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Exogenous Application of Jasmonic Acid Offers Tolerance to Salinity by Altering Stress Responses in *Brassica napus* L. Variety GSC 6

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Abstract: ***Background: Optimum remuneration of seed priming of Brassica napus L. with jasmonic acid were studied under salinity stress. Method: Seeds of Brassica napus L. were primed with jasmonic acid (JA) at four levels (0, 6, 9, 12 M). Then primed (P) and non-primed (NP) seedlings were supplied with different concentrations of saline solutions consisting of 0 (control), 140, 160, 180 mM NaCl. Results: The results showed that the JA reduced the toxicity of salt stress on seedling growth by accumulating proline content and by reducing electrolyte leakage and lipid peroxidation. Pre-treatment of jasmonic acid was found to be significantly effective in increasing the content of protein. Conclusions: The present study revealed that under salinity seed priming with JA could be used as a method to improve productivity in Brassica napus. However, further studies are needed to investigate the effects of JA priming on later growth and development of this crop.***

Keywords: *Brassica napus,* cultivars, MDA Content, JA priming, salinity

1. Introduction

Jasmonic acid (JA) is a lipid-derived plant hormone that mediates diverse biological phenomena. It is one of major goals in JA research field to elucidate the regulatory mechanism of JA level. It 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). MeJA could modulate the expression of two additional wound responsive genes (Robert *et al*., 1992), chalcone synthase (chs,which encodes an enzyme in the phenylpropanoid biosynthetic pathway) and proline-rich cell wall protein (PRP, a cell wall structural protein).

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.

2. Materials and Methods

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, 140, 160, 180 mM) 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/ Biochemical 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.

Electrolyte leakage (EL) was measured as described by Lutts et al. (1996) with a few modifications. Plant material (0.3 g) was washed with deionized water, placed in tubes with 10 ml of deionized water and incubated for 2 h at 25°C. Subsequently, the electrical conductivity of the solution (L1) was determined. Samples were then autoclaved at 120°C for

20 min and the final conductivity (L2) was measured after equilibration at 25°C. The EL was defined as follows:

$$EL\ (\%) = (L1/L2) \times 100$$

Extraction and determination of proline was performed according to the method of Bates *et al* (1973). Leaf samples (1g) were extracted with 3% sulphosalicyclic acid. Extracts 2ml were held for 1 hr in boiling water by adding 2ml ninhydrin and 2 ml glacial acetic acid, after which cold toluene (4ml) was added. Proline content was measured at 520 nm and calculated as $molg^{-1}$ DW against standard proline.

Malondialdehyde (MDA) was extracted with 5% (w/v) trichloroacetic acid and determined according to Heath and Packer (1968). MDA level is routinely used as an index of lipid peroxidation and was expressed as nmol g^{-1} fresh weight.

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.

3. Results and Discussions

Effect of JA and Sodium chloride on Growth

Seedling growth in terms of root and shoot length showed synergistic mechanism of negative effect of jasmonic acid on growth particularly on shoot length. Root length also affected negatively in presence of JA alone or with millimolar solution of NaCl. One interesting observation found in present study was that micro and picomolar JA treatments positively affected root length to 10% and 19% respectively, as compared to control untreated seedlings. Overall JA showed stronger inhibitory effect on seedling growth in presence or absence of sodium chloride.

