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Promotion of physiological resistance in *Phaseolus vulgaris* L. seedlings grown under salinity stress conditions by using ascorbic acid and biofertilizers

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Abstract

This study examined interaction effect between NaCl, *Trichoderma harzianum*, and non enzymatic antioxidant ascorbic acid on growth parameters, chlorophyll content, malondialdehyde (MDA), soluble sugars, proteins, amino acids, proline, phenols, flavonoids, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), ascorbic acid, peroxidase enzyme profile, and some minerals in *Phaseolus vulgaris* cv. Nebraska grown for 60 days from sowing. Fresh and dry weight, plant height, and leaf area were reduced with increasing salinity but enhanced with *Trichoderma* or ascorbic acid treatment. Photosynthetic pigment content (chlorophyll a, b, a+b and carotenoids) decreased with increased salinity, especially at 200 mM NaCl, but ascorbic acid or *Trichoderma* remarkably increased all pigments. Pigment contents had higher values with ascorbic acid than *Trichoderma*, especially carotenoids. Salinity increased soluble proteins, amino acids, MDA, and proline in shoots and roots, ascorbic acid or *Trichoderma* reduced these components. Phenols and flavonoids slightly decreased with increasing salinity, ascorbic acid or *Trichoderma* slightly increased both components, especially at higher salt concentrations. Shoots and roots exhibited a marked increase in DPPH activity by increasing salinity, ascorbic acid or *Trichoderma* decreased DPPH activity in shoots and slightly increased it in roots. Shoots and roots exhibited a marked increase in Na+ content, but K+ content increased in shoots and decreased in roots with increasing salt concentrations, ascorbic acid or *Trichoderma* decreased Na+ in shoots and roots and increased K+ in all organs or treatments compared to control. Peroxidase isozymes are enhanced with various densities and bands under salinity, which more obvious with ascorbic acid and *Trichoderma* treatment under selected salt concentrations, moreover two isoforms of peroxidase (px1 and px2) were appeared under all treatments.

Keywords: Ascorbic acid; Lipid peroxidation ; Peroxidase; Phenols; Salinity; *Trichoderma*.

1. Introduction

Soil salinity is seriously considered one of the most limiting factors affecting crop production and growth in arid and semiarid regions [1]. Poor water quality for irrigation and soil salinization are the most influential factors. This reduction in plant growth could be attributable to inhibited photosynthetic processes and carbohydrate biosynthesis that reduced stomatal conductance, decreased water use efficiency and induced nutritional deficiency [2, 3]. Salinity stress affects many physiological activities connected to the accumulation of ions and osmolytes, such as proline and antioxidants [4]. Biological molecules, such as proline, proteins, and antioxidant enzymes, are important physiological indicators for evaluating plant osmotic adjustment capability [5].

Antioxidant enzymes, such as catalase and peroxidase, are the most important components in reactive oxygen species (ROS) scavenging systems [6]. ROS are highly reactive and can seriously disrupt normal metabolism through oxidative damage on lipids, proteins, and nucleic acids [7, 8]. ROS production under stress is mainly attributable to increased photorespiration, β-oxidation of fatty acids, and mitochondrial electron transport chain [9]. These enzymes play an important role in plant tolerance against stress conditions, and there are higher concentrations of these antioxidative enzymes in tolerant species than sensitive ones [5].

Recent studies have shown that chlorophyll and its derivatives act as antioxidants to prevent oxidative DNA damage and lipid peroxidation by chelating reactive ions and scavenging free radicals [10].

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Several strategies have been developed to decrease the toxic effects caused by high salinity on plant growth. Among them, biofertilizers, such as plant growth-promoting rhizobacteria (PGPR; *Trichoderma*), play an important role in yield improvement. The use of PGPR and *Trichoderma* harzianum biofertilizers may help develop strategies to facilitate plant growth in saline soils [11, 12].

The effect of ascorbate on plant growth has been extensively studied [13]. Ascorbate is an important non enzymatic antioxidant in plants, and many studies have shown that it plays an essential role in several physiological processes in plants, including growth, differentiation, and metabolism [14, 15]. Ascorbate is a reductant for many free radicals because of its ability to donate electrons in many enzymatic and non-enzymatic reactions [16]. Through ascorbate peroxidase, ascorbate detoxifies H2O2 to water and oxygen and is oxidized to monodehydroascorbate and dehydroascorbate radicals. This is usually the main product of ascorbate oxidation in biological systems [15, 17].

Ascorbate enhances plant tolerance to environmental stressors, such as saline stress [18, 19, 20]. For instance, the exogenous application of ascorbate generally reduces the inhibitory effects of salt stress on the net photosynthetic rate, pigments biosynthesis, and membrane integrity [21]. The protective effect of ascorbate pretreatment under control and/or stress conditions is also highly dependent on the rate of ascorbate uptake by plants [22].

Trichoderma spp. represents a fundamental component of the rhizosphere microbiome. These fungi help plants overcome numerous environmental constraints by stimulating defense responses, including the secretion of antimicrobial ROS, production of secondary metabolites [23], and fitness improvement and development [24]. These abilities have supported the application of *Trichoderma* strains as biocontrol agents or plant biostimulants in agriculture.

Phaseolus vulgaris L. is one of the most important legume crops grown worldwide (Subclass, Rosidae, Order, Fabales, Family, Fabaceae (Leguminosae). It is used as food and fodder because it is rich in proteins, carbohydrates, minerals, vitamins, and fibers [25]. A better understanding of the common bean (P. vulgaris cv. Nebraska) physiological responses under salinity may help in programs that aim to improve grain yield under salinity levels. Therefore, this study aimed to evaluate the effects of biofertilizers and ascorbic acid on the physiological responses [fresh and dry weight, chlorophyll, leaf area, malondialdehyde (MDA), phenols, ascorbic acid, flavonoids, antioxidant enzyme activity, soluble proteins, soluble carbohydrates, amino acids, proline, and some minerals] of common bean under salinity stress conditions.

2. Materials and Methods

Seeds of common bean (*Phaseolus. vulgaris* cv. Nebraska) came from the Seed Center affiliated with the Directorate of Agriculture in Minia. Seeds were selected for uniformity by choosing equal-sized and with the same color. The experiment started at October 2019 till December 2019, whereas, the selected seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min, and thoroughly washed again with distilled water and then dried at room temperature for two days. The seeds were divided into three groups: one was soaked in water for 3 h, the second group was soaked in ascorbic acid (200 ppm for 3 h, and the third group was coated with *Trichoderma harzianum* (T24) during sowing. The first two groups were left to dry at room temperature (28-30) ^{o}C on filter paper. Five uniform air-dried common bean seeds were sown along a center row in each pot at 30 mm depth in plastic pots, each filled with about 4 kg mixed clay sandy soil at a proportion of 2:1 (v/v) to reduce compaction and improve drainage. Plants were left to grow for 3 weeks and treated with different NaCl concentrations (0.0, 50, 100, 150, and 200 mM) with top irrigation (tap water), according to its measured field capacity, and left to grow further for 60 days from sowing at ambient temperature and humidity at the garden of Botany & Microbiology Deptartment, Faculty of Science, Minia University, El-Minia, Egypt.

