Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/psaexc82

AMELIORATION OF CADMIUM TOXICITY BY EXOGENOUSLY APPLIED SALICYLIC ACID IN TWO CONTRASTING BARLEY GENOTYPES

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Highlights:
- Cd reduced growth and yield of barley and all SA levels behaved differently
- SA increase the yield and yield parameters of barley under Cd stress
- Low and high levels of SA reduced the yield than 0.5 mM SA

Abstract
The mitigating role of salicylic acid in growth, yield and various yield parameters in barley (*Hordeum vulgare*), genotypes viz., Jau-83 and Jau-87 under cadmium induced stress was studied. The pot study was carried out in the wire house, University of Sargodha, Lyallpur Campus, Faisalabad. Two (0 and 500 µM) levels of Cd metal and three (0.1, 0.5 0.9 mM) levels of salicylic acid alone and in combinations were applied directly in river sand medium. The experiment was designed as Completely Randomized (CRD), with factorial arrangement. Results revealed a marked reduction in shoot and root length, fresh and dry biomass, number of leaves and total leaf area and shoot root ratio under cadmium stress and increased with salicylic acid, but all the three level of SA behaved differently. Maximum increase in growth parameters were noted at 0.5 mM SA compared to 0.1 and 0.9 mM in both the barley genotypes. Although both genotypes statistically behaved similarly, Jau-83 behaved better than Jau-87. The results of yield provided drastic effect of Cd metal on all the recorded yield and yield parameters. The addition of salicylic acid alone in the irrigation medium increased the yield parameters. The results noted about the mitigating effect of salicylic acid under Cd stress exhibited an increase in yield parameters but the increase was less at low and high level of SA than 0.5 mM. SA suppressed the effect of cadmium induced stress and showed increase in growth and yield and yield parameters when applied exogenously through rooting. Results showed presence of SA in the irrigation medium containing cadmium metal mitigated the toxic effect of cadmium.

Keywords: Salicylic acid; Cd; Barley; Yield; PCA

Introduction
Cadmium (Cd) is known as potentially toxic and widespread pollutant commonly entered the environment from application of fertilizers, industrial processes, urban activities and widespread sewage sludge. For plants, it has no benefit and act as a non-essential element. It may cause death of

plants even at very low concentrations (Khan et al., 2022). Plants easily taken up Cd and accumulate it into different parts such as leaves, seeds and fruits (Soni et al., 2023), therefore it enter into food chain and cause harmful effects to humans health (Suhani et al., 2022). Many symptoms appeared at molecular, biochemical and physiological level in plants due to Cd stress. These symptoms include leaf chlorosis, growth retardation, stomatal functional changes, root and leaf necrosis (Haider et al. 2021), Ultrastructure changes and inhibition of photosynthesis and transpiration (Soni *et al*., 2024). It causes serious physiological diseases, oxidative stress such as growth inhibition, root tip damage and reduction of photosynthesis (Haider et al. 2021).

Kaleem *et al*. (2022) showed reduction in root, shoot length and root dry weight of maize under cadmium toxicity. The accumulation of cadmium in root and shoot were increased by adding more cadmium in solution medium. The application of cadmium substantially decreased the fresh and dry weight of shoot, plant height, number of leaves, stem diameter, leaf area, number of ears per plant, 100-kernels weight and number of kernels per ear of maize cultivars (Guo *et al*. 2023). Cd perturbed the morpho-physiology of foxtail millet in a concentration dependent manner particularly at 1.5 µM Cd. The negative effect of Cd included decrease in shoot length, root length, number of leaves and shoots, panicle biomass and chlorophyll content (Jadid *et al*., 2022).

Cd tolerance, translocation and accumulation were studied in 288 broomcorn millet under hydroponic medium. Cd tolerance index showed 160 Cd tolerant varieties among them five varieties were considered hyperaccumulators with shoot Cd concentrations beyond threshold level of 100 $mgkg^{-1}$. The essential elements Mg and Zn significantly decrease in roots under Cd stress (Liu *et al*., 2021). Greenhouse experiment indicated that cadmium had negative effects on growth and biomass production. Roots accumulated more cadmium than areal parts of both plants. It was concluded that barley had maximum tolerance index (Zhang *et al*., 2022). A fluctuation in growth characteristics of two rice cultivars under cadmium stress was investigated. It was noted that plant biomass was reduced at different growth stages under cadmium stress in both cultivars of rice and Si application improved the growth in rice (Cai *et al*., 2020).

