



**Full Length Article**

# Salicylic Acid Seed Priming Modulates Morphology, Nutrient Relations and Photosynthetic Attributes of Wheat Grown under Cadmium Stress

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## Abstract

Cadmium (Cd) is a toxic metal even at micromolar concentration because of its mobility towards edible parts of plants and its negative effects on plant growth and development. Salicylic acid (SA) being a phytohormone is known for its positive actions against abiotic stresses. Therefore, this study was conducted to evaluate the role of SA seed priming in improving Cd stress tolerance in different wheat genotypes. Seeds of two wheat genotypes (Fsd-08 and MH-97) were primed in aerated solutions of SA (0.25 and 0.5 mM) along with control (water soaked; hydropriming) for 24 h. Hydro-primed and SA primed seeds of both genotypes were sown in sand filed pots and subjected to Cd stress (0, 20 and 40 mg/kg) after one week of germination. Results indicated that shoot fresh weight, root/shoot length and dry weights, chlorophyll a and b, and carotenoid contents were decreased in both genotypes with increasing Cd concentration. Likewise, uptake of cations like sodium (Na), calcium (Ca) and potassium (K) was decreased while that of Cd was increased with increase in Cd stress in both genotypes; however, genotype Fsd-08 was found more tolerant against Cd stress in all attributes except for chlorophyll a contents and K uptake. Nonetheless, seed priming with 0.5 mM SA counteracted the damage caused by Cd stress on morphological attributes and plant pigments in both genotypes. Salicylic acid as a priming agent also reduced the Cd toxicity by increasing the uptake of micro as well as macronutrients in both genotypes. In crux, Cd stress, 40 mg/kg was more toxic, decreased the morphological traits due to substantial decrease in photosynthetic pigments and nutrients uptake; however, seed priming with 0.5 mM SA reversed these effects to a certain content. Wheat genotype Fsd-08 proved more tolerant than MH-97, so recommended to grow in Cd-contaminated soils. © 2020 Friends Science Publishers

**Keywords:** Cadmium; Ions; Photosynthetic pigments; Seed priming; Salicylic acid; Wheat

## Introduction

Wheat (*Triticum aestivum* L.) being a staple food, makes up to 1/3<sup>rd</sup> of food requirement worldwide. Due to rapidly growing world population, wheat consumption is also increasing with the similar proportion. In Pakistan, according to an estimate the annual crop production has shown an upward trend during the last 10 years (FAO, 2018). Wheat is a vital element of agriculture sector and a widely cultivated crop in Pakistan. Hence, various techniques and efforts are made through scientific research to improve its production potential while keeping it safe from possible hazardous factors as well.

Cadmium (Cd) is considered as a toxic pollutant (Jarup and Akesson 2009) which finds its way to the environment through waste produced as a result of industrial processes, urban activities, uncontrolled use of fertilizers and sewage sludge (Liu *et al.* 2007). It is one of many factors responsible for stress in plants and

remains hazardous even at low concentration because of its non-essential role in plants (White and Brown 2010). Once absorbed by the plants, Cd gathers in edible parts of plants (Fang *et al.* 2014) and after passing through intermediate stages of the food chain, ultimately becomes part of human diet leading to serious human health issues (Recatala *et al.* 2010).

The negative effects of Cd are evident at biochemical, physiological and molecular levels. It leads to amendments in structures and ultra-structures of photosynthetic apparatus, thus reduces the photosynthetic rate (Shi and Cai 2008; Xue *et al.* 2013). Moreover, it also causes chlorosis of leaf in addition to necrosis and influences vital minerals distribution and retards photosynthetic processes by lowering the chlorophyll contents (Liu *et al.* 2011). Cadmium toxicity results in notable cut in morphological attributes and disturbs water and ionic relations in wheat (Hassan *et al.* 2016; Farhat *et al.* 2018). Moreover, Cd stress leads to impaired germination along with substantial

decrease in chlorophyll and carotenoid contents in wheat (Ahmad *et al.* 2012; Nikolic *et al.* 2014).

