determined based on atomic absorption.

### 2.5. Measurement of Growth and Yield Parameters

Five randomly selected plants were uprooted from each replicate to record the root and shoot lengths, fresh and dry weights. For fresh weight, the uprooted plants were immediately weighed using a top-loading balance. For dry weight, the plants were transferred to paper bags and dried in an oven at 70 °C for one week and then dry weights were measured using a top-loading balance. The panicle length and dry weight was recorded when the plants were fully ripened. For 1000 grain weight, the grains were counted and weighed. Harvest index was calculated by dividing grain yield with above-ground dry mass and expressed as a percentage.

### 2.6. Statistical Analysis

Collected data were analyzed and evaluated statistically using a statistical package (Statistix 8.1). Randomized complete block design (RCBD) with four replicates was used to conduct the experiment. Comparison among treatments were made by analysis of variance (ANOVA) technique at a confidence interval of 95%. Microsoft Excel was used for calculation and graphical presentation.

## 3. Results

### 3.1. Photosynthetic Pigments and Gas Exchange Attributes

Significant levels of photosynthetic pigments, gas exchanges attributes, activities of antioxidants, mineral elements of shoot and root, and growth and yield parameters as affected by foliar spray of plant stimulant are presented in Table 1. Results obtained for chlorophyll contents demonstrated that there was significant difference between the foliar treatments. Data revealed that foliar use of MLE was more effective in increasing chlorophyll content (Figure 2A). Carotenoids were also significantly reduced in control plants (unsprayed), while maximum improvement of carotenoids was noted by the foliar application of MLE (Figure 2B). Data obtained for stomatal conductance ( $g_s$ ) and substomatal  $CO_2$  concentration ( $C_i$ ) revealed that foliar treatments significantly improved these attributes (Figure 2C,D).

### 3.2. Metabolomics

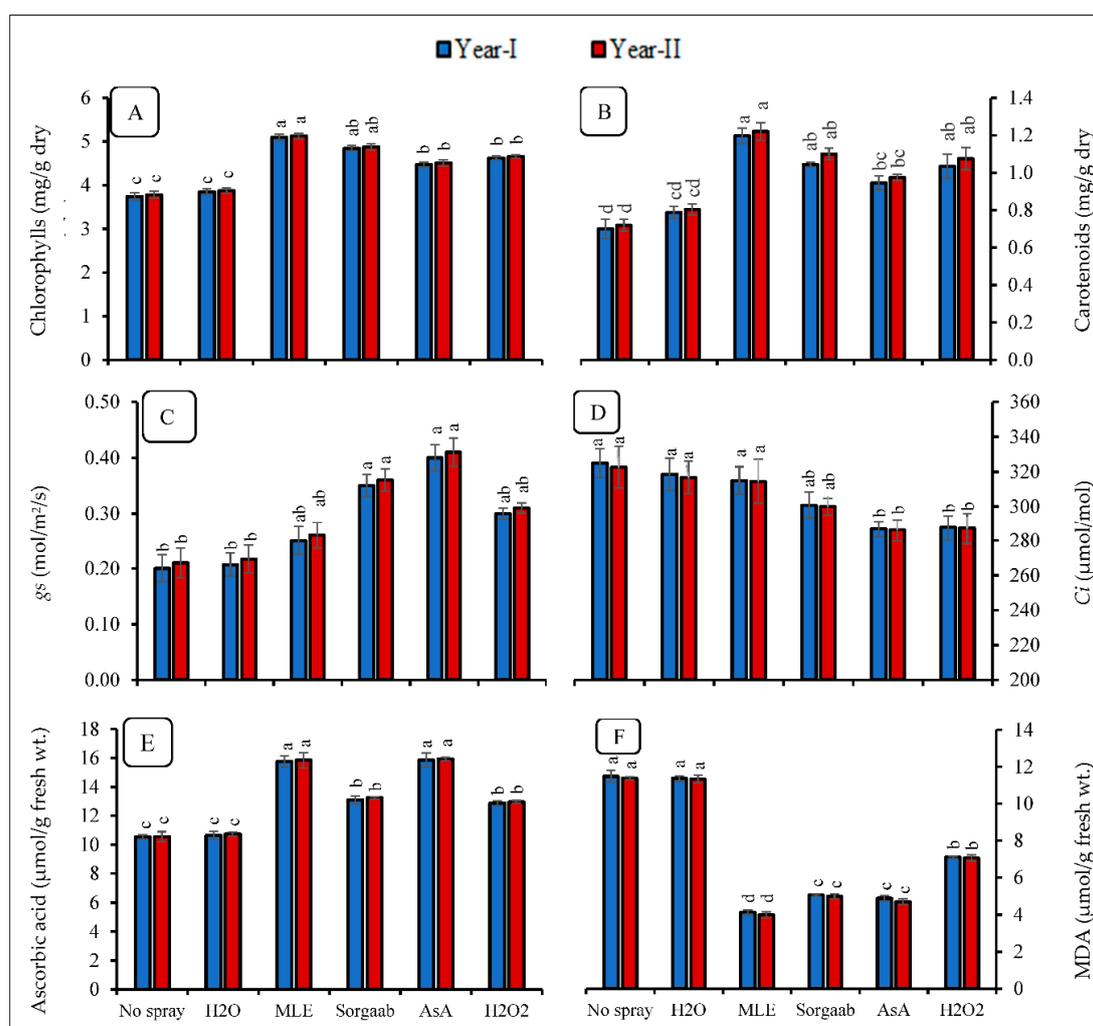
In the current study, statistical analysis of ascorbic acid (AsA) revealed significant differences between treated and non-treated quinoa plants during both experimental years. The highest concentration of AsA was recorded by foliar application of AsA which was statistically at par with MLE (Figure 2E). Foliar treatments of plant growth promoters significantly reduced the malondialdehyde (MDA) level compared with the control group. Data further explored that MLE treatment was more effective in lowering the MDA level (Figure 2F).

