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EFFECT OF JASMONATES ON OSMO-PROTECTANTS IN *BRASSICA OLERACEA* L. VAR. *ITALICA, CAPITATA* AND *BOTRYTIS*

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The present study focuses on the impact of Jasmonic acid and Methyl jasmonate (JA; Me-JA;1 µM, 1 nM and 1 pM) supplementation on osmo-protectants in the form of proline and glycine betaine in 10 day old seedlings of *Brassica oleracea* L. var. *italica*, *capitata* and *botrytis*. Results suggested that priming treatments of both JA and Me-JA enhanced the proline content in all the three varieties of *Brassica oleracea* L. in comparison to untreated control seedlings. However in case of glycine betaine, supplementation of JA and Me-JA raised the content only in var. *capitata* over control distilled water seedlings. Variety *italica* and *var. botrytis* followed the opposite trend and decrease in glycine betaine content was found by JAs application. These results indicated that JAs are potent enough to upsurge the osmoprotectants in *Brassica oleracea* L. seedlings, thus maintaining the process of osmoregulation, but at the same time it depends on dose of JAs used and variety as well.

Key words: *Brassica oleracea*, Jasmonic Acid, Methyl Jasmonate, Proline, Glycine betaine.

Plants produce osmoprotectants like glycine betaine and proline in order to maintain osmoregulation and stabilize macromolecules (Ahmad *et al.,* 2012 a, b and Ashraf and Foolad, 2007). Proline, constituting an energy source is known to be involved in activation of Krebs cycle and reconstruction of chlorophyll (Ramon *et al.,* 2003). Its ability to scavenge reactive oxygen species (ROS) protects the cell from the oxidative damage (Ahmad *et al.*, 2012 a, b and 2015 a, b). Apart from osmotic adjustment glycine betaine plays a role in stabilizing PSII complex, maintaining integrity of membrane, protecting the activity of Rubisco as well as detoxification of harmful ions (Ashraf and Foolad, 2007).

Jasmonates, found ubiquitously in higher plants is the youngest candidate of plant growth regulators and is known for the induction of wide array of developmental processes (Wasternack and Hause, 2013 and Engelberth *et al.*, 2001). JAs play a pivotal role as signalling molecule in plants (Wasternack and Hause, 2013, Wasternack, 2014 and Kamal, and Komatsu, 2016). The supplementation of JAs in minute amounts augment plant growth, gene expression and provide tolerance against various biotic stresses (Creelman and Mullet, 1995 and Cheong and Choi 2003). The present work was aimed to study the effect of exogenously applied two derivatives of jasmonates viz. Jasmonic acid (JA) and methyl jasmonate (Me-JA) on proline and glycine betaine content in *Brassica oleracea* L. (var. *italica, capitata* and *botrytis*).

MATERIALS AND METHODS

Experimental set up: Certified seeds of three varieties of *B. oleracea* L. var. *italica, capitata,* and *botrytis* were procured from the IARI, Katrain, Himachal Pradesh, India. Seeds were surface sterilized by dipping in 0.01% $HgCl_2$ for 2 minutes followed by washing under free-flowing tap water. Pre-soaking treatments of 6 hours in distilled water and different concentrations (1 µ M, 1 nM and 1 pM) of jasmonic acid and methyl jasmonate were given to the seeds. The seeds were arranged as follows:

- Control
- 1 µM JA/1 µM Me-JA
- 1 nM JA/1 nM Me-JA and
- 1pM JA/1pM Me-JA.

Presoaked seeds were germinated in plant growth chamber in petriplates lined with What man's filter paper for 3 days under controlled laboratory conditions of 25°C temperature, photoperiod of 16 h Dark/Light period; PPFD of 200 µ mol

(photon $m^{-2}s^{-1}$) and 80% humidity in randomised block manner with three replicas each. After the emergence of plemule, seedlings were transferred on growth papers upto 10 days. After 10 days, the seedlings were sampled for the analysis of various parameters.

Estimation of Proline: Proline content was estimated by adopting the method of Bates *et al.* (1973). Absorbance was taken spectrophotometrically at 520 nm (Beckman 640 D, USA).