Plant samples were collected after 60 days from sowing to measure some growth parameters (i.e., leaf area, plant height, root length, and fresh and dry weight of shoots and roots), where the drying was done for 24 hours at 105 ^{o}C. photosynthetic pigments, soluble sugars, soluble proteins, total free amino acids, MDA, proline, total phenolics, total flavonoids, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH%) activity, ascorbic acid, peroxidase profile, and some minerals. Chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids were determined using the spectrophotometric method of [26]. According to [27], total free amino acids were determined. Soluble sugars were determined by the anthrone sulfuric acid method of [28, 29] and later adopted by [30]. Soluble proteins were determined according to the method adopted by [31]. Leaf areas were determined by [32]. The lipid peroxidation level was measured in terms of MDA content. MDA, a product of lipid peroxidation,

was determined according to the method of [33], expressed as n mol (MDA) g^{-1}F.W.). Total phenolic content was determined according to [34]. Total flavonoids were determined according to [35]. DPPH% was assayed according to [36]. Ascorbic acid was assayed, according to [37]. The electrophoretic profile of peroxidase followed the method adopted by [38].

For statistical analysis, data from all experiments were subjected to one-way analysis of variance. Means were compared using the least significant difference test using the statistical program (Sta. Base.Exe.) on computer [39].

3. Results

The effects of NaCl on the growth of 60-day-old plants of common bean presoaked in water or 200 ppm ascorbic acid or coated with *T. harzianum* (T24) under field experimental conditions were summarized. Table 1 reveals that salinity reduced fresh and dry weight and plant length (shoots and roots), especially at 200 mM NaCl in water presoaked seeds (control). Seed presoaked with ascorbic acid or coated with *T. harzianum* resulted in a pronounced increase in all these parameters, especially with ascorbic acid. In shoots presoaked with ascorbic acid, the percentage increase in fresh and dry matter at 50 mM reached 126.08% and 135.15%, respectively; however, at the same treatment with T. harzianum, the percentage increase was 121.65% and 118.18%, respectively, compared to control. Both shoot and root lengths increased with ascorbic acid or *Trichoderma* treatment compared to control. There were a pronounced increase in percentage fresh and dry weight at almost all treatments with ascorbic acid or *Trichoderma* compared to control. Figs. 1 and 2 show both leaf area and water content (WC) of P. vulgaris. WC slightly increased in shoots and roots with increasing salinity in control plants. However, plants treated with ascorbic acid or *Trichoderma* had a negligible effect on WC compared to control. Leaf area exhibited a marked decrease with increasing salinity in Control plants; however, plants treated with ascorbic acid or *Trichoderma* had a positive effect only at moderate and higher salinity levels (150 and 200 mM NaCl).

Table 1: Fresh and dry matter (gm), length (cm) of shoots and roots *of Phaseolus vulgaris* cv Nebraska presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data means of 3 replications.

	NaCl (mM)	Shoot						Root					
		F.W.	%	D.W.	%	L	%	F.W.	%	D.W.	%	L	%
Absolute	**0**	**9.7**	**100**	**2.02**	**100**	**35.25**	**100**	**2.24**	**100**	**0.32**	**100**	**27.5**	**100**
Control	50	11.58	119.38	2.38	117.82	33.75	95.74	2.76	123.21	0.34	106.25	31.00	112.73
	100	10.36	106.80	1.82	90.10	33.50	95.04	1.92	85.71	0.21	65.63	20.00	72.73
	150	7.63	78.66	1.34	66.34	28.50	80.85	1.23	54.91	0.10	31.25	15.00	54.55
	200	7.16	73.81	1.03	50.99	25.50	72.34	1.43	63.84	0.15	46.88	17.75	64.55
Ascorbic	0	13.85	142.78	2.74	135.64	39.25	111.35	4.86	216.96	0.51	159.38	33.50	121.82
	50	12.23	126.08	2.73	135.15	37.25	105.67	3.25	145.09	0.36	112.50	32.50	118.18
	100	12.83	132.27	1.93	95.54	34.50	97.87	2.58	115.18	0.25	78.13	27.25	99.09
	150	13.43	138.45	1.97	97.52	35.50	100.71	1.83	81.70	0.25	78.13	26.50	96.36
	200	7.50	77.32	1.30	64.36	28.67	81.33	1.62	72.32	0.17	53.13	25.00	90.91
Trichoderma	0	10.44	107.63	2.37	117.33	37.00	104.96	2.54	113.39	0.37	115.63	32.17	116.98
	50	11.80	121.65	2.40	118.81	34.17	96.94	3.17	141.52	0.40	125.00	31.83	115.75
	100	11.60	119.59	2.16	106.93	36.17	102.61	2.73	121.88	0.30	93.75	25.50	92.73
	150	9.94	102.47	1.75	86.63	37.88	107.46	1.25	55.80	0.21	65.63	24.75	90.00
	200	8.80	90.72	1.51	74.75	33.50	95.04	2.58	115.18	0.26	81.25	25.33	92.11
LSD at 5%		**1.08**		**0.14**		**0.89**		**0.64**		**0.04**		**0.94**	
LSD at 1%		**1.59**		**0.22**		**1.33**		**0.95**		**0.060**		**1.36**	

F.W. (fresh weight), D.W. (dry weight) and L. (length)

Table 2 illustrates data concerned with pigment analysis. All pigment contents (Chl a, b, a+b and carotenoids) decreased with increasing salinity, especially at 200 mM NaCl. Seed treated with ascorbic acid or *Trichoderma* resulted in a remarkable increase in all pigments, especially at 100 mM NaCl (128.24%, 169.76%, 136.24%, and 202.1%, respectively) in Chl a, b, a+b and carotenoids, respectively. Of interest, pigment content with ascorbic acid treatment has higher values than other treatments.

Table 3 illustrates free amino acids, soluble carbohydrates, and soluble proteins in shoots and roots of *P. vulgaris*. Salinity positively affected soluble proteins and amino acids in shoots and roots of control plants, which more pronounced in shoots than roots.

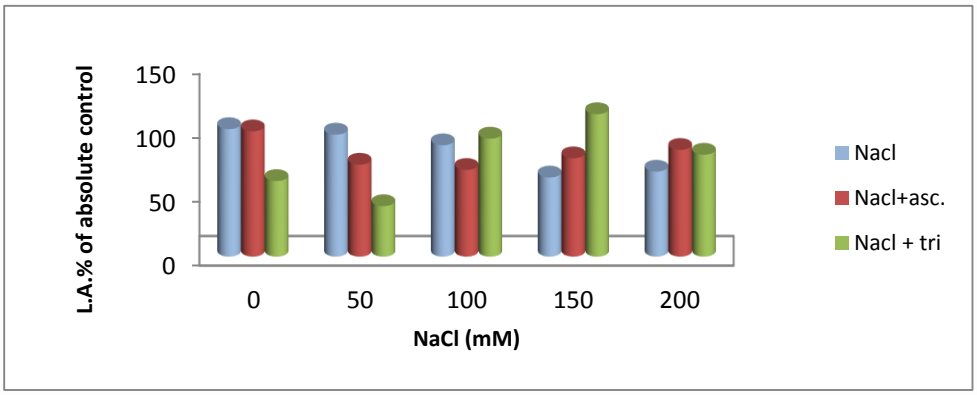

Fig. 1. leaf area (cm2) of *Phaseolus vulgaris* cv Nebraska leaves presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data means of 3 replications.
Asc. (Ascorbic acid), Tri. *(Trichoderma*) and L.A. (leaf area).

