Journal of Stress Physiology & Biochemistry, Vol. 9 No. 4 2013, pp. 53-64 ISSN 1997-0838

ORIGINAL ARTICLE

Sugar Accumulation and its Regulation by Jasmonic Acid in *Brassica napus* L. under Salt Stress

Harpreet Kaur*, Poonam Sharma and Geetika Sirhindi

Departmant of Botany, Punjabi university, Patiala,147002, Punjab, India

*E-Mail: ***sekhon.harpreet123@gmail.com**

Received May 24, 2013

The effects of Jasmonic acid (JA) on sugar accumulation and protein content in 12-days old *Brassica napus* L. seedlings were studied. The seeds pre-treated with different concentrations of jasmonic acid were germinated in Petriplates supplied with various concentrations of salt stress (NaCl). The contents of total chlorophyll, total sugars, reducing sugars, non-reducing sugars, total carbohydrates and total protein content of 12-days old seedlings were analysed. It was observed that JA reduced the toxicity of salt stress on seedling growth by influencing the chlorophyll content and protein content. Pre-treatment of jasmonic acid was found to be significantly effective in increasing the contents of sugars.

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Jasmonic acid is a member of plant growth regulators named jasmonates which are important cellular regulators involved in several developmental processes such as seed germination, root growth, fertility, fruit ripening and senescence. Most of the plant parts contain jasmonates and the highest concentration appears to be present in reproductive tissues whereas much lower levels are found in roots and mature leaves (Lopez *et al*., 1987, Creelman and Mullet, 1995). But these consequences are based mainly on the studies done on excised or intact differentiated leaves after exogenous application of jasmonates (Weidhase *et al*., 1987). Till now it is considered that jasmonates particularly methyl esters of JA (Me-JA) as a chemical stress agent mimicking the effect of that appear in response to external stress factors inducing senescence (Wasternack and Hause, 2002). Me-JA preferentially inhibited chlorophyll accumulation at the level of chlorophyll precursors in the dark as compared to other inhibitory growth regulators as chemicals (Ananiev *et al*., 2004) under different environmental conditions.

Salt stress is one of the common stress, accumulation of salt stress in plants when passes the threshold level, resulted to toxicity in plants lead to many morphological and physiological changes (Dhankar *et al*., 2011). Sodium chloride is a

salt which is essential for structural and functional parts of vital machinery of plant cell. The requirement of NaCl for the plant is very low for normal growth and development. Unfortunately, plants find an ample supply of sodium chloride through their roots from soil and accumulated in system causing stress (Nicholls *et al*., 2011) along with triggering of certain physiological responses (Agarwal and Sharma, 2006).

Brassica napus L. is an important oil crop of the world, which is rich in protein. Canola is major oil yielding crop in India (Kumar *et al*., 2009) and has great area under cultivation, but still the productivity is at very low level and number of endogenous and exogenous factors is responsible for this low yield. Soil pollution and that too salt stress is one of the major factors responsible for this low yield. In present study we analysed the effect of Jasmonic acid and NaCl stress on sugar accumulation and protein content in seedlings of *B. napus* growing under NaCl treatment after priming the seeds with micro, nano and pico-molar concentration of JA.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of *Brassica napus* L. cultivar (GSC-6) were procured from Department of Plant Breeding, Punjab Agriculture University, Ludhiana, India. Seeds were surface sterilized with 0.5% sodium hypochlorite for 15 min, followed by repeated rinses in sterile distilled water. The surface sterilized seeds were then germinated on Whatman No. 1 filter paper lined autoclaved glass Petri dishes containing different concentrations of NaCl (0, 80, 100, 120mM) and JA (10^{-6}, 10^{-9}, 10^{-12}M) alone or in combination. The experiment was conducted under controlled conditions (25±2ºC, 16 h photoperiod) and repeated twice with three replications for each treatment.

