

Effect of Salicylic Acid on the Growth, Photosynthetic Efficiency and Enzyme Activities of Leguminous Plant under Cadmium Stress

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Abstract

The present study was designed with an objective to elucidate the effect of the exogenous application of 10^{-5} M of salicylic acid (SA) to the foliage of the chickpea plants exposed to 0, 25, 50 or 100 mg cadmium (Cd) per kg of soil. The foliage of the plants grown in soil amended with varying doses of Cd was sprayed with 10^{-5} M of SA at 30 days after sowing (DAS). The plant samples were collected at 60 and 90 DAS to assess various parameters. The damage caused due to the Cd exposure was partially overcome by the exogenous application of SA. The SA treatment resulted in a significant increase of growth, photosynthesis, yield and the activity of antioxidative enzymes viz. catalase, peroxidase and superoxide dismutase in the plants exposed to 0, 25 or 50 mg Cd per kg of soil. However, the treatment did not prove to be fruitful in alleviating completely the stress generated by 100 mg Cd per kg of soil.

Keywords: catalase, chickpea, peroxidase, photosynthesis, superoxide dismutase, yield

Introduction

Metal contamination issues are becoming increasingly throughout the world (Foy *et al.*, 1978), with many documented cases of metal toxicity including in mining and agriculture practices. Metals are a natural part of terrestrial systems occurring in soil, rock, air, water and organisms. A few metals, including Cu, Mn and Zn, are however essential to plant metabolism in trace amounts. It has potential to become toxic to plants when metals are present in bioavailable forms at excessive levels. On the other hand, Cd is a non-essential trace element for plants, and has strong toxicity at low concentrations with the ability to readily absorb by roots (Sanita di Toppi and Gabbriellini, 1999). It can suppress the elongation growth of plant cell, inhibit oxidative mitochondrial phosphorylation, induce oxidative stress, inhibit the activities of several antioxidative enzymes, affect photosynthesis by inhibiting ferrous reductase or damaging photosynthesis apparatus, and cause the alteration of chromatin and the change of plasma membrane ATPase activity (Tran and Popova, 2013). Cadmium also causes various direct and indirect effects on plant growth and metabolism (Hasan *et al.*, 2009) by forming complexes with O, N and S ligands (Van Assche and Clijsters, 1990). It interferes with protein metabolism, membrane functioning and water relations (Irfan *et al.*, 2013). Cadmium inhibited net photosynthesis in *Cajanus cajan* (Sheoran *et al.*, 1990) and *Phaseolus vulgaris* (Krupa *et al.*, 1993), O₂ evolution in *Anacystis nedulens* and photosystem II (PS II) in isolated chloroplasts of maize and spinach (Bazzas *et al.*, 1974). Moreover, they

cause metabolic disturbance by altering essential biochemical reactions (Hermans *et al.*, 2004). Exposure to Cd stress disrupt cellular homeostasis and enhances the production of several activated oxygen species designated as reactive oxygen species (ROS) such as superoxide (O₂⁻), hydroxyl radicals (OH[•]), singlet oxygen (¹O₂), and hydrogen peroxide (H₂O₂) that are produced continuously by plants (Foyer and Noctor, 2003). ROS have been shown to damage cell membranes, proteins, lipids, and DNA (causing inter alia DNA base oxidation, DNA protein cross-links, DNA gaps, and breaks) resulting in lipid peroxidation (Skórzyńska-Polit and Krupa, 2006), developmental defects, and genetic instability in plant species (Bal *et al.*, 2000). However, plants possess very efficient enzymatic and non-enzymatic antioxidant defense systems that allow scavenging of ROS and protection of cell to oxidative damage (Gratão *et al.*, 2005).

Salicylic acid (SA) is a phenolic compound synthesized throughout the plant kingdom via the phenylpropanoid pathway (Métraux, 2002). Over the past decade, various workers documented that this molecule plays vital roles in many physiological processes of plants and also have ability to confer tolerance against various abiotic stresses (Janda *et al.*, 2007; Hayat *et al.*, 2013). SA application at a concentration of 0.1 or 0.2 mM reduced the inhibitory effect of Pb²⁺ and Hg²⁺ on the seed germination and seedling growth of two rice (*Oryza sativa* L.) cultivars (Mishra and Choudhuri, 1997). SA increased the fresh and dry mass of shoots and roots in both cultivars under heavy metal stress conditions. The higher concentration of SA was more effective, as evident from a better recovery from metal-

induced growth inhibition. SA moderated the inhibitory effect of lead on the activity of the nitrate reductase enzyme in maize (*Zea mays* L.) plants (Sinha *et al.*, 1994). This suggests that SA has some role in the tolerance of plants to heavy metal stress, though other authors found no increase in the endogenous SA content as the result of Cd treatment, or any difference in the SA content of sensitive and resistant plants of *Salix viminalis* in the control (Landberg and Greger, 2002).

