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CHARACTERIZATION OF JASMONIC ACID-INDUCED PHENOLS IN *VIGNA RADIATA* UNDER SALT STRESS

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Abstract

The present study examined the effect of jasmonic acid (JA) on physio-biochemical attributes, phenylalanine ammonia lyase (PAL) activity, total phenolics, flavonoids, quantitative and qualitative assay of phenolic compounds in 7-day-old *Vigna radiata* sprouts exposed to salt stress (130 mM NaCl). NaCl stress decreases root and shoot length and chlorophyll content as compared to control. However exogenous application of JA was found to ameliorate both root and shoot length and increase chlorophyll content of NaCl fed sprouts. JA enhances the activity of PAL, thus, raising the total levels of phenols and flavonoids. Our study demonstrates that exogenous JA can induce the production of phenolic acids (gallic acid and chlorogenic acid), simple phenols (catechol) and flavonoids (quercetin and flavones). These results support the hypothesis that JA alleviates salt stress by increasing the antioxidant potential of salt-exposed mung bean sprouts, thus, enhancing the health-boosting compounds in food plants.

Key words: Jasmonic acid; NaCl; Phenylalanine ammonia lyase; Phenols; Flavonoids; HPLC.

Introduction

Sprouts are consumed worldwide because of their high nutritional value. Recent research has shown that, in addition to vital nutrients, they contain key phytochemicals that have disease defending and health promoting properties (Pasko *et al*., 2008). Apart from this, sprouts, such as the mung bean also contain specific amounts of phenolics (Kim *et al*., 2012). Mung bean (*Vigna radiata*) sprouts are well-used item in Asian cuisine and are loaded with essential fatty acids, antioxidants, minerals, and proteins. They are also an example of functional foods that lower the risk of various diseases (Anwar *et al*., 2007). More than 100,000 varieties of secondary metabolites have been described in plants (Hadacek, 2002).

Salinity is one of the most detrimental abiotic stresses for plants and is estimated to affect approximately 50% of arable land by the year 2050 (Ashraf & McNeilly, 2004; Blumwald & Grover, 2006). Salinity hinders the ability of plants to absorb water and micronutrients from the soil (Tuna *et al*., 2007; Hashem *et al.*, 2014; Ahmad *et al*., 2015). Simultaneously, it increases the concentration of toxic ions, since Na^+ is not readily sequestered into vacuoles. The result is either nutrient imbalance or deficiency, which ultimately leads to plant death due to growth arrest and molecular damage (Zhu, 2002). In order to survive salinity stress, plants have evolved many mechanisms, such as synthesis and accumulation of compatible solutes (e.g., proline, glycine betaine, and polyols), selective accumulation or exclusion of ions, segregation of ions into separate compartments, induction of antioxidative enzymes and accumulation of secondary metabolites (Parida & Das, 2005; Hashem *et al.*, 2015; Ahmad *et al.*, 2015).Among the secondary metabolites, phenols are powerful antioxidants in plant tissues under stress, having great influence over their oxidative stress tolerance (Sgherri *et al.*, 2004).

Secondary metabolites are known to play a significant role in adaptation of plants to changing environments and in overcoming stress constraints (Edreva *et al.*, 2008). One of the most important groups of metabolites are phenolic compounds, which are mainly synthesized from cinnamic acid, formed by the deamination of phenylalanine (Parr & Bolwell, 2000), with the reaction catalyzed by L-phenylalanine ammonia-lyase (PAL) (EC 4.3.1.5) (Dixon & Paiva, 1995). Phenolic compounds are the most profuse secondary metabolites in plants that possess antioxidant properties and are considered to be more potent than vitamins C and E and carotenoids (Grace & Logan, 2000). Phenolic compounds are usually involved in protection against UV radiation, pathogens, parasites, or predators, and in mechanical support, as well as contributing to the color of plants. They are also beneficial for human health (Boudet, 2007). The biological activity of phenolic compounds, including the antioxidant action of polyphenols, is due to the aromatic ring, which has –OH or OCH_3 substituents. The ability of polyphenols to chelate transition metal ions, directly scavenge molecular species of active oxygen, inhibit lipid peroxidation by trapping the lipid alkoxyl radical, modify lipid packing order, and decrease fluidity of the membranes has made them more efficient than well-known antioxidants (Arora *et al.*, 2000). Since they are ubiquitous in all plant organs, they form an intrinsic part of the human diet. The powerful antioxidant properties of plant polyphenols and their effective role in the prevention of various oxidative stress-associated diseases, such as cancer, have made them a subject of research (Dai & Mumper, 2010). One of the strategies to increase secondary metabolite production is the application of phytohormones, such as jasmonates (JAs).