Growth analysis

Twelve days old seedlings were harvested and their root and shoot length were recorded. Percentage germination was recorded at 3 DAS. Twenty seedlings per Petri dish were used for the determination of morphological parameters.

Biochemical analysis

Chlorophyll estimation

The total Chlorophyll was extracted according to Arnon, 1949. Brassica seedlings (0.5g) were ground using mortar and pestle in 5ml of 80% acetone, and then the developed colour was measured at 645 and 663nm. The amount of pigments was calculated by using Lichtenthaler et al., 1982.

Protein estimation

Protein content was prepared by homogenizing 1gm fresh plant material in 3ml of 100mM phosphate buffer (pH 7.0) by following the method Lowry *et al*., 1951 using bovine serum albumin as a standard.

Sugars estimation

Total sugars content was estimated by following Loewus, 1952. Known weight of dried plant material was homogenised in 80% of ethanol then centrifuged at 3000x g for 15 minutes and the extract was collected for sugars estimation. For total sugars 0.05ml of extract was diluted to 2ml by distilled water and adds 3ml cold anthrone reagent was added into it and mixed thoroughly. Then mixture was heated for 10 min in boiling water bath and cooled rapidly at room temperature. O.D. was recorded at 630 nm. Amount of total sugars was calculated and expressed as mg/g DW tissue.

Reducing sugars content was estimated by following Miller, 1972 0.05ml of extract was diluted with distilled water to make final volume and add 3ml DNSA reagent, then boiled in water bath for 10 min, add 1ml of 40% Rochelle reagent, cooled the reaction mixture. Absorbance was measured at 620nm and expressed as mg/g DW tissue.

Amount of non-reducing sugars were calculated by subtracting the reducing sugars content from the total ones and expressed as mg/g DW tissue.

Carbohydrate estimation

Total carbohydrates content was determined according to Dubois *et al*., 1956. 0.05ml of extract was diluted to 2ml by distilled water and 0.05 ml of phenol reagent was added to it and mixed thoroughly. Then 5ml of H_2SO_4 was added rapidly. Blank was prepared by taking distilled water instead of extract. The samples were allowed to stand at room temperature for 30 minutes and optical density was taken at 485 nm. Standard curve was prepared by using glucose (20-100 mg). The amount of carbohydrates was calculated and expressed as mg/g dry weight.

SDS-PAGE protein analysis

Protein electrophoresis, SDS-polyacrylamide gel electrophoresis was performed using 10% acryl amide slab gel following Laemmli (1970). Gels were photographed, scanned and analyzed using GelDoc 2000 Bio Rad system.

RESULTS

Morphological parameters

We investigated the role of jasmonic acid on the growth of *Brassica napus* L. seedlings. It has been observed that jasmonic acid treated seeds resulted in decreased percentage germination, root length and shoot length of *Brassica napus* as compared to control (Table 1). Maximum decrease in germination rate 63% was observed in seedlings treated with 120 mM salt concentrations. Interestingly seeds grown in presence of 100mM salt after treated with different concentrations of JA shown 2% increase in germination as compared to control distilled water seeds.

Seedling growth in terms of root and shoot length also showed synergistic mechanism of negative effect on growth particularly on root length. Shoot length also affected negatively in presence of JA alone. But on supplementation with different concentrations of NaCl solutions resulted in increased seedling growth. Maximum shoot length 11% and root length 66% was found to increase in 10^{-9}M+100 mM combination. Overall JA showed stronger inhibitory effect on seedling growth.

Biochemical parameters

Total chlorophyll content was decreased with increase in concentrations of NaCl as compared to control (Figure 1). It was 15% lowest as compared to control. The content was further enhanced by applications of different concentrations of JA under salt stress. Maximum total chlorophyll content was observed in case of seedlings treated with 100 mM of NaCl solution supplemented with 10^{-9}M JA and this increase is 43% as compared to control.