On the basis of above reports, the primary objective of this work was to examine whether or not SA could mitigate the Cd-induced oxidative stress in legumes by regulating the antioxidant defense system and also dissect the efficiency of photosynthetic machinery under Cd stress in the presence as well as absence of SA.

Materials and methods

The certified seeds of *Cicer arietinum* L. cv. 'Avarodhi' were purchased from the Chola Beejh Bhandar, Aligarh, India. The seeds were surface sterilized with 0.01% mercuric chloride solution followed by inoculation with *Rhizobium* and were sown in five sets of earthen pots (10 inch diameter) filled with sandy loam soil and farmyard manure (6:1) arranged under a simple randomized block design. At the start of the experiment, out of these five sets of prepared pots, four sets were supplemented with different doses (0, 25, 50 or 100 mg per kg of soil) of Cd in the form of CdCl₂ respectively and one set of pots was left untreated serving as control. At the stage of 30 DAS, the foliage of the resulting plants was sprayed with 10⁻⁵ M of salicylic acid (SA), except control which received double distilled water (DDW) instead of SA. The concentration of SA was selected on the basis of our earlier experiments (Hayat *et al.*, 2010). The plant samples were collected at 60 and 90 DAS to assess various growth and physiological parameters.

Plant growth analysis

The plants were uprooted and washed under running tap water. The root and shoot length of these plants was measured with the help of a graduated scale. These plants were blotted in blotting sheets to remove the adhering water and weighed on electronic balance to record their fresh mass. These plants were kept in an oven run at 80 °C for 72 hours and then weighed to obtain their dry mass.

Carbonic anhydrase (CA) activity

The CA activity in the leaves was measured by following the method described by Dwivedi and Randhawa (1974).

Photosynthetic measurements

The stomatal conductance (g_s), intercellular CO₂ concentration (C_i), transpiration rate (E), water use efficiency (WUE) and net photosynthetic rate (P_N) in intact leaves were measured using a portable photosynthetic system, between 11:00 and 12:00 h.

Assay of antioxidative enzymes

The activities of peroxidase (POX) and catalase (CAT) were analysed as per the method described by Chance and Maehly (1956) whereas the superoxide dismutase (SOD) was measured by the method of Beauchamp and Fridovich

(1971).

Proline content in leaves

The proline content in the fresh leaf sample was measured following the method described by Bates *et al.* (1973).

Yield Characteristics

At harvest (160 DAS), three plants from each treatment were randomly sampled and counted for the number of pods per plant. The pods from five plants, representing each treatment, were crushed, cleaned to assess the seed weight per plant and 100 seed mass.

Seed protein content

The total protein content in the dry seeds, at harvest, was estimated by adopting the method of Lowry *et al.* (1951).

Statistical analysis

Each observation was replicated three times. The treatment means were compared by analysis of variance using SPSS software version 10 (SPSS, Chicago, IL, USA). Least significant difference (LSD) was calculated at 5% level of probability. Standard error (SE) due to replicates was also calculated.

Results

Length of root and shoot

Foliar application of 10⁻⁵ M of SA significantly increased the length of root and shoot by 33.3%, 40.0% and 29.4%, 30.2% in unstressed plants, 22.5%, 26.1% and 16.7%, 18.0% in the plants exposed to Cd at the rate of 25 mg per kg of soil and 13.0%, 14.5% and 7.5%, 8.2% in the plants exposed to a Cd stress of 50 mg per kg of soil, at 60 and 90 DAS, respectively, over their controls (Tab. 1). However, the spray proved to be inefficient in alleviating the stress generated by Cd, supplemented at the rate of 100 mg per kg of soil.

