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SEED PRIMING WITH SALICYLIC ACID INDUCES TOLERANCE AGAINST CHROMIUM (VI) TOXICITY IN RICE (*ORYZA SATIVA* L.)

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Abstract

Anthropogenic activities caused release of toxic heavy metals including chromium (Cr) in environment all over the world, which pollute agricultural lands leading to reduction in growth of crop plants. Plant growth regulators like salicylic acid (SA) control growth and development and seed priming treatments with SA have been proven beneficial for plant growth. Therefore, the present study was focused on the potential mediatory role of SA under Cr (VI) toxicity in rice. For priming, seeds of two rice varieties, namely, Basmati-385 (B-385) and Shaheen Basmati (SB) were soaked in 0 (hydroprimed), 0.25 and 0.50mM solution of SA for 24hrs at room temperature. SA and hydroprimed seeds were grown in petriplates and later transferred to trays containing Hoagland's nutrient medium up to 24 days and then 0 (control), 25, 50 and 100µM Cr (VI) stress was applied for one week. Cr treatments resulted in reduction of physiological parameters like germination rate and percentage, seedling vigor and seedling dry biomass while priming with SA treatments showed protection against Cr. Biochemical analysis of rice leaves showed that Cr stress alleviated chlorophyll content, accelerated cell membrane damage, decreased content of Na^+, K^+ and Ca^{+2} ions and also reduced the total nitrogen content but SA priming mitigated the negative effect on these parameters. Cr content was lowered in leaves of SA primed plants in comparison to non SA primed plants under the respective Cr concentrations. Chromium (VI) stress applied to SA unprimed plants elevated the levels of stress metabolites i.e. total soluble proteins, total soluble sugars, glycinebetaine and proline content while SA priming lowered the toxicity of Cr manifested by alleviation in level of different stress metabolites. The important role of SA priming in inducing tolerance against environmental stresses may be due to its ability in expressing defense related proteins, which may provide tolerance against Cr. It can be suggested from the results that SA seed priming enhances resistance in rice against Cr.

Key words: Seed Priming, Salicylic Acid, Chromium, *Oryza sativa*

Introduction

Chromium (Cr) like other heavy metals such as cadmium, lead has threatened our crop production and human health (Daud *et al*., 2014), which is mostly released by anthropogenic activities i.e. leather tanning, paint and fertilizer industries (Nriaguand & Neiborer, 1988). Cr as hexavalent form, Cr (VI) is considered very toxic for crop plants (Shanker *et al.,* 2009) and there is no clear evidence of Cr (VI) involvement in any beneficial role in plant metabolism (Lin *et al.,* 2004). In polluted soil environment of rice crop, Cr (VI) readily solubilizes in soil solution and thus maximizes the chances of its high uptake causing detrimental Cr toxicity (Bhattacharyya *et al.,* 2005). The Cr damaging effect results in inhibition of germination of seed, growth of seedlings and development due to damaging of cells by high oxidative stress (Suwalsky *et al.,* 2008). Cr alters many metabolic processes like photosynthesis, nutrient uptake, enzymatic activities, water relations and ions imbalance resulting in phytomass reduction, chlorosis, stunting and ultimately death of plant (Dixit *et al.,* 2002). Panda (2007) reported that the damaging effects of Cr in rice seedlings induced oxidative stress, which is the primary mechanism regarding Cr toxicity.

Seed priming is the easiest and economical strategy used to limit the damaging effects of abiotic stresses (Iqbal & Ashraf, 2007). Seed priming i.e. pre sowing treatment of seeds is very effective in enhancing performance of seed by getting better germination and seedling growth (Farooq *et al.,* 2007; Arif *et al.,* 2008). Rice seed priming is done for healthier crop stand and increased yields (Iqbal & Ashraf, 2007). Priming techniques that increase seed performance during stress conditions involves use of certain plant growth regulators like salicylic acid (SA) and plant hormones like gibberellins and abscissic acid (Farooq *et al.,* 2007).

Salicylic acid i.e. a phenolic compound, is an endogenous growth regulator influencing a wide range of mechanisms in plants like seed germination, stomatal closure, ion uptake and translocation, membrane permeability, photosynthesis and growth elevation (Khan *et al.,* 2003). In plants, it has been showed that SA acts as a signaling molecule responsible for developing tolerance against abiotic stress (Gunes *et al.,* 2007). Incorporating plant growth regulators like SA during presoaking and priming increase the seed activity in many crops like rice (Basra *et al.,* 2006). SA may affect directly on function of specific enzymes and might also switch on the genes responsible for protective processes (Horvat *et al.,* 2009).

SA arbitrates positive adaptive responses to abiotic stresses such as heavy metals, salinity and low temperatures (Metwally *et al.,* 2003). In plants like maize and barley, the SA seed pretreatment lowers cadmium toxicity (Metwally *et al.,* 2003, Krantev *et al.,* 2008). SA application ameliorates the harmful consequences of heavy metals like mercury and lead (Mishra & Choudhuri, 1999).

Rice (*Oryza sativa* L.) is a vital cereal crop being utilized as necessary food source all over the world particularly in Asian countries (Ebrahimi *et al.,* 2012). In many parts of the world, rice production is largely affected by drought, heavy metals, high & low temperatures and salt

stress (Oerke *et al.,* 1994). Abiotic stresses harmfully manipulate the growth, biomass production and yield of crop (Zhu, 2001) by induction of variations at biochemical and molecular level via alterations of metabolic pathways and gene expressions (Hasegawa *et al.,* 2000).

In rice, little consideration has been given to avoid Cr stress while it is very damaging factor for its growth and overcoming Cr toxicity can be difficult. Therefore, new and effective strategies should be considered to overcome damages of Cr, so keeping in view the value of rice the current study was focused to explore the potential mediatory role of SA priming for inducing Cr tolerance by investigation of physiological, biochemical and metabolic parameters.

Materials and Methods

Plant materials: Seeds of two rice varieties Basmati -385 (B-385) and Shaheen Basmati (SB) were obtained from National Agriculture Research Center (NARC), Islamabad. Seeds were kept stored at room temperature in paper bags before being utilized for experiments. After carefully hand sorting healthy seeds of both rice varieties, the seeds were surface sterilized with 3.5% (v/v) sodium hypochlorite for five minutes and then washed thrice thoroughly with distilled water (DW) and air dried on sterilized filter paper at room temperature.