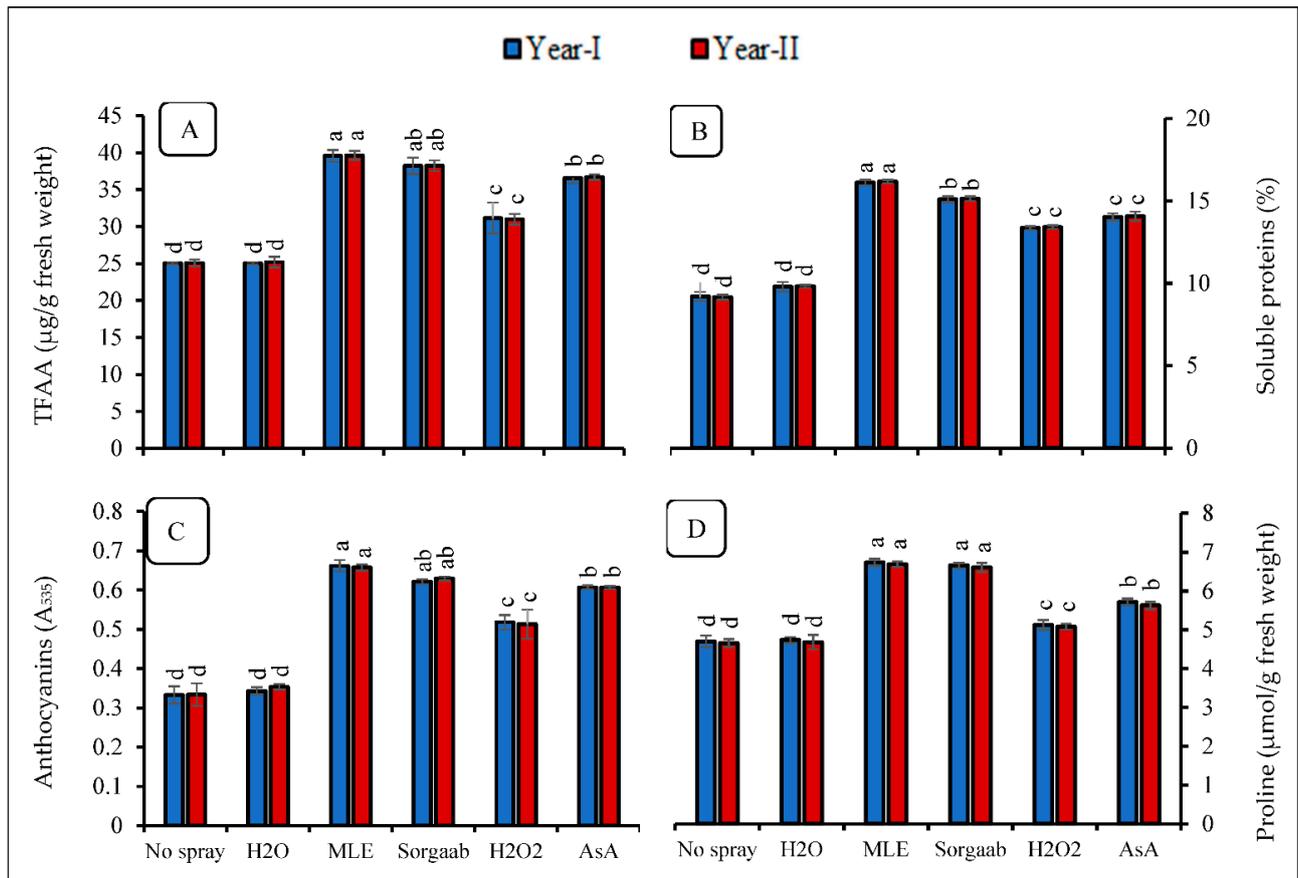
Data regarding shoot total free amino acid (TFAA), total soluble proteins, anthocyanins and proline are presented in Figure 3A–D. Foliar spray of natural and synthetic plant growth promoters significantly improved these attributes compared with the control group. Maximum improvement of shoot TFAA and total soluble proteins were observed by the foliar application of MLE, while minimum improvement was observed in the control. Statistical data for anthocyanins and free proline content revealed significant differences in foliar sprayed, and unsprayed plants. Maximum anthocyanin level and free proline contents were recorded with the use of MLE (Figure 3C,D).

**Table 1.** Mean sum of squares of growth, physiological and yield parameters of quinoa in response to foliar applied plant growth promoters.

SOV	DF	T. Chlo	Caro	gs	Ci	AsA	MDA	TFAA	Protein	Antho
Stimulants (S)	1	1.76 **	0.208 **	0.039 **	1547.6 **	33.0 **	66.5 **	254.6 **	48.54 **	0.122 **
Year (Y)	1	0.011 NS	0.009 NS	0.892 NS	10.13 NS	0.059 NS	0.087 NS	0.025 NS	0.009 NS	0.00003 NS
S × Y	1	0.00002 NS	0.0018 NS	0.043 NS	1.03 NS	0.002 NS	0.0051 NS	0.018 NS	0.0022 NS	0.00006 NS
		<b>Proline</b>	<b>Root P</b>	<b>Shoot P</b>	<b>Root S</b>	<b>Shoot S</b>	<b>RFW</b>	<b>RDW</b>	<b>SFW</b>	<b>SDW</b>
Stimulants (S)	1	5.04 **	186.3 **	211.2 **	40.91 **	188.8 **	45.18 **	15.57 **	405.5 **	482.5 **
Year (Y)	1	0.025 NS	0.02 NS	0.067 NS	0.028 NS	0.56 NS	0.27 NS	0.0013 NS	3.1 NS	0.032 NS
S × Y	1	0.0004 NS	0.0004 NS	0.002 NS	0.0005 NS	0.045 NS	0.009 NS	0.0015 NS	1.5 NS	0.012 NS
		<b>Root L</b>	<b>Shoot L</b>	<b>PL</b>	<b>PW</b>	<b>TGW</b>	<b>HI</b>	<b>Mg</b>	<b>Fe</b>	<b>Zn</b>
Stimulants (S)	1	51.34 **	440 **	66.5 **	91.6 **	2.96 **	96.13 **	10.14 **	34.35 **	207.34 **
Year (Y)	1	0.17 NS	1.58 NS	0.034 NS	0.036 NS	0.0005 NS	0.063 NS	0.043 NS	0.013 NS	0.0001 NS
S × Y	1	0.007 NS	0.204 NS	0.089 NS	0.0006 NS	0.0019 NS	0.002 NS	0.0003 NS	0.003 NS	0.013 NS

SOV = source of variance, DF = degree of freedom, T.Chlo = total Chlorophyll, A = net photosynthetic rate, E = transpiration rate, WUE = water use efficiency, POD = peroxidase, TFAA = total free amino acid, Antho = anthocyanin, RFW = root fresh wt., RDW = root dry wt., SFW = shoot fresh wt., SDW = shoot dry wt., Root L = root length, Shoot L = shoot length, Root P = root phosphate, Shoot P = shoot phosphate, Root S = root sulphate, Shoot S = shoot sulphate, PL = panicle length, PW = panicle wt., TGW = thousand grain wt., HI = harvest index, Mg = magnesium, Fe = iron, Zn = zinc, NS = non-significant, \*\* = significant at  $p \leq 0.01$ .

**Figure 2.** Influence of plant growth promoters on chlorophyll contents (A), carotenoids (B), stomatal conductance (C), sub-stomatal CO<sub>2</sub> concentration (D), ascorbic acid (E), and MDA (F) of quinoa cultivated during the growing seasons of 2016–2017 and 2017–2018. Bars sharing the same letter did not differ significantly at  $P = 0.05$ .



**Figure 3.** Influence of plant growth promoters on total free amino acid (A), total soluble proteins (B), anthocyanin (C) and proline (D) of quinoa cultivated during the growing seasons of 2016–2017 and 2017–2018. Bars sharing the same letter did not differ significantly at  $P = 0.05$ .

### 3.3. Mineral Nutrients

Considering root and shoot mineral contents of control and foliar treated plants, it was observed that during both years of study, MLE-treated plants had higher root and shoot P contents compared with other treatments (Table 2). The sulfur (S) level (i.e., root and shoot) demonstrated significant difference between foliar sprayed and unsprayed plants during both experimental years. It was observed that MLE treatment was more effective for increasing root and shoot S level compared with all other foliar applied treatments, while only a minimum concentration of S was observed in the control treatment (Table 2).

**Table 2.** Impact of foliar applied plant growth enhancers on grain quality of quinoa cultivated during the growing seasons of 2016–2017 (Year I) and 2017–2018 (Year II).

Treatments	Root P			Shoot P			Root S			Shoot S		
	Year I	Year II	Mean (FT)	Year I	Year II	Mean (FT)	Year I	Year II	Mean (FT)	Year I	Year II	Mean (FT)
No spray	19.40	19.34	19.37	30.52	30.55	30.53	11.12	11.19	11.15	21.01	21.02	21.01
Water spray	19.65	19.62	19.62	30.66	30.79	30.72	11.35	11.39	11.37	21.37	21.37	21.37
MLE	32.17	32.10	32.14	44.60	44.69	44.64	17.07	17.10	17.09	35.20	35.18	35.19
Sorgaab	29.82	29.78	29.80	42.36	42.46	42.41	16.45	16.52	16.48	30.86	30.85	30.85
H <sub>2</sub> O <sub>2</sub>	22.16	22.11	22.14	39.86	39.97	39.92	15.90	15.96	15.93	26.05	26.07	26.06
AsA	28.63	28.60	28.61	38.56	38.64	38.60	15.11	15.17	15.14	29.84	29.84	29.84
Mean (Y)	25.31	25.26		37.76	37.85		14.50	14.56		27.39	27.39	
HSD	Y = ns, FT = 2.9, Y × FT = ns			Y = ns, FT = 2.8, Y × FT = ns			Y = ns, FT = 0.66, Y × FT = ns			Y = ns, FT = 1.7, Y × FT = ns		

Means sharing the same letter did not differ significantly at  $p = 0.05$ . MLE = moringa leaf extract, AsA = ascorbic acid, Y = sowing year, FT = foliar treatments, Y × FT = interaction, ns = non-significant.