Total protein content was decreased with increased concentrations NaCl stress (Figure 2). Minimum protein content was observed in case of seedlings treated with 120 mM NaCl 75% as compared to control. Seedlings treated with JA alone showed significant increase in total protein content (Figure 2) in comparison to untreated seedlings. The protein content was significantly higher in the seedlings treated NaCl supplemented with JA than NaCl alone.

Total soluble sugars content was decreased with increased concentrations of NaCl as compared to control (Figure 3). It was 43% highest as compared to control. The content was further enhanced by applications of different concentrations of JA under salt stress. Maximum total soluble sugars content was observed in case of seedlings treated with 80 mM of NaCl solution supplemented with 10^{-12}M JA and this increase is 62% as compared to control.

The observations on reducing sugars revealed that the content of reducing sugars decreased with increased NaCl concentrations (Figure 4). Maximum reducing sugars content (Figure 4) 24% was observed in case of 100 mM of NaCl as compared to that of control. The content of reducing sugars was further enhanced by applications of different concentrations of JA under salt stress and maximum reducing sugars content 50% was observed in seedlings treated with 80 mM of NaCl solution supplemented with 10^{-9}M JA.

A similar trend was observed when effect of JA was studied on the contents of non reducing sugars under NaCl stress (Figure 5). Non reducing sugars content (Figure 5) was 64 % highest at 100 mM NaCl. Non reducing sugars content was 92% maximum in seedlings treated with 10^{-12}M JA supplemented with 100 mM of NaCl.

Similarly, total carbohydrates content (Figure 6) decreased significantly under 120 mM of sodium chloride 41% as compared to control. JA alone was not able to alleviate the decreased contents of JA but on supplementation with NaCl solutions resulted in increased levels of carbohydrates contents (Figure 6). Total carbohydrate content was reported to maximum in case of seedlings treated with 10^{-9}M JA supplemented with 80 mM sodium chloride 59%.

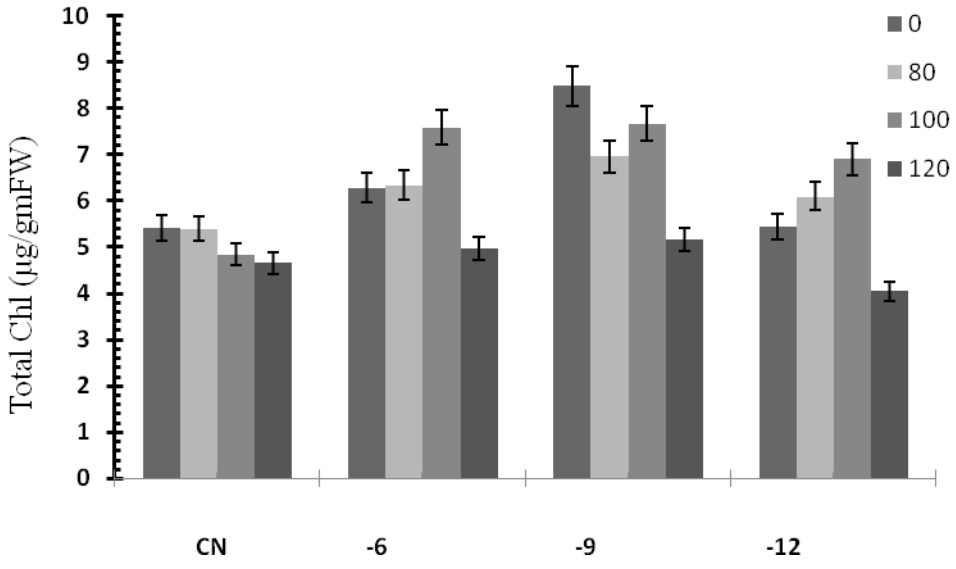

Figure 1. Effect of JA on total chlorophyll content on 12-days old seedlings of *B. napus* L. plants under NaCl stress [Bars represent the SE (n=3)]