SA seed priming: Sterilized seeds of both rice varieties were soaked in solutions of salicylic acid of concentrations 0.25 and 0.50mM and in DW (hydroprimed) at room temperature for 24hrs. The primed seeds were then air dried at room temperature on sterilized filter paper and used for further process.

Seed germination and seedling growth: SA primed seeds (n=10) were germinated in triplicates per treatment on double layered filter paper (Whatman No.1) in sterilized Petri plates treated with 0 (control), 25, 50 and 100µM potassium dichromate ($K_2Cr_2O_7$) solutions referring to increasing Cr (VI) stress. DW primed seeds were used as control. Seeds were germinated in dark conditions in incubator at 30±2°C. Seed germination was recorded at periods of 12hrs up to 96hrs upon emergence of nearly 2mm radical (Jamil *et al.,* 2012). Germination percentage and rate were calculated when germination of 50% of seeds occurred per plate of each treatment. After ten days of growth in petri plates, the seedlings were separated into roots and shoots and their lengths were measured followed by weighing of fresh roots and shoots to determine the biomass weight. For dry weights of roots and shoots, the fresh materials were oven dried for 48hrs at 80°C.

Hydroponic culture experiment: The seeds (SA and hydroprimed) were first germinated in DW in petri plates with double layered filter papers. Early seedlings at two leaves stage were then transferred to plastic trays. For two weeks, the seedlings were provided with Hoagland nutrient solution (Hoagland & Arnon, 1950) by continuously maintaining pH of 5.5-5.9 and changing nutrient solution on weekly basis. After two weeks, chromium stress ($K_2Cr_2O_7$) treatments of 0 (control), 25, 50 and 100µM were applied for seven days with full strength Hoagland solution. The entire experiment was carried under greenhouse environment (temperature 30-35°C, humidity 60-70% and 16hrs photoperiod). After one week of Cr stress, fresh leaves of each treatment were randomly selected from three replicates and properly grinded in liquid nitrogen with mortar and pistil, stored at -20°C in eppendrof tubes for various biochemical and metabolic analysis. The remaining material was dried in incubator at 80°C for 2 days and was powdered manually for usage in different biochemical tests.

Cell membrane stability: After stress treatment, twenty strips of 1cm^2 from fresh leaves were cut and placed in glass test tubes containing 20ml DW and were incubated at 10ºC for 24hrs and measurement of the electrical conductivity (EC1) was done. After that the tubes were autoclaved and EC2 measurement was done by using conductivity meter (Jamil *et al.,* 2012). The net cell injury was calculated by formula: EC-1 / EC-2 ×100.

Chlorophyll and carotenoid content: The method of Lichtenthaler & Wellburn, (1985) was used for the determination of chlorophyll and carotenoids pigments by extraction with methanol and absorbance was found with UV-visible spectrophotometer at three wavelengths: 666, 653 and 470nm. The chlorophyll "a, b" and total carotenoids contents were determined by formula suggested by Lichtenthaler & Wellburn, (1985).

Determination of ions (Na^+, K^+ and Ca^{+2}& Cr): The contents of ions were determined by slightly modifying the method of Awan & Salim (1997). Well powdered dry leaf material (25mg) was heat digested with conc. H_2SO_4 and hydrogen peroxide in a 2:1 ratio (v/v). After digestion, each sample was diluted with 20ml deionized. Ion contents (Na^+, K^+ and Ca^{+2}) were analyzed by flame photometer. Cr content was determined by method of Garraaud *et al.,* (1996). First dry leaves samples of 100mg were heat digested by acid mixture of sulfuric acid and nitric acid. The digested ash content was suitably diluted with deionized water to 20ml volume in all treated samples and used further for atomic absorption spectrophotometer.

Determination of Nitrogen content: Nitrogen content was determined by using the Micro-Kjeldahl apparatus. Dry leaf material (100mg) was taken with 0.1g catalyst mixture ($CuSO_4$, $FeSO_4$ and K_2SO_4) and heated with 2ml conc. H_2SO_4 in Kjeldhal digestion tubes for 2hrs and then transferred to titration unit of Micro-Kjeldhal apparatus for titration process. At the end, the nitrogen content was determined by the formula recommended by Pellet & Young (1980).

Proline and glycinebetaine analysis: Proline content was determined by slightly modified method of Bates *et al.,* (1973). Fresh leaf sample (100mg) was homogenized in 3.5% sulphosalicylic acid and centrifuged. In 1ml supernatant, 1ml of acid ninhydrin was added followed by 1ml of glacial acetic acid and heated at 100ºC for 1hr. The mixture was then extracted with 2ml toluene and proline was determined spectrophotometrically at 520nm from upper colored phase after toluene extraction. L-Proline standard curve was used for comparing sample values.

Glycinebetaine was extracted by mechanical shaking finely ground-dried samples of leaves with deionized water for 24hrs. Glycinebetaine contents were determined spectrophotometrically after reaction with KI-I_2 at 365 nm and dissolving in 1-2, dicholoroethane (Grieve & Grattan, 1983). Final amounts were calculated from standard curve of glycinebetaine.

Total soluble sugar and proteins: Total soluble sugar content was analyzed by phenol-sulphuric acid method of Dey *et al.*, (1990) with slight modification by using 50mg fresh sample. Absorbance values were determined at 485nm wavelength. D-Glucose was used to make standard curve for determination of sample total sugar values. Total soluble protein was determined by following the method of Bradford (1976) by using Comassie G-250 as protein binding dye. Final amount of protein was calculated from standard curve obtained from bovine serum albumin.

Statistical analysis: All the data was analyzed by the statistical software IBM SPSS v.20, presented as mean of triplicates and with standard error bars.