JAs is a phytohormone thatactually promotes the production of secondary metabolites in various plant species, from angiosperms to gymnosperms (Wasternack, 2007). JAs can elicit the synthesis and accumulation of molecules, such as terpenoids, phenylpropanoids, and anthocyanins (Yan *et al*., 2012). Also, JAs can modulate particular primary metabolic pathways to supply connected secondary metabolite pathways with the necessary substrates (Spitzer-Rimon *et al.,* 2010). Numerous reports show the regulatory role of JAs in plants under various biotic and abiotic stresses, such as UV radiation (Mackerness *et al.,* 1999), low temperature (Wilen *et al.,* 1994), metal stress (Tao *et al.,* 2011), drought stress (Tani *et al.,* 2008), and salt stress (Riemann*et al.,* 2015).This has established JA as a master control for adaptation to biotic and abiotic factors in plants (Wasternack & Hause, 2003). Moreover, many stress-related proteins that are synthesized in response to abiotic stress are induced by JAs (Pauwels *et al.,* 2010). This study analyzed the change in phenol metabolism in NaCl-exposed *Vigna radiata* supplemented with JA.

Materials and Methods

Plant material and experimental design: Mung bean (*Vigna radiata* L. Wilczek, 'SML-668') seeds were procured from the Department of Plant Breeding, Punjab Agriculture University, Ludhiana, India. Viable and certified seeds were surface sterilized in 0.01% $HgCl_2$ for 2 min, followed by washing under free-flowing tap water. Then, pre-soaking treatments were applied to the surface-sterilized seeds for 6 h, using either distilled water or different concentrations (1 µM, 1 nM, or 1 pM) of jasmonic acid (JA). The pre-soaked seeds were then arranged as *viz* (1) Control; (2) 130 mM NaCl; (3) 1 µM JA; (4) 1 µM JA+130 mM NaCl; (5) 1 nM JA; (6) 1 nM JA+130 mM NaCl; (7) 1 pM JA; and (8) 1 pM JA+130 mM NaCl.

Jasmonic acid treated and untreated seeds were then grown in petri dishes lined with Whatman's filter paper within a plant growth chamber under controlled conditions [25°C temperature, photoperiod of 16/8 h dark/light; photosynthetic photon flux densityof 200 µmol (photon) $m^{-2}s^{-1}$,and 80% humidity], and were organized in a randomized block, with three replicates of each. Control plants were supplemented with distilled water only while treatment plants were supplemented with 130 mM NaCl. The sprouts were analyzed after 7 days of treatment.

Growth and biomass yield: The root and shoot length were measured manually using a scale. The dry weight was taken after drying the sprouts in an oven at 70°C for 48 hours.

Estimation of total chlorophyll (Total Chl): Total Chl content in leaves was estimated by following the method of Lichtenthaler (1987). The optical density was taken at 645, 663 nm by spectrophotometer (Beckman 640 D, USA) against 80% acetone used as a blank.

Phenylalanine ammonia lyase (PAL) activity: The PAL (EC 4.3.1.24) activity was estimated using the method given by Sadasivam & Manickam (1992). One gram of plant material was homogenized in 5 ml of 25 mM borate-HCl buffer (pH 8.8) containing 5 mM mercaptoethanol. The supernatant was collected to measure enzyme activity after centrifuging the homogenate at 12,000 g for 30 min at 1°C. Then, 0.5 ml of 0.2 M sodium borate buffer (pH 8.7) was collected, and 0.2 ml of enzyme extract was added. The enzyme activity was initiated by adding 1 ml L-phenylalanine solution. After incubation for 30 min at 32°C, the reaction was stopped by adding 1 ml of trichloro acetic acidand absorbance was measured at 290 nm.