Plants adapt different tolerance mechanisms to deal with negative effects of Cd stress. Various techniques are currently in practice to elevate tolerance in plants against stress and seed priming is one of them. Seed priming is a novel technique which is used to induce stress tolerance in plants (Farooq *et al.* 2019). Using different chemicals (ascorbic acid) and hormones (kinetic, SA, gibberellic acid) for seed priming may improve the performance of plants (Jafar *et al.* 2012; Farooq *et al.* 2019). Salicylic acid is a phytohormone that is effective against various stresses in plants (Metwally *et al.* 2003; Arfan 2009). Seed pretreatment with SA decreased the negative effect of Cd toxicity in wheat (Shakirova *et al.* 2016). Seed priming alters the physiological status of the seed prior to germination (Farooq *et al.* 2019). The changes in physiological status, activates the plant's defense responses to counter different stresses without compromising the plant's health (Hulten *et al.* 2006).

Chemically, SA belongs to phenolic class of compounds and acts as phytohormone. It is important signaling molecules which modifies the physiological and biochemical characteristics of the plants and make them tolerant against stresses (Arfan *et al.* 2007). Hence it was hypothesized that SA has the potential to ameliorate the damaging effects of Cd stress in wheat. Therefore, this study was designed to evaluate the role, if any, of SA used as seed priming agent to improve morphology, nutrient relations and photosynthetic attributes of wheat grown under cadmium stress.

## Materials and Methods

A sand cultured experiment was carried out to examine the role of SA seed priming on two wheat genotypes grown under Cd stress. Before sowing wheat seeds, a plastic pot filled with 5 kg fully rinsed sand was thoroughly irrigated with full strength Hoagland solution (Epstein 1972). Two wheat genotype seeds, namely Fsd-08 and MH-97 were chosen for this experiment. Prior to sowing, seeds of both wheat genotypes were soaked in aerated solution of SA (0, 0.25 mM and 0.5 mM) and water (hydropriming; control) for 24 h by maintaining 1:5 (w/v) seed to solution ratio. After 24 h, manually dried with filter paper and 12 seeds of wheat were sown per sand pots and irrigated with full strength Hoagland nutrient solutions. The seeds of both genotypes were allowed to germinate for a week and the subjected to Cd treatments as 0, 20 and 40 mg/kg of soil by dissolving CdCl<sub>2</sub>.H<sub>2</sub>O in Hoagland medium. Moreover, thinning was also done up to 8 plants per pot to maintain homogeneity of experiment and all culture experiment was regularly irrigated with Hoagland medium with an interval of 10 days till the 70 days after sowing. The experiment was laid down in completely randomized design with factorial arrangement and replicated four times.

## Growth attributes

Two plants per pot were harvested from the sand at vegetative stage. Root and shoot lengths were determined in cm(s) by using a meter scale. Shoot fresh weights of two more plants collected from each pot were measured in gram (g) immediately after harvesting. For recording dry weight of shoot and root, samples were placed in an oven at 65°C till the weight get constant in gram (g) using an electronic balance. Maximum width and length of flag leaf was measured by meter scale for leaf area calculation in cm<sup>2</sup>.

## Photosynthetic pigments

Chlorophyll a, b and carotenoid were determined by using a method devised by Arnon (1949). Fresh leaf sample (0.1 g) was extracted in 5 mL of 80% acetone and kept overnight in a dark room. The extract was then centrifuged at 10,000 rpm for 5 min. Absorbance was then recorded at 663, 645 and 480 nm by using a spectrophotometer (Hitachi-U2001, Tokyo, Japan).

## Determination of ions concentrations

Extraction of metals was done by following method of Allen *et al.* (1976). Leaves and roots of the wheat plant dried in the oven at 65°C were dipped for 3 h in HNO<sub>3</sub> (5 mL). Later, the samples were placed on a hot digestion block at temperature of 250°C till complete digestion of dried samples. Digested solution was then diluted with 25 mL distilled water and later filtered. The resulting solution was used for ion analysis (Ca, Na and K) by using a flame photometer (Sherwood model 410, U.K.). Same solution was used for the Cd estimation in roots and leaves by atomic absorption spectrum (PerkinElmer, Waltham, MA, USA).