Results

Seed germination: Germination pattern of both the rice varieties Basmati-385 and Shaheen Basmati showed variations under the 0.25 & 0.50mM SA priming and at Cr stress treatments. Germination percentage (GP) decreased in both the varieties under increasing Cr stress (Fig.1). Maximum GP (100%) under non stress conditions was observed after 48hrs of interval in control (hydroprimed) and SA primings in B-385 variety while in SB, 100% GP was noted at SA 0.50mM priming treatment. In both varieties Cr stress treatments reduced the GP as the stress was increased and lowest GP of 57% and 50% were recorded at 100µM of stress in B-385 & SB respectively. In SA treatments, GPs were higher in comparison to respective stress treatments. Germination rate (GR) ($1/t_{50}$) i.e. the time required for 50% seeds to germinate was also lowered in both the varieties under varying Cr stresses (Fig. 1). Maximum GR was noted in control and SA priming treatments. Reduction in GR was prominent at 25, 50 and 100µM Cr stress in B385 and SB; however, with SA priming obvious elevations in GR were noted

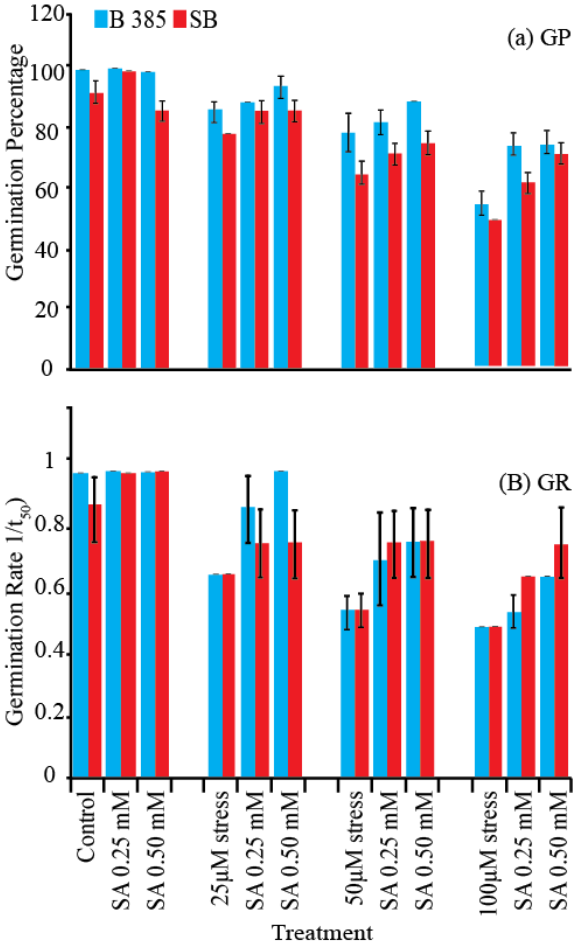

Fig. 1. Effects of SA priming (0.25 & 0.5mM) on germination percentage (after 48 hrs) & germination rate of rice under varying concentrations of Cr (0, 25, 50 & 100µM). The data represented as mean value of observations (n=3) ± SE.

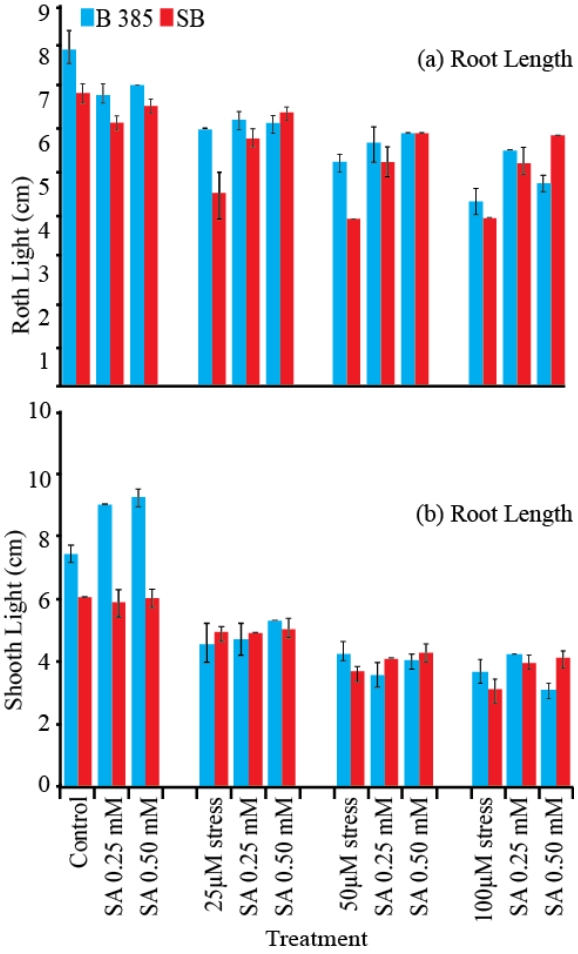

Fig. 2. Effects of SA priming (0.25 & 0.5mM) on (a) root length & (b) shoot length of rice under varying concentrations of Cr (0, 25, 50 & 100µM). The data represented as mean value of observations (n=3) ± SE.

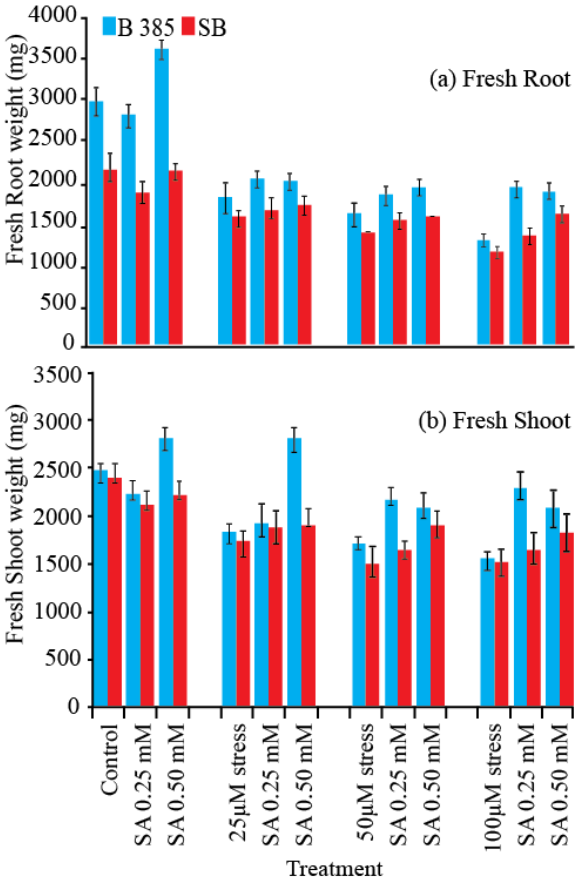

Fig. 3. Effects of SA priming (0.25 & 0.5mM) on fresh biomass of seedlings (a) root& (b) shoot of rice under varying concentrations of Cr (0, 25, 50 & 100µM). The data represented as mean value of observations (n=3) ± SE.