### 3.4. Growth Parameters

In the current study, data regarding growth attributes (fresh and dry weights of root and shoot, and root and shoot lengths) were recorded. Data showed that foliar spray of MLE, sorgaab, H<sub>2</sub>O<sub>2</sub> and AsA significantly affected the growth attributes of quinoa plants during both years (Table 1). Longer root and shoot were recorded in the foliar sprayed group compared with the control group (Table 3). Data regarding fresh and dry root weights revealed that MLE application showed highest improvement regarding fresh and dry root weights while minimum improvement occurred in the control (Table 3). The highest improvement was recorded regarding the fresh and dry shoot weights with the use of MLE during both years of study. Similarly, significant difference between foliar sprayed and control plants was observed regarding root and shoot lengths; less root and shoot lengths were recorded in control plants, while more length was obtained by the foliar application of natural and synthetic plant growth promoters. During both experimental years, highest root and shoot lengths were observed with foliar spray of MLE while minimum under control treatment and water spray (Table 3).

**Table 3.** Impact of foliar applied plant growth enhancers on growth and yield of quinoa plants cultivated during the growing seasons of 2016–2017 (Year I) and 2017–2018 (Year II).

Treatments	Root Fresh Weight			Root Dry Weight			Shoot Fresh Weight		
	Year I	Year II	Mean (FT)	Year I	Year II	Mean (FT)	Year I	Year II	Mean (FT)
No spray	19.76	20.06	19.91c	8.08	8.05	8.07 c	326.29	326.73	326.5 c
Water spray	20.16	20.33	20.25 c	8.70	8.72	8.71 c	331.61	331.66	331.6 c
MLE	26.75	26.90	26.82 a	12.43	12.42	12.43 a	489.38	487.13	488.2 a
Sorgaab	25.30	25.50	25.40 b	11.18	11.19	11.18 ab	388.20	388.46	388.3 b
H <sub>2</sub> O <sub>2</sub>	23.26	23.36	23.31 b	10.59	10.52	10.55 b	363.37	360.53	361.9 b
AsA	23.50	23.60	23.55 b	9.79	9.79	9.79 bc	482.53	482.33	482.4 a
Mean (Y)	23.12	23.29		10.13	10.11		396.90	396.14	
HSD	Y = ns, FT = 2.5, Y × FT = ns			Y = ns, FT = 1.8, Y × FT = ns			Y = ns, FT = 28.1, Y × FT = ns		
Treatments	Shoot Dry Weight			Root Length			Shoot Length		
	Year I	Year II	Mean (FT)	Year I	Year II	Mean (FT)	Year I	Year II	Mean (FT)
No spray	63.20	63.30	63.25 d	12.90	13.00	12.95 c	78.93	79.75	79.34 c
Water spray	64.83	64.94	64.88 d	13.90	14.06	13.98 c	80.36	81.00	80.68 c
MLE	84.46	84.56	84.51 a	20.56	20.66	20.61 a	100.36	100.81	100.59 a
Sorgaab	81.25	81.32	81.28 ab	18.30	18.40	18.35 b	95.76	96.16	95.96 ab
H <sub>2</sub> O <sub>2</sub>	71.45	71.55	71.50 c	17.40	17.66	17.53 b	92.93	93.41	93.17 b
AsA	79.89	79.77	79.83 b	18.40	18.50	18.45 b	93.26	93.00	93.13 b
Mean (Y)	74.18	74.24		16.91	17.05		90.27	90.69	
HSD	Y = ns, FT = 3.7, Y × FT = ns			Y = ns, FT = 5.9, Y × FT = ns			Y = ns, FT = 2.01, Y × FT = ns		

Means sharing the same letter did not differ significantly at  $p = 0.05$ . MLE = moringa leaf extract, AsA = ascorbic acid, Y = sowing year, FT = foliar treatments, Y × FT = interaction, ns = non-significant.

### 3.5. Grain Yield and Quality Attributes

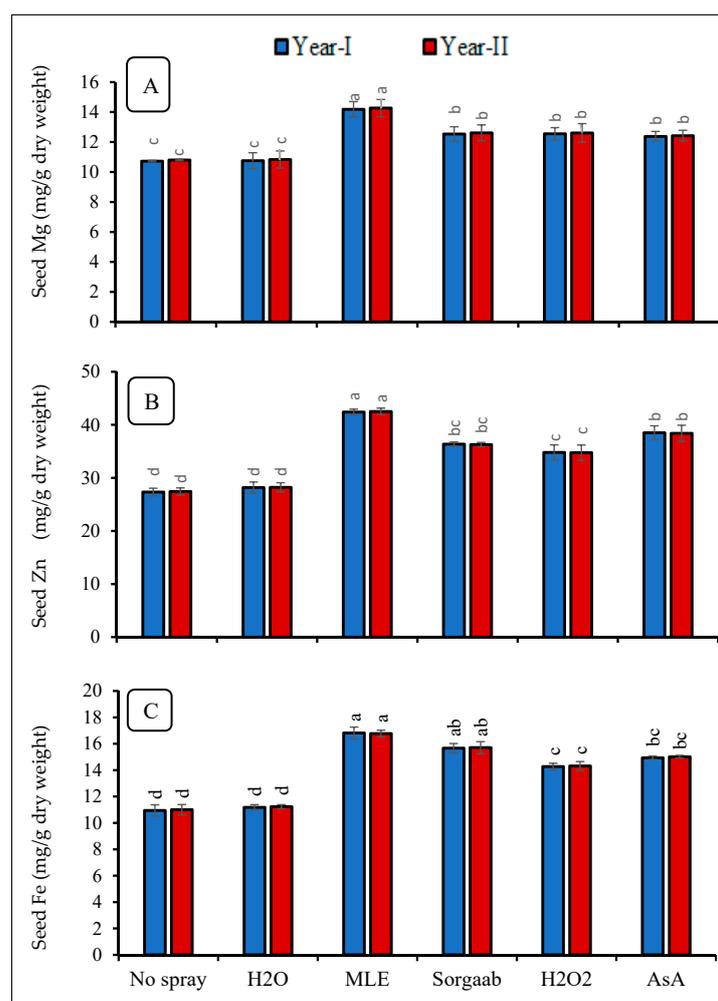
Collected data regarding panicle length and weight demonstrated that yield attributes improved where plants were sprayed with natural and synthetic growth promoters compared with the control group throughout the course of experimentation. The maximum enhancement in panicle length and weight was recorded by foliar application of MLE, while minimum enhancement was recorded in the control treatment (Table 4). Data recorded for thousand grain weight showed that the highest improvement was observed by the foliar application of MLE, which was statistically at par with sorgaab (Table 4). Similarly, maximum harvest index (HI) was observed by application of MLE, which was statistically at par with AsA and sorgaab, respectively (Table 4). During both experimental years, natural and synthetic plant growth promoters also had significant effect on grain mineral

contents compared with the control group. Statistical analysis for grain Mg showed that MLE application produced the highest effect on grain Mg, while minimum effect occurred under control conditions (Figure 4A). Moreover, application of MLE was found to produce a maximum response regarding the concentrations of Zn and Fe in quinoa grains compared with all other treatments throughout the course of experimentation (Figure 4B,C).