Maize showed different responses against cadmium toxicity that depends on varieties, stress duration and nature of growth medium. Exposure of higher level of cadmium considerably decreased seed germination, growth and yields (An *et al*., 2023). The elevated levels of Cd in nutrient medium increased dry mass in inbred while remained unaffected in hybrid maize. The growth was significantly reduced at higher levels of cadmium in both maize plants (Deng *et al.,* 2024). The application of SA (100, 200 and 300 mol L^{-1}) alleviate Cd toxicity symptoms in tartary buckwheat under Cd stress. It significantly promotes ryegrass plant growth, decrease Cd uptake, increase chlorophyll contents and improve nutrient absorption (Luo *et al*., 2022). Similarly, in rice plants exogenous SA application reduce Cd translocation from leaves to grains under Cd stress (Wang *et al*., 2021). SA foliar application is not only helpful in Cd uptake reduction but also promote nutrient absorption and growth of radish plants (Raza *et al*, 2022).

The SA applications to melon seedlings significantly improve pigment contents and increase Fv/Fm under Cd stress. SA may also protect PSII from Cd stress to promote photochemical activity (Moustakas *et al*., 2022). In the Cd-treated plants showed chlorosis due to Fe deficiency and the synthesis of chlorophyll inhibition in crop plants. SA significantly enhanced the concentration of Fe in both the roots and shoots lead to increase in the chlorophyll contents (Bagautdinova *et al*., 2022; Altaf *et al*., 2023). Barley belongs to genus *Hordeum* and grass family *Poaceae* (Blattner *et al*., 2018). This grass family contains many important crop plants for example barley, wheat, maize, oat and rye. The grain crops are primary source of energy that enables humans to move from hunting animals to agriculture and cultivation. In Pakistan, the cultivation of resilient crops is in demand for the future agriculture. But due to heavy metal infected soil, it is difficult to achieve the world's commercial demand of healthy, viable and good vigor seeds.

The present study is planned to examine the effect of Cd metal on the growth and yield of two barley varieties and how application of different treatments of salicylic acid be able to overcome the damaging effect of this metal on quality and yield.

Objectives

- To observe the growth and yield of two different barley varieties (Jau-83 and Jau-87) under Cd stress
- To study the growth and yield of two different barley varieties (Jau-83 and Jau-87) under different concentrations of SA
- To select one of the applied level of SA which is able to mitigate the adverse effect of Cd on two barley varieties

Materials and Methods

A pot trial was conducted to study the effect of Cd on various growth and yield parameters of barley cultivars and how and which dose of salicylic acid will mitigate the damaging effect of Cd. The experiment was arranged in the wire house, University of Sargodha, Lyallpur Campus, Faisalabad during October. Grains (seeds) of two barley genotypes (Jau-83 and Jau-87), were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad. The trial was designed as CRD (Completely Randomized) with two factorials.

a. Plan of Work

Grains of barley were sown in pots (10 kg sand), consecutively washed with tap water and distilled water thrice. After complete germination, the uniformity of plants were maintained to five (5) per pot by thinning. The plants were irrigated twice with Hoagland's solution of ½ strength to fulfill the nutritive demand of young growing barley plants, along with corresponding treatment of metal (Cadmium) and growth regulator SA, till the harvest of plants.