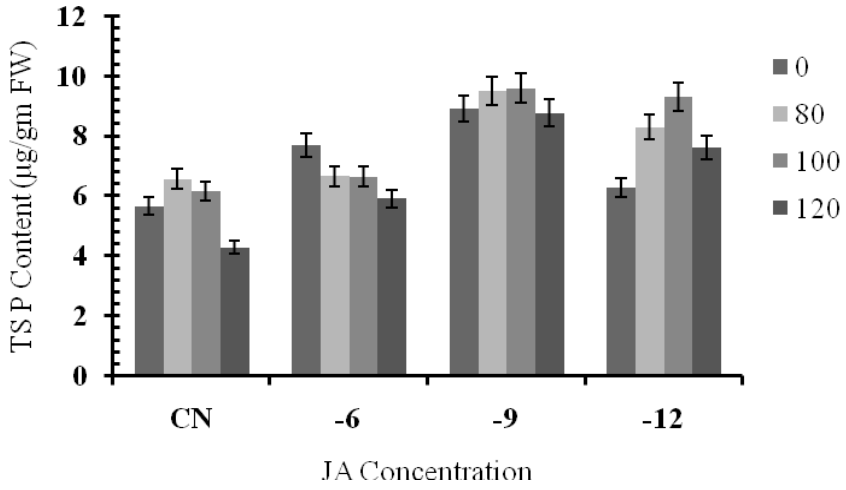

Figure 2. Effect of JA on total soluble proteins content on 12-days old seedlings of *B. napus* L. plants under NaCl stress [Bars represent the SE (n=3)]

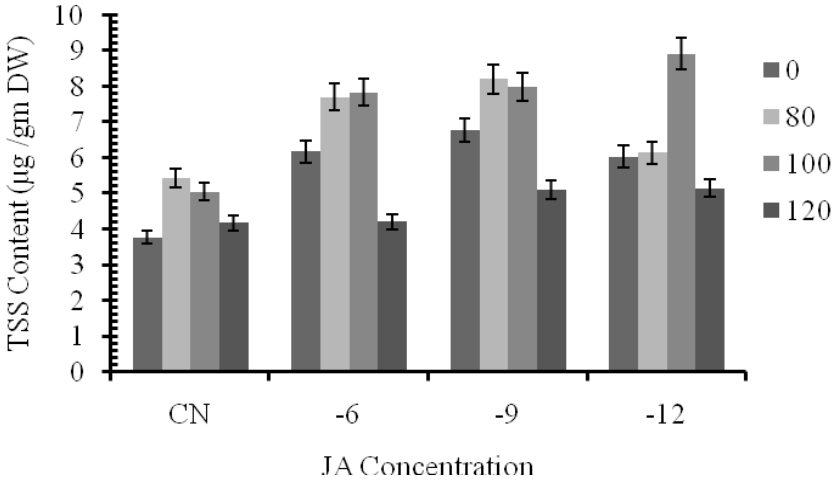

Figure 3. Effect of JA on total soluble sugars content on 12-days old seedlings of *B. napus* L. plants under NaCl stress [Bars represent the SE (n=3)]