**Table 4.** Impact of foliar applied plant growth enhancers on grain quality of quinoa cultivated during the growing seasons of 2016–2017 (Year I) and 2017–2018 (Year II).

Treatments	PL			PW			TSW			HI		
	Year I	Year II	Mean (FT)	Year I	Year II	Mean (FT)	Year I	Year II	Mean (FT)	Year I	Year II	Mean (FT)
No spray	36.40	36.66	36.53 c	17.13	17.20	17.16 d	4.01	4.04	4.03 c	25.07	25.14	25.10 c
Water spray	38.20	38.30	38.25 bc	20.00	20.10	20.05 c	4.06	4.10	4.08 c	25.63	25.70	25.67 c
MLE	42.16	42.23	42.20 a	27.30	27.35	27.32 a	5.79	5.75	5.77 a	34.71	34.76	34.73 a
Sorgaab	45.33	45.06	45.20 a	25.56	25.61	25.59 ab	5.41	5.44	5.42 a	32.12	32.17	32.14 ab
H <sub>2</sub> O <sub>2</sub>	44.10	43.73	43.91 ab	23.80	23.85	23.82 b	4.63	4.66	4.64 b	31.36	31.45	31.40 b
AsA	42.13	42.96	42.05 ab	25.80	25.86	25.83 ab	4.77	4.73	4.75 b	32.94	33.09	33.02 ab
Mean (Y)	41.38	41.32		23.26	23.33		4.78	4.79		30.30	30.39	
HSD	Y = ns, FT = 4.1, Y × FT = ns			Y = ns, FT = 2.4, Y × FT = ns			Y = ns, FT = 0.53, Y × FT = ns			Y = ns, FT = 2.9, Y × FT = ns		

Means sharing the same letter did not differ significantly at  $p = 0.05$ . MLE = moringa leaf extract, AsA = ascorbic acid, Y = sowing year, FT = foliar treatments, Y × FT = interaction, ns = non-significant.



**Figure 4.** Influence of plant growth promoters on seed magnesium (A), zinc (B), and iron (C) of quinoa cultivated during the growing seasons of 2016–2017 and 2017–2018. Bars sharing the same letter did not differ significantly at  $P = 0.05$ .

#### 4. Discussion

Plants produce dry matter as a result of photosynthesis and partitioning of assimilates. In the photosynthetic mechanism, both primary (chlorophyll) and secondary (carotenoids) photosynthetic pigments are responsible for proper functioning. The maintenance and improvement in their concentration is mandatory for maximum efficiency [72]. Exogenous use of MLE significantly improved the chlorophyll pigments and carotenoids, resulting in improvement of plant strength [73]. To improve the photosynthetic process, plant growth promoters (PGPs) play a vital role by protecting chloroplasts from light-induced photoinhibition of photosynthesis [74]; in addition, they also act as a co-factor in photosynthetic enzymatic reactions such as AsA [51]. The application of inorganic fertilizers and mineral elements is considered a helpful practice in maintaining crop productivity with improved soil fertility, to achieve maximum plant growth and economical yield under stressful conditions [75,76].

External use of various plant extracts promotes plant growth and development under normal and stressful conditions, for instance, foliar spray of sorgaab delays leaf senescence and increases chlorophyll concentration because its extract consists of phenolics and secondary metabolites [77]. Moreover, foliar application of 3% MLE improves chlorophyll pigments [13,78] as it is a rich source of zeatin—a natural type of cytokinins [79]. External use of AsA significantly increases the photosynthetic pigments in plants, because AsA spray secures photosynthetic apparatus from ROS produced during oxidative stress, as chloroplast is one of the main sites of ROS formation, but it lacks ROS scavenger catalase enzymes [51]. Consequently, AsA acts as a substrate for ascorbate peroxidase to eliminate ROS. This also encourages the biosynthesis of photosynthetic pigments [80]. In the present study, the gas exchange properties (stomatal conductance and sub-stomatal CO<sub>2</sub> concentration) of quinoa leaves were studied and it was reported that the gas exchange attributes reduced in the control plants, compared with the treated plants. Similarly, in a previous study on rocket plants, MLE application enhanced the gas exchange parameters [44].

Reactive oxygen species (ROS) are continuously produced in plants under both stressful and normal conditions, but their over-production has an adverse effect on cellular organelles because they disturb the cell membrane system [81]. To overcome the harmful effects of ROS antioxidants and other metabolites such as TFAA, total soluble protein, anthocyanin and free proline contents play a vital role. As was previously reported that foliar application of AsA on wheat improved the antioxidants activity [82], similarly, at a low concentration, H<sub>2</sub>O<sub>2</sub> use protects plants from oxidative damage [83]. Proline is considered as an important factor involved in osmoregulation and ROS scavenging [84], thus it plays a vital role in plant defense mechanisms under both stressful and normal conditions [85]. Maswada and El-Rahman [86] observed that an exogenous spray of H<sub>2</sub>O<sub>2</sub> significantly improved the anthocyanin and free proline contents, and these results were similar to the current study.

The popularity of natural and synthetic plant growth promoters is associated with the possibility of obtaining higher fresh and dry biomass of crops. In the current study, it was noted that in the control group, the growth parameters of quinoa reduced in both experimental years. However, foliar application of MLE, sorgaab, H<sub>2</sub>O<sub>2</sub> and AsA significantly improved the fresh and dry biomass of plants, i.e., shoot and root length, their fresh and dry weight, to differential extent. Overall, MLE and sorgaab were more effective, while water had the least effect. Earlier, it was reported that PGPs are an excellent source of growth enhancing compounds which help plant growth and development, cell division, and cell elongation, both in normal and stressful environmental conditions [87]. According to Abdalla [44], foliar application of MLE on rocket plants significantly enhanced the plant length, fresh and dry weight, which may be due to its enriched contents of protein and growth promoting substances such as auxin and cytokinins which aid in the formation of cell protoplasm, cell division and cell elongation. Moreover, Dolatabadian et al. [88] noted that foliar use of different PGPs increased the fresh and dry weight of maize due to the increased photosynthetic rate and cell division. Previously, Hussein and Alva [89] reported

that exogenous use of AsA on millet plants significantly enhanced the plant height and dry biomass due to an enhancement in photosynthetic activities.

Plant growth and development is dependent on the availability of the mineral nutrients and the plant's ability to absorb and assimilate them. The prevailing unusual conditions are likely to decrease the plant's efficiency to absorb and assimilate essential nutrients in the plant body, resulting in growth being affected [38]. Plants show the operation of a number of metabolic pathways, and mineral nutrients of important components, to play a crucial role in these metabolic steps. Due to the important role of nutrients, data were recorded for root, shoot P and S at the optimum sowing period and the role of both organic and synthetic plant growth promoters were found; the results showed that there was a significant difference in mineral nutrients between foliar sprayed treatments and the control group. Plant extracts such as MLE and sorgaab are natural sources of essential minerals, which become available to cereals when they are exogenously applied, eventually balancing the plants' nutrient status [13,78]. The availability of nutrients, either from the soil solution or from foliar fertilization, is beneficial for plant growth and development, since the nutrients measured in this experiment have great metabolic roles in plant life. Phosphorus and sulfur have both structural and functional roles in plant cells, thus their deficiency disturbs the normal cellular function, and in case of severe deficiency, causes chlorosis, necrosis or yellowing of leaves [72].

