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RESEARCH ARTICLE

Elevated ozone phytotoxicity ameliorations in mung bean {*Vigna radiata* (L.) Wilczek} by foliar nebulization of silicic acid and ascorbic acid

Eram Shahzadi[1] · Muhammad Nawaz[1] · Muhammad Adrees[2] · Muhammad Jawad Asghar[3] · Naeem Iqbal[1]

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Abstract

The present work provides an insight into the development of biochemical adaptations in mung beans against ozone (O_3) toxicity. The study aims to explore the O_3 stress tolerance potential of mung bean genotypes under exogenous application of growth regulators. The seeds of twelve mung bean genotypes were grown in plastic pots under controlled conditions in the glasshouse. Six treatments, control (ambient ozone level 40–45 ppb), ambient O_3 with ascorbic acid, ambient ozone with silicic acid, elevated ozone (120 ppb), elevated O_3 with ascorbic acid (10 mM), and elevated ozone with silicic acid (0.1 mM) were applied. The O_3 fumigation was carried out using an O_3 generator. The results revealed that ascorbic acid and silicic acid application decreased the number of plants with foliar O_3 injury symptoms in different degrees, i.e., zero, first, second, third, and fourth degrees; whereas 0–4 degree symptoms represent, no symptoms, symptoms occupying < 1/4, 1/4–1/2, 1/2–3/4, and > 3/4 of the total foliage area, respectively. Application of ascorbic acid and silicic acid also prevented the plants from the negative effects of O_3 in terms of fresh as well as dry matter production, leaf chlorophyll, carotenoids, soluble proteins and ascorbic acid, proline, and malondialdehyde (MDA) contents. Overall, silicic acid application proved more effective in reducing the negative effects of O_3 on mung bean genotypes as compared to that of the ascorbic acid. Three mung bean genotypes (NM 20–21, NM-2006, and NM-2016) were identified to have a better adaptive mechanism for O_3 toxicity tolerance and may be good candidates for future variety development programs.

Keywords Antioxidants · Growth regulators · Plant biomass · Tropospheric ozone

Introduction

Global climate change; an essential ecological concern is associated with many factors like temperature rise, alterations in precipitation patterns, and rising levels of greenhouse gases (Xu et al. 2021). Amongst greenhouse gases, the ever-increasing level of tropospheric O_3 is causing significant losses in the productivity of crops as compared to any other air pollutant (Ren 2021). The highly oxidative gas O_3 has established damaging effects on both plant growth and human health (Pires et al. 2014; Nuvolone et al. 2018). The O_3 concentration has become doubled in the Northern Hemisphere since the preindustrial period (Yeung et al. 2019) and is currently increasing at a rate of 0.5–2% per year due to changes in the release of precursor compounds from industrial activities (Saunier and Blande 2019). Tropospheric O_3 level is predicted to rise by a further 20% over the next 50 years (Prather et al. 2003). Rising O_3 is also a hidden danger for food security and is believed to result in global yield losses of up to 16% depending upon crop species (Sharps et al. 2021).

The problem of climate change linked with rising greenhouse gas levels is particularly serious in developing countries like Pakistan (Hussain et al. 2020). Moreover, O_3 damage is expected to expand more widely and efficiently in densely populated areas of Indo-Pakistan (Khan et al. 2016). Studies

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✉ Naeem Iqbal
drnaeem@gcuf.edu.pk

[1] Department of Botany, Government College University Faisalabad, Faisalabad 38000, Pakistan

[2] Department of Environmental Sciences & Engineering, Government College University Faisalabad, Faisalabad 38000, Pakistan

[3] Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad 38000, Pakistan

involving the O_3 effects have mainly been focused on cereals and legumes in this (Indo-Pak) region (Tiwari and Agrawal 2009). Despite the fact that developing countries have a minimum contribution to greenhouse gas emissions, these are more threatened by climate change than developed countries. Hence, mitigation strategies to combat O_3 pollution are of more importance to these regions (Sofia et al. 2020). The mitigating strategies may include exploration of adoptive germplasm, increasing tolerance to O_3 pollution, uncovering plant tolerance mechanisms, nutrient management in plants under global change scenario, and exogenous supply of plant growth-promoting substances (Iriti et al. 2003; Soares et al. 2019).

Exogenous application of growth regulators to improve plant performance under unfavorable conditions has gained considerable attention from scientists (Khan et al. 2020). Many chemical compounds are known to provide protection to plants from O_3 injury (Chaudhary and Rathore 2020). These include antioxidants, growth regulators, anti-senescent agents, and pesticides (Daripa et al. 2016). Significant progress has been reported regarding the use of protective chemicals in reducing the toxic effects of ozone during the last 2 decades (Tiwari and Agrawal 2009).

Silicic acid is found effective for plant growth and yield parameters when applied as a foliar spray at very minute quantities (Jardin 2015). It is cost-effective as well as eco-friendly and may act as a physical barrier for toxic elements after depositing the cell wall of different plant tissues (Muneer and Jeong 2015). Moreover, ascorbic acid is an influential antioxidant, which eradicates the stress-induced reactive oxygen species (ROS) and is recommended for improvement in crop plants, particularly under unfavorable environments (Bellini and Tullio 2019).

Mung bean {*Vigna radiate* (L.) Wilczek} is a preferred legume crop across Asia and other continents due to the presence of easily digestible proteins (Rani et al. 2018). In Pakistan, mung bean is grown on an estimated area of 127,500 ha. Keeping in view the rising global O_3 concentration, it is time to consider the options for increasing the tolerance of crops to this abiotic stress. We need to explore or develop crop varieties with high productivity potential, suitable for predicted future climate changes. Hence, the present study has been conducted to investigate the relative performance of mung bean genotypes under rising O_3 levels and to find out whether and up to what extent the applied silicic acid and ascorbic acid can ameliorate the negative effects of O_3 on mung bean plants.

Materials and methods

Seeds of twelve available approved mung beans {*Vigna radiate* (L.) Wilczek} genotypes, NM-28, NM 13–1, NM19-19, NM 20–21, NM121-25, NM-51, NM-54, NM-92, NM-98, NM-2006, NM-2011, and NM-2016 were obtained from Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The experiment was conducted in soil-filled pots under controlled conditions of a glasshouse at NIAB Faisalabad with six treatments and three replicates. The seed samples were sterilized with 0.2% $HgCl_2$ before sowing. The study was conducted in a split-plot design with three replications of each experimental unit. After 3 weeks of germination, ascorbic acid (10 mM) and silicic acid (0.1 mM) were sprayed with the addition of 0.1% tween-20 as surfactant. Tween-20 is a nonionic hydrophilic surfactant that helps in the wetting of the leaf surface. The aqueous solution applied to the leaf surface alone would have fallen off and not adhered to the surface, whereas tween-20 helps in spreading the spray uniformly and sticking to the surface (Hassan et al. 2021). The plants were then exposed to elevated O_3 levels (120 ppb) using an O_3 generator (AOT-MD-500 model) for 4 h per day except for three treatments; control (O_3 untreated ones), ambient O_3 with ascorbic acid, and ambient O_3 with silicic acid. The level of O_3 was monitored in the glasshouse using an O_3 meter UV photometry O_3 analyzer (model: 0342e) and was maintained for 15 days (Table 4 provided in the supplementary file). Normal air was considered a control treatment.