Estimation of MDA and Proline Content

There are number of factors, plants showed at morphological and biochemical level which can be taken as stress indicators under inadequate environmental conditions. Lipid peroxidation (MDA) (Fig) and electrolyte leakage (Fig) increases in presence of NaCl Figure 1(c). Environment indicated that 180 mM NaCl is lethal and responsible for disintegration of plasma membrane and thus caused decrease in growth of cell. In present study 139 % of lipid peroxidation and 109 % electrolyte leakage increase was observed in presence of 180 mM NaCl. JA treatment resulted in about 23% decrease in MDA content and 14% decrease in electrolyte leakage. JA increases lipid peroxidation that was harmful to the cells (Orozoc cardenas *et al*., 1999). Enhancement of MDA content subjected to 24, 100 and 250 µM JA was noticed in peanut seedlings (Kumari *et al*., 2006) and in Scenedesmus incrassatulus (Fedina *et al*., 2000). Drought stress increased membrane leakage in *Ocimum basilicum* L. (Sorial and Gendy. 2010). JA application protects membranes from damage by various stress factors by reducing MDA content (Bandurska *et al*., 2003). From present study it was observed that this protection of cell membrane by JA is very much dose dependent along with absence or presence of any stress factor such as sodium chloride in present case. Proline is an amino acid which starts accumulating in higher amount in plants under inadequate environmental conditions which can be taken as stress marker as well as it helped the plant in protecting from harmful effect of stress. Presence of 180mM Figure 1(d) in seedlings growth environment enhanced proline content upto 533% as compared to control (untreated distilled water). On the other hand the promoting effect of NaCl on proline content stimulation was lowered in presence of JA which decreased with decrease in JA concentration. These results showed that in contrast to JA, the NaCl stimulatory effect on proline content was sustainable and that for the neutralization of its effect higher concentration of JA was needed. JA application can promote the biosynthesis of proline and putrascine under environmental stresses (Chen and Kao. 1993; Gao *et al*., 2004). 10^{-9} JA was most effective in proline accumulation in which about 158% of more proline accumulated as compared to untreated control distilled water seedlings. These results are in accordance with Walters (2002) and Seo (2001).

Effect of NaCl and JA on Sugars content

Involvement of soluble sugars in osmotic adjustment has been proposed by Mansour (2000) in alleviating the adverse effect of salt stress. In our results, total soluble sugars content was increased with increased concentrations of NaCl as compared to control (Figure 3). Similar results were also reported in *Chenopodium quinoa* Willd. seeds (Fernando *et al*., 2000). Soluble sugars was 84% highest as compared to control. The content was further enhanced by applications of different concentrations of JA under salt stress. 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) 83 % was observed in case of 180 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 239% was observed in seedlings treated with 140 mM of NaCl solution supplemented with 10^{-12}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 80 % highest at 180 mM NaCl. Non reducing sugars content was 380 % maximum in seedlings treated with 10^{-12}M JA supplemented with 140 mM of NaCl.

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).

4. Conclusion

The influence of JA on proline, MDA, 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 accumulation levels of proline, MDA 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.

5. Acknowledgements

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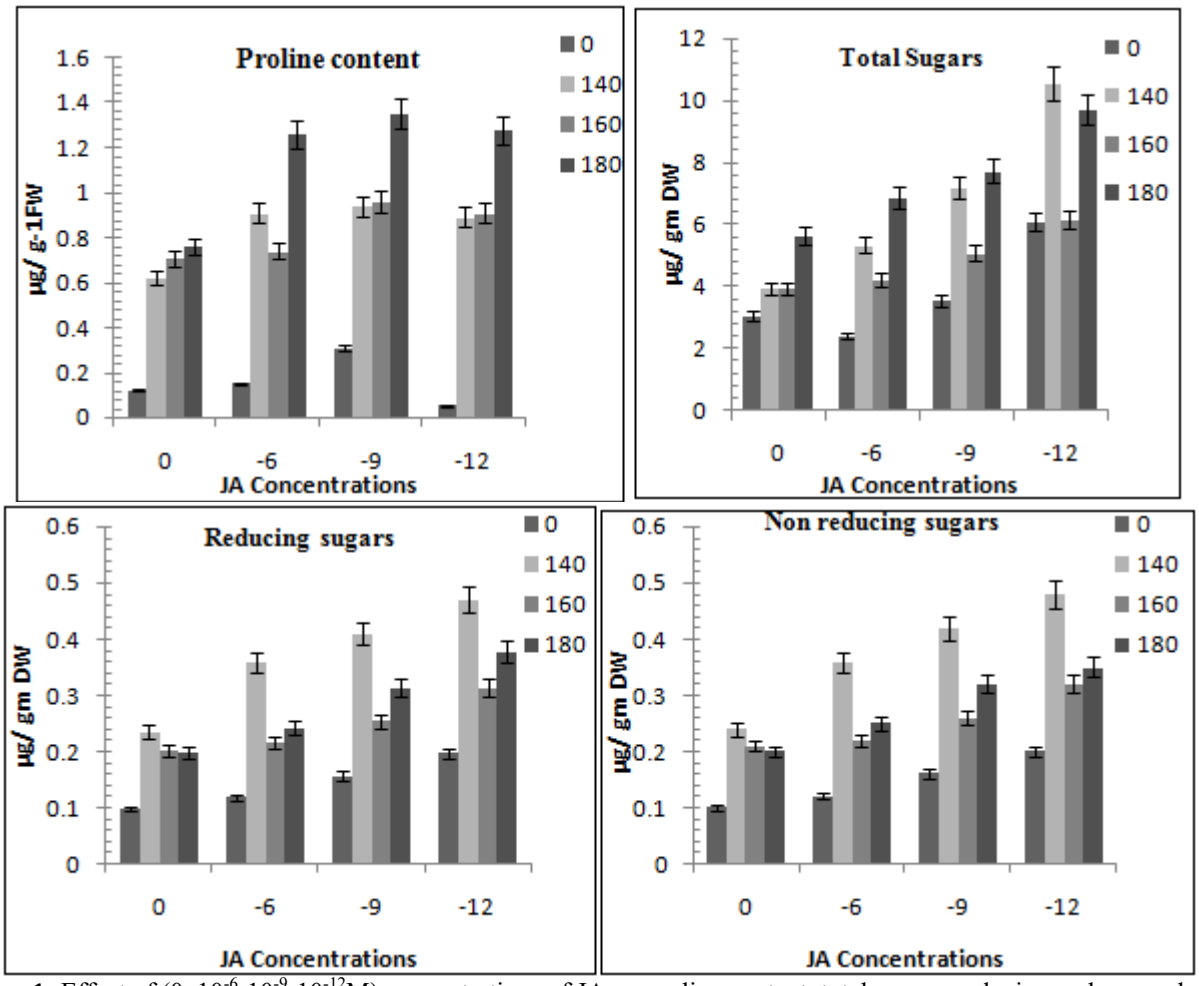