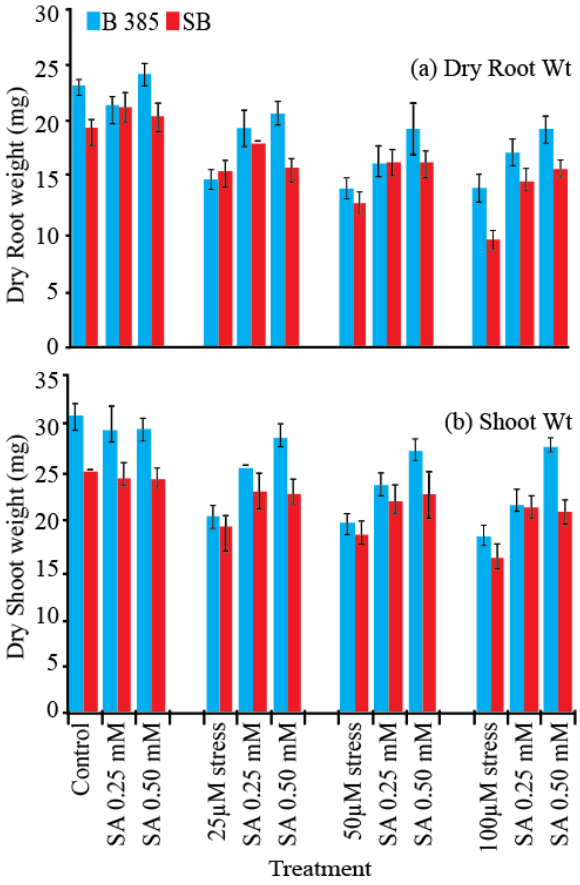

Fig. 4. Effects of SA priming (0.25 & 0.5mM) on dry biomass of seedlings (a) root & (b) shoot of rice under varying concentrations of Cr (0, 25, 50 & 100µM). The data represented as mean value of observations (n=3) ± SE.

Seedling vigor: In both varieties, for SA priming treatments and control the root and shoot lengths improved sufficiently while gradual reduction in lengths were observed under increasing concentrations of Cr (Fig. 2). In B385, lowest root length was ~4cm under 100µM stress and 3.9cm in SB. SA priming alone and under stress had improved lengths at respective Cr concentrations. Same pattern was seen in shoot length measurements. SA priming prominently improved shoot lengths and the highest values 9.35 & 6.15cm at 0.50mM for B-385 and SB were noted. Under stress condition, shoot length decreased significantly. The fresh and dry biomass of roots and shoots decreased significantly but SA treatments were effective enough in alleviation of stress and helped in development of fresh and dry biomass as presented in Figs. 3 & 4.

Cell membrane injury: Cell membrane injury elevated prominently with increase in Cr stress for B-385 and SB in comparison to control and SA priming treatments (Fig. 5). Significant elevation of cell injury was noted at 100µM stress of 57.8 and ~60µS/cm^2 as compared to control values of 24µS/cm^2 and 25µS/cm^2 in B-385 and SB respectively. SA showed a positive response in tolerating the damaging effects of Cr.

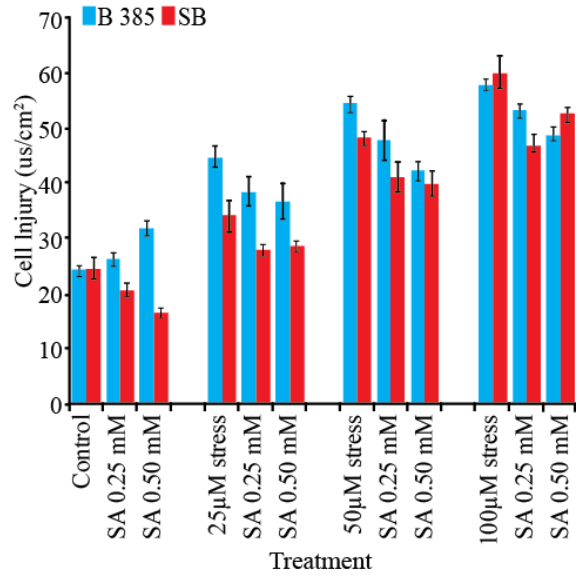

Fig. 5. Effects of SA priming (0.25 & 0.5mM) on cell membrane injury of rice under varying concentrations of Cr (0, 25, 50 & 100µM). The data represented as mean value of observations (n=3) ± SE.

Photosynthetic pigments: Chlorophyll and carotenoids contents decreased largely with increasing concentration of Cr. In control the chlorophyll a, b and carotenoids values were 6.7, 9.2 and ~660mg/g respectively which reduced to 0.72, 1.2 and 63 mg/g at 100µM Cr stress in B-385. Same results pattern were observed in SB (Fig. 6). The SA priming under the subjected stress parameters showed higher values of photosynthetic pigments revealing the SA protective role during stress.

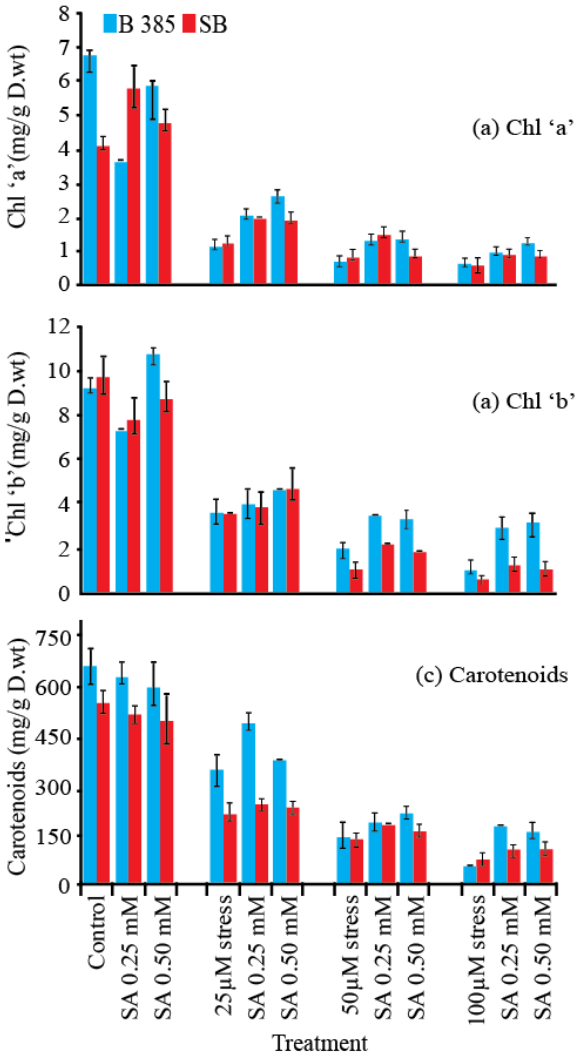

Fig. 6. Effects of SA priming (0.25 & 0.5mM) on photosynthetic pigments (a) Chl 'a', (b) Chl 'b' & (c) Carotenoids of rice under varying concentrations of Cr (0, 25, 50 & 100µM). The data represented as mean value of observations (n=3) ± SE.