Fresh and dry mass per plant

Exogenous application of 10⁻⁵ M of SA resulted in a significant increase of 44.4%, 46.9% and 64.5%, 64.9% in the fresh and dry mass of unstressed plants and 17.8%, 21.2% and 19.3%, 13.5% in those exposed to Cd supplemented at the rate of 25 mg per kg of soil, at 60 and

Tab. 1. Effect of 10⁻⁵ M salicylic acid (SA) on the Cd (0, 25, 50 or 100 mg kg⁻¹ of soil) induced changes in root and shoot length plant⁻¹ in *Cicer arietinum* at 60 and 90 DAS. Data are the mean of three independent replicates (± SE)

Treatments	Root length (cm)		Shoot length (cm)	
	60 DAS	90 DAS	60 DAS	90 DAS
Control (DDW)	14.34 ^b ±1.6	17.12 ^b ±3.8	36.54 ^a ±0.11	41.25 ^a ±0.15
Cd (0 mg)+SA	18.53 ^a ±1.7	22.50 ^a ±3.5	47.25 ^a ±0.15	52.35 ^a ±0.17
Cd (25 mg)+SA	17.32 ^a ±3.5	20.15 ^b ±3.2	41.25 ^b ±0.18	46.92 ^b ±0.20
Cd (50 mg)+SA	15.84 ^b ±4.0	17.38 ^b ±2.9	39.11 ^b ±0.1	41.78 ^b ±0.12
Cd (100 mg)+SA	12.78 ^c ±2.9	14.65 ^c ±4.0	32.15 ^c ±0.2	35.56 ^c ±0.11
LSD 5%	1.32	1.56	2.95	3.25

90 DAS, respectively, compared to the control (Tab. 2). Further, the fresh mass per plant was recorded to be statistically equal at day 60 to that of control in the plants fed with Cd at the rate of 50 mg per kg of soil, followed by foliar spray of 10^{-5} M of SA, whereas, at 90 DAS, the fresh mass per plant was recorded to be 10.0% higher compared to that of control. The hormonal spray resulted in a significant increase of 12.9% and 5.4% at 60 and 90 DAS, respectively, compared to the control, in the dry mass of the plants supplemented with 50 mg Cd per kg of soil. However, like that of root length and shoot length foliar spray of SA proved to be inefficient in alleviating the stress (Cd 100 mg per kg of soil) generated in plants, where a percent reduction of 12.6, 17.5 and 16.1, 18.9 was recorded in fresh and dry mass per plant at 60 and 90 DAS, respectively, compared to the control.

Tab. 2. Effect of 10^{-5} M salicylic acid (SA) on the Cd (0, 25, 50 or 100 mg kg⁻¹ of soil) induced changes in fresh mass and dry mass plant⁻¹ (g) in *Cicer arietinum* at 60 and 90 DAS. Data are the mean of three independent replicates (\pm SE)

Treatments	Fresh mass per plant (g)		Dry mass per plant (g)	
	60 DAS	90 DAS	60 DAS	90 DAS
Control (DDW)	14.25 ^a ±3.5	15.82 ^a ±4.6	3.54 ^a ±1.15	3.88 ^a ±0.80
Cd (0 mg) + SA	19.35 ^b ±3.8	23.21 ^b ±4.0	5.25 ^b ±0.72	6.11 ^b ±0.75
Cd (25 mg) + SA	15.87 ^b ±2.3	18.75 ^b ±4.3	3.76 ^b ±0.61	4.11 ^b ±1.15
Cd (50 mg) + SA	14.92 ^b ±4.6	16.95 ^b ±5.2	3.55 ^b ±0.78	3.89 ^b ±0.75
Cd (100 mg) + SA	12.65 ^a ±5.5	14.25 ^a ±4.0	2.65 ^a ±0.58	2.88 ^a ±0.60
LSD 5%	1.6	1.8	0.35	0.45

Carbonic anhydrase (CA) activity in leaves

The plants received 100 mg Cd and also sprayed with SA exhibited a lower activity of CA compared to the control, at both the sampling stages (Tab. 3). The application of SA to the foliage, however, resulted in a significant increase of 47.3%, 58.0% (unstressed); 28.1%, 33.9% (25 mg Cd) and 12.9%, 13.9% (50 mg Cd) at the two sampling stages, respectively, in the activity of CA compared to the control (Tab. 3).

Tab. 3. Effect of 10^{-5} M salicylic acid (SA) on the Cd (0, 25, 50 or 100 mg kg⁻¹ of soil) induced changes in CA activity [mol (CO₂) Kg⁻¹ leaf (F.M)] and gs (mol m⁻² sec⁻¹) in *Cicer arietinum* at 60 and 90 DAS. Data are the mean of three independent replicates (\pm SE)