Cadmium metal toxicity was induced by using Cadmium Sulphate ($CdSO_4$). A single dose (500 µM) was used during the course of study (Kepova *et al*., 2006). There were three doses (0.1, 0.5, 0.9 mM) of salicylic acid were used. The Cd and SA were applied alone and in combination and each treatment was replicated thrice.

b. Growth parameters

After 60 days of treatment application, the plants were harvested and growth attributes were studied. Shoot length was measured from the stem base to the top of the plant and mean values was calculated. Root length was measured from the stem base the root tip and mean values was calculated. Fresh weight of shoots of three plants per replicate, per treatment were determined immediately after uprooting the plants with the help of top loading balance and mean values were calculated. Fresh weight of roots of three plants per replicate, per treatment were determined immediately after uprooting the plants with the help of top loading balance and mean values were calculated. Dry weight of shoots and root of three plants per replicate, per treatment were determined after keeping the fresh samples in oven at 65^0 C for 48 hours to get constant dry weight and mean values were calculated. Numbers of leaves per plant and numbers of tillers per plant were counted and mean values were calculated. Leaf area was calculated by measuring the maximum width and length and then leaf area of each plant of each treatment was calculated according to the formula Total Leaf Area = Maximum Leaf Length × Maximum Leaf Width × Correction Factor

Where Correction Factor (C.F) = 0.68

i. Shoot Water Contents (%)

Shoot fresh weight / Shoot dry weight x 100

ii. Root Water Contents (%)

Root fresh weight / Root dry weight x 100

iii. Tolerance Index

Tolerance index was calculated by Chen *et al*. (2011).

$$\text{T.I.} = \frac{\text{Mean Root Length in Matal Solution}}{\text{Mean Root Length in Control}} \times 100$$

iv. Shoot / root ratio

Shoot root ratio was calculated by the following formula.

Ratio = Shoot Length / Root Length

c. Yield Parameters

At maturity, the crop was harvested and yield parameters were determined. Number of spikes per replicate, per treatment was counted at maturity stage and mean values were calculated. The length of each spike per replicate and per treatment was measured from base to the top of the spike and mean values were calculated. Number of spikelets per spike per replicate per treatment was counted and means values were calculated. Number of grains per spike per replicate, per treatment was counted after threshing the individual spike and means were calculated. Hundred grains weight per replicate, per treatment was measured and mean values were calculated.

i. Total Seeds per Plant

Total number of seeds per spike per plant

ii. Yield per Plant (g)

Total weight of seeds per spike per plant

iii. Harvest index

Harvest index was calculated by Mahboob *et al*. (2015).

$$\text{H.I.} = \frac{\text{Economical Yield}}{\text{Biological Yield}} \times 100$$

H.I. = Dry Mass of Harvested Components / Total Shoot Dry Mass

d. Statistical Analysis

A two way analysis of variance of data for all the parameters will be computed, using COSTAT computer package (Cohort Software Berkeley, California). The least significant differences between mean values were calculated. Principal component analysis (PCA) was carried out to understand the relationship of variables.

Results and Discussion

Abiotic stress like salt, drought, nutritional and heavy metals caused adverse effects on crop plants, led to influence their growth and development. A common adverse effect of cadmium toxicity on crop plants is in the form of reduction both in fresh and dry biomass production (Fatima and Javed, 2016).