Total phenolic content: Phenolic content was determined following the method iven by Malick & Singh (1980). Fresh plant material (100 mg) was homogenized in ethanol (80%) and centrifuged at 10,000 g for 20 min. After collecting the supernatant, the residue was re-extracted with 80% ethanol, centrifuged, and the supernatants were pooled and evaporated to dry. The remaining residue after drying was dissolved in a known volume (3ml) of distilled water. Taking an aliquot of 0.5 ml in a test tube, the volume was made up to 3 ml with distilled water, and 0.5 ml of Folin-Ciocalteuwas added. After 3 min, 2 ml of 20% Na_2CO_3 solution was added to each test tube and mixed thoroughly. The test tubes were placed in boiling water for 1 min, allowed to cool, and absorbance was measured at 650 nm against a blank. A standard curve was prepared using different concentrations of gallic acid to calculate the amount of total phenols, which were expressed as mg/g fresh weight.

Total flavonoid content: Flavonoid content was determined following the aluminum chloride method suggested by Mervat *et al.* (2009). The supernatant required to quantify total flavonoid content was collected by crushing 500 mg dry plant material in ethanol (80%) and centrifuging at 10,000 rpm for 10 min. A 0.2-ml aliquot of the extract solution was collected in 10-ml test tubes,and the volume was made up to 3 ml with methanol. Then, 0.1 ml $AlCl_3$ (10%), 0.1 ml Na–K tartrate, and 2.8 ml distilled water were addedconsecutively, and the solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 min of incubation at room temperature. A standard calibration plot was generated using known concentrations of quercetin to calculate total flavonoid content and expressed as mg quercetin equivalent/g of sample.

Quantitative and qualitative assay of phenolic compounds

Sample preparation: The method adopted by Tang *et al.* (2014) was followedfor HPLC analysis of phenolic compounds. Fresh plant material (1 g) was extracted by crushing in 10 ml of 75% (v/v) methanol. The homogenate was then ultrasonicated for 30 min, and the extracts were filtered through a 0.45-µm filter and injected (20 µl) into HPLC for analysis.

Chemicals and reagents: HPLC-grade methanol and acetonitrile were procured from Sigma-Aldrich and Ultra-pure water from Merck. The phenolic reference compounds were purchased from Himedia. All the references were 98% pure for HPLC analysis. For the quantitative analysis, a mixed standard solution containing quercetin (20 ppm), coumarin (20 ppm), flavone (20 ppm), gallic acid (20 ppm), chlorogenic acid

(20 ppm), and catechol (20 ppm) was prepared. Each standard was prepared by dissolving 2 mg in 100 ml 75% methanol (v/v). The solutions were stored in dark glass bottles at 4 °C. The working standard solutions were freshly prepared by diluting suitable amounts of the above solutions with 75% (v/v) methanol before injection. The standard solutions were filtered through a 0.45-µm filter and injected (20 µl) into the HPLC for analysis.

Analytical determinations: Samples were analyzed using HPLC on a Waters system, comprising of an HPLC pump, auto-sampler, photodiode array detector, and Nucleosil 100–5 C18 column 5.0 µm (250 × 4.6 nm). Two mobile phases, one containing methanol-0.1% phosphoric acid (20:80) and another containing aetonitrile-0.2% acetic acid (60:40) were conducted. The flow rate was 1 ml/min, and the injection volume was 20 µl. The methanol-0.1% phosphoric acid produced a full separation of gallic acid, chlorogenic acid, and catechol, while the acetonitrile-0.2% acetic acid produced a complete separation of quercetin, coumarin, and flavone. Once the maximum absorption of each standard had been identified, the detection wavelength was set from 220 nm to 370 nm for analysis of mung bean extracts.

Statistical analysis: All of the data were analyzed statistically using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Each treatment was represented by the mean of five replicates (n = 5) and $P \leq 0.05$ were considered as significantly different from the control.

Results

Effect of JA on growth parameters and photosynthetic pigments: The results related to the effect of NaCl and JA on germination and growth of *Vigna radiata*are presented in Table 1. A significant decline in root length by 51.06% was observed in NaCl-stressed plants comparative to control. Supplementation of JA in all the three concentrations enhanced the root length as compared to NaCl treated plants alone, and maximum enhancement of 8.97% was by pico molarconcentration. In NaCl fed plants shoot length decreased by 81.65% over control. However, these plants in supplementation with JA showed an increase in shoot length, maximum of 27.56% by 1 pM JA over NaCl treated plants alone (Table 1). JA treatment alone in 1 pM concentration showed utmost augmentation in root and shoot length as compared to other two concentrations relative to control (Table 1).