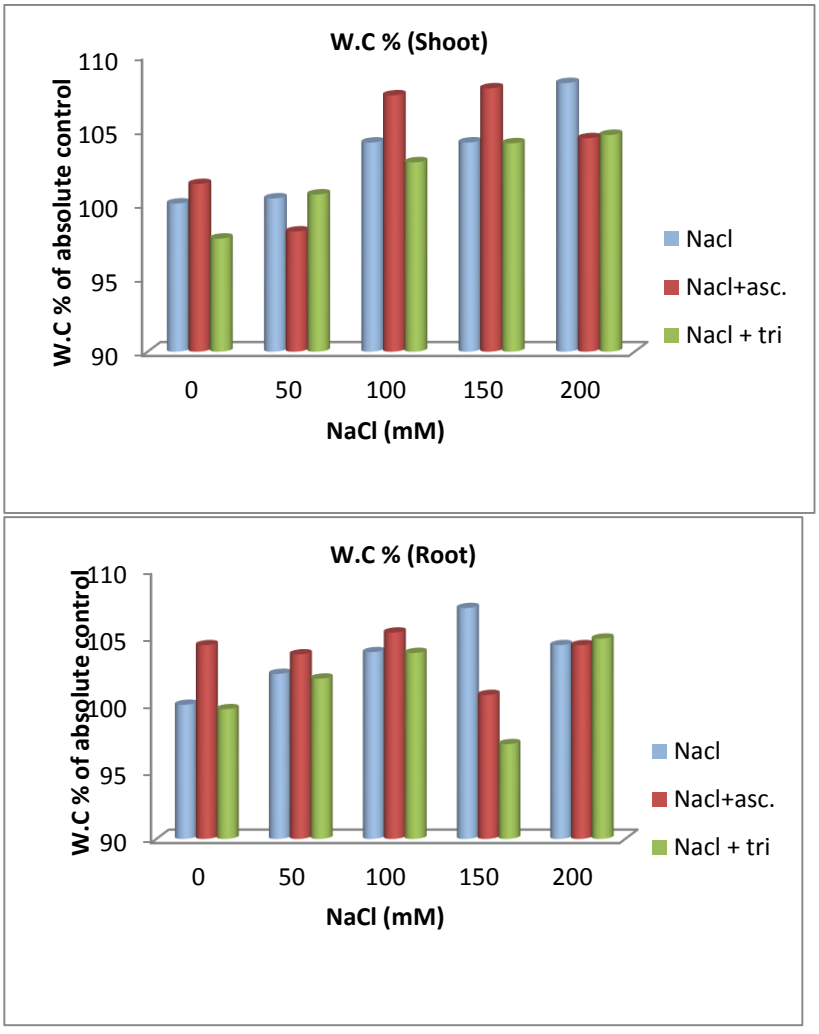

Fig. 2. Water content of shoots and roots of *Phaseolus vulgaris* cv Nebraska presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data means of 3 replications.

Asc. (ascorbic acid), Tri. (*Trichoderma*) and W.C (water content).

However, treatment with ascorbic acid or *Trichoderma* negatively affected soluble proteins and amino acids in both organs under different salinity levels compared to control. Soluble carbohydrates decreased in shoots and increased in roots with increasing salinity in control plants (Table 3); however, treatment with ascorbic acid or *Trichoderma* had an increasing effect at almost treatments, especially in roots. In shoots, *Trichoderma*-treated plants showed a depressive effect on soluble carbohydrates compared to control.

Table 2: Pigment contents (mg/gm DW) of *Phaseolus vulgaris* cv Nebraska presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data means of 3 replications.

	NaCl (mM)	chl.a	%	chl.b	%	chl. (a&b)	%	carotenoids	%
Absolute	0	**3.96**	**100**	**0.95**	**100**	**4.89**	**100**	**1.13**	**100**
Control	50	2.90	73.22	1.34	141.54	4.23	86.38	1.17	103.85
	100	3.54	89.54	0.40	42.50	3.94	80.48	1.61	142.65
	150	2.42	61.17	0.75	79.50	3.17	64.70	0.91	80.23
	200	1.97	49.87	0.84	89.39	2.81	57.48	0.78	68.86
Ascorbic	0	4.15	104.77	1.37	144.51	5.50	112.42	1.87	165.33
	50	3.50	88.49	2.44	258.02	5.93	121.15	1.76	156.07
	100	5.07	128.24	1.60	169.76	6.67	136.24	2.28	202.09
	150	4.49	113.57	1.46	154.72	5.95	121.50	1.98	175.31
	200	2.15	54.22	1.66	176.14	3.80	77.71	1.20	106.17
Trichoderma	0	4.28	108.07	0.55	58.01	4.82	98.43	1.39	123.23
	50	4.24	107.06	0.47	49.41	4.70	95.96	1.17	103.56
	100	4.39	110.88	0.88	92.91	5.26	107.42	1.91	169.31
	150	3.67	92.65	0.77	81.79	4.43	90.56	1.64	145.07
	200	3.85	97.30	0.57	60.53	4.42	90.21	1.62	143.93
LSD at 5%		**0.07**		**0.08**		**0.12**		**0.04**	
LSD at 1%		**0.10**		**0.11**		**0.17**		**0.05**	

chl.a (chlorophyll a), chl.b (chlorophyll b) and chl.(a& b)chlorophyll (a&b).

Table 3: Soluble proteins, amino acids, and soluble sugars (mg/gm DW) of shoots and roots of *Phaseolus vulgaris* cv Nebraska presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data means of 3 replications.

	NaCl (mM)	Shoot						Root					
		Soluble protein	%	Amino acids	%	Soluble carbohydrate	%	Soluble protein	%	Amino acids	%	Soluble carbohydrate	%
Absolute	**0**	**42.52**	**100.00**	**14.66**	**100.00**	**50.09**	**100.00**	**13.19**	**100.00**	**5.72**	**100.00**	**22.27**	**100.00**
Control	50	45.61	107.28	14.11	96.27	56.00	111.80	13.13	99.60	9.63	168.20	35.55	159.59
	100	53.35	125.46	20.14	137.38	43.18	86.21	13.25	100.51	13.32	232.77	27.27	122.45
	150	54.45	128.07	19.77	134.89	32.86	65.61	11.66	88.42	13.22	230.95	29.18	131.02
	200	59.40	139.70	12.78	87.22	51.00	101.81	14.52	110.11	12.93	225.99	30.68	137.76
Ascorbic	0	37.36	87.86	12.40	84.59	67.64	135.03	9.13	69.26	6.87	120.10	85.00	381.63
	50	32.04	75.35	17.05	116.33	69.27	138.29	6.38	48.38	5.40	94.45	80.59	361.84
	100	36.67	86.23	15.39	104.97	103.63	206.90	8.25	62.59	4.00	69.97	53.36	239.59
	150	43.69	102.76	13.82	94.28	137.72	274.95	9.61	72.90	5.44	95.04	69.23	310.82