Figure 1: Effect of (0, 10^{-6},10^{-9},10^{-12}M) concentrations of JA on proline content, total sugars, reducing and non-reducing sugars of *B. napus* L. under NaCl salt (140, 160 and 180 mM) stress in laboratory conditions.

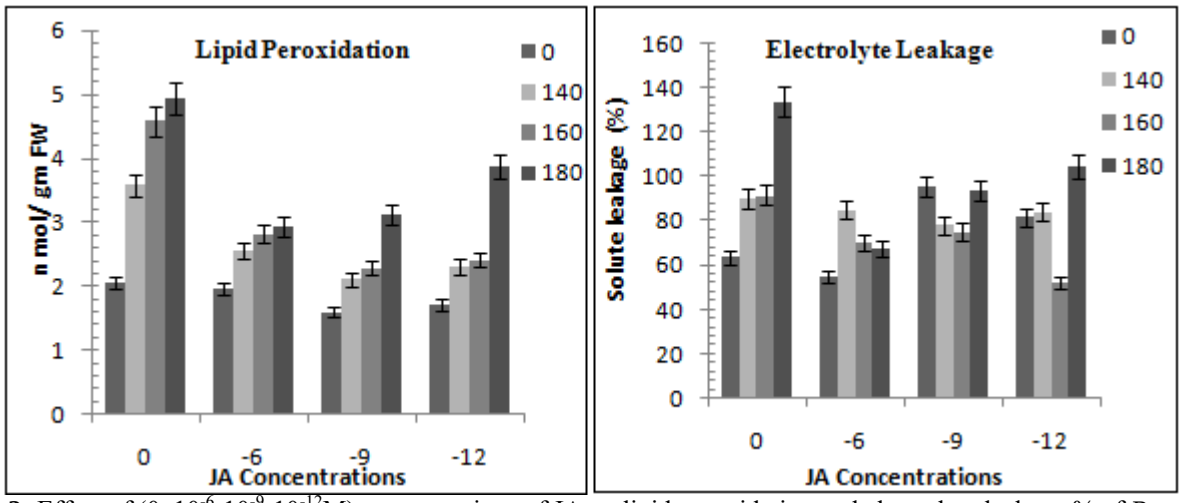

Figure 2: Effect of (0, 10^{-6} ,10^{-9} ,10^{-12}M) concentrations of JA on lipid peroxidation and electrolyte leakage % of *B. napus* L. under NaCl salt (140, 160 and 180 mM) stress in laboratory conditions.