Total Nitrogen: Total leaf nitrogen content in both varieties was higher in control and SA seed primed plants but the contents was lowered in stress conditions i.e. 1.19, 0.91 & 0.63 g/g in B-385 and 1.26, 0.9 & 0.63 g/g in SB at 25, 50 and 100µM Cr stresses respectively as presented in Fig. 8. SA primed treatments had higher N content at different Cr stress with respect to the stress applied to non SA primed rice plants. B-385 was more responsive to SA priming than SB under Cr toxicity.

Ion contents: Considerable variations were observed in content of ions in leaves (Na^+, K^+ and Ca^2) for rice plants treated under Cr stress. Under stress conditions, the content of Na^+, K^+ and Ca^{2+} ions decreased in both the varieties but with priming approach the ions contents were affected as in stress circumstances (Fig. 7). At 100µM Cr stress, lowest values of Na^+, K^+ and Ca^{2+} ions noted in B-385 and SB were 10 & 9.33ppm, 18 & 18.66ppm and 7.66 & 5.33ppm respectively.

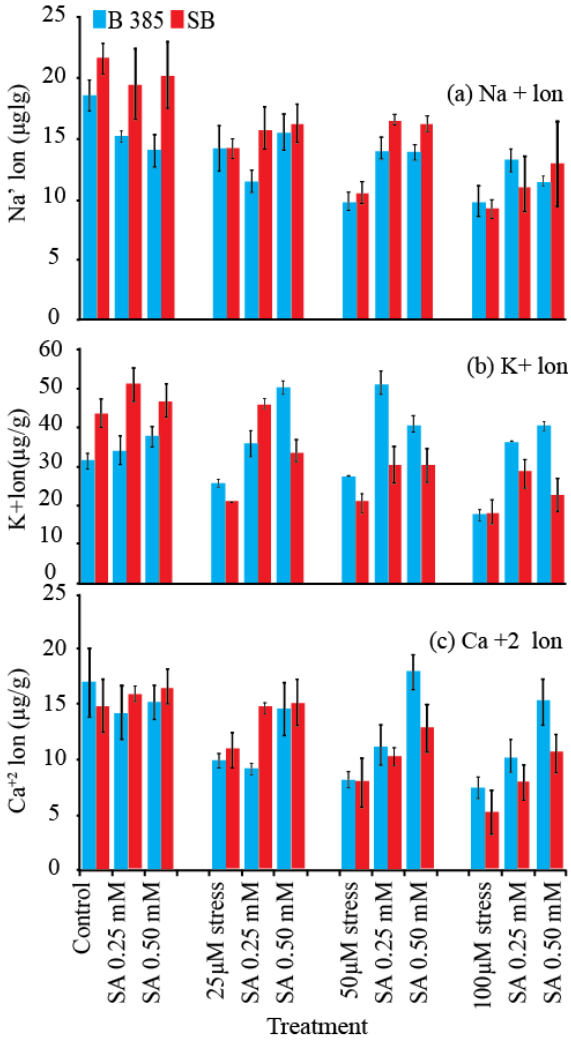

Fig. 7. Effects of SA priming (0.25 & 0.5mM) on Ion contents (a) Na^+, (b) K^+ and (c) Ca^{+2} of rice under varying concentrations of Cr (0, 25, 50 & 100µM). The data represented as mean value of observations (n=3) ± SE.

Chromium content: The increase in chromium content of leaves of both varieties was significant with the elevation of Cr stress as observed from Fig. 9. In control and SA priming, very low Cr content was detected i.e. ~0.012ppm for both varieties while the highest values were 1.7 and 2.0 ppm in100µM stress for B-385 and SB respectively. SA priming treatments under stress had lowered the Cr level to some extent while SB accumulated more Cr than B-385 at 100 µM stress.

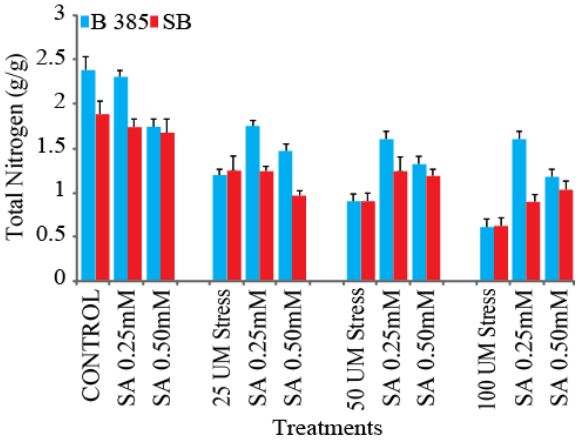

Fig. 8. Effects of SA priming (0.25 & 0.5mM) on total nitrogen content of rice under varying concentrations of Cr (0, 25, 50 & 100µM). The data represented as mean value of observations (n=3) ± SE.

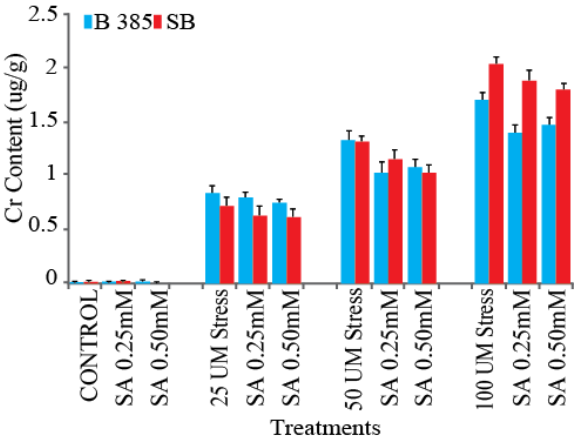

Fig. 9. Effects of SA priming (0.25 & 0.5mM) on chromium contentof rice under varying concentrations of Cr (0, 25, 50 & 100µM). The data represented as mean value of observations (n=3) ± SE.