	200	37.12	87.30	19.21	131.05	116.86	233.30	8.88	67.34	5.83	101.96	23.32	104.69
	0	39.16	92.10	12.80	87.31	52.55	104.90	13.43	101.82	7.13	124.58	68.64	308.16
	50	32.68	76.86	16.36	111.62	41.18	82.21	11.25	85.34	3.45	60.27	89.45	401.63
Trichoderma	100	47.52	111.76	13.31	90.78	33.45	66.79	8.00	60.67	2.06	35.95	79.86	358.57
	150	35.59	83.69	10.33	70.48	28.63	57.17	9.92	75.23	2.54	44.47	80.27	360.41
	200	22.48	52.87	12.41	84.65	22.73	45.37	9.01	68.35	1.18	20.55	82.59	370.82
LSD at 5%		**1.16**		**1.97**		**1.77**		**0.25**		**0.03**		**0.18**	
LSD at 1%		**1.68**		**2.86**		**2.57**		**0.36**		**0.04**		**0.26**	

Table 4 shows both MDA in shoots and proline contents in shoots and roots of P. vulgaris under different treatments. MDA increased in shoots and decreased in roots except at higher concentrations (200 mM NaCl), in control plants. Seed treatment with ascorbic acid or *Trichoderma* had a remarkable decreasing effect on MDA, especially in shoots. Proline content significantly increased with increasing salt concentrations (115.30% and 241.38%) in shoots and roots, respectively, at 150 mM NaCl in control plants. Treatment of plants with ascorbic acid or *Trichoderma* decreased proline content in roots and slightly increased it at certain concentrations in shoots with increasing salinity compared to untreated plants.

Table 4: MDA content (n mol g^{-1} Fw) and Proline (mg/gm DW) of shoots and roots of *Phaseolus vulgaris* cv Nebraska presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data means of 3 replications.

	NaCl (mM)	Shoot				Root	
		MDA	%	Proline	%	Proline	%
Absolute	**0**	**256.96**	**100**	**2.26**	**100**	**0.58**	**100**
Control	50	199.26	77.55	2.30	102.04	0.73	125.34
	100	208.39	81.10	2.51	111.14	1.01	172.56
	150	214.07	83.31	2.61	115.30	1.40	241.38
	200	141.22	54.96	2.33	103.28	1.84	316.79
Ascorbic	0	174.63	67.96	2.36	104.55	0.55	94.83
	50	169.46	65.95	2.39	105.70	0.62	107.35
	100	164.30	63.94	2.33	103.28	0.86	148.58
	150	164.30	63.94	2.40	106.32	0.76	130.73
	200	115.73	45.04	2.52	111.64	1.58	271.96
Trichoderma	0	135.88	52.88	2.51	110.83	0.63	109.15
	50	116.25	45.24	2.59	114.91	0.61	105.55
	100	100.75	39.21	2.34	103.85	0.53	92.20
	150	103.33	40.21	2.39	105.78	0.58	100.00
	200	111.60	43.43	2.57	114.14	0.61	106.00
LSD at 5%		**5.34**		**0.018**		**0.02**	
LSD at 1%		**7.73**		**0.026**		**0.03**	

MDA (malondialdehyde)

Table 5 demonstrates the effect of different treatments with ascorbic acid or *Trichoderma* on phenols, flavonoids, and ascorbic acid contents under different NaCl concentrations. In shoots, both phenols and flavonoids slightly decreased with increasing salinity; however, in roots, phenols decreased, and flavonoids markedly increased. Treatment with *Trichoderma* slightly decreased both components, especially at higher concentrations. Ascorbic acid treamtment enhanced phenols only at

higher concentrations in shoots. Ascorbic acid content decreased in roots and remained unchanged in shoots under Control conditions. Plants treated with ascorbic acid or *trichoderma* showed a marked increase in ascorbic acid with increasing salinity, especially in shoots that reached 131.4% at 150 mM NaCl with ascorbic acid compared to control.

DPPH activity was measured in shoots and roots of P. vulgaris (Fig. 3). Both shoots and roots exhibited a marked increase in DPPH% activity by increasing salinity. Treatment of plants with ascorbic acid or *Trichoderma* decreased the percentage of DPPH% activity in shoots and slightly increased it in roots which more pronounced with *Trichoderma* treatment.

Table 5: Total phenolics (mg Ga /g DW) , flavonoids (mg Qu /g DW) and ascorbic acid (mg/gm DW) of shoots and roots of *Phaseolus vulgaris* cv Nebraska presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data means of 3 replications.

	NaCl (mM)	Shoot						Root					
		phenols	%	flavonoids	%	ascorbic acid	%	phenols	%	flavonoids	%	ascorbic acid	%
Absolute	**0**	**51**	**100**	**43.58**	**100**	**0.70**	**100**	**24.26**	**100**	**23.75**	**100**	**1.43**	**100**
Control	50	42.54	83.41	39.58	90.82	0.70	100.00	27.40	112.94	38.00	160.00	1.10	76.92
	100	47.01	92.18	36.28	83.25	0.70	100.00	23.12	95.30	38.00	160.00	0.88	61.54
	150	46.41	91.00	37.60	86.29	0.70	100.00	23.82	98.19	35.25	148.42	0.66	46.15
	200	46.44	91.06	37.00	84.91	0.70	100.00	23.61	97.32	33.00	138.95	0.38	26.92
Ascorbic	0	47.79	93.71	35.95	82.50	0.86	122.66	21.08	86.89	35.00	147.37	0.77	53.85
	50	44.67	87.59	32.38	74.30	0.79	113.22	21.39	88.17	21.75	91.58	0.82	57.69
	100	43.35	85.00	32.78	75.22	0.79	112.86	21.72	89.53	29.50	124.21	1.11	76.92
	150	56.25	110.29	34.88	80.03	0.92	131.43	21.24	87.55	24.25	102.11	1.11	76.92
	200	50.07	98.18	37.00	84.91	0.92	131.43	20.42	84.17	25.00	105.26	1.21	84.62
Trichoderma	0	58.89	115.47	40.53	93.00	0.79	112.86	22.72	93.65	39.00	164.21	1.43	100.00
	50	32.07	62.88	39.40	90.42	0.66	94.29	20.10	82.85	29.50	124.21	1.32	92.31
	100	36.48	71.53	38.48	88.30	0.86	122.66	16.94	69.83	28.75	121.05	0.99	69.23
	150	47.31	92.76	26.28	60.30	0.86	122.66	21.94	90.44	29.75	125.26	0.66	46.15
	200	28.53	55.94	16.28	37.35	0.53	75.71	19.98	82.36	27.50	115.79	0.33	23.08
LSD at 5%		**1.06**		**0.69**		**0.01**		**0.50**		**1.04**		**0.02**	
LSD at 1%		**1.57**		**0.99**		**0.02**		**0.72**		**1.51**		**0.03**	

Figs. 4 and 5 summarize Na^+ and K^+ contents. Both shoots and roots exhibited a marked increase in Na+ content and reached 145% from Control values at 200 mM NaCl in shoots and 122.58% at the same treatment in roots; however, K^+ content increased in shoots and decreased in roots with increasing salt concentrations. Treatment with ascorbic acid or *Trichoderma* decreased Na^+ in both organs (shoots and roots) and increased K+ in all organs analyzed or treatments used compared to control.