Accruing better yields is the prime purpose of growing crops. The final seed yield is dependent upon plant yield components such as panicle characteristics, seed weight and harvest index. There are various strategies to improve plant economic yield—it is argued that under the exogenous supply of growth promoting substances, the seed yield and yield related attributes can be increased. In the current study, foliar application of the selected treatments improved the quinoa yield and yield related parameters, but exogenous use of MLE produced the greatest improvement in almost all yield regarding attributes of both years' study. The increase in the yield and its attributes occurred, possibly, because aqueous plant extracts are excellent sources of minerals and secondary metabolites, which help the plant withstand harsh conditions by improving source and sink activity, and water uptake pathway [90,91]. As a result, an improved thousand seed weight and harvest index was accomplished with the foliar spray in both years. Moreover, in the present study, mineral contents, including Mg, Zn and Fe, of quinoa seeds were also improved with the foliar spray of the selected growth enhancers. Results showed that nutritional level of quinoa grains were decreased under the control group, while exogenous application of Sorgaab, MLE, AsA and low level H<sub>2</sub>O<sub>2</sub> enhanced these attributes in the treated group to great extent, thus improving the quality of quinoa seed for consumption. The increase in these attributes may be due to better absorption of nutrients via roots and thus nutrients were efficiently available towards seed filling by partitioning of assimilates from proximal leaves of the panicles [72].

Furthermore, it was quite evident from the data that the plants sprayed with the growth enhancing agents produced longer and more compact panicles than those which were not sprayed, or foliar sprayed with water only. The increase in the length and weight of panicle strongly corroborated with the 1000 seed weight, possibly due to the ability of the foliar sprayed growth promoters to divert more assimilates during seed filling [92]. These data further resulted in an improved harvest index (HI), which was undoubtedly related to the improved seed yield, but least changed the aboveground dry matter yield irrespective of the foliar spray of growth enhancers. A greater effectiveness of sorgaab can be attributed to the presence of phenolic and terpenoid compounds in the aqueous extract [93], which when used in appropriately diluted concentration can improve plant yield [77,94]. Similarly, MLE, with more cytokinins and vitamins, is important in enhancing the yield of plants [95,96].

## 5. Conclusions

Application of biostimulants is a sustainable and ecofriendly approach to enhance the productivity of field crops. All treatments, either natural or synthetic, significantly improved the growth and productivity of quinoa crop. However, maximum improvement in yield and grain quality of quinoa was observed by foliar application of moringa leaf extract at stem elongation and anthesis stages.

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## References

1. Navruz-Varli, S.; Sanlier, N. Nutritional and health benefits of quinoa (*Chenopodium quinoa* Willd.). *J. Cereal Sci.* **2016**, *69*, 371–376. [CrossRef]
2. Shahbandeh, M. Global Quinoa Production 2010–2019. 2021. Available online: <https://www.statista.com/statistics/486442/global-quinoa-production/> (accessed on 7 November 2021).
3. Vega-Gálvez, A.; Miranda, M.; Vergara, J.; Uribe, E.; Puente, L.; Martínez, E.A. Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* Willd.), an ancient Andean grain: A review. *J. Sci. Food Agric.* **2010**, *90*, 2541–2547. [CrossRef] [PubMed]
4. Prakash, D.; Pal, M. Chenopodium: Seed protein, fractionation and amino acid composition. *Int. J. Food Sci. Nutr.* **1998**, *49*, 271–275. [CrossRef]
5. Bhargava, A.; Shukla, S.; Ohri, D. Genetic variability and heritability of selected traits during different cuttings of vegetable Chenopodium. *Ind. J. Genet. Plant Breed.* **2003**, *63*, 359–360.
6. James, A.L.E. Quinoa (*Chenopodium quinoa* Willd.): Composition, chemistry, nutritional, and functional properties. *Adv. Food Nutr. Res.* **2009**, *58*, 1–31.
7. Repo-Carrasco, R.; Espinoza, C.; Jacobsen, S.E. Nutritional value and use of the Andean crops quinoa (*Chenopodium quinoa*) and kaniwa (*Chenopodium pallidicaule*). *Food Rev. Int.* **2003**, *19*, 179–189. [CrossRef]
8. Jenkins, D.J.A.; Kendall, C.W.C.; Augustin, L.S.A.; Franceschi, S.; Hamidi, M.; Marchie, A.; Jenkins, A.L.; Axelsen, M. Glycemic index: Overview of implications in health and disease. *Am. J. Clin. Nutr.* **2002**, *76*, 266–273. [CrossRef]
9. Berti, C.; Riso, P.; Monti, L.D.; Porrini, M. In vitro starch digestibility and in vivo glucose response of gluten-free foods and their gluten counterparts. *Eur. J. Nutr.* **2004**, *43*, 198–204. [CrossRef]
10. Jacobsen, S.E. The worldwide potential of quinoa (*Chenopodium quinoa* Willd.). *Food Rev. Int.* **2003**, *19*, 167–177. [CrossRef]
11. Saddiq, M.S.; Wang, X.; Iqbal, S.; Hafeez, M.B.; Khan, S.; Raza, A.; Iqbal, J.; Maqbool, M.M.; Fiaz, S.; Qazi, M.A.; et al. Effect of Water Stress on Grain Yield and Physiological Characters of Quinoa Genotypes. *Agronomy* **2021**, *11*, 1934. [CrossRef]
12. Iqbal, H.; Yaning, C.; Waqas, M.; Rehman, H.; Shareef, M.; Iqbal, S. Hydrogen peroxide application improves quinoa performance by affecting physiological and biochemical mechanisms under water-deficit conditions. *J. Agron. Crop Sci.* **2018**, *204*, 541–553. [CrossRef]
13. Khan, S.; Basra, S.M.A.; Nawaz, M.; Hussain, I.; Foidl, N. Combined application of moringa leaf extract and chemical growth-promoters enhances the plant growth and productivity of wheat crop (*Triticum aestivum* L.). *S. Afr. J. Bot.* **2020**, *129*, 74–81. [CrossRef]