The chlorophyll content decreased by 79.14% in salt-stressed sprouts over control. In our study JA alone reduced the chlorophyll content but in combination with NaCl it increased the content and 1 pM JA has increased it by 117.9% over NaCl-treated sprouts (Table 1).

JA enhances activity of PAL in NaCl-treated sprouts: Our results showed that 130 mM NaCl increased PAL activity by 30% over the control plants (Fig. 1A). JA ameliorated PAL activity in nano molar concentration but only by 2%. However, all the concentrations of JA ameliorated PAL activity in combination with NaCl, and the effect was dose-dependent. The most effective combination was found to be 1 pM JA + 130 mM NaCl (Fig. 1A), as the enzymatic activity increased by 46% over the control seedlings followed by 1nM + 130 mM where it was enhanced by 34% over control plants.

JA increases total phenol and flavonoid content under NaCl stress: In the present study, although the total phenolic content of the mung bean sprouts increased after 1 nM JA supplementation alone, there was no significant difference in the total phenolic content between the control and the seeds supplemented with 1 nM JA (9%). However, all of the combined treatments of JA and NaCl enhanced phenol content over control, and the maximum enhancement (126 %) was observed in 1 pM JA + 130 mM NaCl treated sprouts (Fig. 1B) followed by1nM + 130 mM where it was enhanced by 39% over control.

In this study, flavonoid content in 130 mM NaCl treated sprouts was increased by 245% compared to control (Fig. 1C). Conversely, the sprouts raised from JA supplemented seeds showed enhancement over control although they had lower flavonoid content than that of NaCl fed sprouts, and the most effective concentration was found to be 1 nM JA (29%). However, JA in combination with NaCl has shown the positive results, and 1 pM JA + 130 mM NaCl has proven to be the best combination with 289% enhancement over control and 13 % over NaCl fed sprouts (Fig. 1C).

Table 1. Effect of JA(1µM, 1nM and 1pM) and NaCl (130 mM) individually and in combination on germination, root length, shoot length and total chlorophyll in *Vigna radiata* sprouts.

Treatments	Germination	Root Length (cm)	Shoot Length (cm)	Total Chl ($mgg^{-1}FW$)
Control	99 ± 3.73a	6.11 ±0.56b	12.32 ± 0.92a	1.87 ± 0.11a
1 µM JA	95 ± 3.65b	6.58 ± 0.67c	10.60 ± 0.85c	1.42 ± 0.06b
1 n M JA	98 ± 3.70a	5.90 ± 0.50d	11.31 ± 0.89b	1.55 ± 0.07b
1 p M JA	99 ±3.72a	6.73 ±0.75a	12.36 ± 0.93a	1.28 ± 0.04c
NaCl	85 ± 2.88d	3.12 ± 0.18f	2.26 ± 0.26e	0.39 ± 0.003g
1 µM JA + NaCl	86 ± 2.88d	3.17 ± 0.20g	2.87 ± 0.27e	0.44 ±0.004f
1 n M JA + NaCl	88 ± 2.91c	3.23 ± 0.22g	2.90 ± 0.28e	0.47 ±0.005e
1 p M JA + NaCl	93 ± 3.63b	3.40 ± 0.39e	3.13 ± 0.35d	0.85 ±0.008d

Data presented are the means± SE (n= 5). Different letters next to the number designate significant difference ($p<0.05$)