## Statistical analysis

The design of experiment was completely randomized with four replicates. MSTAT-C (CoHort software, Berkeley C.A.) was used for analysis of variance (ANOVA) of all parameters. DMRT was applied for determination of difference between various factors and their interactions.

## Results

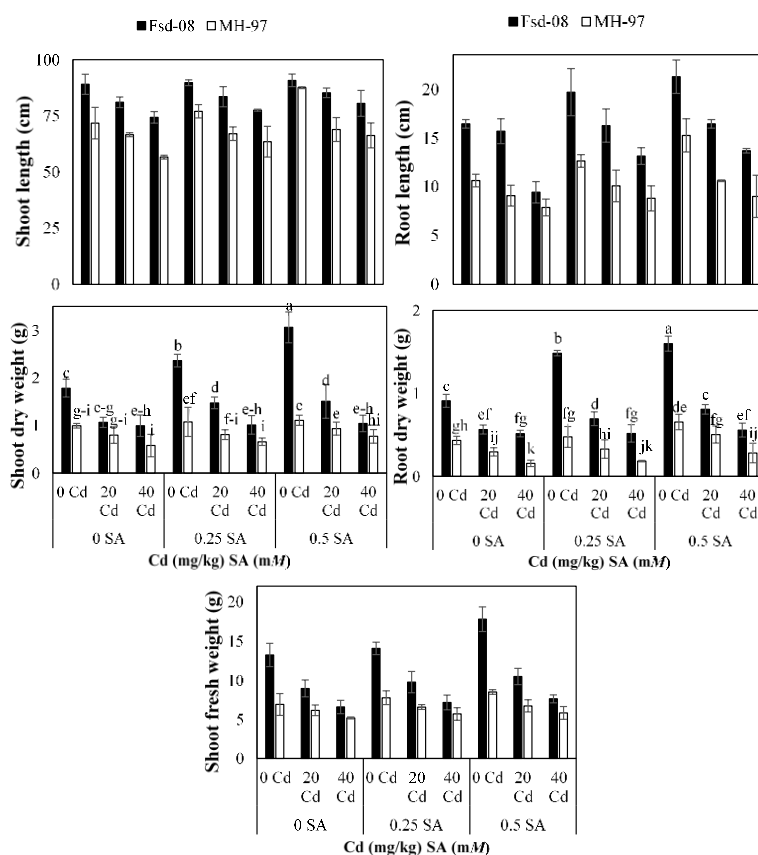
### Growth attributes

Various growth parameters such as root /shoot length and dry weight and shoot fresh weight decreased significantly ( $P \leq 0.05$ ) with the increase in Cd (Fig. 1 and Table 1). Maximum reduction was observed in all these morphological parameters when 40 mg/kg Cd was applied to MH-97 genotype and the opposite was true when 0.5 mM SA was applied to the Fsd-08 genotype (Fig. 1). Exogenous application of SA as well as genotypic differences were

**Table 1:** Statistical summary of root and shoot traits of wheat genotypes grown under varying levels of Cd stress and SA seed priming

Sources of variation	DF	Root length (cm)	Shoot length (cm)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
Cd	2	192.23***	1286.9***	156.12***	1.9739***	5.0194***
SA	2	50.603***	266.55***	16.679***	0.3907***	0.8163***
Genotype	1	512***	3570.1***	292.89***	4.1913***	9.6128***
Cd × SA	4	6.9472**	21.504ns	3.5853**	0.0816***	0.1806**
Cd × Genotype	2	14.861***	39.865ns	52.342***	0.4851***	1.7242***
SA × Genotype	2	2.285ns	41.267ns	3.7959*	0.0637***	0.2991***
Cd × SA × Genotype	4	2.3904ns	36.344ns	1.3591ns	0.0565***	0.2404***
Error	54	1.6781	15.253	0.9232	0.0064	0.0375