14. Brazales-Cevallos, D.K.; Romero-Contreras, Y.J.; Vences-Guzmán, M.A.; Torres, M.; Aviles-Baltazar, N.Y.; Sohlenkamp, C.; Serrano, M. Transcriptional characterization of the biostimulant effect of *Moringa oleifera* leaf extracts using *Arabidopsis thaliana* as a model. *S. Afr. J. Bot.* **2022**, *144*, 250–256. [[CrossRef](#)]
15. Calvo, P.; Nelson, L.; Klopper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [[CrossRef](#)]
16. Kocira, S.; Szparaga, A.; Kocira, A.; Czerwinska, E.; Wójtowicz, A.; Bronowicka-Mielniczuk, U.; Koszel, M.; Findura, P. Modeling biometric traits, yield and nutritional and antioxidant properties of seeds of three soybean cultivars through the application of biostimulant containing seaweed and amino acids. *Front. Plant Sci.* **2018**, *9*, 388. [[CrossRef](#)]
17. Tanga, M.; Lewu, F.B.; Oyedeji, A.O.; Oyedeji, O.O. Yield and morphological characteristics of Burdock (*Arctium lappa* L.) in response to mineral fertilizer application. *Asian J. Agric. Biol.* **2020**, *8*, 511–518. [[CrossRef](#)]
18. Niewiadomska, A.; Sulewska, H.; Wolna-Maruwka, A.; Ratajczak, K.; Waraczewska, Z.; Budka, A. The Influence of Bio-Stimulants and Foliar Fertilizers on Yield, Plant Features, and the Level of Soil Biochemical Activity in White Lupine (*Lupinus albus* L.) Cultivation. *Agronomy* **2020**, *10*, 150. [[CrossRef](#)]
19. Campobenedetto, C.; Grange, E.; Mannino, G.; van Arkel, J.; Beekwilder, J.; Karlova, R.; Garabello, C.; Contartese, V.; Berteza, C.M. A Biostimulant Seed Treatment Improved Heat Stress Tolerance During Cucumber Seed Germination by Acting on the Antioxidant System and Glyoxylate Cycle. *Front. Plant Sci.* **2020**, *11*, 836. [[CrossRef](#)]
20. Makhaye, G.; Aremu, A.O.; Gerrano, A.S.; Tesfay, S.; Du Plooy, C.P.; Amoo, S.O. Biopriming with Seaweed Extract and Microbial-Based Commercial Biostimulants Influences Seed Germination of Five *Abelmoschus esculentus* Genotypes. *Plants* **2021**, *10*, 1327. [[CrossRef](#)] [[PubMed](#)]
21. Sobarzo-Bernal, O.; Gómez-Merino, F.C.; Alcántar-González, G.; Saucedo-Veloz, C.; Trejo-Téllez, L.I. Biostimulant Effects of Cerium on Seed Germination and Initial Growth of Tomato Seedlings. *Agronomy* **2021**, *11*, 1525. [[CrossRef](#)]
22. Hassan, S.M.; Ashour, M.; Sakai, N.; Zhang, L.; Hassanien, H.A.; Ammar, G.A.G.; Ammar, G. Impact of Seaweed Liquid Extract Biostimulant on Growth, Yield, and Chemical Composition of Cucumber (*Cucumis sativus*). *Agriculture* **2021**, *11*, 320. [[CrossRef](#)]
23. Mutale-joan, C.; Rachidi, F.; Mohamed, H.A.; Mernissi, N.E.; Aasfar, A.; Barakate, M.; Mohammed, D.; Sbabou, L.; Arroussi, H.E. Microalgae-cyanobacteria-based biostimulant effect on salinity tolerance mechanisms, nutrient uptake, and tomato plant growth under salt stress. *J. Appl. Phycol.* **2021**, 1–17. [[CrossRef](#)]
24. Ali, S.; Charles, T.C.; Glick, B.R. Delay of flower senescence by bacterial endophytes expressing 1-aminocyclopropane-1-carboxylate deaminase. *J. Appl. Microbiol.* **2012**, *113*, 1139–1144. [[CrossRef](#)]
25. Roupheal, Y.; Lucini, L.; Miras-Moreno, B.; Colla, G.; Bonini, P.; Cardarelli, M. Metabolomic Responses of Maize Shoots and Roots Elicited by Combinatorial Seed Treatments With Microbial and Non-microbial Biostimulants. *Front. Microbiol.* **2020**, *11*, 664. [[CrossRef](#)] [[PubMed](#)]
26. Campobenedetto, C.; Mannino, G.; Beekwilder, J.; Contartese, V.; Karlova, R.; Berteza, C.M. The application of a biostimulant based on tannins affects root architecture and improves tolerance to salinity in tomato plants. *Sci. Rep.* **2021**, *11*, 354. [[CrossRef](#)] [[PubMed](#)]
27. Maignan, V.; Bernay, B.; Géliot, P.; Avice, J.-C. Biostimulant Effects of Glutacetine R and Its Derived Formulations Mixed With N Fertilizer on Post-heading N Uptake and Remobilization, Seed Yield, and Grain Quality in Winter Wheat. *Front. Plant Sci.* **2020**, *11*, 607615. [[CrossRef](#)]
28. Mannino, G.; Campobenedetto, C.; Vigliante, I.; Contartese, V.; Gentile, C.; Berteza, C.M. The Application of a Plant Biostimulant Based on Seaweed and Yeast Extract Improved Tomato Fruit Development and Quality. *Biomolecules* **2020**, *10*, 1662. [[CrossRef](#)]
29. Bulgari, R.; Franzoni, G.; Ferrante, A. Biostimulants application in horticultural crops under abiotic stress conditions. *Agronomy* **2019**, *9*, 306. [[CrossRef](#)]
30. Laurent, E.-A.; Ahmed, N.; Durieu, C.; Grieu, P.; Lamaze, T. Marine and fungal biostimulants improve grain yield, nitrogen absorption and allocation in durum wheat plants. *J. Agric. Sci.* **2020**, *158*, 279–287. [[CrossRef](#)]
31. Hassanein, Y.Z.; Abdel-Rahman, S.S.A.; Soliman, W.S.; Salaheldin, S. Growth, yield, and quality of roselle (*Hibiscus sabdariffa* L.) plants as affected by nano zinc and bio-stimulant treatments. *Hortic. Environ. Biotechnol.* **2021**, *53*, 397–403. [[CrossRef](#)]
32. Zulfqar, F.; Casadesus, A.; Brockman, H.; Munne-Bosch, S. An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. *Plant Sci.* **2020**, *295*, 110194. [[CrossRef](#)] [[PubMed](#)]
33. Shareef, H.J. Organic fertilizer modulates IAA and ABA levels and biochemical reactions of date palm *Phoenix dactylifera* L. Hillawi cultivar under salinity conditions. *Asian J. Agric. Biol.* **2020**, *8*, 24–30. [[CrossRef](#)]
34. Makawita, G.I.P.S.; Wickramasinghe, I.; Wijesekara, I. Using brown seaweed as a biofertilizer in the crop management industry and assessing the nutrient upliftment of crops. *Asian J. Agric. Biol.* **2021**. [[CrossRef](#)]
35. Hussain, M.U.; Saleem, M.F.; Hafeez, M.B.; Khan, S.; Hussain, S.; Ahmad, N.; Ramzan, Y.; Nadeem, M. Impact of soil applied humic acid, zinc and boron supplementation on the growth, yield and zinc translocation in winter wheat. *Asian J. Agric. Biol.* **2022**. [[CrossRef](#)]
36. Anwar, Z.; Basharat, Z.; Hafeez, M.B.; Khan, S.; Zahra, N.; Rafique, Z.; Maqsood, M. Biofortification of Maize with Zinc and Iron not only Enhances Crop Growth but also Improves Grain Quality. *Asian J. Agric. Biol.* **2021**. [[CrossRef](#)]
37. Tabaxi, I.; Zisi, C.; Karydogianni, S.; Folina, A.E.; Kakabouki, I.; Kalivas, A.; Bilalis, D. Effect of organic fertilization on quality and yield of oriental tobacco (*Nicotiana tabacum* L.) under Mediterranean conditions. *Asian J. Agric. Biol.* **2021**. [[CrossRef](#)]