Treatments	CA activity			
	g		g	
	60 DAS	90 DAS	60 DAS	90 DAS
Control (DDW)	2.56 ^a ±0.02	2.74 ^a ±0.06	0.307 ^a ±0.003	0.328 ^a ±0.004
Cd(0mg)+SA	3.77 ^b ±0.02	4.33 ^b ±0.06	0.400 ^b ±0.002	0.432 ^b ±0.005
Cd(25mg)+SA	3.28 ^b ±0.05	3.67 ^b ±0.07	0.359 ^b ±0.002	0.388 ^b ±0.006
Cd(50mg)+SA	2.89 ^b ±0.03	3.12 ^b ±0.06	0.330 ^b ±0.004	0.346 ^b ±0.006
Cd(100mg)+SA	2.30 ^a ±0.02	2.46 ^a ±0.07	0.288 ^a ±0.001	0.305 ^a ±0.004
LSD 5%	0.15	0.22	0.01	0.01

Photosynthetic parameters

The foliar application of 10^{-5} M of SA resulted in a significant increase of 30.3%, 31.7% (g); 33.1%, 35.6% (C_i); 86.8%, 92.7% (WUE); 37.7%, 40.1% (E) and 59.5%, 55.7% (P_N) at 60 and 90 DAS, respectively in unstressed plants, over their controls (Tabs. 3-5). The SA treatment alleviated the stress generated by Cd supplemented at the rate of 25 or 50 mg per kg of soil. An increase of 16.9%, 18.3% (g); 17.1%,

15.9% (C_i); 36.8%, 41.5% (WUE); 11.3%, 10.2% (E) and 35.0%, 28.9% (P_N) at 60 and 90 DAS, respectively, over the control was observed in the plants fed with Cd at the rate of 25 mg per kg of soil and also sprayed with SA. Further the increase in g, C_i, WUE, E and P_N in the plants supplemented with Cd at the rate of 50 mg per kg of soil followed by foliar spray of SA at day 30, after sowing, was found to be 7.5%, 5.5%; 7.3%, 6.2%; 18.4%, 14.6%; 5.03%, 2.4% and 19.0%, 20.0% at 60 and 90 DAS, respectively, over control. However, the spray did not prove to be fruitful in alleviating the stress generated by Cd supplemented at the rate of 100 mg per kg of soil (Tabs. 3-5).

Tab. 4. Effect of 10^{-5} M salicylic acid (SA) on the Cd (0, 25, 50 or 100 mg kg⁻¹ of soil) induced changes in C_i (ppm) and WUE (mM M⁻¹) in *Cicer arietinum* at 60 and 90 DAS. Data are the mean of three independent replicates (\pm SE)

Treatments	C _i		WUE	
	60 DAS	90 DAS	60 DAS	90 DAS
Control (DDW)	275 ^a ±4.1	289 ^a ±3.2	0.38 ^a ±0.02	0.41 ^a ±0.02
Cd (0 mg) + SA	366 ^b ±3.2	392 ^b ±2.3	0.71 ^b ±0.01	0.79 ^b ±0.02
Cd (25 mg) + SA	322 ^b ±3.8	335 ^b ±2.9	0.52 ^b ±0.06	0.58 ^b ±0.006
Cd (50 mg) + SA	295 ^b ±2.9	307 ^b ±3.2	0.45 ^b ±0.02	0.47 ^b ±0.03
Cd (100 mg) + SA	260 ^a ±3.5	271 ^a ±3.5	0.26 ^a ±0.01	0.27 ^a ±0.006
LSD 5%	17	16	0.05	0.04

Tab. 5. Effect of 10^{-5} M salicylic acid (SA) on the Cd (0, 25, 50 or 100 mg kg⁻¹ of soil) induced changes in E (m mol m⁻² sec⁻¹) and P_N (m mol CO₂ m⁻² sec⁻¹) in *Cicer arietinum* at 60 and 90 DAS. Data are the mean of three independent replicates (\pm SE)

Treatments	E		P _N	
	60 DAS	90 DAS	60 DAS	90 DAS
Control (DDW)	1.59 ^a ±0.01	1.67 ^a ±0.01	5.83 ^a ±0.03	6.36 ^a ±0.02
Cd (0 mg) + SA	2.19 ^b ±0.01	2.34 ^b ±0.006	9.30 ^b ±0.06	9.90 ^b ±0.02
Cd (25 mg) + SA	1.77 ^b ±0.006	1.84 ^b ±0.02	7.87 ^b ±0.06	8.20 ^b ±0.01
Cd (50 mg) + SA	1.67 ^b ±0.02	1.71 ^b ±0.03	6.94 ^b ±0.02	7.63 ^b ±0.01
Cd (100 mg) + SA	1.35 ^a ±0.02	1.40 ^a ±0.01	5.26 ^a ±0.6	5.92 ^a ±0.02
LSD 5%	0.07	0.08	0.50	0.49