\*\*\*, \*\*, \*\*\*= significant at 0.001, 0.01 and 0.05 probability level, respectively; ns= non-significant; Cd= Cadmium; SA= Salicylic acid

**Fig. 1:** Effect of salicylic acid seed priming on root and shoot length and dry weight and shoot fresh weight of two wheat genotypes under Cd stress ± SE

observed significant ( $P \leq 0.05$ ) for these growth attributes (Table 1). Genotypes treated with 0.5 mM SA performed better as compared to 0.25 mM (Fig. 1). Overall, genotype Fsd-08 performed better as compared to MH-97 with respect to all growth attributes (Table 1). Leaf area also decreased significantly ( $P \leq 0.05$ ) with the increase in Cd stress (Table 2). The lowest and highest values for leaf area were seen in same genotype MH-97 with Cd stress of 40 mg/kg alone and SA priming with 0.5 mM SA in control plants, respectively (Fig. 2).

### Photosynthetic pigments

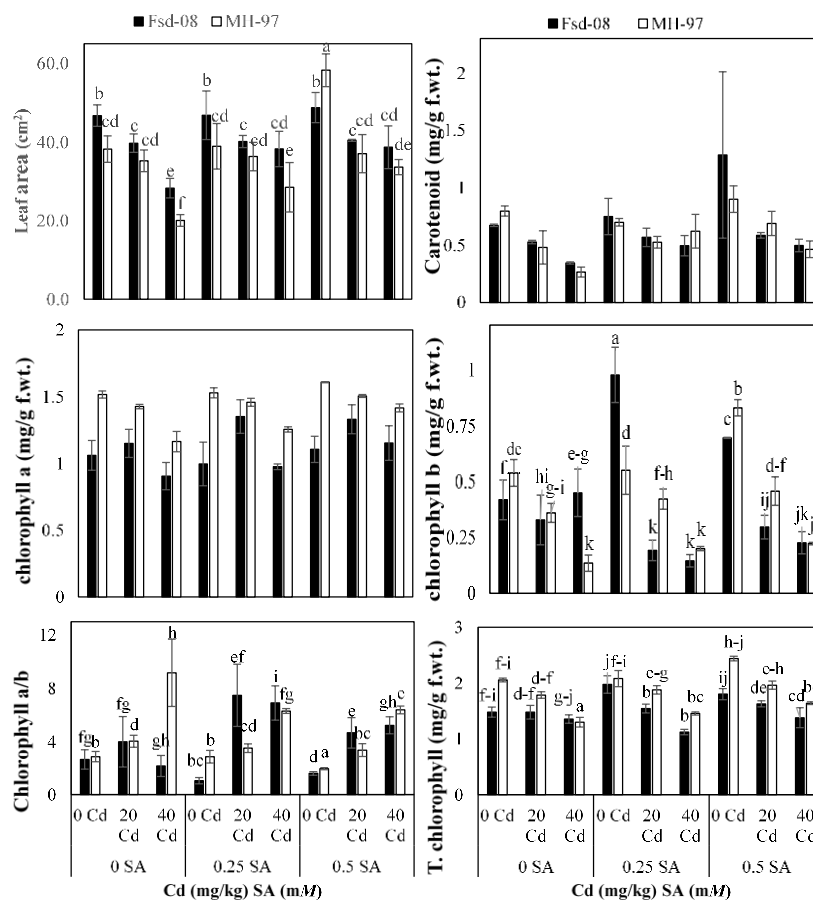
Application of Cd stress caused a significant ( $P \leq 0.05$ ) reduction in contents of chlorophyll a (Fig. 2; Table 3). The

maximum increase in chlorophyll a was observed in MH-97 when seeds were primed with 0.5 mM SA, whereas minimum chlorophyll a content was observed in Fsd-08 plants treated with 40 mg/kg Cd (Fig. 2). Chlorophyll b decreased significantly ( $P \leq 0.05$ ) under stress conditions (Table 3). Maximum increase was observed when 0.25 mM SA was applied in Fsd-08 whereas, minimum increase was observed in MH-97 when 40 mg/kg Cd was applied (Fig. 2). Salicylic acid application and genotypic difference were significant for total chlorophyll (Table 3). Wheat genotypes treated with Cd caused significant ( $P \leq 0.05$ ) decline in the total chlorophyll contents of both genotypes (Table 3). Amount of total chlorophyll was highest when MH-97 primed with 0.5 mM SA was grown in unstressed condition. Total chlorophyll was lowest in Fsd-08 when combination of