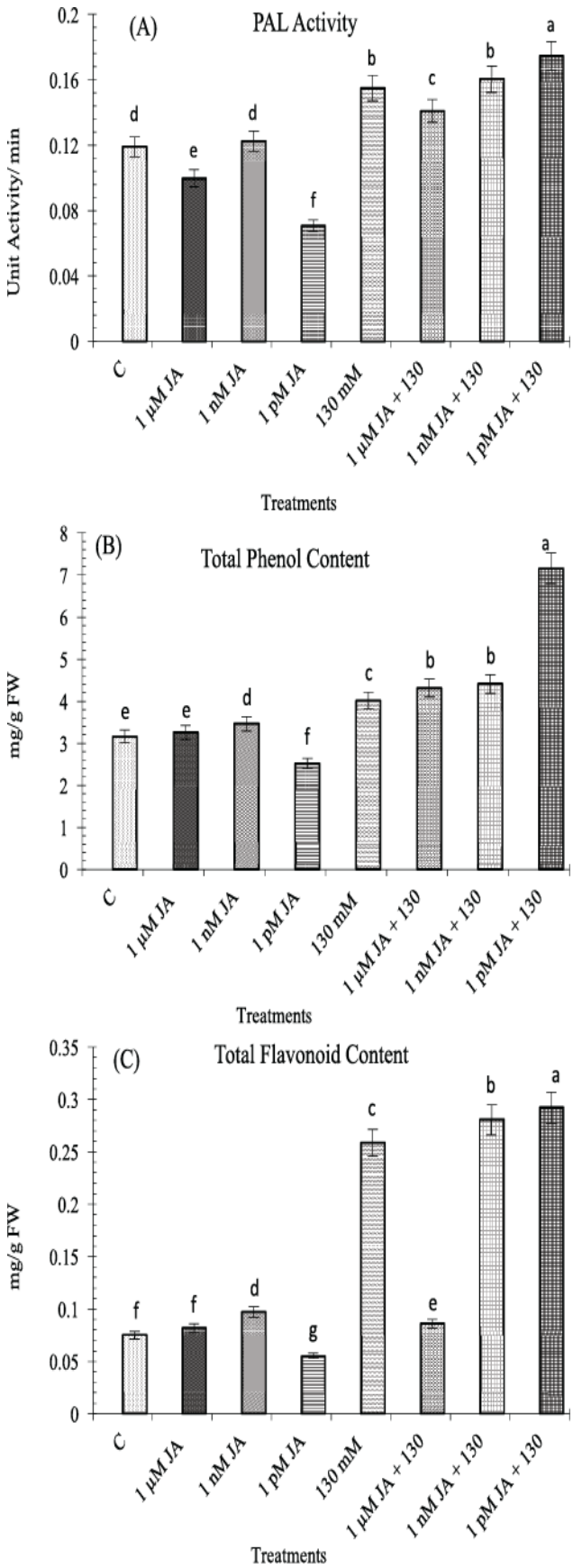

Fig. 1. Effect of Jasmonic acid on (A) PAL activity (B) total phenol and (C) total flavonoid contentof *Vigna radiata* under Salt (130 mM NaCl) stress. Values are means ± SE (n= 5). Different letters indicate significant difference between means at p≤0.05 (DMRT).

JA induces production of phenolic compound under salt stress: In this study, we used HPLC technique to examine the production and enhancement of phenolic compounds in JA primed and non-primed seeds of *Vigna radiata* under salt (NaCl) stress. Six phenolic reference compounds (viz, gallic acid, chlorogenic acid, catechol, quercetin, flavone, and coumarin) were examined (Fig. 2 A–B). Of these, 1 pM JA primed seeds supplemented with 130 mM NaCl showed the highest number of phenolic compounds, followed by 1 nM JA primed seeds supplemented with 130 mM NaCl (Table 2). Among the phenolic acids, gallic acid was found to be the most common phenolic acid in *Vigna radiata* sprouts. The maximum content (69%) was found in 1 µM primed seeds, which further increased by 94% over the control when supplemented with 130 mM NaCl (Table 2). Conversely, 1 pM JA priming decreased gallic acid content by 21% over the control (Table 2). In contrast, chlorogenic acid was induced in 1 nM JA primed seeds, which increased multiple times when supplemented with 130 mM NaCl (Table 2). Catechol, which is a simple phenol has been induced in only 1 pM primed seeds supplemented with 130 mM NaCl. For flavonoids, quercetin was dominant in all of the treatments, except the control and 1 nM primed seeds, followed by flavones. However, quercetin and flavone content was lower than that of 130 mM supplemented sprouts (Table 2). The production of coumarin, which is a phenylpropanoid, considerably increased by NaCl stress over the control and 1 pM primed seeds supplemented with 130 mM NaCl, and showed the maximum content (53.09%).