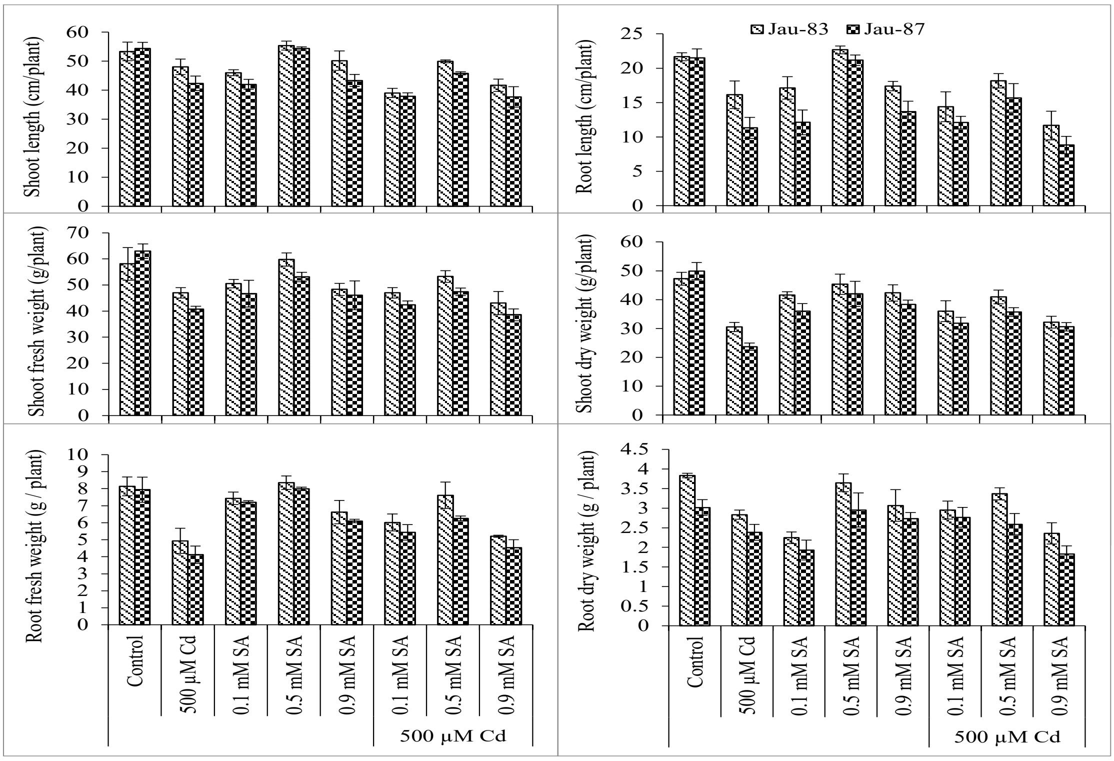

Fig. 1. Growth parameters were studied under Cd and SA treatments in two barley genotypes Jau-83 and Jau-87

Many workers reported that presence of cadmium metal in soil or in nutrient medium caused negative influence on almost all the growth parameters (Fig. 1 and 2), like shoot length (Yourtchi and Bayat, 2013; Akhtar *et al*., 2017; Anjum *et al*., 2017; Peng *et al*., 2017), root length in sorghum (Da-lin *et al*., 2011), in maize (Akhtar *et al*., 2017), in barnyard grass (Peng *et al*., 2017), their fresh and dry weights (Akhtar *et al*., 2017; Anjum *et al*., 2017; Rizk *et al*., 2014), number of leaves per plant (Mohammad, 2011; Anjum *et al*., 2017), leaf area (Fathi *et al*., 2012; Anjum *et al*., 2017), tolerance index (Jamali *et al*., 2014; Gonzalez *et al*., 2017), shoot root ratio (Dikkaya and Ergun, 2014) and number of tillers (Mohammad, 2011; Ymin *et al*., 2011) that was due to reduction in their cell elongation. Reasons for the reduction are the reduction in plant photosynthesis led to reduce water and nutrients absorption that affected the normal growth of plants (Liu *et al*., 2003).