**Table 2:** Statistical summary of leaf area, carotenoid and Cd concentration in roots and leaves of wheat genotypes grown under varying levels of Cd stress and SA seed priming

Sources of variation	DF	Leaf area (cm <sup>2</sup> )	Carotenoid (mg/g f. wt.)	Cd in roots (mg/kg)	Cd in leaves (mg/kg)
Cd	2	1363.1***	1.0338***	250.94***	140.29***
SA	2	397.24***	0.2996***	16.912***	17.346***
Genotype	1	386.10***	0.0178ns	2.8451**	19.661***
Cd × SA	4	117.73***	0.1127*	5.4938***	6.2850***
Cd × Genotype	2	45.833ns	0.0235ns	0.8255*	7.9258***
SA × Genotype	2	111.12**	0.0247ns	3.4495***	5.1994***
Cd × SA × Genotype	4	54.891*	0.0735ns	5.2083***	2.1525***
Error	54	15.361	0.0360	0.2386	0.1379

\*\*\*, \*\*, \* = significant at 0.001, 0.01 and 0.05 probability level, respectively; ns = non-significant; Cd = Cadmium; SA = Salicylic acid; DF = Degree of freedom

**Fig. 2:** Effect of salicylic acid seed priming on chlorophyll a, chlorophyll b, total chlorophyll and ratio of chlorophyll a/b of two wheat genotypes under Cd stress ± SE

0.25 mM SA and 40 mg/kg Cd stress was applied (Fig. 2).

The ratio of chlorophyll a and b was affected by the Cd alone and in combination with SA (Table 3). The highest level of Cd applied to MH-97 gave the highest value for the ratio of chlorophyll a and b. The Fsd-08 primed with 0.25 mM SA alone gave the lowest value for the ratio of chlorophyll a and b (Fig. 2). Salicylic acid treatment was significant ( $P \leq 0.05$ ) for the ratio of chlorophyll a and b (Table 3). A significant ( $P \leq 0.05$ ) reduction in carotenoid contents was observed under stress condition (Table 2). Maximum reduction was observed when the highest level of Cd (40 mg/kg) was applied to MH-97 whereas, minimum reduction in carotenoid content was

observed in Fsd-08 primed with 0.5 mM SA (Fig. 2). Effect of SA priming was observed to be significant ( $P \leq 0.05$ ) with respect to this parameter. There was a non-significant genotypic difference for the respective parameter (Table 2).