Fig. 6 showed peroxidase profile (PX). The electrophoresis profile of PX showed two isoforms (px1 and px2) under salinity and treatments with ascorbic acid or *trichoderma*. In peroxidase profile, px1 was appeared under all different salt concentrations and with other treatments. On the other hand, px2 appeared only at 200 mM NaCl and with all selected *trichoderma* treatments. The band intensity of px1 was enhanced under different concentrations of NaCl and more pronounced at 200 mM NaCl. The treatment of plants with ascorbic acid had more or less unchanges in band intensity of px1 at lower and moderate salinity levels, but at 200 mM NaCl the band was vanishingly appeared. Treatments with *trichoderma* resulted in enhanced Px1 intensity in control plants only.

4. Disscussion

In this study, salinity had negative effects on the plant growth of *P. vulgaris*. Both fresh and dry weight of shoots decreased to 73.81% and 50.99%, respectively, compared to absolute control plants (Table 1). However, ascorbic acid or *T. harzianum* (T24) improved fresh weight by 132.27% and 119.59% with ascorbic acid or *Trichoderma* at 100 mM NaCl. Salinity stress induces osmotic and ionic stress, leading to retarded growth in shoot and root length, fresh and dry weight, reduced pigment content, and hampers the uptake of mineral elements [40]. Similar results were obtained by [41], who stated that the growth of common bean plants considerably decreased, by salt stress. Salinity can inhibit plant growth by altering the water potential, increasing ion toxicity, inhibiting cell division and expansion, or causing an ion imbalance [42].

Similarly, [43] reported that growth reduction caused by salinity stress is due to the inhibition of apical growth in plants and the imbalance of endogenous

hormones. In addition, a secondary cause of salinity stress in plants is the stress-induced production of ROS [44]. The enhanced production of ROS during salinity stress leads to progressive oxidative damage and ultimately cell death and growth suppression [45].

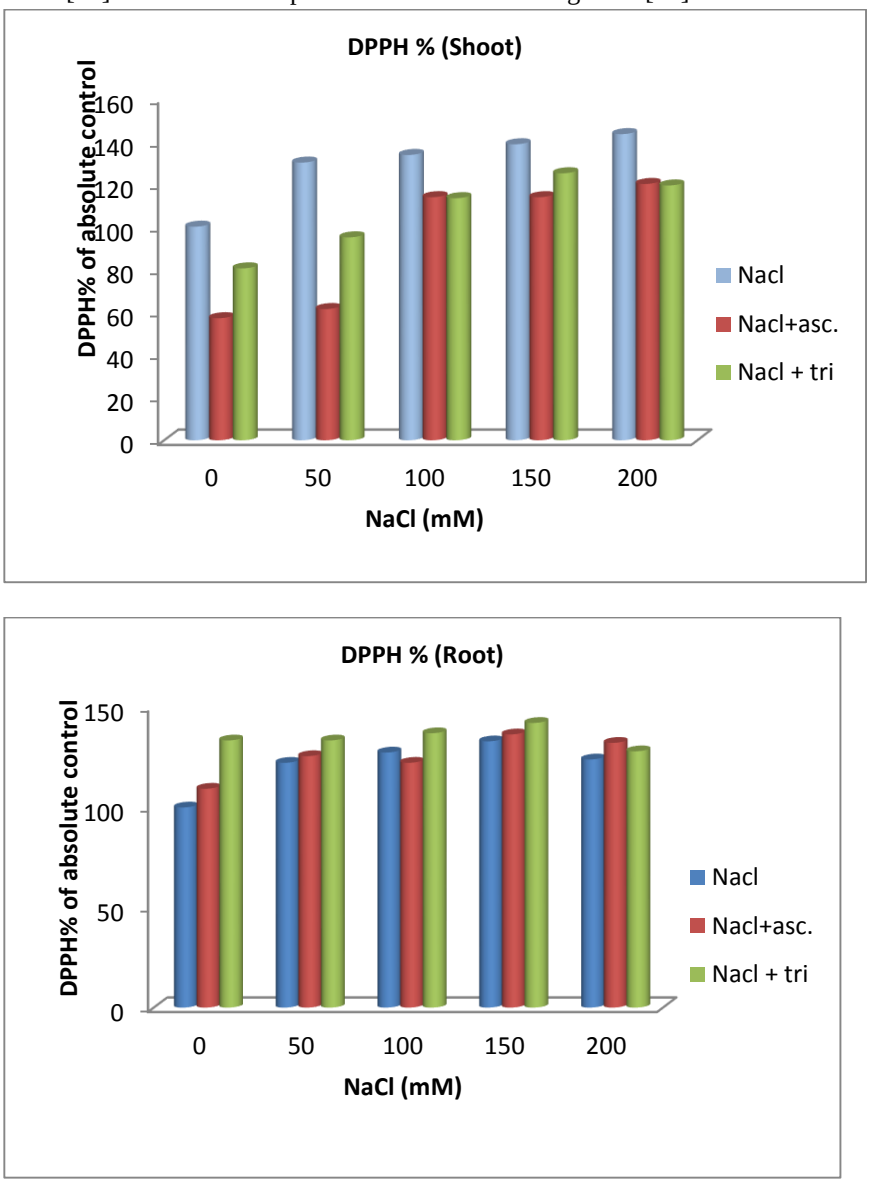

Fig. 3. Antioxidant activity (DPPH %) of shoots and roots of *Phaseolus vulgaris* cv Nebraska presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data means of 3 replications.
Asc. (ascorbic acid), Tri. (*Trichoderma*) and DPPH (2, 2-Diphenyl-1-picrylhydrazyl).

In contrast, the beneficial effects of ascorbic acid (AsA) on plant growth (Table 1) reached 138.45% at 150 mM NaCl compared to control in shoots. AsA is involved in root elongation, cell vacuolation, and cell expansion [17]. Moreover, AsA increased indole-3-acetic acid (IAA) content, stimulating cell division and/or cell enlargement, thus improving plant growth [20]. Ascorbate is also involved in controlling intracellular ROS levels by direct scavenging or via the ascorbate-glutathione cycle. This might provide the means to protect the cell against uncontrolled oxidation and improved growth [46].

Treatment of plants with ascorbic acid or *Trichoderma* resulted in the promotion of dry weight in shoots and roots at almost all salinity levels used. This promotion reached 135.15% and 118.81% with ascorbic acid and *Trichoderma*, respectively, at 50 mM NaCl in shoots. In roots, enhancement in dry

weight at 50 mM NaCl with ascorbic acid or *Trichoderma* reached 112.5% and 125%, respectively, compared to control. [47] found that bean plants inoculated with *Trichoderma velutinum* showed a significant increase in dry weight in shoots and roots by inducing the expression of defense-related genes. The enhancement of plant biomass by promoting lateral root growth has been observed in many plant species treated with *Trichoderma* spp.