38. Rashid, N.; Khan, S.; Wahid, A.; Basra, S.M.A.; Alwahibi, M.S.; Jacobsen, S.-E. Impact of natural and synthetic growth enhancers on the productivity and yield of quinoa (*Chenopodium quinoa* willd.) cultivated under normal and late sown circumstances. *J. Agron. Crop Sci.* **2021**, 1–15. [[CrossRef](#)]
39. Phiri, C.; Mbewe, D.N. Influence of *Moringa oleifera* leaf extracts on germination and seedling survival of three common legumes. *Int. J. Agric. Biol.* **2010**, *12*, 315.
40. Toscano, S.; Ferrante, A.; Branca, F.; Romano, D. Enhancing the Quality of Two Species of Baby Leaves Sprayed with Moringa Leaf Extract as Biostimulant. *Agronomy* **2021**, *11*, 1399. [[CrossRef](#)]
41. Leone, A.; Spada, A.; Battezzati, A.; Schiraldi, A.; Aristil, J.; Bertoli, S. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An Overview. *Int. J. Mol. Sci.* **2015**, *16*, 12791–12835. [[CrossRef](#)]
42. El-Serafy, R.S.; El-Sheshtawy, A.-N.A.; Abd El-Razek, U.A.; Abd El-Hakim, A.F.; Hasham, M.M.A.; Sami, R.; Khojah, E.; Al-Mushhin, A.A.M. Growth, Yield, Quality, and Phytochemical Behavior of Three Cultivars of Quinoa in Response to Moringa and Azolla Extracts under Organic Farming Conditions. *Agronomy* **2021**, *11*, 2186. [[CrossRef](#)]
43. Yasmeen, A.; Basra, S.M.A.; Farooq, M.; Rehman, H.; Hussain, N.; Athar, H.R. Exogenous application of moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. *Plant Growth Regul.* **2013**, *69*, 225–233. [[CrossRef](#)]
44. Abdalla, M.M. The potential of *Moringa oleifera* extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eruca vesicaria* subsp. *sativa*) plants. *Int. J. Plant Physiol. Biochem.* **2013**, *5*, 42–49.
45. Alsaadawi, I.S.; Dayan, F.E. Potentials and prospects of sorghum allelopathy in agroecosystems. *Allelopath. J.* **2009**, *24*, 255–270.
46. Cheema, Z.A.; Khaliq, A. Use of sorghum allelopathic properties to control weeds in irrigated wheat in a semi arid region of Punjab. *Agric. Ecosyst. Environ.* **2000**, *79*, 105–112. [[CrossRef](#)]
47. Wazir, I.; Sadiq, M.; Baloch, M.S.; Awan, I.U.; Khan, E.A.; Shah, I.H.; Nadim, M.A.; Khakwani, A.A.; Bakhsh, I. Application of bioherbicide alternatives for chemical weed control in rice. *Pak. J. Weed Sci. Res.* **2011**, *17*, 245–252.
48. Farooq, O.; Ali, M.; Sarwar, N.; Rehman, A.; Iqbal, M.M.; Naz, T.; Asghar, M.; Ehsan, F.; Nasir, M.; Hussain, Q.M.; et al. Foliar applied brassica water extract improves the seedling development of wheat and chickpea. *Asian J. Agric. Biol.* **2021**. [[CrossRef](#)]
49. Rehman, A.; Hassan, F.; Qamar, R.; Rehman, A.U. Application of plant growth promoters on sugarcane (*Saccharum officinarum* L.) budchip under subtropical conditions. *Asian J. Agric. Biol.* **2021**, *2*, 202003202. [[CrossRef](#)]
50. Shah, T.; Latif, S.; Khan, H.; Munsif, F.; Nie, L. Ascorbic Acid Priming Enhances Seed Germination and Seedling Growth of Winter Wheat under Low Temperature Due to Late Sowing in Pakistan. *Agronomy* **2019**, *9*, 757. [[CrossRef](#)]
51. Wang, J.; Zhang, Z.; Huang, R. Regulation of ascorbic acid synthesis in plants. *Plant Signal. Behav.* **2013**, *8*, e24536. [[CrossRef](#)] [[PubMed](#)]
52. Gao, Y.; Guo, Y.K.; Lin, S.H.; Fang, Y.Y.; Bai, J.G. Hydrogen peroxide pretreatment alters the activity of antioxidant enzymes and protects chloroplast ultrastructure in heat-stressed cucumber leaves. *Sci. Hortic.* **2010**, *126*, 20–26. [[CrossRef](#)]
53. Hossain, M.A.; Bhattacharjee, S.; Armin, S.-M.; Qian, P.; Xin, W.; Li, H.-Y.; Burritt, D.J.; Fujita, M.; Tran, L.-S.P. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: Insights from ROS detoxification and scavenging. *Front. Plant Sci.* **2015**, *6*, 420. [[CrossRef](#)] [[PubMed](#)]
54. Cerny, M.; Habánová, H.; Berka, M.; Luklová, M.; Brzobohatý, B. Hydrogen Peroxide: Its Role in Plant Biology and Crosstalk with Signalling Networks. *Int. J. Mol. Sci.* **2018**, *19*, 2812. [[CrossRef](#)]
55. Farooq, M.; Nawaz, A.; Chaudhary, M.A.M.; Rehman, A. Foliage-applied sodium nitroprusside and hydrogen peroxide improves resistance against terminal drought in bread wheat. *J. Agron. Crop Sci.* **2017**, *203*, 473–482. [[CrossRef](#)]
56. Sarwar, M.; Saleem, M.F.; Ullah, N.; Rizwan, M.; Ali, S.; Shahid, M.R.; Alamri, S.A.; Alyemeni, M.N.; Ahmad, P. Exogenously applied growth regulators protect the cotton crop from heat-induced injury by modulating plant defense mechanism. *Sci. Rep.* **2018**, *8*, 17086. [[CrossRef](#)] [[PubMed](#)]
57. Akram, M.Z.; Basra, S.M.A.; Hafeez, M.B.; Khan, S.; Nazeer, S.; Iqbal, S.; Saddiq, M.S.; Zahra, N. Adaptability and yield potential of new quinoa lines under agro-ecological conditions of Faisalabad-Pakistan. *Asian J. Agric. Biol.* **2021**, *2*, 202005301. [[CrossRef](#)]
58. Khan, S.; Basra, S.M.A.; Afzal, I.; Wahid, A. Screening of moringa landraces for leaf extract as biostimulant in wheat. *Int. J. Agric. Biol.* **2017**, *19*, 999–1006. [[CrossRef](#)]
59. Bhatti, M.Q.L.; Cheema, Z.A.; Mahmood, T. Efficacy of Sorgaab as Natural Weed Inhibitor in Raya. *Pak. J. Biol. Sci.* **2000**, *3*, 1128–1130. [[CrossRef](#)]
60. Jahangeer, A. Response of Maize (*Zea mays* L.) to Foliar Application of Three PLANT water Extracts. Master's Thesis, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan, 2011.
61. Khan, S.; Basra, S.M.A.; Afzal, I.; Nawaz, M.; Rehman, H.U. Growth promoting potential of fresh and stored *Moringa oleifera* leaf extracts in improving seedling vigor, growth and productivity of wheat crop. *Environ. Sci. Pollut. Res.* **2017**, *24*, 27601–27612. [[CrossRef](#)]
62. Arnon, D.I. Copper enzyme in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **1949**, *24*, 1–15. [[CrossRef](#)]
63. Davies, B.H. Carotenoids. In *Chemistry and Biochemistry of Plant Pigments*, 2nd ed.; Goodwin, T.W., Ed.; Academic Press: Cambridge, MA, USA, 1976; Volume 2, pp. 38–165.
64. Mukherjee, S.P.; Choudhuri, M.A. Implication of water stress-induced changes in levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedling. *Physiol. Plant.* **1983**, *58*, 166–169. [[CrossRef](#)]
65. Heath, R.L.; Packer, L. Photoperoxidation in isolated chloroplasts. *Arch. Biochem. Biophys.* **1968**, *125*, 189–198. [[CrossRef](#)]