Estimation of Glycine Betaine: The content of glycine betaine was determined by following the method of Grieve and Grattan (1983). The OD was taken by spectrophotometer (Beckman 640 D, USA) at 365 nm.

Statistical analysis: All data were subjected to one-way analysis of variance (ANOVA) for scrutinizing interactions of each concentration of JA and Me-JA and expressed as the mean ± standard error of three replicates. Tukey's test ($P<0.05$) was applied for the multiple comparisons using GraphPad Prism Version 7.

RESULTS AND DISCUSSION

In variety *italica* proline content was enhanced in its level by treatment of JA as well as Me-JA. It has been observed that JA in 1 µM and 1 nM treatment showed enhancement in free amino acid proline accumulation which was 32.00% and 42.00 %, respectively as compared to control distilled water seedlings (0.33±0.01 µg g^{-1} FW) (Table 1). The enhancement by Me-JA in proline content of variety italica was significantly higher than JA and the maximum increase by 598.00% was seen in seedlings grown from 1 µM treated seeds followed by 1 pM and 1 nM treatment which was 243.00% and 157.00% more than control distilled water seedlings (Table 1). In variety *capitata* JA and Me-JA again showed positive impact on proline content. Among JA treatments the highest proline accumulation was noted in 1 µM treated seedlings (1.22±0.09 µg g^{-1} FW) followed by 1 pM (0.79±0.13 µg g^{-1} FW) which was 192.25% and 88.73% over control distilled water seedlings (0.42±0.01 µg g^{-1} FW) (Table 1). Among Me-JA treatments, 1 pM has shown maximum proline content followed by 1 µM and 1 nM which was 171.83%, 147.18% and 97.18% over control distilled water seedlings (Table 1). In variety botrytis 1 µM and 1 pM JA supplemented seedlings showed increased proline content by 9.28% and 19.53% as compared to control distilled water seedlings (1.72±0.02 µg g^{-1} FW). On the other hand the exogenous application of Me-JA enhanced proline content in 1 pM concentration by 17.79% over control (Table 1). Our results are supported by Sirhindi *et al*., (2016) and Ali *et al.,* (2007) who reported accumulation of proline by JA and Me-JA and in *Glycine max* and *Panax ginseng* bioreactor root suspension cultures respectively. The present results are also in accordance to Poonam *et al.,* (2013) who reported an increased content of proline in *Cajanus cajan* under copper stress after supplementation of JA at different concentrations and the reason might be that JA stimulates the enzymes responsible for proline biosynthesis. In addition to this proline is having antioxidant property and might be induced by JA to protect the cell from oxidative burst. A similar role of Me-JA in proline production has been discussed by Pazirandeh *et al.,* (2015) in barley genotypes.

In variety *italica* the exogenous application of JA and Me-JA was not found to be effective in glycine betaine accumulation as all the treatments of both JA and Me-JA decreased the glycine betaine content to a considerable level as compared to control untreated seedlings (Table 2). On the other hand both JA and Me-JA had dynamic impact on uplifting the accumulation of glycine betaine content in variety *capitata* seedlings. With the decrease in concentration of JA from 1 µM to 1 nM, glycine betaine content increased from 6.54±0.03 mg g^{-1} DW to 9.6±0.06 mg g^{-1} DW but decreased to 4.61±0.10 mg g^{-1} DW at 1 pM JA (Table 2). Among Me-JA treatments increase in concentration from 1 pM JA to 1 nM JA and 1 µM JA, increased glycine betaine content by 67.49%, 69.83% and 100.24% respectively was recorded over control distilled water seedlings (2.70±0.08 mg g^{-1} DW) (Table 2). In variety *botrytis* all the priming treatments of JA decreased glycine betaine content supplementation of Me-JA also reduced glycine betaine content except at 1 µM treatment where a petite increase (2.11%) in glycine betaine content was recorded over control unprimed seedlings (Table 2). Our results revealed that the three varieties behaved differently towards exogenous application of both JA and Me-JA. In variety *capitata* both JA and Me-JA enhanced glycine betaine content thus corroborating with the findings of Gao *et al.,* (2004) by upregulating the expression of betaine aldehyde dehydrogenase (BADH). In addition to this our results are also supported by Sirhindi *et*

Table 1: Showing the statistical analysis of JA and Me-JA on Proline Content in *B. oleracea* var. *italica, capitata* and *botrytis*. Values are means ± SD of three independent replications (n = 3).