Discussion

The present study has shown a decrease in percentage germination and growth by NaCl toxicity (Table 1) and the results substantiate with the results of Khan & Weber, (2008) and Kaveh *et al.* (2011) in *Solanum lycopersicum* species. The reason being that NaCl lowers the osmotic potential of germination media which ultimately alters the water imbibition by seeds. Salt fed sprouts showed poor root growth due to water deficit thus reducing their overall growth caused by the turning down the cellular expansion (Kramer 1974). Our results are in conformity with Farsiani & Ghobadi, (2009) and Khodarahmpour *et al.* (2012) in *Zea mays.* Both root and shoot length of the plant have been improved by JA supplementation, and it may be due to the reduction in NaCl accumulation by the roots. Modulation of root hydraulic conductivity by JA is the main cause of water uptake from soil and has been reported by Sánchez-Romera *et al.* (2014). Exogenous supplementation of JA enhances endogenous JA which may help the plant to withstand the salt stress (Kang *et al.* 2005). Fedina & Tsonev (1997) reported that JA pre-treatment to *Pisum sativum* declines the accumulation of Na^+ ions in shoot. Application of JA have been reported to enhance many physiological properties like, leaf water potential, maximum quantum yield of PSII and uptake of mineral nutrients in rice plants (Kang *et al.* 2005).