Antioxidative enzyme activities

Application of 10^{-5} M of SA to the foliage of unstressed plants resulted in a significant increase of 14.9%, 17.1% (CAT); 30.3%, 34.9% (POX) and 27.7%, 30.0% (SOD) at 60 and 90 DAS, respectively, compared to the control (Tabs. 6-7). Further, when SA was applied exogenously to the Cd stressed plants, a sharp increase in the activity of these antioxidant enzymes was observed that was proportionate with the concentration of the metal. The activity of CAT, POX and SOD was found to be highest under the influence of exogenous SA in the plants fed with Cd at the rate of 100 mg per kg of soil, showing a significant increase of 33.4%, 38.1%; 85.6%, 95.2% and 61.3%, 70.0% at 60 and 90 DAS, respectively, compared to the control. The values revealed for these parameters, in the plants fed with Cd at the rate of 25 mg per kg of soil followed by a foliar spray of SA was found to be significantly higher by 20.5%, 23.8%; 40.9%, 43.8% and 36.5%, 40.0% at 60 and 90 DAS, respectively, over control (Tabs. 6-7).

Proline content in leaves

The exogenous application of SA significantly increased

the endogenous level of proline over control, irrespective of the Cd concentration used (Tab. 7). The endogenous proline level in unstressed plants was found to be 50.0% and 48.2% higher at 60 and 90 DAS, respectively, compared to the control, in response to exogenous SA. Further, the proline level in response to the foliar applied SA, increased linearly with increasing concentration of Cd and was found to be 57.0%, 56.8% (25 mg Cd); 68.1%, 62.0% (50 mg Cd) and 92.4%, 90.6% (100 mg Cd) higher, compared to the control at 60 and 90 DAS, respectively (Tab. 7).

Tab. 6. Effect of 10⁻⁵ M salicylic acid (SA) on the Cd (0, 25, 50 or 100 mg kg⁻¹ of soil) induced changes in CAT [μ mol H₂O₂ decomposed g⁻¹ (FM)] and POX [units g⁻¹ (FM)] activities in *Cicer arietinum* at 60 and 90 DAS. Data are the mean of three independent replicates (\pm SE)

Treatments	CAT		POX	
	60 DAS	90 DAS	60 DAS	90 DAS
Control (DDW)	395 ^a ±3.5	415 ^a ±4.6	13.2 ^a ±1.15	14.6 ^a ±0.80
Cd (0 mg) + SA	454 ^d ±3.8	486 ^d ±4.0	17.2 ^d ±0.72	19.7 ^d ±0.75
Cd (25 mg) + SA	476 ^c ±2.3	514 ^c ±4.3	18.6 ^c ±0.61	21.0 ^c ±1.15
Cd (50 mg) + SA	497 ^b ±4.6	545 ^b ±5.2	21.8 ^b ±0.78	25.3 ^b ±0.75
Cd (100 mg) + SA	527 ^a ±5.5	573 ^a ±4.0	24.5 ^a ±0.58	28.5 ^a ±0.60
LSD 5%	16	18	2.0	2.8

Tab. 7. Effect of 10⁻⁵ M salicylic acid (SA) on the Cd (0, 25, 50 or 100 mg kg⁻¹ of soil) induced changes in SOD [units g⁻¹ (FM)] activity and proline content [mg g⁻¹ (FM)] in *Cicer arietinum* at 60 and 90 DAS. Data are the mean of three independent replicates (\pm SE)

Treatments	SOD		Proline content	
	60 DAS	90 DAS	60 DAS	90 DAS
Control (DDW)	137 ^d ±3.2	150 ^d ±3.8	10.00 ^d ±0.11	11.81 ^d ±0.15
Cd (0 mg) + SA	175 ^c ±2.6	195 ^c ±3.5	15.00 ^c ±0.15	17.50 ^c ±0.17
Cd (25 mg) + SA	187 ^b ±3.5	210 ^b ±3.2	15.7 ^b ±0.18	18.52 ^b ±0.20
Cd (50 mg) + SA	200 ^b ±4.0	225 ^b ±2.9	16.81 ^b ±0.1	19.13 ^b ±0.12
Cd (100 mg) + SA	221 ^a ±2.9	255 ^a ±4.0	19.24 ^a ±0.2	22.51 ^a ±0.11
LSD 5%	18	23	0.5	0.6

Number of pods per plant

Application of SA to the foliage of unstressed plants resulted in a significant increase of 57.8% (number of pods) over control. Further the treatment also proved to be fruitful in increasing the number of pods in the plants fed with Cd (25 or 50 mg) and also received SA where a significant increase of 32.4% and 13.0% in the number of pods per plant was observed respectively over control. Further, like other parameters the foliar application of SA proved to be inefficient in alleviating the stress generated by Cd (100 mg), where 11.7% reduction in this parameter was observed compared to the control (Tab. 8).