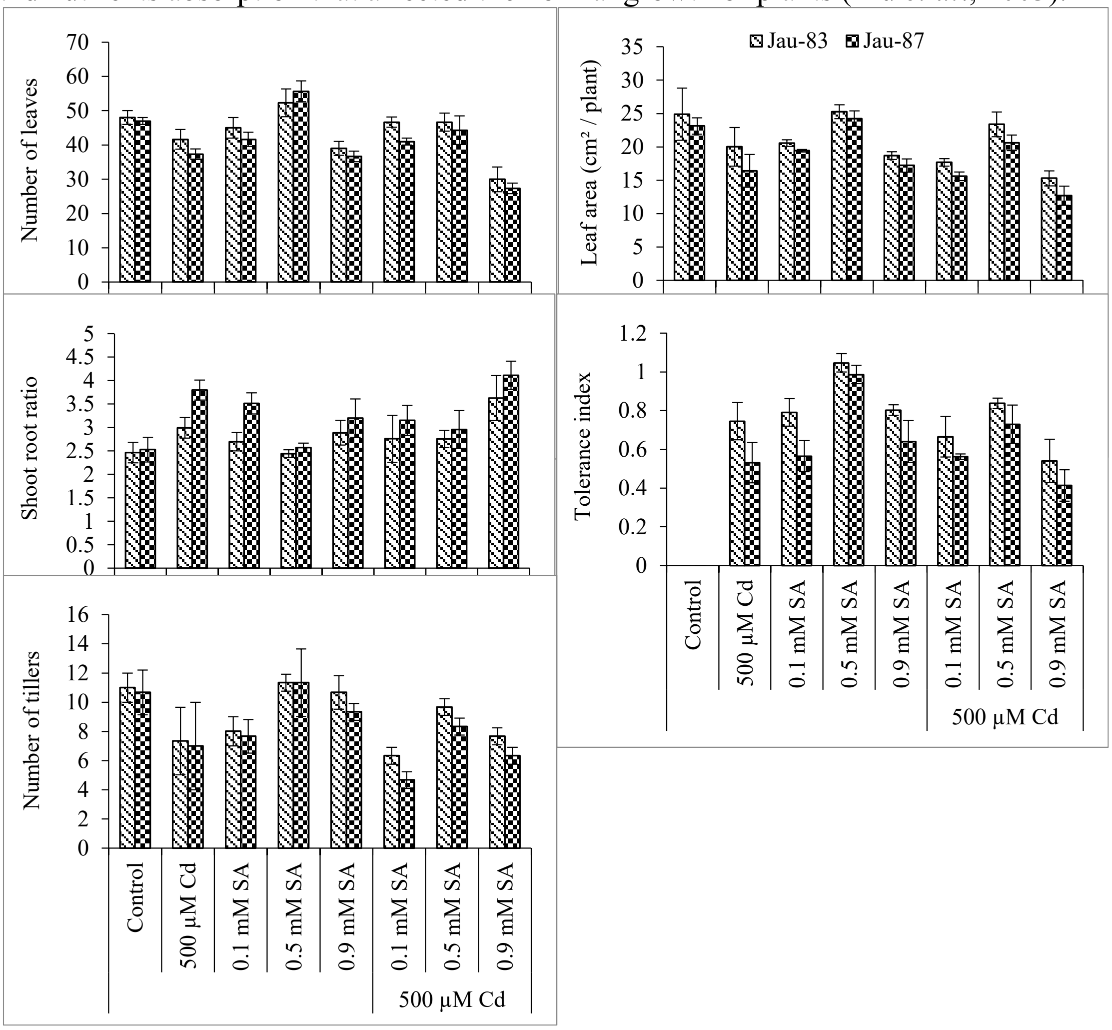

Fig. 2. Growth parameters were studied under Cd and SA treatments in two barley genotypes Jau-83 and Jau-87

Our studies on barley genotypes showed the same effects on growth parameters. Shoot length of both the genotypes reduced when cadmium metal present in the irrigation medium. This reduction in the plant length caused in shoot and root fresh and their dry weights, number of leaves per plant, leaf area per plant, shoot root ratio and tolerance index. Although both barley genotypes behave statistical similar but Jau-83 respond better to cadmium stress than Jau-87.

Plant Yield and yield parameters (Fig. 3) also affected under metal stresses. Many reports revealed reduction in spike length (Shu-juan *et al*., 2008), number of spike per plant (Wang-da, 2005; Anjum *et al*., 2017), number of spikelet per spike (Wang-da, 2005; Ymin *et al*., 2011), hundred grain weight (Wang-da, 2005; Shu-juan *et al*., 2008; Ymin *et al*., 2011), total number of grains (Mohammad, 2011; Fathi *et al*., 2012; Amin *et al*., 2013) and yield per plant (Wang-da, 2005; Ymin *et al*., 2011; Anjum *et al*., 2017; Rizwan *et al*., 2017). In the present study same effect of cadmium toxicity found about yield and various yield parameters of both the barley genotypes when cadmium metal added exogenously in the irrigation medium. This may be due to the reason that cadmium stress effects the physiological reactions in barley particularly photosynthesis, that decreased the production of carbohydrates.