The O_3 damages were measured on the basis of percent injury to mature fully enlarged leaves. The foliage injury was calculated according to the equation used by Chang and Yu (2001) as follows:

$$\text{Foliage injury } \% = \frac{(\mathrm{N1} \times 1) + (\mathrm{N2} \times 2) + (\mathrm{N3} \times 3)(\mathrm{N4} \times 4)}{(\mathrm{N0} + \mathrm{N1} + \mathrm{N2} \times + \mathrm{N3} + \mathrm{N4}) \times 4} \times 100$$

where N0, N1, N2, N3, and N4 were the numbers of leaves with zero, first, second, third, and fourth degree symptoms of ozone injury, respectively; whereas 0–4 degree symptoms represent no symptoms, symptoms occupying < 1/4, 1/4–1/2, 1/2–3/4, and > 3/4 of the total foliage area, respectively. The injury was rated by visual estimation. The percentage of the injured area on each leaf was recorded according to a 0–100% scale in 5% increments as adopted by Madkour and Laurence (2002).

The harvested shoots and roots of all subjects were washed with tap water, blotted with brown paper towels, and dried at 70 °C for 72 h. The dry weight was recorded using an electric balance**.** Chlorophyll a, b, and total chlorophyll contents were determined using the method described by Arnon (1949). The optical density of acetone extracted samples was recorded at 645 and 663 nm using a spectrophotometer (Hitachi U-2001, Japan). Total carotenoid contents were calculated using the formula of Arnon (1949).

Protein estimation was made by the Bradford method (Bradford 1976). The absorbance of Bradford reagent treated samples was noted at 595 nm using the spectrophotometer (Hitachi U-2001, Japan). Total soluble sugars were measured following the Yemm and Willis (1954) method. The ethanolic extracted samples were treated with anthrone reagent, boiled and cooled. The absorbance was noted at 625 nm using a spectrophotometer (Hitachi U-2001, Japan).

MDA contents were calculated following the method of Cakmak and Horst (1991). Total phenolic contents (TPC) were calculated using the Folin–Ciocalteu reagent with some modifications of the Wolfe et al. (2003) method. Ascorbic acid was determined using the protocol of Mukherjee and Choudhuri (1983). For ascorbic acid estimation, TCA (6%) extracted material was treated with 2, 4-dinitrophenyl hydrazine (2%) and one drop of 10% thiourea. The mixture was boiled in a water bath, cooled and treated with sulfuric acid (80%). Absorbance was recorded at 530 nm using a spectrophotometer (Hitachi U-2001, Japan) with 6% TCA as blank. Leaf proline contents were determined following the method of Bates et al. (1973). The absorbance of the reaction solution was taken at 520 nm.

Statistical analysis

The collected data were subjected to statistical analysis to test the significance of differences among mean values using CoStat software version 6.303. Logarithmic transformations were carried out for data normalization, where necessary, prior to analysis. The relationships between various analyzed variables (Fig. 4) and principal component analysis (Fig. 5) were carried out using RStudio software.

Results

The shoot fresh weight value of all mung bean genotypes declined significantly under high tropospheric O_3 levels as compared to control. Foliar application of ascorbic acid and silicic acid significantly improved ($P \leq 0.05$) shoot fresh weight at ambient O_3 level by 53 and 57%, respectively, while these also improved shoot fresh weight by 22 and 24%, respectively, in elevated O_3 stressed plants as compared to O_3 untreated plants. Results indicated that mung bean genotypes, NM 20–21, NM 2006, and NM 2016 showed a minimum reduction in shoot fresh weight under O_3 stress (Fig. 1).

Analysis of variance of data showed that root fresh weight of O_3-treated plants decreased in mung bean genotypes used in the study, but the interaction between O_3 level, varieties, and treatments was not found significant (Table 1). Foliar application of silicic acid and ascorbic acid increased root fresh weight up to 54 and 42%, respectively, in elevated O_3 stressed plants while 64 and 62% in O_3 unstressed plants (Fig. 1).

Shoot dry weight and root dry weight of all mung bean genotypes were decreased significantly under elevated O_3. Foliar application of ascorbic and silicic acid under elevated O_3 levels increased the shoot dry weight ($P < 0.01$) up to 32 and 41%, as compared to untreated plants (50 and 51%), respectively (Fig. 1). But the interaction between O_3 level, varieties, and treatments was not found significant on root dry weight. The silicic acid foliar application was found to be more effective in improving the biomass of mung bean genotypes than that of ascorbic acid application.

Elevated O_3 levels produced a remarkable decrease in chlorophyll a and b contents of mung bean genotypes. Results revealed that mung bean variety NM-51 and NM-98 showed a decrease in chlorophyll a and b contents, respectively. However, foliar application of ascorbic acid and silicic acid exhibited more significant improvement ($P < 0.001$) in chlorophyll a and chlorophyll b contents at an ambient level in NM-54 and NM-2011varieties, while genotype NM- 51 and NM 13–1 showed maximum improvement in chlorophyll a and chlorophyll b content under elevated O_3 level, respectively (Fig. 2). Total chlorophyll contents were decreased in mung bean genotypes under a higher level of O_3 treatment. Mean values of the attribute in all subjects indicated that NM-2016 produced maximum, while NM-13–1 produced minimum total chlorophyll contents under O_3 stress. Foliar application of both growth regulators increased significantly ($P \leq 0.001$) total chlorophyll contents under O_3 stress effectively (Fig. 2).

All the mung bean genotypes displayed differential responses against elevated O_3 levels regarding carotenoid accumulation. Elevated O_3 decreased the carotenoid contents in NM 13–1, NM 51, and NM 28, while all other genotypes depicted an increase in carotenoid contents. Both growth regulators helped to increase ($P < 0.01$). Carotenoid contents (20%) in O_3 stressed and unstressed (43 and 33%) plants (Fig. 2). The silicic acid application was found to be more effective in improving the pigment contents of mung bean genotypes than that of the ascorbic acid application.

Exposure of mung bean plants to elevated O_3 levels increased the foliar injury on the upper surface leaves. The foliar injury became more severe as plants grew older. Severe injury symptoms under elevated O_3 stress were observed in the variety NM-51. The foliar injury symptoms in variety ranged between 36.45%. Foliar application of both growth regulators significantly ($P < 0.001$) proved to be helpful in reducing the O_3-induced injury symptoms. Ascorbic acid and silicic acid application resulted in less injury in variety NM-92 (18.24%) and NM-2006 (17.33%), respectively, at ambient O_3 levels, while NM-2016 (20.48%) and NM-2006 (20.83%) varieties showed less injury by ascorbic acid and silicic acid application under elevated O_3 levels (Table 2).