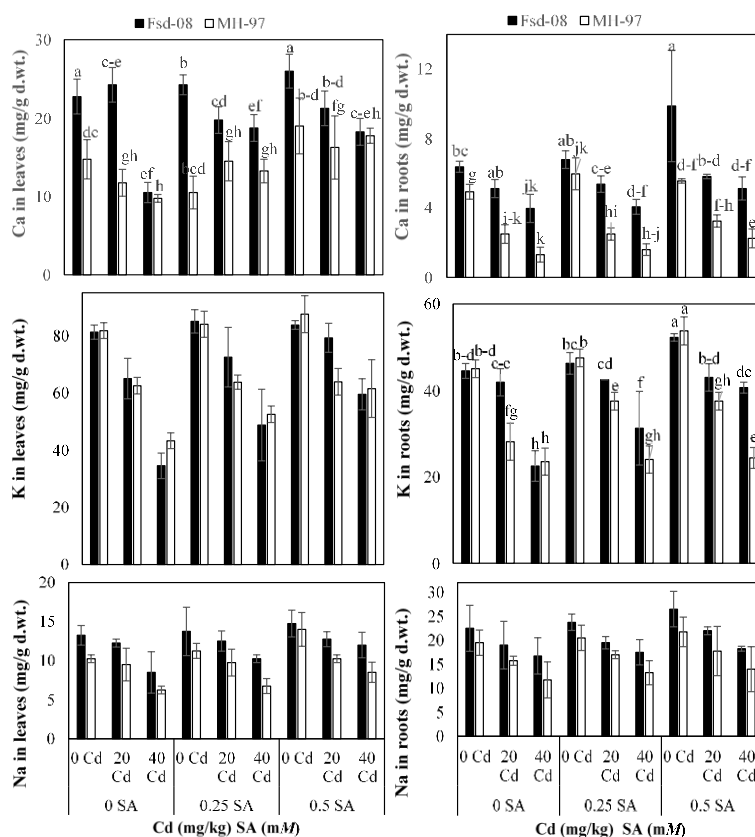
### Ion concentrations

Imposition of Cd stress caused a significant ( $P \leq 0.05$ ) reduction in Na, Ca in roots and leaves (Fig. 3 and Table 4). A minimum reduction in Ca was observed in roots and leaves of Fsd-08 when 0.5 mM SA was exogenously applied under control condition (Fig. 3), while the maximum

**Table 3:** Statistical summary of chlorophyll contents of wheat genotypes grown under varying levels of Cd stress and SA seed priming

Sources of variation	DF	Chlorophyll a (mg/g f. wt.)	Chlorophyll b (mg/g f. wt.)	Total Chlorophyll (mg/g f. wt.)	Ratio of Chlorophyll a & b
Cd	2	0.3210***	1.2458***	2.1482***	90.656***
SA	2	0.1362***	0.0416***	0.3267***	4.1692*
Genotype	1	1.8011***	3.9e-5ns	1.7843***	4.8721*
Cd × SA	4	0.0268**	0.1068***	0.0524***	2.9720ns
Cd × Genotype	2	0.1559***	0.0924***	0.1021***	27.652***
SA × Genotype	2	8.9e-4ns	0.0440***	0.0416*	17.727***
Cd × SA × Genotype	4	0.0083ns	0.1298***	0.1070***	12.020***
Error	54	0.0069	0.0045	0.0085	1.1747

\*\*\*, \*\*, \* = significant at 0.001, 0.01 and 0.05 probability level, respectively; ns= non-significant; Cd= Cadmium; SA= Salicylic acid; DF= Degree of freedom

**Fig. 3:** Effect of salicylic acid seed priming on Ca, K and Na in leaves and roots of two wheat genotypes under Cd stress ± SE

reduction of Ca was observed in leaves and roots of MH-97 under stress condition (40 mg/kg Cd). Maximum decrease in K of roots and leaves was observed when 40 mg/kg stress was applied to cv. Fsd-08. Minimum reduction in K of leaves and roots was observed when 0.5 mM SA primed seed of MH-97 were grown under controlled environment (Fig. 3). Sodium was significantly ( $P \leq 0.05$ ) higher in roots and leaves of non-stressed plants (Table 4). Maximum Na was observed in the leaves and in the roots of Fsd-08 when 0 mg/kg Cd was applied, and 0.5 mM SA was applied as a priming agent. On the other hand, minimum Na was observed in hydro-primed MH-97 under 40 mg/kg Cd stress (Fig. 3). Moreover, Cd contents were increased with the increase in applied Cd to plant in both genotypes (Fig. 4 and Table 2). The amount of Cd was less in leaves as compare to roots. Cadmium contents were increased on the expense

of other cations (Na, K and Ca). The highest Cd contents were in leaves and roots of MH-97 when 40 mg/kg Cd stress was applied alone; whereas, lowest Cd contents were in Fsd-08 roots and leaves when 20 mg/kg Cd stress was applied in presence 0.5 mM SA (Fig. 4).