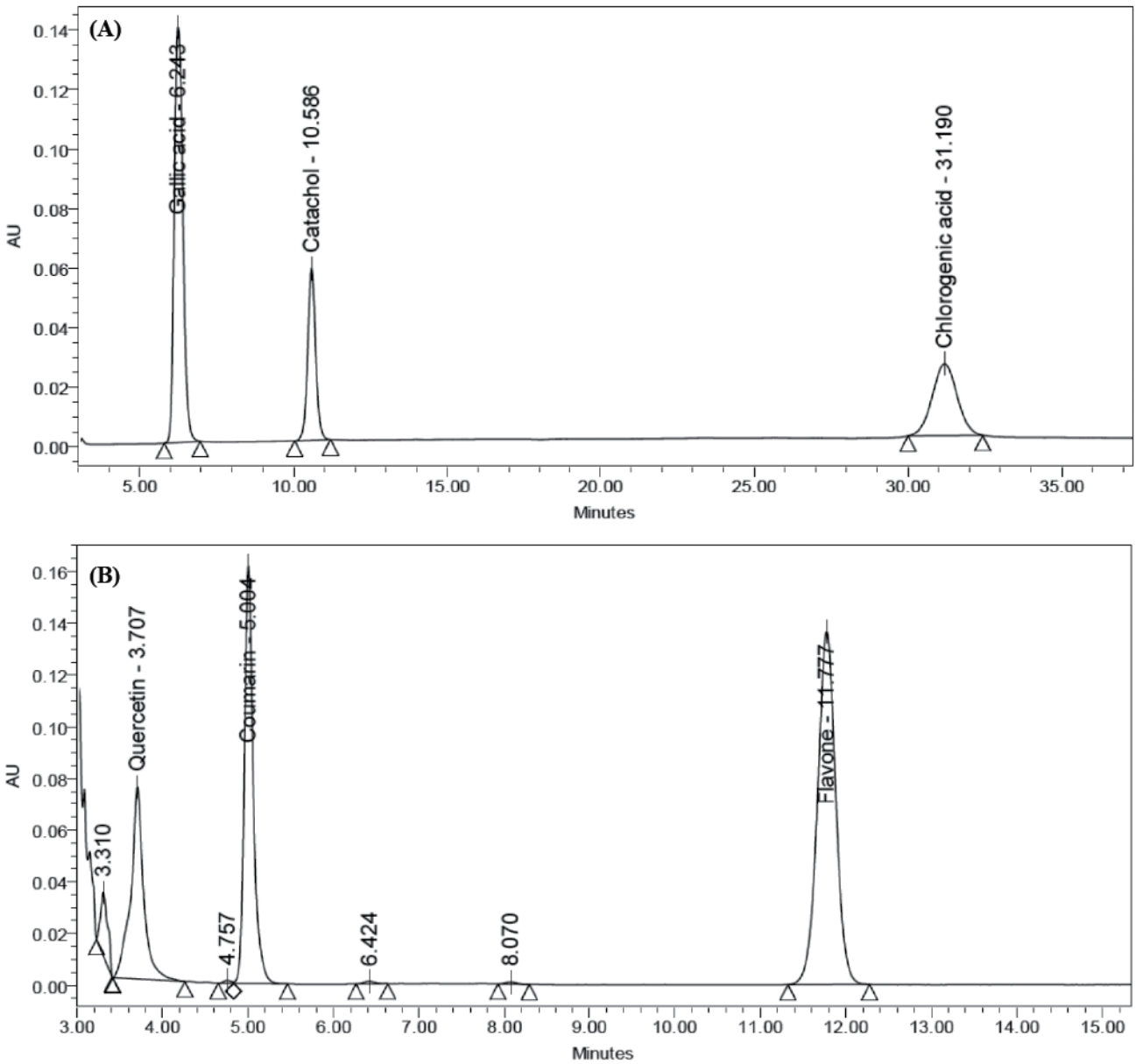

Fig. 2. HPLC chromatogram of standards using two mobile phases: methanol-0.1% phosphoric acid (20:80), giving the complete separation of gallic acid, catechol, chlorogenic acid (A) and acetonitrile-0.2% acetic acid (60:40) separating quercetin, coumarin and flavones (B).

Table 2. Effect of JA (1µM, 1nM and 1pM) and NaCl (130 mM) individually and in combination on different phenolic compounds in *Vigna radiata* sprouts.

Treatment	Gallic acid (µg g^{-1}FW)	Catechol (µg g^{-1}FW)	Chlorogenic acid (µg g^{-1}FW)	Quercetin (µg g^{-1}FW)	Coumarin (µg g^{-1}FW)	Flavone (µg g^{-1}FW)
CN	0.86 ± 0.12f	ND	ND	ND	41.07 ± 2.15d	ND
1 µM JA	1.45 ± 0.26c	ND	ND	2.45 ± 0.28f	59.04 ± 2.82b	0.10 ± 0.003a
1 nM JA	1.40 ± 00.23c	ND	0.52 ± 0.05b	ND	47.86 ± 2.37c	ND
1 pM JA	0.68 ± 0.08g	ND	ND	3.06 ± 0.32e	42.99 ± 2.19d	ND
130 mM	1.08 ± 0.15e	ND	ND	5.10 ± 0.61a	62.11 ± 2.95a	0.11 ± 0.003a
1 µM+130	1.67 ± 0.39a	ND	ND	3.58 ± 0.44d	52.40 ± 2.48c	0.05 ± 0.0003c
1 nM +130	1.56 ± 0.31b	ND	5.56 ± 0.06a	4.24 ± 0.50b	61.37 ± 2.89b	ND
1 pM+130	1.38 ± 0.24d	0.66 ± 0.07a	ND	3.87 ± 0.45c	62.88 ± 2.99a	0.09 ± 0.0006b

ND- not detected

Data presented here are the means± SE (n= 5). Different letters next to the number specify significant difference ($p<0.05$)

NaCl toxicity has affected the pigment system severely in the present study (Table 1). Our results are supported by Kumari *et al*. (2006) in *Arachis hypogaea* seedlings, Amirjani (2011) in *Oryza sativa* and Saha *et al.* (2010) in *Vigna radiata*. Reduction in pigments due to NaCl toxicity may be due to activation and/or synthesis of enzymes involved in the degradation of photosynthetic pigments (Ahmad *et al.,* 2015, 2016). However, JA application has improved photosynthetic and growth parameters in the present study (Table 1). Similar finding has been reported in *Capsicum frutescens* (Yan *et al*., 2013) and *Kandelia obovata* under cadmium stress (Chen *et al*., 2014) by the supplementation of JA. Enhanced chlorophyll content and better growth by JA supplementation might be due to: (i) JA upregulates the expression of genes involved in chlorophyll synthesis. (ii) Prevents NaCl uptake by roots and (iii) enhances cellular expansion.

PAL is a crucial enzyme of phenylpropanoid metabolism involved in the defense response of plant cells and catalyzes the deamination of L-phenylalanine to generate trans-cinnamic acid and ammonia (Koukol & Conn, 1961). NaCl toxicity enhances the activity of PAL in the present study (Fig. 1A) and the results are in close conformity with Baque *et al.* (2010) in *Morinda citrifolia*, where PAL activity increased with an increase in NaCl supplementation. JA further increases the activity of PAL and an augmentation in PAL activity is key to the production of many defense compounds (Dixon & Paiva, 1995). Our results are similar to Campos-Vargas & Saltveit, (2002) where PAL activity was increased in lettuce after applying Methyl Jasmonate (MeJ). JAs are known to trigger plant defense responses, together with the induction of PAL (Gundlach *et al.*, 1992; Gadzovska *et al.*, 2007).