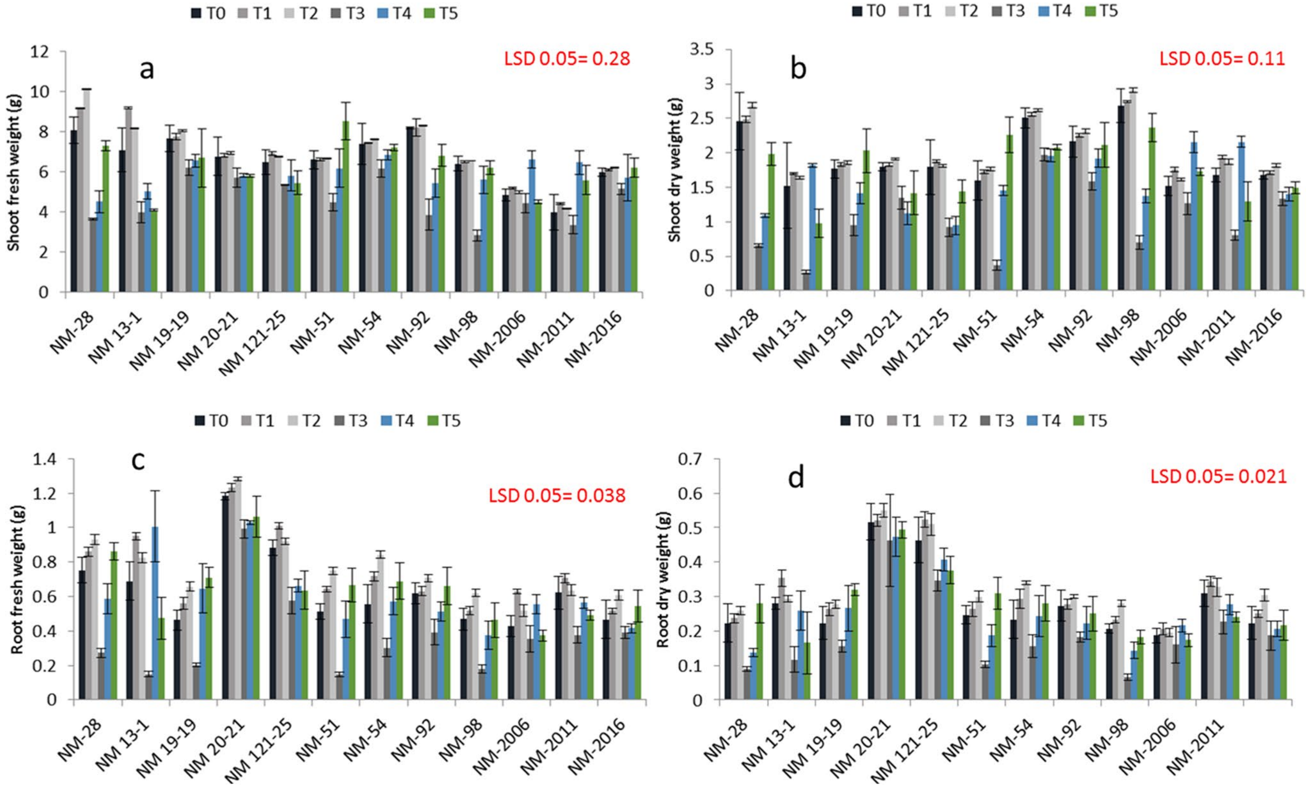

Fig. 1 Effect of ascorbic acid and silicic acid on shoot fresh weight (**a**), shoot dry weight (**b**), root fresh weight (**c**), and root dry weight (**d**) of mung bean varieties under elevated O_3 levels. T0 = ambient O_3 level 40–45 ppb, T1 = ambient O_3 + ascorbic acid, T2 = ambient O_3 + silicic acid, T3 = elevated O_3 level 120 ppb, T4 = elevated O_3 level + ascorbic acid, T5 = elevated O_3 level + silicic acid

Exogenous application of ascorbic acid and silicic acid enhanced the soluble protein contents in O_3 treated plants as compared to untreated plants (Fig. 3), but the interaction between O_3 level, varieties, and treatments was not found significant. Total soluble sugar contents decreased by 37% under O_3 stress than that of O_3 untreated plants. Ascorbic acid and silicic acid foliar application increased significantly ($P \leq 0.001$) total soluble sugar contents by 33 and 29% in plants exposed to elevated O_3 levels, while 56 and 45% in ambient O_3 level exposed plants, respectively. Mung bean genotype NM-2016 displayed higher total soluble sugar contents, while NM-98 showed lower sugar contents as compared to all other genotypes (Fig. 3).

The elevated level of O_3 impacted the proline content in all twelve mung bean varieties. In variety NM- 98, a substantial increase due to O_3 pollution was seen. Foliar application of ascorbic acid and silicic acid influenced this variable, but the interaction between O_3 level, varieties, and treatments was not found significant (Fig. 3). MDA contents were increased by19% under O_3 stress conditions. The response of all cultivars was different regarding leaf MDA contents under O_3 stress with and without foliar application of growth regulators, but the interaction between O_3 level, varieties, and treatments was not found significant in the study (Fig. 3). An increase in total phenolic contents (TPC) was recorded in all mung bean genotypes under O_3 stress as compared to plants growing in the ambient O_3 environment. Mung bean variety NM 20–21 showed the maximum value for TPC, while NM-51 showed the minimum value of TPC, but the effect of foliar application of ascorbic acid and silicic acid on TPC contents under elevated O_3 levels was not found significant (Table 3). A higher value of ascorbic acid was recorded in variety NM-2006 than all other genotypes. Ascorbic acid contents of plants increased up to 27% under O_3 stress as compared to the plants growing under the ambient level of O_3; however, foliar application of growth regulators significantly ($P \leq 0.01$) enhanced the ascorbic acid content (Table 3).

A Pearson's correlation graph was also developed to depict a relationship between various growth and physio-biochemical parameters of different mung bean varieties under elevated O_3 using the foliar application of ascorbic acid and silicic acid (Fig. 4). Leaf injury was positively correlated with each other but negatively correlated with all other morphological and physiological attributes in mung bean varieties. Although MDA contents were also

Table 1 Analysis of variance for the parameter of growth, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, leaf injury, protein content, soluble sugars, proline, MDA, phenolics, and ascorbic acid contents of mung bean (*Vigna radiata* (L.) Wilczek) plants foliarly sprayed with ascorbic acid and silicic acid under elevated O_3 levels

		Shoot (FW)	Shoot (DW)	Root (FW)	Root (DW)	Chl. a	Chl.b	T. Chl	Car. contents	(%) Foliar injury	Protein contents	Sugar contents	MDA contents	Proline contents	Total phenolic contents	Ascorbic acid contents
Source	df	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
O_3 levels	1	96.28***	16.95***	1.77***	0.25***	512,513***	612,272***	224,514***	3566***	40,896***	424,358***	51,850***	343,127***	249.59***	4.67***	776,580***
Varieties	11	11.4****	1.54***	0.63***	0.17***	27,738***	131,808***	252,025***	309***	2473***	106,497***	8728***	82,916***	14.02***	2.98***	62,567***
Treatments	2	21.02***	3.79***	0.0120. 0.90***	0.083***	76,293***	96,615***	329,193***	1178***	2849***	8549***	14,108***	73,650***	0.380***	2.80***	34,040***
O_3 levels * varieties	11	10.28***	0.70***	0.03*	0.006 ns	1810***	4737***	10,896***	11.28 ns	949***	13,046***	539.84 ***	5128***	8.76***	1.525***	14,399***
O_3 levels * treatments	2	6.73***	1.83***	0.121***	0.008 ns	272***	2304***	1020***	5.08 ns	181***	259.33 ns	78.08 ns	1761***	0.015 ns	17,945,767 ns	14,165***
Varieties * treatments	22	1.81***	0.32***	0.05***	0.004 ns	2905***	5184***	9026***	58.37***	105***	422 ns	768***	1773**	0.030***	13,035,344 ns	3342***
O_3 levels * varieties * T	22	1.27*	0.22**	0.018 ns	0.001 ns	1613***	4326***	9899***	20.68**	32.69***	355 ns	199***	854 ns	0.008 ns	18,014,503 ns	2052**
Error	144	0.74	0.11	0.013	0.0043	9.78	10.58	21.03	9.59	5.36	372	43.51	754.7	0.009	2,736,686	978.79