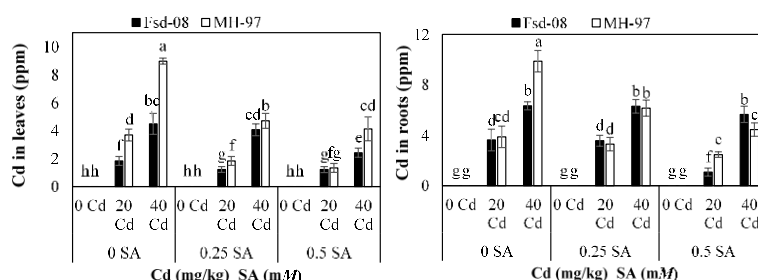
## Discussion

Cadmium stress, 40 mg/kg was the most toxic, negatively impaired the morphological traits, photosynthetic pigments, and nutrients uptake of both genotypes, MH-97 was more sensitive, of wheat. Nonetheless, seed priming with SA, 0.5 mM in particular, counteracted the damaging effects of Cd stress to considerable extent on all above cited traits in both genotypes (Fig. 1–4). Mane *et al.* (2010) also observed the decrease in growth characters in wheat in the presence of Cd.

**Table 4:** Statistical summary of Ca, K and Na concentrations (mg/g d. wt.) in roots and leaves of wheat genotypes grown under varying levels of Cd stress and SA seed priming

Source of variance	DF	Ca in leaves (mg/g d. wt.)	Ca in roots (mg/g d. wt.)	K in leaves (mg/g d. wt.)	K in roots (mg/g d. wt.)	Na in leaves (mg/g d. wt.)	Na in roots (mg/g d. wt.)
Cd	2	145.72***	78.939***	6891.2***	2523.1***	105.29***	309.05***
SA	2	107.93***	10.434***	75.843***	352.72***	25.791***	37.847*
Genotype	1	754.01***	113.80***	17.503ns	420.5***	122.72***	264.5***
Cd × SA	4	43.847***	0.9902ns	170.406**	22.388ns	3.2083ns	1.0972ns
Cd × Genotype	2	86.222***	0.4745ns	301.14***	158.04***	1.5138ns	2.1666ns
SA × Genotype	2	25.680**	2.4888ns	49.315ns	16.791ns	0.6805ns	1.7916ns
Cd × SA × Genotype	4	26.513***	2.3010*	34.961ns	90.458***	1.5972ns	0.9583ns
Error	54	4.6435	0.8111	35.508	10.449	2.3888	10.111

\*\*\*, \*\*, \*\*\*= significant at 0.001, 0.01 and 0.05 probability level, respectively; ns= non-significant; Cd= Cadmium; SA= Salicylic acid; DF= Degree of freedom; Ca= Calcium; K= Potassium; Na= Sodium

**Fig. 4:** Effect of salicylic acid seed priming on Cadmium in leaves and roots of two wheat genotypes under Cd stress ± SE

The reduction of plant biomass as well as length of wheat can be considered as Cd toxicity as it retards the normal physiological plant functions. Due to inhibition of the proton pump caused by Cd effect, the decline in division and elongation of cells leads to reduce the overall plant's biomass (Howladar 2014). The decrease in photosynthetic rate due to Cd also retards growth of plants (Metwally *et al.* 2003). Salicylic acid seeds pretreatment improved the growth attributes such as plant height, fresh/dry weights under non-stressed as well as stress condition which coincides with the findings of Abdolahi and Shekari (2013). Due to increase in photosynthetic pigments and ionic concentrations in the presence of SA, improvements in plant growth occur in both wheat genotypes. Application of SA improves the morphological character also by protecting the enzymes affected by Cd stress (Hassan and Mansoor, 2017). Nazarian *et al.* (2016) reported that leaf area is significantly affected by Cd application and Fahraji *et al.* (2014) stated that increased leaf area was observed in plants treated with SA as compared to control. Leaf area is directly related to the interception of light; so, it can be a significant parameter for the determination of productivity of plant (Koester *et al.* 2014).