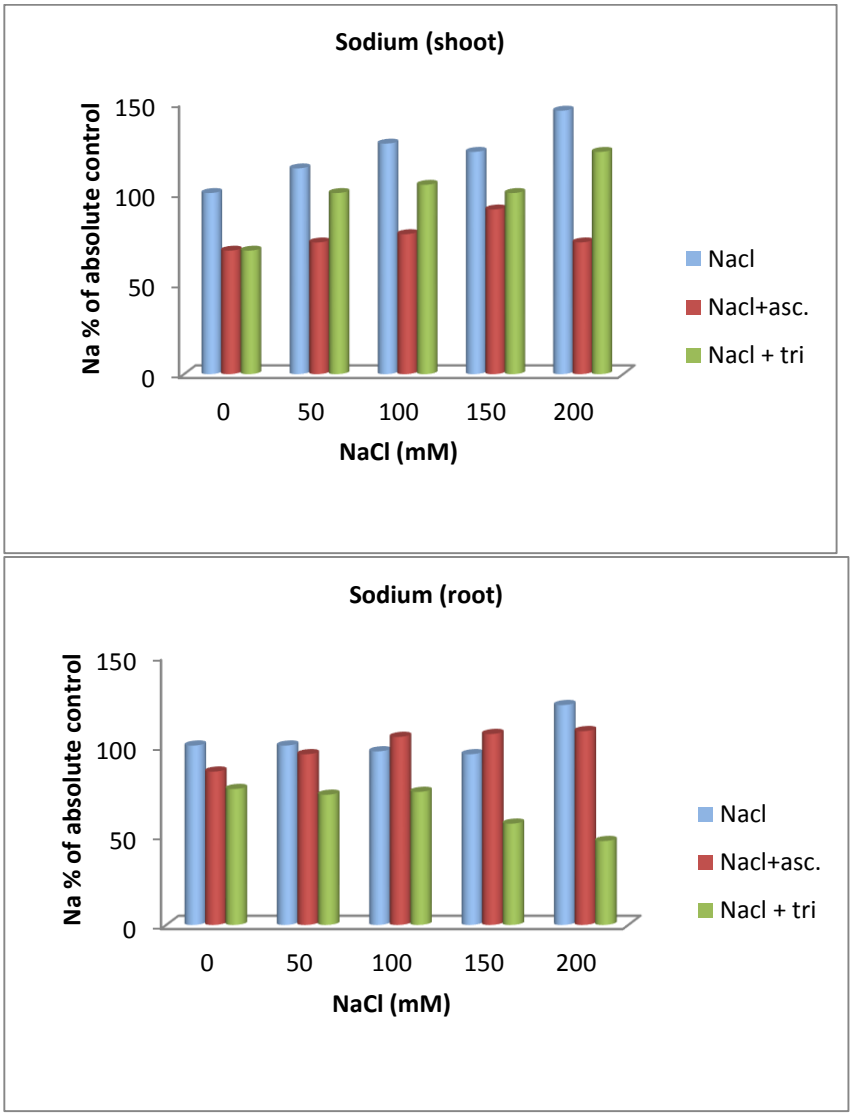

Fig. 4. Sodium (mg/gm dry matter) of shoots and roots of *Phaseolus vulgaris* cv Nebraska presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data .means of 3 replications.
Asc. (ascorbic acid) and Tri. (*Trichoderma*).

This effect has also been related to IAA or auxin analogs [48]. According to [49], the application of *T. harzianum* restored mustard plant height.

Fig. 1 and 2 show the leaf area and WC in shoots and roots of P. vulgaris under different treatments. Leaf area decreased with increasing salinity. Treatment with ascorbic acid or *Trichoderma* caused a slight increase in leaf area, especially at higher salt concentrations (150 and 200 mM NaCl). [23] reported that *Trichoderma*-inoculated plants had increased foliar area and secondary root development, modulated root architecture, and increased growth in plants, such as strawberries, tomatoes, and soybeans. [54] reported that seed biopriming treatment with *T. harzianum* isolates in rice (*Oryza sativa* L.) subjected to salt stress significantly increased the length and fresh weight of shoots and roots, number of leaves, and leaf area compared to control at all stress levels.

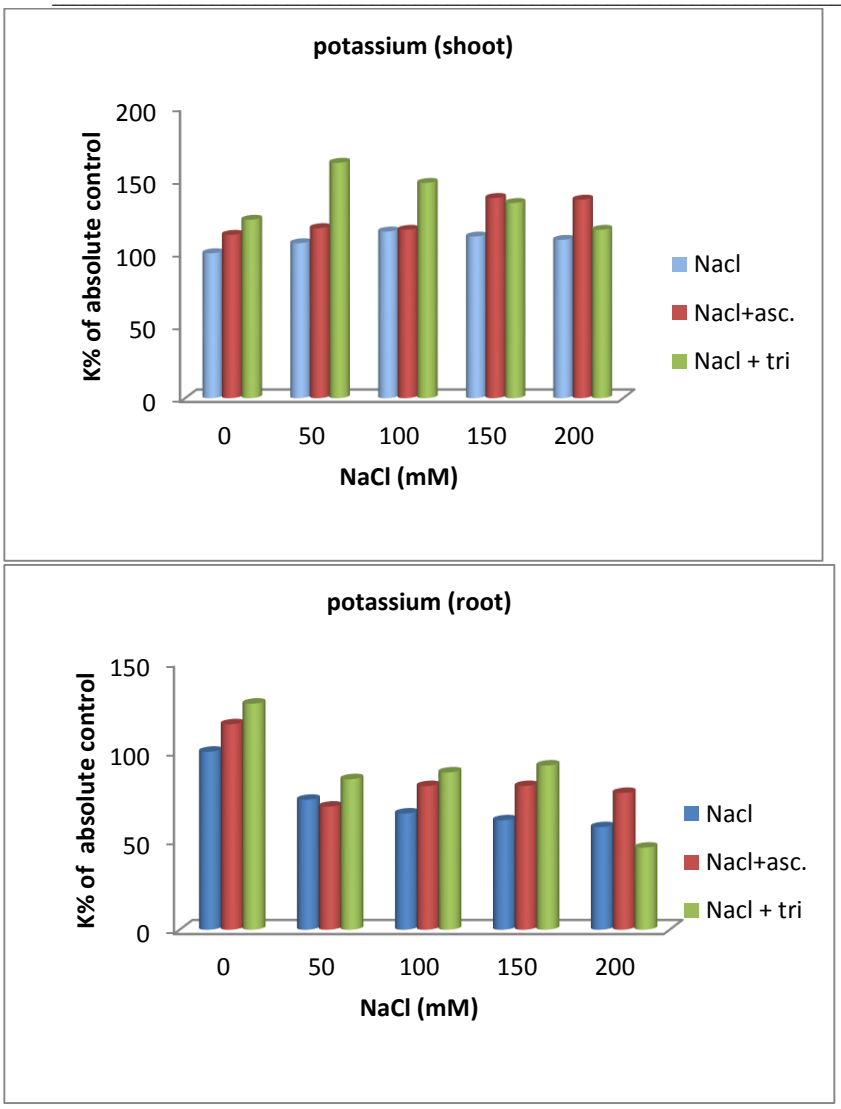

Fig. 5. Potassium (mg/gm DW) of shoots and roots of *Phaseolus vulgaris* cv Nebraska presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data means of 3 replications. Asc. (ascorbic) and Tri. (*Trichoderma*).