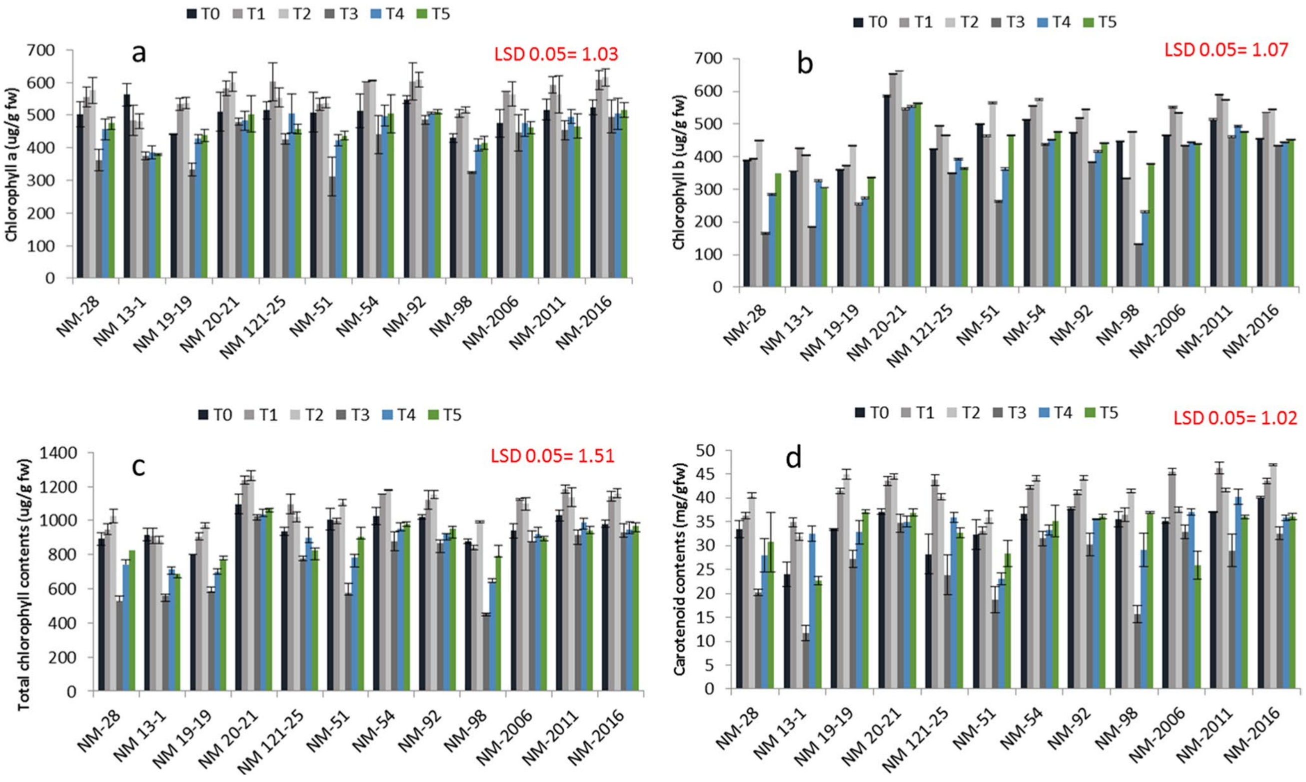

Fig. 2 Effect of ascorbic acid and silicic acid on chlorophyll a (**a**), chlorophyll b (**b**), total chlorophyll (**c**), and carotenoids content (**d**) of mung bean varieties under elevated O_3 levels. T0 = ambient O_3 level 40–45 ppb, T1 = ambient O_3 + ascorbic acid, T2 = ambient O_3 + silicic acid, T3 = elevated O_3 level 120 ppb, T4 = elevated O_3 level + ascorbic acid, T5 = elevated O_3 level + silicic acid

negatively correlated with plant growth and biomass, photosynthetic efficiency positively correlated with leaf injury and soluble protein. This relationship depicted a close connection between plant growth and composition in mung bean varieties under O_3 stress conditions.

The loading plots of PCA to illustrate the effect of O_3 toxicity under the application of ascorbic acid and silicic acid in various varieties of mung beans are presented in Fig. 5. In the whole database, Dim1 and Dim2 exhibited maximum contribution and occupy more than 61% of all

Table 2 Foliar injury percentage of tested mung bean (*Vigna radiata* (L.) Wilczek) varieties after 120 ppb O_3 exposure

(%) Foliar injury						
Varieties	Ambient O_3	Ambient O_3 + ascorbic acid	Ambient O_3 + silicic acid	Elevated O_3 (120 ppb)	Elevated O_3 + ascorbic acid	Elevated O_3 + silicic acid
NM-28	34.3 ± 1.23d	28.6 ± 0.30e	23.41 ± 0.39f	81.56 ± 1.49a	73.45 ± 0.78b	66.46 ± 1.07c
NM 13–1	36.96 ± 0.90d	22.01 ± 1.58f	27.48 ± 0.30e	84.74 ± 0.70a	64.54 ± 0.33c	77.43 ± 1.09b
NM 19–19	30.49 ± 1.50d	24.4 ± 1.20e	21.08 ± 1.07f	65.23 ± 1.37a	57.13 ± 1.90b	46.40 ± 1.29c
NM 20–21	26.92 ± 1.18d	20.88 ± 1.055e	18.85 ± 0.95f	35.65 ± 0.70a	32.41 ± 1.96b	27.85 ± 1.07c
NM 121–25	28.31 ± 0.85d	19.94 ± 0.75f	21.92 ± 1.18e	61.81 ± 0.66a	42.62 ± 1.08c	55.40 ± 1.13b
NM-51	40.91 ± 1.24d	32.81 ± 1.15e	27.42 ± 1.63f	86.87 ± 1.13a	73.49 ± 1.60b	61.73 ± 1.22c
NM-54	30.62 ± 1.58d	25.65 ± 0.66e	21.75 ± 1.12f	64.91 ± 0.53a	56.64 ± 1.39b	41.64 ± 1.09c
NM-92	27.41 ± 1.37d	21.96 ± 0.87e	17.79 ± 0.82f	59.07 ± 0.96a	44.29 ± 1.26b	35.78 ± 1.03c
NM-98	42.76 ± 0.99d	31.12 ± 1.64e	25.83 ± 1.31f	83.63 ± 1.21a	78.31 ± 1.54b	59.683 ± 5.61c
NM-2006	22.11 ± 0.88d	16.92 ± 1.35f	19.92 ± 1.66e	31.94 ± 0.88a	20.64 ± 1.14c	25.85 ± 0.54b
NM-2011	28.12 ± 1.17d	19.24 ± 0.52f	22.99 ± 0.85e	59.83 ± 1.39a	33.66 ± 1.08c	46.68 ± 0.97b
NM-2016	21.8 ± 1.22c	18.72 ± 1.57e	16.36 ± 1.91f	28.27 ± 1.25a	23.63 ± 0.41b	19.94 ± 0.11d