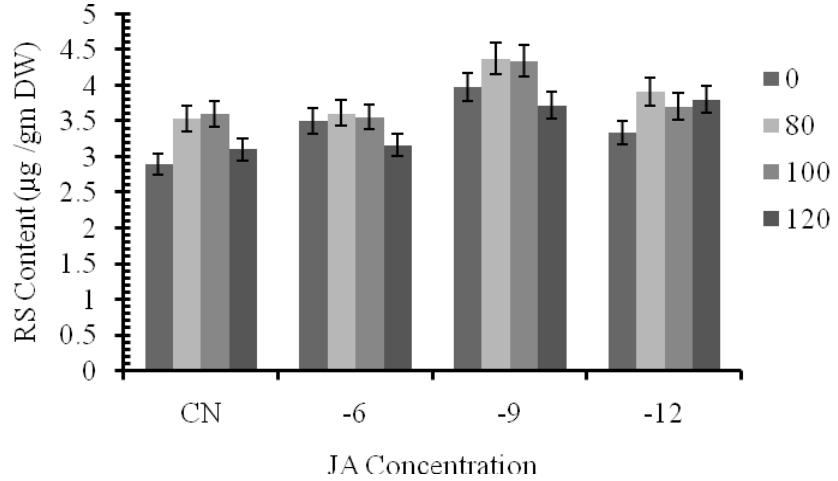

Figure 4. Effect of JA on reducing sugars content on 12-days old seedlings of *B. napus* L. plants under NaCl stress [Bars represent the SE (n=3)]

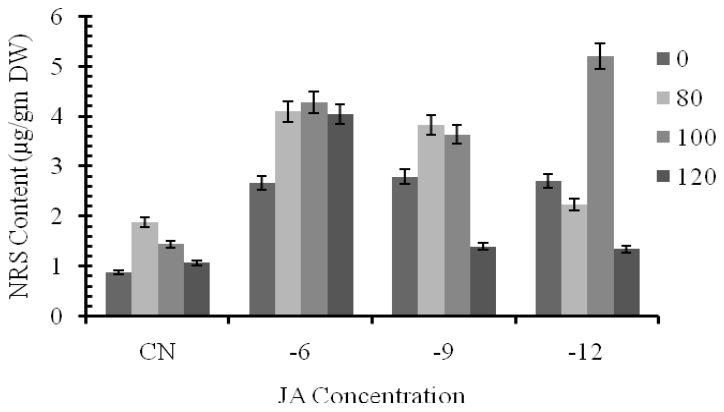

Figure 5. Effect of JA on non reducing sugars content on 12-days old seedlings of *B. napus* L. plants under NaCl stress [Bars represent the SE (n=3)]

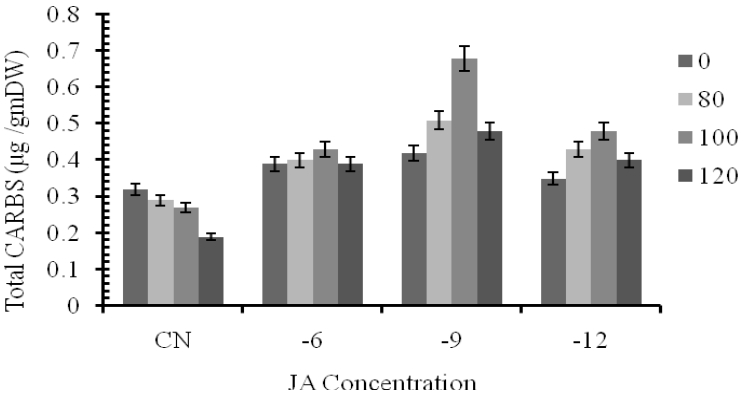

Figure 6. Effect of JA on total carbohydrates content on 12-days old seedlings of *B. napus* L. plants under NaCl stress [Bars represent the SE (n=3)]