The reduction in chlorophyll a and b might be due to non-availability of many essential minerals which actively participate in chlorophyll pigment biosynthesis (Azevedo *et al.* 2005). Shukla *et al.* (2003) mentioned a decrease in the photosynthetic pigments due to Cd exposure in the wheat seedling and Zhang *et al.* (2002) in cucumber (*Cucumis sativus*). Deng *et al.* (2014) concluded that photosynthesis is negatively affected due to Cd stress on chlorophyll pigments

in plants. Salicylic acid resulted in the improvement of the chlorophyll content under stress condition. Xu *et al.* (2015) indicated an improvement of chlorophyll content by exogenous SA application in peanuts (*Arachis hypogaea*) under Cd stress. An important aspect of SA is improvement of nutrients uptake which stabilized chlorophyll content against Cd stress (Liu *et al.* 2016). Pretreatment of plants with SA before subjecting it to Cd stress decreased the Cd toxicity on carboxylating enzymes, this may be a reason of increase in chlorophyll contents in presence of SA (Krantev *et al.* 2006).

Carotenoid contents in plants reduced due to high Cd content (Cai *et al.* 2010). Literature (Amani 2008) confirmed that chlorophyll and carotenoid contents are crucial to photosynthetic manifestation of energy. Thus, any fluctuation in numbers of these compounds may cause significant effect on metabolic processes in plants. Amani (2008) also reported that carotenoids conserved chlorophyll compounds against photo-oxidation; therefore, reduction in these contents may damage the chlorophyll pigments. Moreover, carotenoids being potent antioxidants serve as ROS scavengers against photo-oxidative disruption of photosystems. Hence, reduced carotenoid content results in deterioration of D1 protein accompanied with PSII damage. It eventually inhibits the synthesis of chlorophyll contents (Huang and Wang 2010). In another study, Sayyari *et al.* (2013) reported that SA pretreated plants showed increased photosynthetic pigments under stress. It explains the protective role of SA specifically under stressful environmental conditions. Shinwari *et al.* (2015) also reported that stability and functionality of chloroplast

membrane is maintained by SA during stressful conditions. This also demonstrates defensive role of SA against stressful conditions in plants.

The nutrients balance is necessary for growth of plants and for its overall development. Cd uptake causes the mineral deficiency and lowers the metabolic functions (Gomes *et al.* 2013). Elements such as Ca, K and Na decreased with the increase in Cd concentration which clearly showed that Cd acts as a dominating cation which inhibits the activities of other cation channels and carriers. The decrease in Ca and K due to Cd was also in wheat (Shukla *et al.* 2003) and *Arabidopsis* (Suzuki 2005). In current study Ca, K and Na ion content increased in SA primed plants. While experimenting with soya bean (*Glycine max*) seedlings, Drazic and Mihailovic (2005) found that SA lessened the Cd toxicity by regulating the distribution of cations. Szalai *et al.* (2013) reported that treatment of seeds with SA before exposing it to Cd decrease Cd injury within the plant by affecting the phytochelatin in plants. The findings of the research work and afore-mentioned citing proved that the presence of SA plays an important role in increasing the availability of important nutrients to plants.

Accumulation of Cd was higher in roots as compared to leaves; moreover, uptake and subsequently accumulation was increased overall in different plant parts following increased in Cd application (Rizk *et al.* 2014). Cadmium contents were found lower in roots primed with SA as compared to SA-free roots which agree with the results of Moussa and El-Gamal (2010). This Cd differential accumulation in plant parts can be attributed to the physiological effects of SA. It is reported in literature that Cd could form a complex with SA that may also increase Cd tolerance (Choudhury and Panda 2004).

## Conclusion

Cadmium stress, 40 mg/kg was the most toxic, negatively impaired the morphological traits due to substantial decrease in photosynthetic pigments and nutrients uptake in both genotypes; However, genotypes differ in this regard. Seed priming with 0.5 mM SA counteracted the damaging effects of Cd stress on wheat morphology due to increase in photosynthetic pigments and nutrients uptake. Wheat genotype Fsd-08 proved more tolerant than MH-97, so recommended to grown in Cd-contaminated soils.

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