66. Hamilton, P.B.; Van-Slyke, D.D. Amino acid determination with ninhydrin. *J. Biol. Chem.* **1943**, *150*, 231–233. [[CrossRef](#)]
67. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye binding. *Anal. Biochem.* **1976**, *72*, 247–254. [[CrossRef](#)]
68. Stark, D.; Wray, V. Anthocyanins. In *Methods in Plant Biology, Plant Phenolics*; Harborne, J.B., Ed.; Academic Press/Harcourt Brace Jovanovich: London, UK, 1989; Volume 1, pp. 32–356.
69. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water stress studies. *Plant Soil* **1973**, *39*, 205–207. [[CrossRef](#)]
70. Yoshida, S.; Forno, D.A.; Cock, J.H.; Gomez, K.A. *Laboratory Manual for Physiological Studies of Rice*; International Rice Research Institute (IRRI): Los Banos, Philippines, 1976.
71. Tendon, H.L.S. (Ed.) *Methods of Analysis of Soil, Plants, Water and Fertilizers*; Fertilization Development and Consultation Organisation: New Delhi, India, 1993.
72. Taiz, L.; Zeiger, E.; Moller, M.I.; Murphy, A. *Plant Physiology and Development*, 6th ed.; Sinauer Associates: Sunderland, MA, USA, 2015.
73. Azzedine, F.; Gherroucha, H.; Baka, M. Improvement of salt tolerance in durum wheat by ascorbic acid application. *J. Stress Physiol. Biochem.* **2011**, *7*, 27–37.
74. Miyaji, T.; Kuromori, T.; Takeuchi, Y.; Yamaji, N.; Yokosho, K.; Shimazawa, A. AtPHT4;4 is a chloroplast-localized ascorbate transporter in Arabidopsis. *Nat. Commun.* **2015**, *6*, 5928–6928. [[CrossRef](#)]
75. Safdar, M.E.; Aslam, A.; Qamar, R.; Ali, A.; Javaid, M.M.; Hayyat, M.S.; Raza, A. Allelopathic effect of prickly chaff flower (*Achyranthes aspera* L.) used as a tool for managing noxious weeds. *Asian J. Agric. Biol.* **2021**, *3*, 202006370. [[CrossRef](#)]
76. Zahid, N.; Ahmed, M.J.; Tahir, M.M.; Maqbool, M.; Shah, S.Z.A.; Hussain, S.J.; Khaliq, A.; Rehmani, M.I.A. Integrated effect of urea and poultry manure on growth, yield and postharvest quality of cucumber (*Cucumis sativus* L.). *Asian J. Agric. Biol.* **2021**. [[CrossRef](#)]
77. Maqbool, N.; Wahid, A.; Farooq, M.; Cheema, Z.A.; Siddique, K.H.M. Allelopathy and abiotic stress interaction in crop plants. In *Allelopathy: Current Trends and Future Applications*; Cheema, Z.A., Farooq, M., Wahid, A., Eds.; Springer: Berlin/Heidelberg, Germany, 2013; pp. 113–143.
78. Rashid, N.; Wahid, A.; Basra, S.M.A.; Arfan, M. Foliar spray of moringa leaf extract, sorgaab, hydrogen peroxide and ascorbic acid improve leaf physiological and seed quality traits of quinoa (*Chenopodium quinoa*) under terminal heat stress. *Int. J. Agric. Biol.* **2020**, *23*, 801–810.
79. Basra, S.; Iftikhar, M.; Afzal, I. Potential of moringa (*Moringa oleifera*) leaf extract as priming agent for hybrid maize seeds. *Int. J. Agric. Biol.* **2011**, *13*, 1006–1010.
80. Akram, N.A.; Shafiq, F.; Ashraf, M. Ascorbic Acid-A Potential Oxidant Scavenger and Its Role in Plant Development and Abiotic Stress Tolerance. *Front. Plant Sci.* **2017**, *8*, 613. [[CrossRef](#)]
81. ElSayed, A.I.; Rafudeen, M.H.; Gomaa, A.M.; Hasanuzzaman, M. Exogenous melatonin enhances the reactive oxygen species metabolism, antioxidant defense-related gene expression, and photosynthetic capacity of *Phaseolus vulgaris* L. to confer salt stress tolerance. *Physiol. Plant.* **2021**, 1–13. [[CrossRef](#)]
82. Siddiqui, M.H.; Alamri, S.A.; Al-Khaishany, M.; Al-Qutami, M.A.; Ali, H.M. Ascorbic acid application improves salinity stress tolerance in wheat. *Chiang Mai J. Sci.* **2018**, *45*, 1296–1306.
83. Zhang, X.L.; Jia, X.F.; Yu, B.; Gao, Y.; Bai, J.G. Exogenous hydrogen peroxide influences antioxidant enzyme activity and lipid peroxidation in cucumber leaves at low light. *Sci. Hortic.* **2011**, *129*, 656–662. [[CrossRef](#)]
84. Heuer, B. Role of proline in plant response to drought and salinity. In *Handbook of Plant and Crop Stress*, 3rd ed.; Pessarakli, M., Ed.; Taylor and Francis Press: New York, NY, USA, 2010; pp. 213–238.
85. Siddiqui, M.H.; Al-Khaishany, M.Y.; Al-Qutami, M.A.; Al-Wahaibi, M.H.; Grover, A.; Ali, H.M.; Al-Wahibi, M.S. Morphological and physiological characterization of different genotypes of faba bean under heat stress. *Saudi J. Biol. Sci.* **2015**, *22*, 1–20. [[CrossRef](#)] [[PubMed](#)]
86. Maswada, H.F.; Abd-El-Rahman, L.A. Inducing salinity tolerance in wheat plants by hydrogen peroxide and lithovit a nano-caco3 fertilizer. *J. Agric. Res. Kafir. El-Sheikh Univ.* **2014**, *40*, 693–716.
87. Chaiwanon, J.; Wang, W.; Zhu, J.-Y.; Oh, E.; Wang, Z.-Y. Information integration and communication in plant growth regulation. *Cell* **2016**, *164*, 1257–1268. [[CrossRef](#)]
88. Dolatabadian, A.; Mohammad, S.A.; Asilan, K.S. Effect of ascorbic acid foliar application on yield, yield component and several morphological traits of grain corn under water deficit stress conditions. *Not. Sci. Biol.* **2010**, *2*, 45–50. [[CrossRef](#)]
89. Hussein, M.M.; Alva, A.K. Effects of Zinc and Ascorbic Acid Application on the Growth and Photosynthetic Pigments of Millet Plants Grown under Different Salinity. *Agric. Sci.* **2014**, *5*, 1253–1260. [[CrossRef](#)]
90. Garcia-Martinez, A.M.; Diaz, A.; Tejada, M.; Bautista, J.; Rodriguez, B.; Santa-Maria, C.; Revilla, E.; Parrado, J. Enzymatic Production of an Organic Soil Biostimulant from Wheat-Condensed Distiller Solubles: Effects on Soil Biochemistry and Biodiversity. *Process Biochem.* **2010**, *45*, 1127–1133. [[CrossRef](#)]
91. Yasmeen, A.; Basra, S.M.A.; Wahid, A.; Nouman, W.; Rehman, H. Exploring the potential of Moringa oleifera leaf extract (MLE) as a seed priming agent in improving wheat performance. *Turk. J. Bot.* **2013**, *37*, 512–520.
92. Maheswari, U.M.; Karthik, A. Effect of foliar nutrition on growth, yield attributes and seed yield of pulse crops. *Adv. Crop. Sci. Technol.* **2017**, *5*, 278. [[CrossRef](#)]

93. Shah, S.H.; Khan, E.A.; Shah, H.; Ahmad, N.; Khan, J.; Sadozai, G.U. Allelopathic sorghum water extract helps to improve yield of sunflower (*Helianthus annuus* L.). *Pak. J. Bot.* **2016**, *48*, 1197–1202.
94. Basra, S.M.A.; Lovatt, C. Exogenous applications of Moringa oleifera leaf extract and cytokinins improve plant growth, yield and fruit quality of cherry tomato (*Solanum lycopersicum*). *HortTechnology* **2016**, *26*, 327–337. [[CrossRef](#)]
95. Khan, S.; Basit, A.; Hafeez, M.B.; Irshad, S.; Bashir, S.; Bashir, S.; Maqbool, M.M.; Saddiq, M.S.; Hasnain, Z.; Aljuaid, B.S.; et al. Moringa leaf extract improves biochemical attributes, yield and grain quality of rice (*Oryza sativa* L.) under drought stress. *PLoS ONE* **2021**, *16*, e0254452. [[CrossRef](#)]
96. Iqbal, J.; Irshad, J.; Bashir, S.; Khan, S.; Yousaf, M.; Shah, A.N. Comparative study of water extracts of Moringa leaves and roots to improve the growth and yield of sunflower. *S. Afri. J. Bot.* **2020**, *129*, 221–224. [[CrossRef](#)]