Tab. 8. Effect of 10⁻⁵ M of salicylic acid on the Cd (0, 25, 50 or 100 mg per of soil) induced changes in yield characteristics in chickpea. Data are the mean of three independent replicates. LSD is the least significant difference

Treatments	Number of pods per plant	Seed yield (g plant ⁻¹)	100 seeds mass (g)	Seed protein content (%)
Control (DDW)	18.50 ^a ±1.7	6.18 ^a ±0.7	14.55 ^a ±1.3	19.20 ^a ±2.1
Cd (0 mg) + SA	29.20 ^b ±3.2	12.85 ^b ±1.3	22.81 ^b ±1.9	21.89 ^b ±2.4
Cd (25 mg) + SA	24.50 ^b ±2.6	9.51 ^b ±1.0	20.50 ^b ±1.8	20.74 ^b ±2.1
Cd (50 mg) + SA	20.91 ^a ±2.1	7.11 ^a ±0.8	17.50 ^a ±1.4	20.10 ^a ±1.9
Cd (100 mg) + SA	16.33 ^a ±1.7	4.90 ^a ±0.6	13.80 ^a ±1.1	17.50 ^a ±1.6
LSD 5%	1.2	0.5	0.9	0.5

Seed yield, 100 seed mass and seed protein content

The exogenous foliar application of 10⁻⁵ M of SA significantly increased the seed yield, 100 seed mass and seed protein content in unstressed plants by 108%; 56.8% and 14.0% respectively, over control (Tab. 8). Further, the SA treatment to the plants already fed with Cd (25 or 50 mg per kg soil) significantly increased the values of seed yield, 100 seeds mass and seed protein content over that of control. The percent increase recorded in these parameters was found to be 53.9%, 40.9% and 8.0% (Cd 25 mg) and 15.0%, 20.3% and 5.0% (Cd 50 mg), respectively, over that of the control. However, the foliar spray of SA to the plants already supplemented with 100 mg Cd exhibited 20.0%, 5.0% and 8.8% reduction in seed yield, 100 seeds mass and seed protein content, respectively, compared to the control (Tab. 8).

Beside this promising response of SA on photosynthesis, SA has ability to conferred tolerance to barley seedlings and maize plants against oxidative stress induced by Pq (Paraquat) and Cd respectively (Kranterev *et al.*, 2008). Accumulation of reactive oxygen species i.e. oxidative stress that cause extensive damage including lipid peroxidation, chlorophyll breakdown, loss of photosynthetic activity and membrane integrity, as well as electrolyte leakage. However, Kranterev *et al.* (2008) observed in maize plants that pre-treated with 0.5 mM SA before exposure to 10-25 mM cadmium, protection of photosynthesis conferred by SA could be the result of a very rapid detoxification of ROS. It has been demonstrated in different plants species that pre-treatment with low concentrations of SA enhances tolerance toward most kinds of abiotic stresses due to an enhanced antioxidant capacity (Horváth *et al.*, 2007). Similar response was observed in the present study where SA (10⁻⁵ M) significantly increased the antioxidant enzymes (CAT, POX, and SOD) compared to control (Tabs. 6-7) in the presence and absence of Cd along with increased accumulation of proline in SA treated plants (Tab. 7). Moreover, proline plays vital role in wide range of protective responses including osmotic adjustment, stabilizer for cellular structure and reducing damage to photosynthetic apparatus (Nounjan *et al.*, 2012). Hossain *et al.* (2011) demonstrated that exogenous proline provided a protective action against abiotic stress-induced oxidative damage by reducing H₂O₂ and lipid peroxidation level and by enhancing antioxidant defense and methylglyoxal detoxification systems. In addition to this, exogenous proline also prevents protein aggregation and stabilization of M4 lactate dehydrogenase during extreme temperatures, protection of nitrate reductase during heavy metal and osmotic stress (Szabados and Savaouré, 2010).