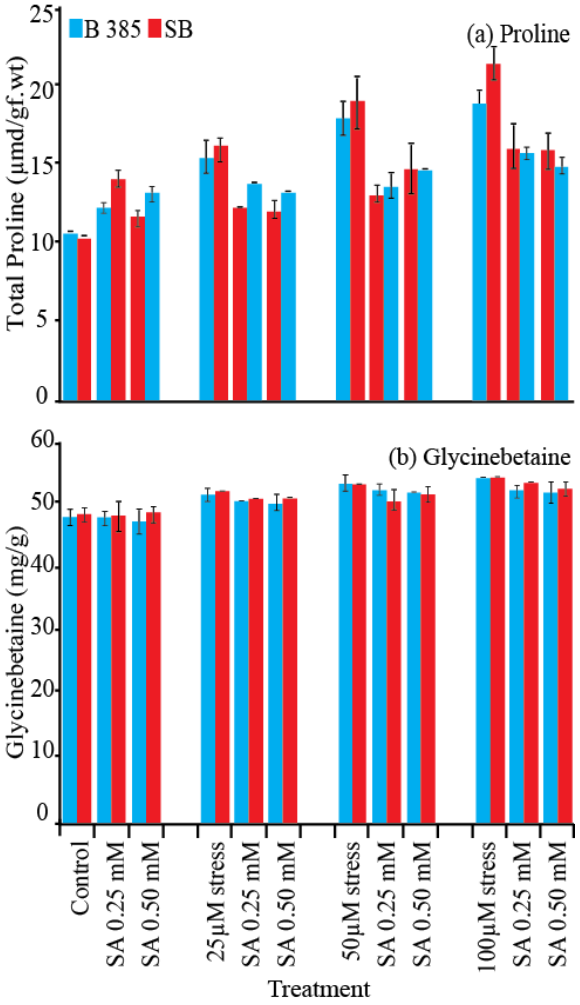

Fig. 10. Effects of SA priming (0.25 & 0.5mM) on (a) proline content& (b) glycinebetaine of rice under varying concentrations of Cr (0, 25, 50 & 100µM). The data represented as mean value of observations (n=3) ± SE.

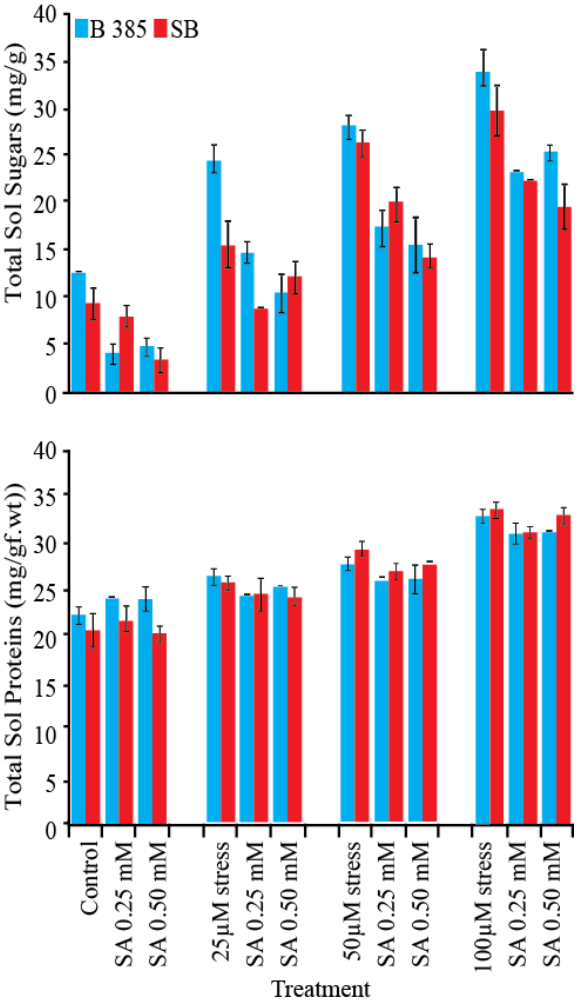

Fig. 11. Effects of SA priming (0.25 & 0.5mM) on total soluble sugars of rice under varying concentrations of Cr (0, 25, 50 & 100µM). The data represented as mean value of observations (n=3) ± SE.

Total proline and glycinebetaine: Our results showed that proline accumulation elevated under increasing Cr toxicity as compared to control and SA priming treatments. In B-385, proline content increased in control from 10 to ~15, 18 and 19µmol/g in 25, 50 and 100µM stress respectively while in SB similar values were observed with increase in stress (Fig. 10). But in both the varieties, the SA primed treatments showed a tolerating response to Cr stress and their proline contents were lower as compared to the respective Cr concentration applied. The glycinebetaine content of leaves under the highest Cr concentration (100µM) was 54.4 & 55mg/g in B-385 and SB (Fig. 10). The glycinebetaine level in SA priming was not significantly varied in comparison to control and Cr stress treatments.

Total soluble sugar and protein: The total soluble sugars content increased with increase of stress treatments which represented stress counteracting effects by both the varieties in comparison to control and SA primed plants (Fig. 11). The values in control increased from 12 to 36mg/g and 2 to 29 mg/g in 100µM stress for B-385 and SB correspondingly. However, under SA priming treatments, significant reductions in values were seen under stress in both varieties. Total soluble proteins content was also observed to be higher at Cr stress alone i.e. maximum level was noted at 100µM in B-385 & SB varieties i.e. 33.3 & 33.5 mg/g individually (Fig. 11). Both SA priming approaches mitigated the effects of Cr toxicity and showed significantly lower values at 25, 50 & 100µM Cr concentrations.