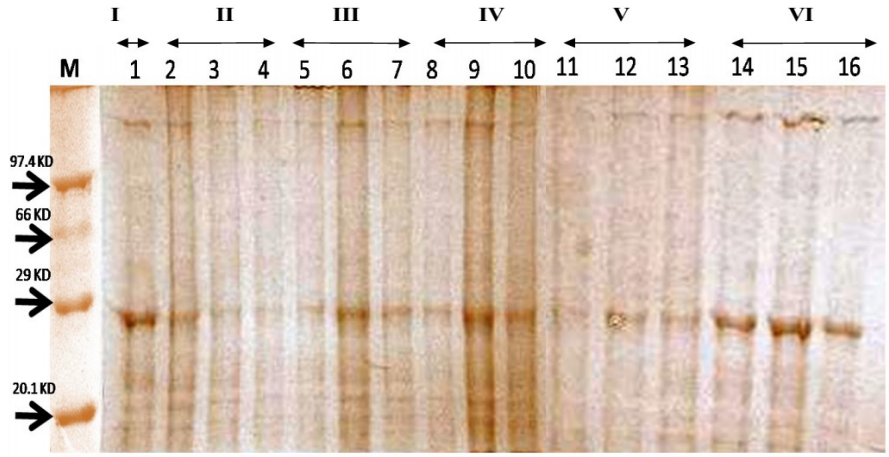

Figure 7 Protein Profiling on the basis of Molecular Weight in *Brassica napus* L. (cv. GSC-6) seedlings raised from seed primed with different concentrations of Jasmonic acid (JA) under different salt (NaCl) concentrations.

M	Marker (Mol. Wt.)			
I	Control	1. Distilled Water		
II	Salt Treatment	2. 80 mM	3.100mM	4. 120 mM
III	JA Treatment	5. 10^{-6} M	6. 10^{-9} M	7. 10^{-9} M
IV	10^{-6} JA + NaCl	8. 80 mM	9. 100 mM	10. 120mM
V	10^{-9} JA + NaCl	11. 80 mM	12. 100 mM	13. 120 mM
VI	10^{-12} JA + NaCl	14. 80 mM	15. 100 mM	16. 120 mM

Table 1. Influence of pre-treatment of jasmonic acid on germination, shoot length and root length under NaCl stress.

Treatments	% Germination	Shoot length (cm)	Root length (cm)
Control	48±0.33	4.97±0.02	6.16±0.04
10^{-6}M	32±0.33	4.28±0.06	4.27±0.03
10^{-9}M	36±0.57	4.57±0.17	4.90±0.05
10^{-12}M	30±1.20	4.58±0.47	4.01±0.04
80 mM	28±1.15	4.81±0.05	3.16±0.02
100 mM	24±0.88	4.03±0.03	4.16±0.07
120 mM	18±1.45	3.06±0.08	2.60±0.02
10^{-6}+80mM	38±0.88	4.97±0.01	3.27±0.03
10^{-6}+100mM	46±2.02	5.05±0.08	3.88±0.05
10^{-6}+120mM	30±1.52	4.86±0.03	2.49±0.01
10^{-9}+80mM	46±2.64	5.99±0.08	3.60±0.01
10^{-9}+100mM	49±2.33	5.55±0.03	4.11±0.02
10^{-9}+120mM	38±1.76	4.01±0.11	2.95±0.03
10^{-12}+80mM	43±1.15	4.97±0.12	3.40±0.04
10^{-12}+100mM	45±1.45	5.00±0.09	3.91±0.01
10^{-12}+120mM	41±1.11	4.61±0.06	3.05±0.02

Values are the mean of three replicates measurements

SDS-PAGE protein analysis

Five bands were scored in the protein profile of *Brassica napus* L. seedlings (Figure 7). Band having about 29 KDa molecular weight showed variation in thickness. Salt stress 120 mM NaCl concentration reduced maximum thickness of band (Lane 4). Regarding JA treatments maximum thickness was observed in 10^{-9} M JA treated seedlings (Lane 6). In combined treatment of JA + NaCl, it was found that 10^{-12} JA + 100 mM NaCl treatment showed maximum band thickness (Lane 15). However combined treatment of 10^{-12}M JA with different concentration of NaCl showed increased band thickness in comparison to control seedlings.

DISCUSSION

Salinity stress accounted as a major stress due to interference with metabolic processes of plants such as nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity (Hasegawa *et al*., 2000, Munns 2002, Zhu 2007).

In present study decrease in seed germination, root and shoot length (Table 1) was observed under salinity stress. However, supplementation of JA improved the seed germination and seedling growth. Overall JA showed stronger inhibitory effect on seedling growth in absence of NaCl.