The accelerated photosynthesis and its related attributes, enhanced proline accumulation coupled with the increased activity of antioxidant enzymes in Cd stressed plants in the presence of exogenously applied 10⁻⁵ M of SA is naturally expected to increase the growth of plants which was reflected in the form of increased length, fresh and dry mass of root and shoot per plant (Tabs. 1-2). Further, the enhanced growth characteristics of Cd fed plants sprayed with 10⁻⁵ M of SA might also be due to the fact that SA acts at the level of transcription and/or translation thereby increasing the activity of various other enzymes necessary for growth of plants (Hayat *et al.*, 2010). Moreover, SA also

promotes cell division and cell enlargement (Hayat *et al.*, 2013). This increased growth under the influence of exogenous SA is likely to increase the yield characteristics (Tab. 8). The plausible reason for increasing the crop yield might be due to delayed senescence of plant organs (particularly leaves and flowers) in response to exogenous SA (Imran *et al.*, 2007) that will automatically help the plant in extending the duration of photosynthetically active sites and also prevent the premature loss of flowers and fruits. This consequently resulted in the observed increase in the number of pods per plant (Tab. 8). It gets additional support from the observations of Marschner (2012) that phytohormones increase the degree of sink at the level of seeds, directing the flow of metabolites to the developing seeds increasing the seed protein content (Tab. 8) consequent to an improvement in the seed mass (Tab. 8) and seed yield per plant at harvest (Tab. 8).

Conclusions

The present investigation revealed that exogenously applied 10^{-5} SA plays significant role to protect the photosynthetic machinery under Cd-stressed plants. In addition to this, SA also enhanced the activities of antioxidant enzymes and proline accumulation that confer tolerance to Cd-stressed plants and enhanced the yield characteristics.

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References

- Arfan M, Athar HR, Ashraf M (2007). Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *J Plant Physiol* 164(6):685-694.
- Bal W, Liang R, Lukszo J, Lee S-H, Dizdaroglu M, Kasprzak KS (2000). Ni (II) specifically cleaves the C-terminal tail of the major variant of histone H2A and forms an oxidative damage-mediating complex with the cleaved-off octapeptide. *Chem Res Toxicol* 13(7):616-624.
- Barcelo J, Poschenrieder C (1990). Plant water relations as affected by heavy metal stress: a review. *Journal of Plant Nutrition* 13(1):1-37.
- Bates LS, Walden RT, Tearse ID (1973). Rapid determination of free proline for water stress studies. *Plant Soil* 39:205-207.
- Bazzas FA, Carlson RW, Rolfe GL (1974). The effect of heavy metals on plants. *Environ Poll* 7:241-246.
- Beauchamp C, Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry* 44(1):276-287.
- Chance B, Maehly AC (1956). Assay of catalase and peroxidase. *Method Enzymol* 2:764-775.
- Dwivedi RS, Randhawa NS (1974). Evolution of a rapid test of the hidden hunger of zinc in plants. *Plant Soil* 40:445-451.
- Foy C, Chaney Rt, White M (1978). The physiology of metal toxicity in plants. *Ann Rev Plant Physio* 29(1):511-566.
- Foyer CH, Noctor G (2003). Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol Plantarum* 119(3):355-364.
- Gémes K, Poór P, Sulyok Z, Szepesi Á, Szabó M, Tari I (2008). Role of salicylic acid pre-treatment on the photosynthetic performance of tomato plants (*Lycopersicon esculentum* Mill. L. cv. Rio Fuego) under salt stress. *Acta Biol Szeg* 52(1):161-162.
- Gratão PL, Polle A, Lea PJ, Azevedo RA (2005). Making the life of heavy metal-stressed plants a little easier. *Funct Plant Biol* 32(6):481-494.
- Hasan SA, Fariduddin Q, Ali B, Hayat S, Ahmad A (2009). Cadmium: Toxicity and tolerance in plants. *J Environ Biol* 30(2):165-174.
- Hasan SA, Hayat S, Ali B, Ahmad A (2008). 28-homobrassinolide protects chickpea (*Cicer arietinum*) from cadmium toxicity by stimulating antioxidants. *Environmental pollution* 151(1):60-66.
- Hayat Q, Hayat S, Irfan M, Ahmad A (2010). Effect of exogenous salicylic acid under changing environment: A review. *Env Exp Bot* 68(1):14-25.
- Hayat S, Ali B, Hasan SA, Ahmad A (2007). Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. *Env Exp Bot* 60(1): 33-41.
- Hayat S, Ahmad A, Alyemeni MN (2013). Salicylic Acid: Plant Growth and Development. Springer, Dordrecht, The Netherlands.
- Hermans C, Johnson GN, Strasser RJ, Verbruggen N (2004). Physiological characterisation of magnesium deficiency in sugar beet: acclimation to low magnesium differentially affects photosystems I and II. *Planta* 220(2):344-355.
- Horváth E, Szalai G, Janda T (2007). Induction of abiotic stress tolerance by salicylic acid signaling. *J Plant Growth Regul* 26(3):290-300.
- Hossain MA, Hasanuzzaman M, Fujita M (2011). Coordinate induction of antioxidant defense and glyoxalase system by exogenous proline and glycinebetaine is correlated with salt tolerance in mung bean. *Front Agric China* 5(1):1-14.
- Irfan M, Hayat S, Ahmad A, Alyemeni MN (2013). Soil cadmium enrichment: Allocation and plant physiological manifestations. *Saudi J Biol Sci* 20:1-10.
- Imran H, Zhang Y, Du G, Wang G, Zhang J (2007). Effect of salicylic acid (SA) on delaying fruit senescence of Huang Kum pear. *Front Agric China* 1(4):456-459.
- Janda T, Horváth E, Szalai G, Paldi E (2007). Role of salicylic acid in the induction of abiotic stress tolerance, p. 91-150. In: Hayat S, Ahmad P (eds) Salicylic acid: a plant hormone. Springer, Dordrecht, The Netherlands.