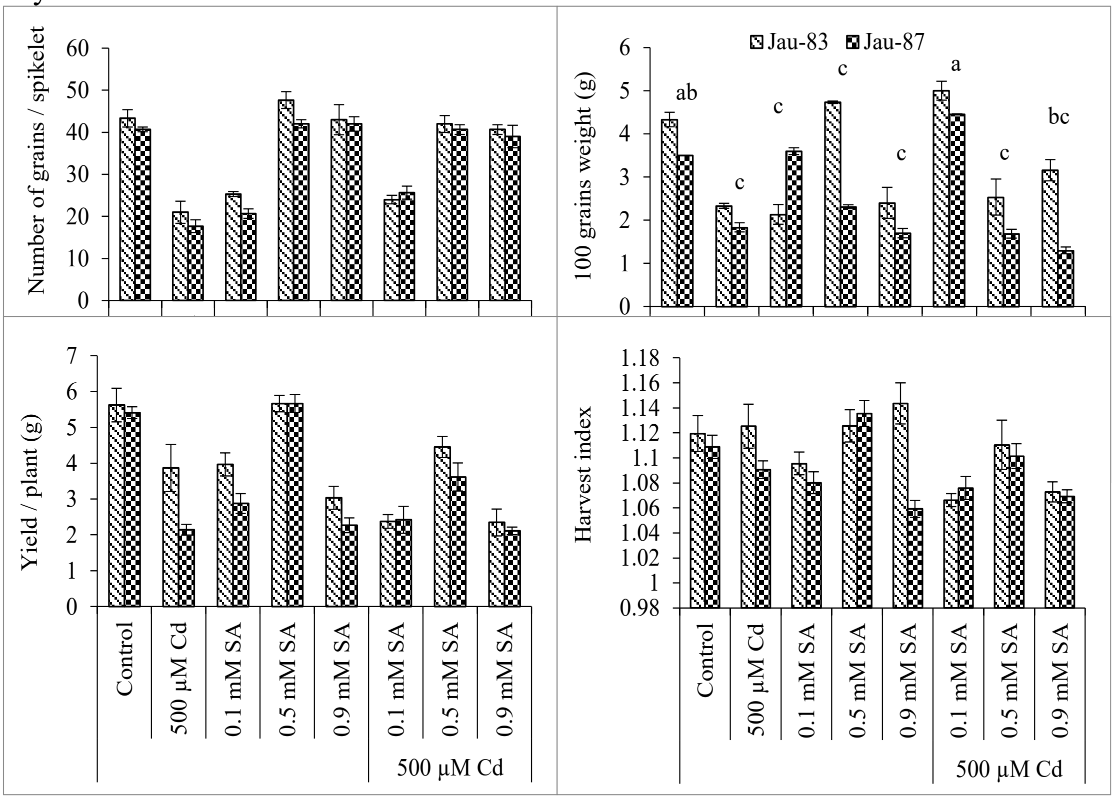

Fig. 3. Plant Yield and yield parameters were studied under Cd and SA treatments in two barley genotypes Jau-83 and Jau-87