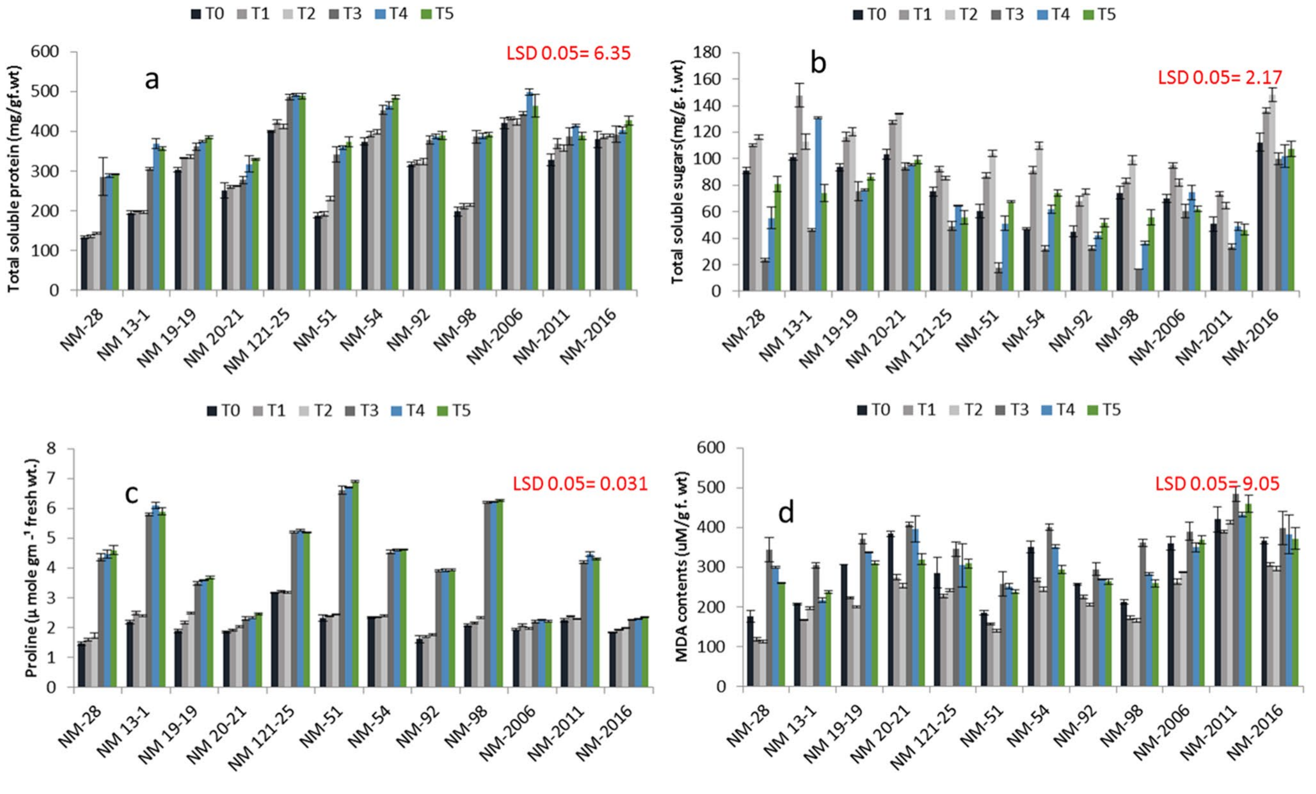

Fig. 3 Effect of ascorbic acid and silicic acid on total soluble protein (a), total soluble sugars (b), proline (**c**), and MDA content (**d**) of mung bean varieties under elevated O_3 leveld. T0 = ambient O_3 level 40–45 ppb, T1 = ambient O_3 + ascorbic acid, T2 = ambient O_3 + silicic acid, T3 = elevated O_3 level 120 ppb, T4 = elevated O_3 level + ascorbic acid, T5 = elevated O_3 level + silicic acid

databases. Among which, Dim1 exhibits 43.7%, and Dim2 exhibits 17.1%. All studied parameters were distributed successfully in the database, which is giving a clear indication that O_3 stress causes a significant effect on the growth and physiology of all mung bean varieties. From the results, it can be indicated that leaf injuries are positively correlated; malondialdehyde, total protein, and soluble protein contents were almost neutral in the database, and shoot fresh weight, shoot dry weight, total soluble sugar, ascorbic acid content, root fresh weight, root dry weight, total chlorophyll content, and carotenoid content are negatively correlated with other studied attributes in the whole database.

Discussion

The significant reduction in shoot and root fresh and dry matter under elevated O_3 was recorded during the present investigation and it could be related to altered partitioning of assimilates among different plant parts (Ghosh et al. 2020) due to a decrease in photosynthesis and carbon allocation (Emberson et al. 2018). The positive effects of silicic acid on plant growth and biomass production could be due to the involvement of silicon in cell division and elongation processes as well as in improvements in photosynthesis and light interception (Farooq et al. 2015). Moreover, the role of ascorbic acid as a cofactor of dioxygenases during the biosynthesis of auxins (IAA) and gibberellins (GA) had been documented (Mahmood and Dunwell 2020). Hence, enhanced plant growth in terms of biomass production as observed during the present study could be due to stimulation of IAA and GA synthesis by exogenous ascorbic acid supply, which in turn increased plant biomass production.

Pigment determination results of the current study indicated that chlorophyll and carotenoid contents were decreased under O_3 stress in all mung bean genotypes. The decrease in chlorophyll and carotenoid contents under higher O_3 levels is due to damage to mesophyll cells (Maamar et al. 2015; Fatima et al. 2019). Our results also indicated the positive effects of ascorbic acid application on chlorophyll and carotenoids contents are due to the scavenging of reactive oxygen species (ROS) in leaves either by reducing their diffusion or increasing their detoxification under O_3 stress (Akram et al. 2017). Similarly, the silicic acid application was more effective in enhancing chlorophyll and carotenoid contents as recorded during the current investigation and the same has been reported by Maghsoudi et al. (2015).

Table 3 Effect of ascorbic acid and silicic acid on total phenolic and ascorbic acid contents of mung bean (*Vigna radiata* (L.) Wilczek) varieties under O_3 stress. T0 = ambient O_3 level 40–45 ppb, T1 = ambient O_3 level + ascorbic acid, T2 = ambient O_3 level + silicic acid, T3 = elevated O_3 level 120 ppb, T4 = elevated O_3 level + ascorbic acid T5 = elevated O_3 level + silicic acid

	Treatments	NM-28	NM 13–1	NM 19–19	NM 20–21	NM 121–25	NM-51	NM-54	NM- 92	NM-98	NM-2006	NM-2011	NM-2016
Ascorbic acid	T0	204 ± 31.69	359 ± 30.30	456 ± 55.86	518 ± 8.39	432 ± 2.58	302 ± 8.73	403 ± 8.15	330 ± 14.01	248 ± 22.12	561 ± 16.96	409 ± 11.60	476 ± 5.60
	T1	329 ± 1.86	401 ± 32.86	481 ± 32.76	526 ± 2.72	440 ± 0.87	346 ± 19.01	430 ± 15.23	352 ± 26.47	360 ± 42.92	564 ± 10.91	459 ± 18.12	509 ± 5.89
	T2	419 ± 16.09	366 ± 28.18	543 ± 7.41	534 ± 2.72	433 ± 2.17	441 ± 7.17	438 ± 8.62	415 ± 18.49	442 ± 25.56	562 ± 5.17	437 ± 12.29	515 ± 2.58
	T3	450 ± 16.52	539 ± 1.14	583 ± 3.44	563 ± 2.29	512 ± 5.60	565 ± 1.86	515 ± 0.57	475 ± 12.70	524 ± 1.72	638 ± 2.58	547 ± 17.12	550 ± 14.65
	T4	452 ± 20.85	601 ± 1.43	589 ± 10.24	564 ± 32.25	558 ± 4.16	578 ± 13.71	518 ± 2.87	478 ± 15.30	529 ± 14.49	643 ± 12.29	595 ± 11.99	551 ± 9.50
	T5	465 ± 19.96	565 ± 27.29	591 ± 23.10	579 ± 30.11	519 ± 33.22	627 ± 10.12	520 ± 7.28	489 ± 8.32	536 ± 3566	641 ± 9.93	551 ± 13.12	552 ± 5.84
Phenolics	T0	33,500 ± 1522	28,050 ± 2044	42,950 ± 16	69,025 ± 2019	54,900 ± 4333	27,600 ± 2559	58,825 ± 2799	63,950 ± 8796	25,050 ± 2554	68,125 ± 1584	52,875 ± 5152	58,800 ± 3720
	T1	43,108 ± 663	37,916 ± 2392	43,600 ± 2633	70,234 ± 589	56,600 ± 2072	41,175 ± 3106	59,867 ± 2949	66,450 ± 3495	26,825 ± 1149	71,000 ± 1260	54,377 ± 917	60,384 ± 2949
	T2	44,109 ± 877	35,584 ± 2322	46,600 ± 1520	71,133 ± 575	55,533 ± 578	44,392 ± 1182	62,800 ± 949	67,117 ± 1847	27,492 ± 877	69,350 ± 2726	53,100 ± 3589	60,717 ± 1446
	T3	45,475 ± 2485	55,825 ± 2054	49,500 ± 3696	72,800 ± 6264	64,300 ± 2477	50,925 ± 5403	68,600 ± 1836	73,050 ± 4977	37,050 ± 2399	72,000 ± 2158	58,175 ± 3045	61,250 ± 3134
	T4	45,975 ± 1953	59,450 ± 1435	50,775 ± 853	73,200 ± 1929	68,625 ± 3124	51,059 ± 955	69,900 ± 2780	73,850 ± 283	41,350 ± 2603	75,017 ± 963	67,400 ± 9022	62,050 ± 3556
	T5	46,158 ± 1099	56,117 ± 2214	51,042 ± 842	76,258 ± 1867	66,250 ± 3261	52,158 ± 2175	72,200 ± 4819	75,375 ± 754	43,825 ± 1816	74,333 ± 2254	64,125 ± 4612	62,775 ± 3548