- Krantev A, Yordanova R, Janda T, Szalai G, Popova L (2008). Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. *J Plant Physiol* 165(9):920-931.
- Krupa Z, Öquist G, Huner NPA (1993). The effects of cadmium on photosynthesis of *Phaseolus vulgaris* – a fluorescence analysis. *Physiol Plant* 88(4):626-630.
- Landberg T, Greger M (2002). Differences in oxidative stress in heavy metal resistant and sensitive clones of *Salix viminalis*. *J Plant Physiol* 159(1):69-75.
- Larsen PB, Degenhardt J, Tai CY, Stenzler LM, Howell SH, Kochian LV (1998). Aluminum-resistant Arabidopsis mutants that exhibit altered patterns of aluminum accumulation and organic acid release from roots. *Plant physiology* 117(1):9-18.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. *The Journal of biological chemistry* 193(1):265-275.
- Marschner H (2012). Nitrogen fixation, p. 389-408. In: Marschner P (ed). *Marschner's mineral nutrition of higher plants*. Academic Press, United States of America.
- Métraux J-P (2002). Recent breakthroughs in the study of salicylic acid biosynthesis. *Trends Plant Sci* 7(8):332-334.
- Mishra A, Choudhuri M (1997). Ameliorating effects of salicylic acid on lead and mercury: induced inhibition of germination and early seedling growth of two rice cultivars. *Seed Sci Technol* 25(2):263-270.
- Nounjan N, Nghia PT, Theerakulpisut P (2012). Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. *J Plant Physiol* 169(6):596-604.
- Poór P, Gémes K, Horváth F, Szepesi A, Simon M, Tari I (2011). Salicylic acid treatment via the rooting medium interferes with stomatal response, CO₂ fixation rate and carbohydrate metabolism in tomato, and decreases harmful effects of subsequent salt stress. *Plant Biol* 13(1):105-114.
- Reddy MP, Vora AB (1986). Changes in pigment composition, Hill reaction activity and saccharide metabolism in bajra (*Pennisetum typhoides* S&H) leaves under NaCl salinity. *Photosynthetica* 20:50-55.
- Sahu G, Kar M, Sabat S (2002). Electron transport activities of isolated thylakoids from wheat plants grown in salicylic acid. *Plant Biol* 4(3):321-328.
- Sanita di Toppi L, Gabbriellini R (1999). Response to cadmium in higher plants. *Environ Exp Bot* 41(2):105-130.
- Sheoran IS, Signal HR, Singh R (1990). Effect of cadmium and nickel on photosynthesis and the enzymes of photosynthetic carbon reduction cycle in pigeon pea (*Cajanus cajan* L.). *Photosynth Res* 23(3):345-351.
- Singh B, Usha K (2003). Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul* 39(2):137-141.
- Sinha S, Srivastava H, Tripathi R (1994). Influence of some growth regulators and divalent cations on the inhibition of nitrate reductase activity by lead in maize leaves. *Chemosphere* 29(8):1775-1782.
- Skórzyńska-Polit E, Krupa Z (2006). Lipid peroxidation in cadmium-treated *Phaseolus coccineus* plants. *Arch Environ Con Tox* 50(4):482-487.
- Szabados L, Savouré A (2010). Proline: a multifunctional amino acid. *Trends Plant Sci* 15(2):89-97.
- Tran TA, Popova LP (2013). Functions and toxicity of cadmium in plants: recent advances and future prospects. *Turk J Bot* 37(1):1-13.
- Van Assche F, Clijsters H (1990). Effects of metals on enzyme activity in plants. *Plant Cell Environ* 13(3):195-206.