NaCl stress increases phenol content in the present study (Fig. 1B) and a similar response of enhanced total phenolic content has also been observed in *Capsicum annuum* and *Cakile maritima* (Navarro *et al.*, 2006; Ksouri*et al.*, 2007). Phenolic compounds have been known to play a vital role by acting as antioxidants, thus, defending reactive oxygen species produced during the mutilation of aerobic or photosynthetic metabolism by various environmental stresses (Sreenivasulu *et al.*, 2000). They achieve this by adsorbing and deactivating free radicals, satiating singlet oxygen, or decomposing peroxides. Polyphenol production and accumulation in plants are usually stimulated in response to salinity (Navarro *et al.*, 2006). Total phenolic compounds increase with JA treatments under salt supplementation (Fig. 1B). Our results are consistent with the studies of Saniewski *et al.* (1987) and Campos-Vargas & Saltveit (2002), who reported a positive correlation between the synthesis of phenols and MeJbeing applied exogenously. These results indicate that JA supplementation might have considerably enhanced salinity stress tolerance of mung bean sprouts by enhancing the total phenol content, thereby improving their nutritional value. This could be a positive way to intensify health-promoting compounds in food plants.

Flavonoids are potent non-enzymatic antioxidants and increases under salt stress (Fig. 1C). Similar results have been found in indica rice varieties under salinity stress (Chutipaijit *et al.*, 2009). The recurrent induction of flavonoids, which enhance plant protection, is due to abiotic stresses (Grace & Logan, 2000). Their accumulation protects plants from the detrimental effects of reactive oxygen species by acting as free radical scavengers via the hydroxyl group. JA enhances the production of flavonoids in the presentstudy, and the results concur with those shown for *Arabidopsis thaliana* and *Vitis vinifera***,** where externally applied JA increased flavonoid content (Hendrawati *et al.*, 2006; Belhadj *et al.*, 2008). Therefore, we conclude that JA priming promoted the salt tolerance of *Vigna radiata* sprouts by raising the total flavonoid content, which is in general viewed as an indication of an increase in the antioxidant potential. Thisis supported by the fact that flavonoid metabolism appears exceedingly flexible, able to adjust rapidly to environmental changes (Yoshitama, 2000).

JA induces and enhances the production of phenolic compounds (chlorogenic acid, catechol, gallic acid and coumarin) in NaCl-treated sprouts (Table 2). Our results are analogous to Keinanen *et al.* (2001) who conducted rapid HPLC screening of alkaloids, phenolics, and diterpene glycosides using exogenous treatment of JAs in *Nicotiana attenuata* and found that chlorogenic acid isomers increased in JAs treated leaves. Nieves *et al.* (2001) showed that high levels of 4-methylcatechol were induced by JA in sugarcane. Gadzovska *et al*. (2007) showed the production of phenylpropanoids in *Hypericum perforatum* L. cell suspension increased after JA supplementation. Therefore, JA priming during salt stress could be considered as a source of rapid induction, and a means to increase production of phenolic compounds. These results indicate that JA could increase the antioxidant potential of NaCl supplemented mung bean sprouts, thus improving the health virtues of *Vigna radiata* sprouts economically.

Conclusions

JA application was used to evaluate the tolerance mechanism against salinity stress in *Vigna radiata*. JA priming with 1 pM+130 mMNaCl was most effective treatment in combination and showed the presence of five of six reference phenolic compounds. In addition, this concentration was found to contain the highest content of total phenols and flavonoids. This was followed by 1 nM primed sprouts supplemented with 130 mM NaCl, showing the presence of four reference phenolic compounds. Our study supports the hypothesis that NaCl (130 mM) supplemented sprouts no longer remain under stress when supplemented with JA and become a source of dietary antioxidants, thus, providing protection against chronic diseases. These results indicate that JA is potent enough to amelioratethe detrimental effect on plants in an economical way.

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