Discussion

Chromium stress is an important environmental factor which affects various physiological and biochemical parameters of plants including rice (Panda & Choudhury, 2005) but priming approaches offer the best solution for overcoming the drastic effects of stress (Iqbal & Ashraf, 2007). During growth of plants, seed germination is the preliminary physiological process which is affected by Cr stress (Peralta *et al.,* 2001). Our results have shown that under increasing stress of Cr, germination of seeds i.e. germination percentage and germination rates were reduced in comparison to control and primed seeds in B-385 and SB. The reduction in germination is due to the fact that various physiological disturbances occur like immobilization of nutrients in germinating seeds by presence of Cr as an inhibitor entity (Zeid, 2011).Similar findings were observed by Gyawali & Lekhak, (2006) that increase in toxicity of Cr inhibited germination of various rice cultivars at seedling stages by observing 50 to 100% reduction in germination percentages at highest Cr levels. We obtained significant results with SA priming i.e. improvement of GP and GR with respect to control and stress which might be suggested that SA act as signaling molecule in growth processes leading to breakage of seed dormancy and increased metabolic activities in SA primed seeds (Khan *et al.,* 2003). The work of Rehman *et al.,* (2011) proved that salicylic acid priming improves early seedling emergence which is in accordance to our results.

Early seedling development i.e. primary root and shoot development is essential for effective growth of plants. The root and shoot growth i.e. root and shoot lengths decreased significantly in presence of Cr during growth for both varieties B385 and SB as shown. Decrease in lengths might be due to involvement of Cr in inhibition of cell cycle mechanisms that stops elongation of shoots and roots (Ahmad *et al.,* 2011). Dubey *et al.,* (2010) had also found similar results in rice that the shoot and root growth inhibited at 100µM Cr (VI) in comparison to control. Our results also coincide to the reported Cr toxicity in rice seedling growth (Panda, 2007). SA is thought to be growth regulator which positively regulates cell proliferations (Sakhbutdinova *et al.,* 2003). SA priming showed varied response in both varieties but under stress significantly better results were obtained which can be related to findings of Choudhury and Panda, (2004) they found minor reduction in root lengths in SA treated rice under Cd stress in comparison to stress plants. SA primed seedling vigor growth promoting properties were also reported in barley (Metwally *et al.,* 2003).

Abiotic stresses inhibit growth which leads to reduction of seedling biomass. Obvious variations were observed in fresh and dry biomass in terms of fresh and dry weights of roots and shoots for both varieties B-385 and SB under Cr stress. Reduction in biomass could be due to poor development referring to toxic effects of Cr interfering with vital growth processes (Vajpayee *et al.,* 2001). Our findings were related to the findings of Chen *et al.,* (2011) who stated that the total root and shoot weight of wheat was affected by stress of 20 mg Cr (VI)/kg soil. Our results were also similar to Gyawali & Lekhak, (2006) due to the fact that they observed large reduction in weights of roots and shoots under increased Cr concentrations in rice. SA priming showed notable increase in fresh and dry biomass with and without Cr stress. Our results are supported by Ahmed *et al.,* (2011) who concluded that SA treated *Brassica* seedlings had more shoot biomass than untreated seedlings under Cd stress applied. Choudhury & Panda, (2004) had also reported that SA treated rice seedlings had more biomass of roots under Cr stress. It can be suggested from our findings that SA acts as endogenous growth regulator which can tolerate the toxic Cr effects by stimulating developmental processes.

The rigidity of cell membrane, which could be damaged by stress, is important for retaining important processes of plant cells. In the present study it was exhibited that cell membrane integrity is disturbed during Cr stress conditions and as the Cr stress was increased, maximum electrical conductivity measurements for 100µM in both varieties represented cell injury to large extents as shown. It can be argued that since Cr (VI) is toxic specie, it disturbs cell membrane potential, its firmness and functional processes leading to high leakage of electrolytes (Dixit *et al.,* 2002) .Our results are related to Dubey *et al.,* (2010) who found large changes in the physical properties of cell membrane during heavy metal stress of Cr. Regarding our results, the findings of Zeid *et al.,* (2013) also showed that heavy metals like Cr and cobalt had a potential damaging effect on cell membrane leading to electrolyte leakage in alfalfa. In both varieties, SA priming revealed effective response by lowering cell injury under Cr stress. Similar to our findings, Fahad & Bano, (2012) reported that SA effectively elevated osmotic potential and maintained membrane stability in maize under salt stress.

Choudhury & Panda, (2004) also stated that SA treated rice roots were less prone to membrane damages under cadmium stress. It can be argued that SA is stress tolerating hormone which maintains cell turgor pressure and avoid cell membrane from damages.

The present investigation revealed that the important process of plant growth i.e. photosynthesis was largely affected by the findings that the chlorophyll contents i.e. chlorophyll a, b and total carotenoids were reduced gradually with increasing Cr stress but their contents were higher in control and priming treatments for both varieties as presented. The decrease of pigments might be due to the reason that Cr toxicity results in damaging of chloroplast membrane, replacing Mg^{+2} ions and leading to disturbed structure of chlorophyll molecules and also causing reduction of photosynthesis (Singh & Agarwal, 2007). Our results coincide with findings of Ahmad *et al.,* (2011) who reported for rice that Cr reduced the chlorophyll a, chlorophyll b and carotenoids in range from 17-47, 12-43 and 31-50% respectively in comparison to control. Our results are also in accordance to the reported findings that by elevating the concentration of Cr from 0.005 to 0.02M, alleviation in chlorophyll a, b and carotenoids contents occur (Zeid *et al.,* 2013). SA priming was effective enough to tolerate Cr stress by showing high values than stressed plants and SA priming have also shown positive response in increasing chlorophyll contents. Our findings are related to Mousa and El-Gamal, (2010) that SA treatment enhanced photosynthetic activity i.e. presence of high chlorophyll contents in wheat. Moreover, by SA application under salt stress, the total chlorophyll content was elevated significantly in maize (Fahad & Bano, 2012). It can be discussed that SA maintain chloroplast membrane stability and its functionality thus preventing it from harmful effects during stress conditions.