Water content (WC) in shoots and roots of Control plants increased with increasing salt concentrations in the rooting medium; however, treatment with ascorbic acid or *Trichoderma* had a negligible effect in WC in all treatments used. [18] recounted that the effects of wheat seeds with *Trichoderma longibrachiatum* (T6) increased the relative WC in leaves and roots. In the same context, [55, 56] reported that ascorbate mitigated the inhibitory effects of salt stress on plant growth due to increased leaf area, improved chlorophyll, and carotenoid contents, and enhanced antioxidant accumulation.

Measurements of photosynthetic pigments under salinity conditions in *P. vulgaris* showed that NaCl salinity negatively affects these pigments (Table 2). A pronounced decrease in Chl a, b, a+b and carotenoids (49.87%, 89.39%, 57.48%, and 68.86%, respectively, compared to control) at 200 mM NaCl reflects the adverse effects of salt stress on chlorophylls and carotenoids. The application of ascorbic acid or *Trichoderma* to salted plants resulted in a pronounced increase in pigment fractions, especially those treated with ascorbic acid (113.57%, 154.72%, 121.5%, and 175.3% in Chl a, b, a+b and carotenoids, respectively) compared to control at 150 mM NaCl. In ascorbic acid-treated plants, a high level of carotenoids can synergistically function with

ascorbic acid to provide an effective barrier against oxidation under salinity stress.

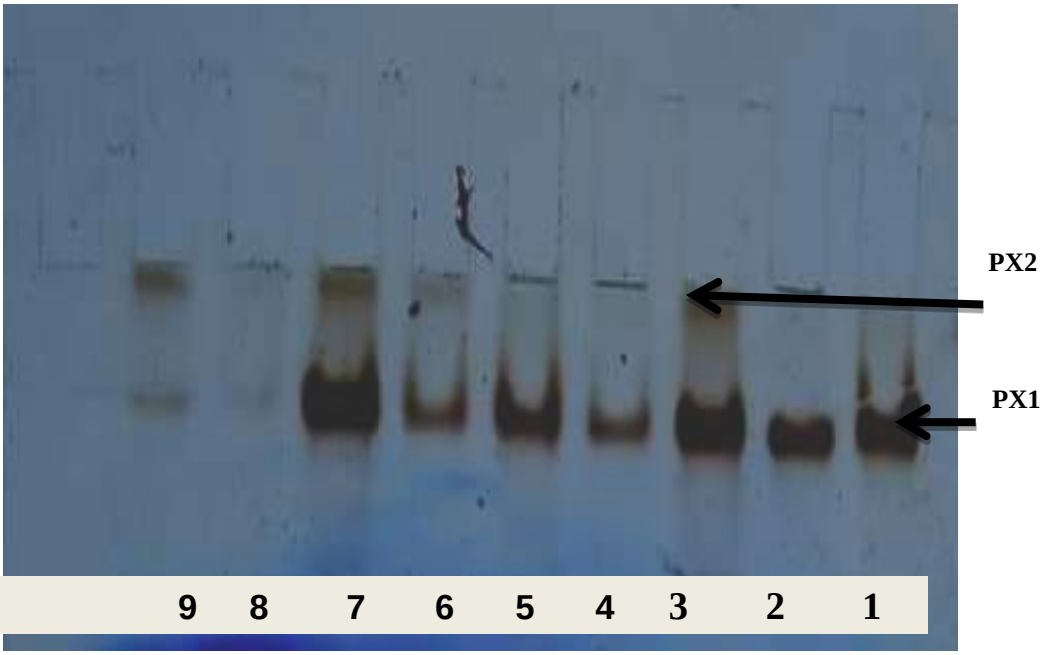

Fig. 6. Electrophoresis banding profile of peroxidase from leaves of *Phaseolus vulgaris* cv Nebraska: lane 1: control, lane 2: control + ascorbic acid, lane 3: control +*Trichoderma*, lane 4: (100 mM NaCl), lane 5: (100 mM NaCl +ascorbic), lane 6: (100 mM NaCl +*Trichoderma*), lane 7: (200 mM NaCl), lane 8: (200 mM NaCl + ascorbic acid) and lane 9: (200 mM NaCl +*Trichoderma*).

PX1 (peroxidase 1) and PX2 (peroxidase 2).

High salinity caused a disturbed chloroplast structure, number, and size, which affected chlorophyll content and/or caused the disruption of chloroplasts by oxidative stress that causes a decrease in chlorophyll content [50]. Also, chlorophyll content reduction under salinity stress is attributable to the salt-induced acceleration of enzymes responsible for chlorophyll degradation and instability of the pigment-protein complex [51].

The positive effects of ascorbic acid on counteracting the adverse effects of salt stress are stabilizing and protecting the photosynthetic pigments and the photosynthetic apparatus from oxidation damage [20]. Ascorbic acid can mitigate salinity's adverse effects by increasing the content of IAA and gibberellin GA3 and decreasing the abscisic acid level [20], which may be involved in protecting the photosynthetic apparatus and consequently increasing photosynthetic pigments.

In this study, the application of *T. harzianum* restored the chlorophyll and carotenoid content to an appreciable level, and the results corroborated with [52]. *T. harzianum* increases the uptake of essential elements, especially Mg^{2+}, which was negatively affected by NaCl stress; hence, chlorophyll synthesis increases in *Trichoderma*-inoculated plants. The increase in photosynthetic pigments by *Trichoderma* colonization in plants may also be due to the inhibition of Na uptake [53], which agreed with the results in (Figs. 4 and 5).

Table 3 shows the amounts of soluble proteins, soluble carbohydrates, and soluble amino acids. All these components increased in shoots and roots under Control conditions. Soluble proteins in shoots and amino acids in roots showed a significant increase, especially at higher salt treatments, reaching 137.70% and 225.99%, at 200 mM NaCl respectively, compared to control. The observed significant increase in soluble carbohydrates, soluble proteins, and free amino acids under ascorbic acid or *Trichoderma* treatment reflects the role of these compounds in osmotic regulation under salt stress conditions.

Ascorbate pretreatment also activates carbohydrate biosynthesis [57], participating in cell osmotic potential regulation [58]. The same was

observed in this study, as illustrated in Table 3. Ascorbate pretreatment increased growth and osmoprotectant accumulation under control and stress conditions (soluble carbohydrates, free amino acids, and proline). [59] also ascribed the accumulation of these compounds to their regulating role of stomatal function. Accumulation of soluble carbohydrates might have a physiologically important role in energy supply, osmotic adjustment to maintain leaf water potential, and relative WC. It can reduce cell osmotic potential and increase stress tolerance [60].