Proline Content (µg g^{-1} FW)				
Sr. No.	Treatments	Var.*italica*	Var.*capitata*	Var.*botrytis*
1.	Control	0.33 ± 0.015^{de}	0.42 ± 0.010^{c}	1.72 ± 0.023^{c}
2.	1 µM JA	0.44 ± 0.090^{de}	1.22 ± 0.090^{a}	1.88 ± 0.035^{b}
3.	1 nM JA	0.48 ± 0.006^{d}	0.40 ± 0.010^{cd}	1.40 ± 0.045^{d}
4.	1 pM JA	0.12 ± 0.025^{e}	0.79 ± 0.130^{bc}	2.06 ± 0.017^{a}
5.	1 µM Me -JA	2.33 ± 0.091^{a}	1.04 ± 0.020^{ab}	1.18 ± 0.037^{e}
6.	1 nM Me -JA	0.86 ± 0.092^{c}	0.83 ± 0.040^{b}	1.33 ± 0.015^{de}
7.	1 pM Me -JA	1.14 ± 0.035^{b}	1.14 ± 0.020^{a}	2.03 ± 0.069^{a}

*** Different letters (a-f) within the column indicate statistically significant differences among the treatments, according to Tukey's test at (P < 0.05).**

Table 2: : **Showing the statistical analysis of JA and Me-JA on Glycine Betaine Content in *B. oleracea* var. *italica, capitata* and *botrytis*. Values are means ± SD of three independent replications (n = 3).**

Glycine Betaine Content (mg g^{-1} DW)				
Sr. No.	Treatments	Var. *italica*	Var. *capitata*	Var. *botrytis*
1.	Control	7.19 ± 0.02^{b}	2.70 ± 0.08^{e}	13.86 ± 0.08^{a}
2.	1 µM JA	3.73 ± 0.00^{f}	6.55 ± 0.04^{b}	11.73 ± 0.04^{d}
3.	1 nM JA	7.84 ± 0.01^{a}	9.60 ± 0.07^{a}	12.33 ± 0.03^{cd}
4.	1 pM JA	3.16 ± 0.00^{g}	4.62 ± 0.11^{d}	12.63 ± 0.02^{c}
5.	1 µM Me-JA	6.54 ± 0.02^{d}	5.40 ± 0.04^{c}	14.15 ± 0.11^{a}
6.	1 nM Me-JA	5.90 ± 0.00^{e}	4.58 ± 0.04^{de}	10.85 ± 0.11^{e}
7.	1 pM Me-JA	6.79 ± 0.00^{c}	4.52 ± 0.05^{de}	12.98 ± 0.22^{b}

*** Different letters (a-f) within the column indicate statistically significant differences among the treatments, according to Tukey's test at (P < 0.05).**

al., (2016). However variety *italica* and variety *botrytis* followed the opposite trend and decrease in glycine betaine content was found by JAs application.

CONCLUSION

Jasmonates (JA and Me-JA) were supplemented to evaluate their impact on the accumulation of osmo-protectants in the form of proline and glycine betaine in *Brassica oleracea* L. var. *italica, capitata* and *botrytis*. Both JA and Me-JA priming was effective in enhacing the accumulation of proline in all the three varieties. However JA and Me-JA was able to augment the glycine betaine content only in variety *capitata.* Our study supports the hypothesis that JAs do so by stimulating and increasing the expression of genes involved in proline and glycine betaine biosynthesis but concurrently it depends on the variety also. These results infer that JAs have much potential in enhancing the accumulation of osmo-protetants, which also possess antioxidant property in *B. oleracea* L. in an economical way and above all variety *capitata* has outperformed.

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