The active uptake of toxic metal ions by plants via different ion channels results in disparity of ion channels. Our findings elaborated that under presence of higher Cr concentrations in growth medium macronutrients i.e. (Na^{+}, K^{+} and Ca^{+2}) in leaves tend to decrease in both B-385 and SB in comparison to control and priming treatments. From our findings it can be assumed that Cr as Cr^{+6} can bind competitively to ion carriers of K^{+}, Ca^{+2} & Na^{+} and thus distorting macronutrient imbalance and their respective ion channel mechanisms. Our results are strongly supported by findings of Zhen *et al.,* (2010) who found that level of macronutrients K^{+}, Ca^{+2} & Na^{+} decrease significantly in rice leaves and stems when Cr stress was applied up to 100µM. Shafaqat *et al.,* (2012) also found that at high Cr level (50µM), K^{+} content decreased but in contrast increase in Ca^{+2} was noted in barley plant. SA priming in both varieties showed more increase in K^{+} and Ca^{+2} ions than Na^{+} content under stress conditions which showed role of SA as a tolerance enhancer entity. Fahad & Bano, (2012) found that application of SA during saline conditions decreased the Na^{+} content; Ca^{+2} remained unchanged whereas K^{+} contents increased in maize. SA treatment might help in stimulating mechanisms involved in regulation of macronutrient ions.

The presence of toxic heavy metals in growth medium of plants leads to their higher uptake and gradual accumulation in different plant parts. From our work it is clear that Cr accumulation in leaves elevates as the stress is increased up to 100 uM concentration in both varieties but Cr concentration was negligible in control and priming treatments. Our results are supported by findings of Zheng *et al.,* (2001) who elaborated that Cr contents were significantly increased by addition of Cr in culture solution. Dubey *et al.,* (2010) also showed that Cr accumulation in roots and shoots increased with increasing concentration of Cr (VI) and similarly to our findings Cr was not detected in any part of the control rice plants. SA treatments also showed little resistance to increasing Cr concentration and Cr contents were lowered less in comparison to respective Cr concentrations in leaves of both varieties. Pertaining to our results, Choudhury & Panda, (2004) reported that SA treated rice plants had reduced accumulation of Cd in roots. It has also been reported that different concentrations of SA reduce accumulation of aluminum in *Cassia tora* (Yang *et al.,* 2013).

Nitrogen is an essential part of all plant biomolecules which gets affected by various stresses. Total nitrogen (N) contents were lowered by Cr stress, both the varieties showed similar pattern of decreasing values. It can be said that interference of Cr with nitrogen results in inhibition of those processes which are involved in assimilation of nitrogen into proteins (Ahmad *et al.,* 2011). Our results correlate with Ahmad *et al.,* (2011) who reported that N contents decreased by 23-82% under increased concentration of Cr (VI) in rice. Our work is also supported by Athar & Ahmad, (2002) who reported obvious lowering in N contents under treatment of heavy metals. SA treatments without stress were less responsive to increase N contents but under Cr stress SA treatments showed better results. Related to our results greater N contents were observed in leaves of cucumber treated with SA under salt stress (Yildirim *et al.,* 2008) and Gunes *et al.,* (2005) reported that SA application showed high uptake of N in maize under salt stress. It can be suggested that under stress conditions SA can induce higher uptakes of N to be used for production of various protective proteins.

Many defense mechanisms are activated during stress conditions in plants in which proline production is essential one as stress indicator. In our findings, when Cr concentration was higher, the leaf proline levels were also higher in comparison to control and priming treatments. It can be argued that proline production increases as stress increases and as a soluble solute it acts as protectant to maintain cell stability (Gajewska *et al.,* 2006). Supporting results were presented by Dubey *et al.,* (2010) who found high levels of proline at 100µM Cr concentrations. But in contrast Zeid *et al.,* (2013) found that proline content was lowered in alfalfa leaves by elevation of Cr concentration. In our findings, plants of SA primed seeds exhibited that leaf proline levels increase to some extent under increasing Cr stress but the proline contents were lowered as compared to plants of SA unprimed seeds. Related to our results, Fahad & Bano, (2012) found in maize that SA treatments under salinity showed significant increase in leaf proline content. The elevation in proline accumulation by SA application under stress has also been showed in seedlings of wheat (Sakhabutdinova *et al.,* 2003). It can be proposed that SA pretreatment activates process of defense mechanisms and make plants to tolerate stresses by modulating levels of osmolytes like proline.

Glycinebetaine (GB) is an important osmolyte which acts as protectant for plants during abiotic stress conditions. In the present study, it was evaluated that GB content did not vary significantly among stress and control treatments in both varieties. Supportively, Dhir *et al.,* (2012) found

that GB levels didn't increase under Cr but it did elevate at other heavy metal stress in spinach. SA treatments were least effective to show any significant variations in GB level. GB plays enzyme protecting role and maintaining osmotic potential (Shirasawa *et al.,* 2006).

Plants respond to abiotic stress by inducing alterations in level of various metabolites like soluble sugars for counteracting stress. In our investigation, total soluble sugar contents of leaves increased with increase in Cr stress in both the varieties. The accumulation of sugars might play vital role in the plant defense mechanisms of osmoregulation and energy preservation (Morsy *et al.,* 2007). Related to our findings, Fahad & Bano, (2012) reported significantly higher soluble sugar contents of leaves in maize plants grown in saline fields. SA seed primed treatments showed that SA ameliorate the effects of Cr by lowering soluble sugar content but in contrast Fahad & Bano, (2012) had reported that SA application further increased soluble sugar contents under salt stress. Gemes *et al.,* (2008) have also accounted that SA application increased the soluble sugar content of tomato plants exposed to salt stress. It can be signified that SA acts as a stress tolerating hormone by regulation of sugar metabolism.

The content of total soluble protein also varies in presence of abiotic factors as it is revealed by our study that total soluble proteins increased under varied Cr concentrations. It can be assumed that Cr toxicity might enhance the proteolytic degradation. Previous works advocated that increasing levels of proteins mediate the plants to sustain their growth even under stress conditions (Agastian *et al.,* 2000; Ali *et al.,* 2012). In the current study for SA treatments gradual decrease in soluble proteins content was observed. Altered protein production occurs in response to abiotic stress and many of these are activated by phytohormones such as salicylic acid (Fahad & Bano, 2012). SA might be able to mediate mechanisms to suppress degradation of proteins.

Conclusion

Salicylic acid seed priming proved helpful in enhancing physiological, biochemical and metabolic parameters of rice under Cr stress and without it. The adverse effects of Cr toxicity can be mitigated by SA. Although the exact molecular mechanisms involving SA application are still to be elaborated but SA might be suggested for farmers to be used for seed priming which is cost effective and eco-friendly and also might improve rice growth even under heavy metal contamination.

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