Lipid peroxidation is estimated through MDA accumulation and has been used as a good criterion for determining the sensitivity of plants to saline stress [40, 61]. MDA content increased under salt stress in shoots of control plants (Table 4). Treatment of plants with ascorbic acid or *Trichoderma* resulted in a significant decrease in MDA content in shoots. Increased MDA content under salt stress was also reported in mulberry [40]. This study observed a decrease in MDA content in plants treated with *Trichoderma*. Similar results were observed for chickpea, with significantly higher MDA accumulation in non-inoculated than inoculated *Trichoderma* plants [62]. *Trichoderma* treatment resulted in a pronounced decrease in lipid peroxidation levels in the leaves of all plants under salt stress. These results suggested that *Trichoderma* can protect *P. vulgaris* plants against oxidative salt damage. These results strongly agreed with [63], who found decreased lipid peroxidation levels in cucumber plants treated with *T. harzianum* under salt stress. *Trichoderma* induced phytohormones, such as salicylic acid (SA) and jasmonic acid [64]. Intrinsic SA may reduce H_2O_2 content due to its role as an antioxidant in counteracting H_2O_2 generation. *Trichoderma* induced the expression of many antioxidant enzymes that directly or indirectly scavenge ROS and minimize the effects on the plasma membrane [65].

Proline content of P. vulgaris increased significantly under salt stress conditions, reaching 103.28% and 316.79%, compared to control in shoots and roots, respectively, at 200 mM NaCl in control plants. Treatment with ascorbic acid or *Trichoderma* enhanced proline accumulation in shoots, and decreased it in roots especially at higher salinity levels. [49] found the proline content in mustard plants increased when treated with *Trichoderma* under salt stress. Proline is an important osmolyte that maintains cell osmoregulation in NaCl stress [66, 67]. Proline has a high antioxidative property that could scavenge ROS like H_2O_2 and protect the cell from oxidative damage [68].

Polyphenols comprise many compounds, such as phenols and flavonoids [69]. This study found differences in flavonoids, phenols, and ascorbic acid accumulation in shoots and roots depending on the interaction conditions used (Table 5). Flavonoids protect plants against various biotic and abiotic stresses and exhibit a diverse spectrum of biological functions, playing an important role in the interaction between the plant and the environment [70]. The differences in flavonoid accumulation patterns observed in the metabolome of beans could reflect the different metabolic pathways induced by the beneficial fungus *Trichoderma* or treatment with ascorbic acid. Application of ascorbic acid increased total phenolics and total flavonoids under salt stress conditions. These results confirmed [71, 72] in *P. vulgaris* under salt stress. Application of ascorbic acid at 200 or 400 mg via foliar spray significantly increased total phenolics, total flavonoids, and total tannins in *P. vulgaris* under water stress conditions [73].

Antioxidant activity is referred to as free radical scavenging activity by DPPH% the proton radical scavenging action is known as an important mechanism of antioxidation. The effect of antioxidants on DPPH% radical scavenging appeared to be due to their hydrogen-donating ability [74]. Treatment of plants with NaCl under different concentrations resulted in a significant increase in DPPH activity, reaching 143.33% and 123.88% compared to control in shoots and roots, respectively, at 200 mM NaCl (Fig. 3). Treatment of plants with ascorbic acid or *Trichoderma* resulted in decreased DPPH activity in shoots and slightly increased it in roots. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [75]. The decrease in absorbance of the DPPH% radical caused by antioxidants can be ascribed to the action between the antioxidant molecule and the radical.

However, increases in DPPH observed in roots despite these increased antioxidant levels. Therefore, the ROS production exceeded even the induced defense capacity of the anti-oxidant systems to remove them. Also in other studies increases in the

antioxidant system were insufficient to effectively protect the plant against ROS accumulation [16, 17].

In addition, a further increase in DPPH activity and endogenous ascorbic acid resulted from strengthening the antioxidative defense system, followed by an increased tolerance of bean plants to salt stress. Ascorbic acid may participate in the up regulation of soluble sugars and proline synthesis to enhance tolerance mechanisms under salt stress. The increased antioxidant activity was attributable to increases in secondary metabolites (phenolic and flavonoids), considered a tolerance advantage of water and other abiotic stresses, as reported by [76].

The content of Na^+ and K^+ in shoots and roots of tested plants showed a marked increase in both ions

treatment could be because decreasing cytosol's water activity by higher Na accumulation in vacuole during stress requires a coordinated increase in compatible solutes (potassium) in the cytosol to balance out osmotic pressure. The role of potassium in osmotic adjustment is due to charge balance [81].

Plants use many enzymatic and nonenzymatic antioxidants to prevent oxidative damage and keep ROS concentrations within a narrow functional range [82]. Plant defense mechanisms against water stress are also associated with peroxidase activity due to the peroxidase (PX) specific role in synthesis of phenols [83]. In our results Table 5 phenols were enhanced by ascorbic acid treatments which reflect the role of peroxidase in synthesis of phenols. Enhancing peroxidase isozymes with various densities and bands under salinity treatments in control plants may be associated with removing excess H_2O_2 caused by salt stress. Peroxidase isozymes play a key role in salt tolerance [33]. Peroxidase reduces H_2O_2 to H_2O using several reductants available to the cells. Elevated antioxidant activity leads to lower lipid peroxidation under salinity, as reported in *Pisum sativum* [84]. In addition, [85] reported that catalase and APX are the most effective antioxidant enzymes in preventing cell damage. These increases in the activity of antioxidant enzymes, such as peroxidase, under treatment with *Trichoderma* may be an effective defense system supported salt-stressed plants to tolerate salt stress and reduce ROS (i.e., $O_2\bullet-$ and H_2O_2) damage. [86] showed that *Trichoderma* increased SOD and peroxidase in rice cultivars provides tolerance to these plants under water stress. Vitamins could be regarded as bioregulators or hormone precursors that are in tiny amounts employ a valuable impact on plant growth. Ascorbic acids in the defense of plants, in shoots and a significant reduction in roots. Treatment with ascorbic acid significantly increased Na^+ and K^+ in shoots and roots. However, plants treated with *Trichoderma* significantly increased Na^+ and K^+ in shoots and roots (Figs. 4 and 5). *Trichoderma*-inoculated plants were also found to increase potassium content in plants [77]. Increasing potassium uptake ameliorates the negative effect of salinity [78]. [79, 80] found that rice plants treated with *T. harzianum* have better uptake of nutrients and significantly increased the ability to tolerate drought and water-deficit conditions. The observed positive K accumulation under ascorbic acid and *Trichoderma* in resistance to oxidative stress, with their antioxidant properties also play an important role as free radical scavengers. So the band intensity of peroxidase with ascorbic acid treatment was very weak under higher salt concentration may be due to the protective role of ascorbic acid under salt stress.

5. Conclusions

The resistance strategy of *P. vulgaris* cultivated under salinity stress conditions can contribute to the high quality of the final product in terms of leaf pigments, antioxidant activity, increased leaf area, carotenoids, soluble sugars, proline, phenolics, flavonoids, and K^+ ions. Using biofertilizers and non-enzymatic antioxidants should alleviate salinity's adverse effects on the growth of the common bean. It is a simple, cost effective technology that leads to more savings for the environment. Its impact is applicable in terms of enhanced yields. It can be a promising alternative crop legume in saline-prone areas.

6. Conflicts of interest

There are no conflicts to declare.

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