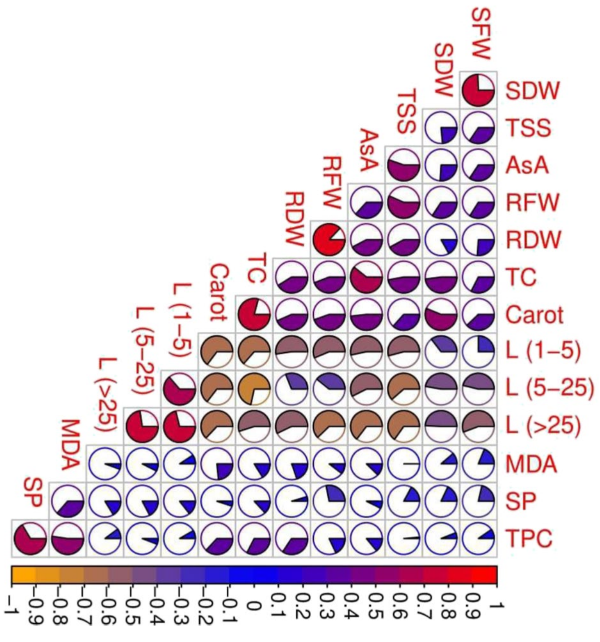

Fig. 4 Relationship between different variables of mung bean varieties under elevated O_3 with application of ascorbic acid and silicic acid. Different abbreviations used are as follows: SFW, shoot fresh weight; SDW, shoot dry weight; TSS, total soluble sugar; AsA, ascorbic acid contents; RFW, root fresh weight; RDW, root dry weight; TC, total chlorophyll content; Carot, carotenoid content; L (1–5), leaves with 1–5% injury; L (2–25), leaves with 2–25% injury; L (>25), leaves with more than 25% injury; MDA, malondialdehyde content; SP, soluble protein

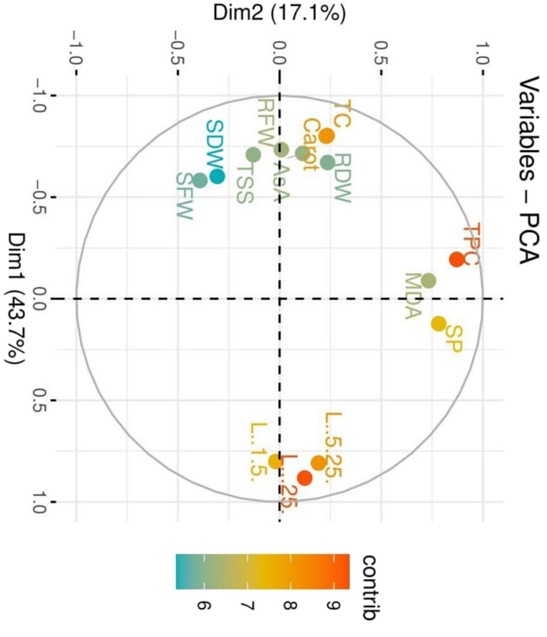

Fig. 5 Loading plots of principal component analysis (PCA) on various attributes (morphological and physiological)of different varieties of mung bean under elevated O_3 with foliar application of ascorbic acid and silicic acid. Different abbreviations are as follows: SFW, shoot fresh weight; SDW, shoot dry weight; TSS, total soluble sugar; AsA, ascorbic acid contents; RFW, root fresh weight; RDW, root dry weight; TC, total chlorophyll content; Carot, carotenoid content; L (1–5), leaves with 1–5% injury; L (2–25), leaves with 2–25% injury; L (>25), leaves with more than 25% injury; MDA, malondialdehyde content; SP, soluble protein

The foliar injury symptoms due to excessive tropospheric O_3 as recorded during the current experiment had also been reported by Leung et al. (2020). The results of the present investigation also indicated purple or brown stipples, necrosis, tip or margin burns, and abscission of leaves due to elevated O_3 levels (Feng et al. 2014). Visible foliar injury symptoms (chlorosis) could be due to the breakdown of chlorophyll molecules as elevated O_3 induced oxidative burst in leaves and excessive accumulation of ROS leading to leaf chlorosis and necrosis (Sharps et al. 2021). Palisade mesophyll cells are the principal target of O_3 damage, according to studies. Furthermore, palisade cells in plants exposed to O_3 were substantially shorter than those in plants kept in filtered air. The presence of clusters of necrotized and collapsed cells in which only the remains of the organelles could be seen was verified by ultrastructural studies of mesophyll. Programmed cell death and other necrosis had occurred in some of the cells that formed the lesion. The aberrant growth of enlarged thylakoids was the most evident change produced by prolonged O_3 exposure, as measured in (Faoro and Iriti 2005; Faoro and Iriti 2009). The protective role of ascorbic acid for photosynthetic pigments and other cellular components from oxidative damage as evident from the results of the current experiment is due to its detoxification capacity of ROS produced under O_3 stress (Ardebili et al. 2015). Moreover, the silicic acid foliar application also increased the activities and accumulation of ROS detoxifying enzymes and antioxidants (Moradtalab et al. 2018) and hence prevented the plants from negative effects of elevated O_3 in terms of foliar injury.

A decrease in total soluble sugar contents of mug beans growing under elevated O_3 levels is likely to be the effect of ozone on photosynthesis, carbohydrates distribution, and gas exchange (Grulke and Heath 2020), which decreases the overall metabolic turnover in the cell. Moreover, Sanches et al. (2019) reported a decrease in carbohydrates of potato leaves at a concentration of 60 ppb O_3 due to the damage to the outer membrane of the cell. The positive effects of the applied growth regulators as recorded during the present study had also been reported by other researchers. For instance, Coskun et al. (2016) reported that silicic acid applications resulted in higher soluble sugar content in plants. Likewise, Mekki et al. (2015) reported that ascorbic acid application increased the total soluble sugars that might be due to the significant increase in photosynthetic pigments, which are reflected in the photosynthesis process and led to increases in sugar contents.

The increasing trend of soluble proteins in mung beans growing under elevated O_3 levels as observed during the current study had also been reported in other crops such as soybean (Ahsan et al. 2010). The silicic acid foliar application further enhanced the protein contents, which may be the result of a reduction in oxidative damage of cells caused by higher O_3 levels as compared to ambient conditions (Ma and Takahashi 2002). In addition, protein contents had also been steadily increased by exogenous application of ascorbic acid that might be due to increased nitrogen; the building block of protein synthesis (Ejaz et al. 2012).