Earlier studies revealed that JA treatments enhanced the protein concentration of peanut seedlings (Kumari *et al*., 2006). JA application may increase cell division and alters the membrane permeability. It is proposed that JA induced changes are mediated through jasmonate induced stress proteins (Rakwl and Komatsu, 2001). In Soybean JA are reported to increase protein content (Anderson, 1991). Similarly, in present study, protein content was found to be significantly increased with JA treatments under salt stressed seedlings (Figure 2).

Preceding reports have shown that exogenous application of Me-JA in excised cotyledons of *Cucurbita pepo* preferentially inhibited accumulation of chlorophyll and when applied in a mixture of cytokinin and Me-JA, neutalization of stimulatory effect of cytokinin on chlorophyll

accumulation (Ananieva *et al*., 2004). The positive effect of JA was enhanced in presence of NaCl in our results (Figure 1). (Sorial and Gendy, 2010) also observed the positive influence of JA in chlorophyll content under NaCl stress. The stronger overall effect of JA on photosynthetic pigment accumulation could be due to its stronger effect on the chlorophyll synthesis pathway specially δ-ALA (aminolevulinic acid) which is the rate limiting step in the biosynthesis of chlorophyll during earliest stage of greening (Beale, 1978). (Kovac and Ravnikar, 1994) demonstrated that JA treatment resulted in an increase of active cytokinin concentration which enhances chlorophyll accumulation in potato plant. Cytokinin treatment enhanced the amount of δ-ALA accumulation in *Cucurbita pepo* (Fletcher and McCullagh, 1971). Cytokinin is the phytohormone promoting light harvesting capacity of the plant, thus exerting a co-operative effect on the process of greening (Reiss and Beale 1995).

Involvement of soluble sugars in osmotic adjustment has been proposed by Mansour (2000) in alleviating the adverse effect of salt stress. In current study, in presence of JA the total soluble sugars, reducing and non reducing sugars content (Figure 3,4 and 5) was increased under NaCl stress and application of exogenous JA has been further reported to increase sugars content. The increase in sugar concentration may be a result from the degradation of starch (Fisher and Holl, 1991).

We also reported that JA treatments enhanced the accumulation of carbohydrates in presence of salt stress. Similar responses were reported in sugar beet, pea and black cumin (El Khallal, 2001, Cherki *et al*., 2002, Murakeozy, 2003). Hajar *et al*., 1996) suggested that carbohydrate accumulation in *Nigella saliva* may increase the ability for water absorption under salt stress, similar carbohydrate accumulation was observed in our study (Figure 6).

In our results difference in the expression of 29 KDa molecular weight band was observed under salt stress. Similar changes were observed by Gomathi *et al*., 2013 in different genotypes of sugarcane under salt stress. He affirmed new salt shock proteins under salt stress. (Khalifa, 2012) also observed four different protein bands in tomato cultivars involved in salt tolerance. In our results changes in the thickness of protein bands in presence of JA alone as well as in combination with NaCl observed. Changes in protein band under salt stress as well as in JA are mainly due to the formation of salt shock proteins in the *Brassica napus* L. seedlings (Figure 7). (Hashimoto *et al*., 2004) confirmed the presence of new proteins, which shows similarity with Rice PR10 proteins, osPR10a/PBZ1 and osPR10b. which rapidly accumulated under different types of abiotic stresses. JA also shows a positive role in the formation of new salt shock protein.

CONCLUSIONS

The influence of JA on photosynthetic pigments, sugars content and seedling growth was more prominent under NaCl stress, suggesting that JA treated seedlings was less affected by NaCl than the untreated seedlings. Also, JA induced elevated levels of photosynthetic pigments and sugars, which increased the tolerance of *Brassica napus* seedlings to NaCl stress. However, the molecular mechanism involved in function of stress protection remains to be explored.

ACKNOWLEDGEMENTS

Authors are highly acknowledged to Head, Department of Botany, Punjabi university Patiala for providing experimental facility and Head, Plant

Breeding and Genetics, Punjab Agriculture University, Ludhiana for providing certified seeds of *Brassica napus* L.

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