PCA Plot

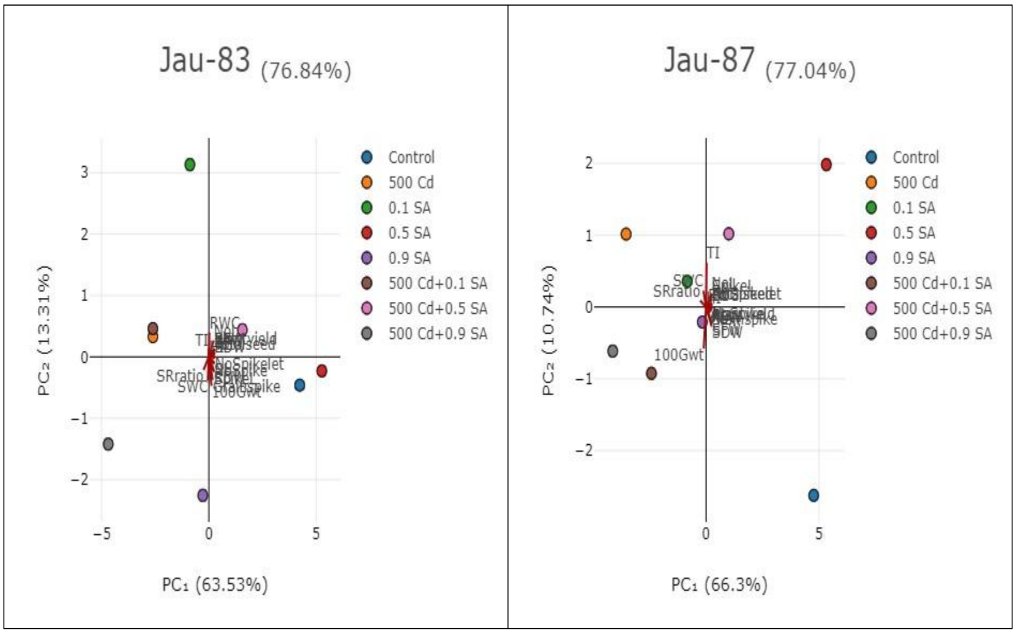

Fig 4: PCA biplot for two barley varieties (Jau-83 and Jau-87)

The biplot Principal component analysis shows the cumulative proportions of the explained variance and the number of principal components is displayed. In this representation, the dots represent the principal components (PC) and lines represent the variables. With the help of multivariate method of PCA, the dimensionality of the data reduced i.e. reduced the eight distinct treatments that can affect the growth of two barley varieties to only 1 or 2 treatments. For Jau-83 variety (a) the PC_1 explains 63.53% of the total variability and for Jau-87 variety (b) the PC_1 explains 63.3% of the total variability. Therefore we might want to stop at the PC_2. Maximum information (variances) contained in the data are retained by the first two principal components. There are high (or positive) correlations between all the studies variables except shoot water content and shoot root ratio. The biplot (Fig 4) shows that 0.5 SA alone and in combination with 500 µM Cd are effective treatments for Jau-83 and Jau-87 varieties.

Salicylic acid (SA) increased plant growth by increasing cell division with the apical meristem of both growth and yield and yield parameters in crop plants like, in rice growth (Herath *et al*., 2014; Mohammad, 2011), in corn (Xinyue, 2013; Fanimayani *et al*., 2014; Anjum *et al*., 2017), rice yield (Amin *et al*., 2013; Mohammad, 2011). The present studies also revealed same an increase in all the studied growth and yield and yield parameters. Maximum growth and yield were observed at 0.5 mM of salicylic acid. Low and high levels of salicylic acid than 0.5 mM showed less increase. Low level (0.1 mM) exhibited less increase than high level (0.9 nM) in both the barley genotypes. Jau-83 behaved to salicylic acid present in irrigation medium better than Jau-87.

Salicylic acid is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants, it plays an important role in abiotic stress tolerance and considerable attention has been focused on the ability of salicylic acid to induce protective effect on plants under stress (Popova *et al*., 2011). Many studies report the role of salicylic acid in inducing stress tolerance in plants (Popova *et al*., 2011; Xinyue, 2013; Zhang *et al*., 2013). For example, salicylic acid has been found to induce chilling tolerance in maize and wheat (Janda *et al*., 2014), salinity tolerance in wheat (Shakirova, 2007), heavy metal stress tolerance in barley (Metwali *et al*., 2013) and drought tolerance in wheat (Singh and Shah, 2015; Shakiorva, 2007), cadmium tolerance in wheat (Mousa and El-Gamal, 2010). Although, a lot of work has been done on different crops to mitigate the adverse effects of exogenous added cadmium metal, little information is available regarding barley. The present studies also revealed the same results that all the growth and yield and yield parameters were increased by exogenously addition of salicylic acid in cadmium containing irrigation medium. Although both the barley genotypes behaved similar to cadmium stress and mitigating effect of salicylic acid, Jau-83 behaved better than Jau-87.

Conclusion

In conclusion, growth and yield parameters of both genotypes showed declined in the presence of cadmium induced stress. When SA applied in combination with Cd, data revealed increase in growth and yield parameters as by reduced the toxic effect of Cd as compared to Cd alone. Although, SA reduced the toxic effect of Cd, root was more affected than shoot due to the direct contact to metal. Genotype Jau-83 exhibited better results as compared to Jau-87.

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