The level of O_3 exposure has been demonstrated to correlate with MDA concentration, which measures the condition and integrity of a membrane by measuring the degree of lipid peroxidation. Furthermore, O_3 treated plants had higher MDA levels, demonstrating that the status of membrane lipid peroxidation is related to the level of O_3 exposure in plants (Iglesias et al. 2006). MDA may harm plant cells by covalently altering the thiol groups of peptides and interacting with other biological components. Moreover, most nonenzymatic lipid peroxidation products initially accumulate within membranes and can disrupt the selective permeability of the lipid bilayer (Sattler et al. 2006). The effectiveness of the exogenous application of ascorbic acid in decreasing the MDA content under O_3 stress as recorded during the current investigation is due to its enhancing membrane characteristics ability and antioxidative potential (Elkeilsh et al. 2019). Similarly, silicon application had been reported to reduce MDA contents by decreasing the plasma membrane permeability of leaf cells (Hasanuzzaman et al. 2020) and hence can protect the plants from the damaging effects of O_3 as indicated by the results of the present study.

Proline levels in O_3 contaminated mung bean leaves increased considerably in the current study because proline helps to stabilize subcellular structures (e.g., membranes and proteins), scavenge free radicals, and buffer cellular redox potential under stress circumstances (Ashraf and Foolad 2007). However, Hemmati et al. (2018) observed that the application of ascorbic acid-induced tolerance to stress reduced the reactive oxygen species by increasing antioxidant enzyme activity. Silicon foliar application increased proline levels in plants as compared to those treated with O_3 stress because proline is considered a biochemical indicator of stress and an osmotic regulator (Mauad et al. 2016). The enhancement in total phenolic contents in mung bean genotypes due to O_3 stress pointed toward a link between increased phenolic content and impaired growth. It had been suggested that the allocation of carbon in O_3 exposed plants was changed toward the formation of phenolics (Wang et al. 2018). Furthermore, phenolics also act as antioxidants and show protective properties against ROS (Phuyal et al. 2020). The increase in total phenolics due to foliarly applied ascorbic could be explained by the fact that ascorbic acid activates some kind of gene regulatory mechanism to stimulate the functioning of nonenzymatic antioxidants (Halimeh et al. 2013). Similarly, silicon application had been found to improve secondary metabolism by promoting the formation of phenolic compounds and structural functions (Filha et al. 2011).

The results obtained from the present study establish differential responses of all twelve mung bean genotypes with the foliar application of two different growth regulators under high O_3 levels (120 ppb). Four genotypes of mung bean (NM 28, NM 13–1, NM-51, and NM-98) were found to be sensitive; five genotypes (NM 19–19, NM 121–25, NM-54, NM-92, and NM-2011) were moderately sensitive, while three genotypes (NM 20–21, NM-2006, and NM-2016) tolerated O_3 stress. Foliar application of ascorbic acid and silicic acid remunerated the negative effects of O_3 pollution by restructuring the metabolic flux in terms of reduced visible injury, growth maintenance, and an upturn of antioxidant profile. The application of 0.1 mM silicic acid was found to be more operative than 10 mM ascorbic acid in reducing the negative effects of O_3 damage in the subject genotypes of mung bean. The O_3 stress tolerance behavior of the signposted genotypes (NM 20–21, NM-2006, and NM-2016) may be explored at a molecular level in the future and could be used as blood in varietal development programs of mung beans in the country.

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Declarations

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Consent for publication Not applicable.

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Competing interests The authors declare no competing interests.

References

Ahsan N, Nanjo Y, Sawada H, Kohno Y, Komatsu S (2010) Ozone stress-induced proteomic changes in leaf total soluble and chloroplast proteins of soybean reveal that carbon allocation is involved in adaptation in the early developmental stage. Proteomics 10(14):2605–2619

Akram NA, Shafiq F, Ashraf M (2017) Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. Front Plant Science 8:613

Ardebili ZO, Ardebili NO, Jalili S, Safiallah S (2015) The modified qualities of basil plants by selenium and/or ascorbic acid. Turk J Bot 39(3):401–407

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta Vulgaris Plant Physiol 24(1):1

Ashraf MFMR, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59(2):206–216

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39(1):205–207

Bellini E, De Tullio MC (2019) Ascorbic acid and ozone: novel perspectives to explain an elusive relationship. Plants 8(5):122

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1–2):248–254

Cakmak I, Horst WJ (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiol Plant 83(3):463–468

Chang YS, Yu MR (2001) Correlation between ozone resistance and relative chlorophyll fluorescence or relative stomatal conductance of bedding plants. Bot Bull Acad Sin 42:265–272

Chaudhary IJ, Rathore D (2020) Relative effectiveness of ethylenediurea, phenyl urea, ascorbic acid and urea in preventing groundnut (*Arachis hypogaea* L) crop from ground level ozone. Environ Technol Innov 19:100963

Coskun D, Britto DT, Huynh WQ, Kronzucker HJ (2016) The role of silicon in higher plants under salinity and drought stress. Front Plant Sci 7:1072

Daripa A, Bhatia A, Ojha S, Tomer R, Chattaraj S, Singh KP, Singh SD (2016) Chemical and natural plant extract in ameliorating negative impact of tropospheric ozone on wheat crop: a case study in a part of semiarid north west india. Aerosol Air Qual Res 16(7):1742–1756

Du Jardin P (2015) Plant biostimulants: definition, concept, main categories and regulation. Sci Hortic 196:3–14

Ejaz B, Sajid ZA, Aftab F (2012) Effect of exogenous application of ascorbic acid on antioxidant enzyme activities, proline contents, and growth parameters of Saccharum spp. hybrid cv. HSF-240 under salt stress. Turk J Biol 36(6):630–640

Elkeilsh A, Awad YM, Soliman MH, Abu-Elsaoud A, Abdelhamid MT, El-Metwally IM (2019) Exogenous application of β-sitosterol mediated growth and yield improvement in water-stressed wheat (*Triticum aestivum*) involves up-regulated antioxidant system. J Plant Res 132(6):881–901

Emberson LD, Pleijel H, Ainsworth EA, Van den Berg M, Ren W, Osborne S, Büker P (2018) Ozone effects on crops and consideration in crop models. Eur J Agron 100:19–34

Faoro F, Iriti M (2005) Cell death behind invisible symptoms: early diagnosis of ozone injury. Biol Plant 49(4):585–592

Faoro F, Iriti M (2009) Plant cell death and cellular alterations induced by ozone: key studies in Mediterranean conditions. Environ Pollut 157(5):1470–1477

Farooq MA, Saqib ZA, Akhtar J (2015) Silicon-mediated oxidative stress tolerance and genetic variability in rice (Oryza sativa L.) grown under combined stress of salinity and boron toxicity. Turk J Agric For 39(5):718–729

Fatima A, Singh AA, Mukherjee A, Dolker T, Agrawal M, Agrawal SB (2019) Assessment of ozone sensitivity in three wheat cultivars using ethylenediurea. Plants 8(4):80

Feng Z, Sun J, Wan W, Hu E, Calatayud V (2014) Evidence of widespread ozone-induced visible injury on plants in Beijing, China. Environ Pollut 193:296–301

Filha MX, Rodrigues F, Domiciano G, Oliveira H, Silveira P, Moreira W (2011) Wheat resistance to leaf blast mediated by silicon. Australas Plant Pathol 40(1):28–38

Ghosh A, Agrawal M, Agrawal S B (2020) Examining the effectiveness of biomass-derived biochar for the amelioration of tropospheric ozone-induced phytotoxicity in the Indian wheat cultivar HD 2967. J Hazard Mater 124968. https://doi.org/10.1016/j.jhazmat.2020.124968

Grulke NE, Heath R (2020) Ozone effects on plants in natural ecosystems. Plant Biol 22:12–37

Halimeh R, Mahlagh G, Maryam P, Pazoki A (2013) Effect of drought interactions with ascorbate on some biochemical parameters and antioxidant enzymes activities in *Dracocephalum moldavica* L. Middle East J Sci Res 13:522–531

Hasanuzzaman M, Bhuyan M, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fotopoulos V (2020) Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. Antioxidants 9(8):681

Hassan A, Amjad SF, Saleem MH, Yasmin H, Imran M, Riaz M, Alyemeni MN (2021) Foliar application of ascorbic acid enhances salinity stress tolerance in barley (*Hordeum vulgare* L.) through modulation of morpho-physio-biochemical attributes, ions uptake, osmo-protectants and stress response genes expression. Saudi J Biol Sci 28(8):4276–4290

Hemmati K, Ebadi A, Khomari S, Sedghi M (2018) Influence of ascorbic acid and 24-epibrassinolide on physiological characteristics of pot marigold under water-stress condition. J Plant Interact 13(1):364–372

Hussain M, Butt AR, Uzma F, Ahmed R, Irshad S, Rehman A, Yousaf B (2020) A comprehensive review of climate change impacts, adaptation, and mitigation on environmental and natural calamities in Pakistan. Environ Monit and Assess 192(1):1–20

Iglesias DJ, Calatayud Á, Barreno E, Primo-Millo E, Talon M (2006) Responses of citrus plants to ozone: leaf biochemistry, antioxidant mechanisms and lipid peroxidation. Plant Physiol Biochem 44(2–3):125–131

Iriti M, Rabotti G, De Ascensao A, Faoro F (2003) Benzothiadiazole-induced resistance modulates ozone tolerance. J Agric Food Chem 51(15):4308–4314

Khan MA, Khan JA, Ali Z, Ahmad I, Ahmad MN (2016) The challenge of climate change and policy response in Pakistan. Environ Earth Sci 75(5):412

Khan N, Bano AM, Babar A (2020) Impacts of plant growth promoters and plant growth regulators on rainfed agriculture. PLoS ONE 15(4):e0231426

Ma JF, Takahashi E (2002) Soil, fertilizer, and plant silicon research in Japan. Elsevier, Amsterdam

Leung F, Pang J, Tai AP, Lam T, Tao DK, Sharps K (2020) Evidence of ozone-induced visible foliar injury in Hong Kong using Phaseolus vulgaris as a bioindicator. Atmosphere 11(3):266

Maamar B, Maatoug M, Iriti M, Dellal A (2015) Physiological effects of ozone exposure on De Colgar and Rechaiga II tomato (Solanum lycopersicum L.) cultivars. Environ Sci Pollut Res 22(16):12124–12132

Madkour SA, Laurence JA (2002) Egyptian plant species as new ozone indicators. Environ Pollut 120(2):339–353

Maghsoudi K, Emam Y, Ashraf M (2015) Influence of foliar application of silicon on chlorophyll fluorescence, photosynthetic pigments, and growth in water-stressed wheat cultivars differing in drought tolerance. Turk J Bot 39(4):625–634

Mahmood AM, Dunwell JM (2020) 2-oxoglutarate-dependent dioxygenases: a renaissance in attention for ascorbic acid in plants. PloS One 15(12):e0242833

Mauad M, Crusciol CAC, Nascente AS, Grassi H, Lima GPP (2016) Effects of silicon and drought stress on biochemical characteristics of leaves of upland rice cultivars. Rev Cienc Agron 47:532–539

Mekki BE-D, Hussien H-A, Salem H (2015) Role of glutathione, ascorbic acid and α-tocopherol in alleviation of drought stress in cotton plants. Int J Chemtech Res 8(4):1573–1581

Moradtalab N, Weinmann M, Walker F, Höglinger B, Ludewig U, Neumann G (2018) Silicon improves chilling tolerance during early growth of maize by effects on micronutrient homeostasis and hormonal balances. Front Plant Sci 9:420

Mukherjee SP, Choudhuri MA (1983) Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiol Plant 58(2):166–170

Muneer S, Jeong BR (2015) Proteomic analysis of salt-stress responsive proteins in roots of tomato (Lycopersicon esculentum L.) plants towards silicon efficiency. Plant Growth Regul 77(2):133–146

Nuvolone D, Petri D, Voller F (2018) The effects of ozone on human health. Environ Sci Pollut Res 25(9):8074–8088

Phuyal N, Jha PK, Raturi PP, Rajbhandary S (2020) Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. The Sci World J. https://doi.org/10.1155/2020/8780704

Pires JCM, Souza A, Pavão HG, Martins FG (2014) Variation of surface ozone in Campo Grande, Brazil: meteorological effect analysis and prediction. Environ Sci Pollut Res 21(17):10550–10559

Prather M, Gauss M, Berntsen T, Isaksen I, Sundet J, Bey I, Stevenson D (2003) Fresh air in the 21st century? Geophys Res Lett 30(2). https://doi.org/10.1029/2002GL016285

Rani S, Schreinemachers P, Kuziyev B (2018) Mungbean as a catch crop for dryland systems in Pakistan and Uzbekistan: a situational analysis. Cogent Food Agric 4(1):1499241

Ren J (2021) Effects of O_3 pollution near formation on crop yield and economic loss. Environ Technol Innov 22:101446

Sanches RFE, Silva APOD, Costa VPD, Carvalho MÂMD, Silva EAD (2019) Changes in carbohydrates accumulation in Viguiera discolor Baker in response to water deficit. Hoehnea 46(4). https://doi.org/10.1590/2236-8906-38/2019

Sattler SE, Mene-Saffrane L, Farmer EE, Krischke M, Mueller MJ, DellaPenna D (2006) Non-enzymatic lipid peroxidation reprograms gene expression and activates defense markers in Arabidopsis tocopherol-deficient mutants. Plant Cell 18(12):3706–3720

Saunier A, Blande JD (2019) The effect of elevated ozone on floral chemistry of Brassicaceae species. Environ Pollut 255:113257

Sharps K, Hayes F, Harmens H, Mills G (2021) Ozone-induced effects on leaves in African crop species. Environ Poll 268:115789

Soares JC, Santos CS, Carvalho SM, Pintado MM, Vasconcelos MW (2019) Preserving the nutritional quality of crop plants under a changing climate: importance and strategies. Plant Soil 443(1):1–26

Sofia D, Gioiella F, Lotrecchiano N, Giuliano A (2020) Mitigation strategies for reducing air pollution. Environ Sci Pollut Res 27(16):19226–19235

Tiwari S, Agrawal M (2009) Protection of palak (Beta vulgaris L. var Allgreen) plants from ozone injury by ethylenediurea (EDU): roles of biochemical and physiological variations in alleviating the adverse impacts. Chemosphere 75(11):1492–1499

Wang H, Zhang L, Ma X, Zou J, Siemann E (2018) The effects of elevated ozone and CO_2 on growth and defense of native, exotic and invader trees. J Plant Ecol 11(2):266–272

Wolfe K, Wu X, Liu RH (2003) Antioxidant activity of apple peels. J Agric Food Chem 51(3):609–614

Xu S, Wang Y, Zhang W, Li B, Du Z, He X, Li M (2021) Experimental warming alleviates the adverse effects from tropospheric ozone on two urban tree species. Environ Pollution 268:115289

Yemm EW, Willis AJ (1954) The estimation of carbohydrate in the plant extract by anthrone reagent. J Biochem 57:508–514

Yeung LY, Murray LT, Martinerie P, Witrant E, Hu H, Banerjee A, Chappellaz J (2019) Isotopic constraint on the twentieth-century increase in tropospheric ozone. Nature 570(7760):224–227