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MODULATION OF ADVERSE IMPACT OF CHILLING IN *VICIA FABA* L. BY METHYL JASMONATE INVOLVES CHANGES IN ANTIOXIDANT METABOLISM AND METABOLITES

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Abstract

We conducted experiments to assess the effect of chilling (10°C) stress on growth, nitrogen and antioxidant components of faba bean (*Vicia faba* L.) and the role of methyl jasmonate (MJ 25 µM) in growth regulation and amelioration of chilling stress. Chilling temperature significantly reduced growth and pigment synthesis which was however significantly improved by application of MJ. Nodule growth, nitrogenase activity and nitrogen content were negatively affected by chilling and MJ application caused significant improvement in these attributes. Application of MJ significantly enhanced activity of antioxidant enzymes resulting in reduced oxidative damage. Chilling stressed plants exhibited higher lipid peroxidation and production of hydrogen peroxide. Ascorbic acid and phenol contents were observed to increase by 8.4% and 7.9% due to MJ providing strength to plants against chilling stress. In addition application of MJ was observed to maintain optimal levels of abscissic acid (ABA) and salicylic acid (SA) resulting in coordinated regulation of defence mechanisms against chilling stress.

Key words: Antioxidants, Nitrogenase, Lipid peroxidation, Phenols, Methyl jasmonate and Chilling.

Introduction

Different environmental factors control distribution and the growth of crop plants. Among the several environmental factors low temperature is the important factor limiting geographical distribution of plants. Indeed low temperature exposure imparts deleterious effects on the normal growth patterns and the productivity. It is well established now that stresses have negative influence on the growth and development of plants (Ma *et al*., 2010; Dumont *et al*., 2011; Bakht*et al*., 2006). Besides water, salt and metal stress, chilling or freezing is also the main determinant of crop plantation over globe and in addition it reduces yield of major food crops. During the course of evolution many plant have developed different strategies to acquire tolerance to the periodically occurring lower temperatures (Ma *et al*., 2010). Prolonged exposure to low temperatures trigger oxidative stress in plants thereby causing metabolic alterations in cells (Krol *et al*., 2015). Most important effect of low temperature is the reduction in the water uptake rate and therefore leading to the alterations in the membrane structures and hence causing distortions in the selective transport (Bray, 2009).Such effects have their obvious consequences on the stabilization of nucleic acid structure and the enzyme activity (Bakht *et al*., 2006; Dumont *et al*., 2011).

Like other stresses, low temperature also interferes with the photosynthetic rate by inducing upsurge in the production of reactive oxygen species (ROS) and hence hampering the redox homeostasis of the cells leading to oxidative stress (Bakht *et al*., 2006). At lower concentrations ROS has an important role in signalling however, their excessive accumulation disrupts electron transport in mitochondria as well as chloroplasts. Besides, ROS induce damage to key cell constituents like proteins, cellular membrane lipids and nucleic acids (Sirhindi *et al*., 2016). Such modulations in the internal cellular makeup and hence the functioning can lead to alterations in the cellular genetic programme. Under such conditions antioxidants including enzymatic as well as non-enzymatic, contribute significantly to growth regulation by mediating the elimination of the ROS. In addition improved synthesis of metabolically compatible compounds like proline, amino acids and phenols help in reinstating homeostasis (Ma *et al*., 2010; Qiu *et al*., 2014). Synthesis of the secondary metabolic products and the osmolytes is intensified under stress and has been reported to act as antioxidants for bringing the stabilization of metabolic processes (Qiu *et al*., 2014; Sirhindi *et al*., 2016). Both enzymatic and non-enzymatic antioxidants protect cells against the negative effects of ROS thereby improving the stability of lipids, proteins and the nucleic acids (Fahimirad *et al*., 2013; Abdelgawad *et al*., 2014).

Jasmonates are the lipid derivatives that act as signaling compounds in mediating diverse responses of plants to stress. These are important cellular regulators actively engaged in several developmental processes like germination, root growth, leaf movement, gravitropism, fertility, embryo development and sex determination, fruit ripening and senescence (Wasternack, 2014; Dar *et al*., 2015). Besides, jasmonates have been recognized to regulate plant growth and development and responses to biotic and abiotic stresses (Wasternack, 2014). Jasmonates

are known to improve defense responses of plants against the herbivores, nematodes, pathogens (Wasternack, 2014; Dar *et al*., 2015). Jasmonates have been reported to share crosstalk network with several important phytohormones such as auxin, gibberellic acid and salicylic acid for bringing the signaling events to regulate growth of plants (Wasternack, 2014).In soybean, jasmonic acid has been reported to regulate leaf and root morphogenesis (Xue & Zhang, 2007).

The present study was focused with the aim: (i) to investigate the impact of chilling (10^{o}C) stress on plant growth and physio-biochemical metabolism of *Vicia faba L*, (ii) to determine the role of methyl jasmonate (MJ 25µM) in improvement of stress tolerance in plants, and (iii) to assess the mechanism of plant stress tolerance. *Vicia faba* (L.) is an important cool-season grain legume species used widely in agriculture but also in plant physiology research, particularly as an experimental model plant for this experiment.

Material and Methods

Plant growth and treatments: Seeds of faba bean (*Vicia faba* L.) (cv. Giza 40) were kindly provided by Field Crop Research Institute, ARC, Giza, Egypt. The biofertilizer bacterium (*Rhizobium leguminosarum* bv. viciae Frank) was provided by Dr. Gamal El-Didamony, Botany & Microbiology Department, Faculty of science, Zagazig University, Egypt. Methyl jasmonate ($C_{13}H_{20}O_3$) used was the product of Sigma chemicals, USA.

The seeds were surface sterilized with NaOCl (0.5%, v/v) for two minutes and rinsed with sterile water several times, then germinated on blotter for one week at 25±1^{o}C. *R. leguminosarum* was applied to similar healthy germinating faba bean seeds at rate of 2×10^{8} CFU/g seeds (carboxymethyl cellulose 0.05%, w/v used as sticker). The inoculated seeds sown in plastic pots (2 Kg capacity, one plant/pot) filled with Peat-moss-vermiculite-sand (1:1:1, v/v/v). The pots incubated for one week more in growth chamber as above until three-leaf-stage, then MJ was applied at the rate of 25 µM as a foliar spray (5 ml/plant). The pot experiment was carried out in split-plot in randomized complete block design with five replications of each treatment: (i) Control without MJ incubated at 20±1^{o}C, (ii) Control without MJ incubated at 10±1^{o}C, (iii) Treated with MJ incubated at 20±1^{o}C, and (iv) Treated with MJ incubated at 10±1^{o}C. Hoagland's solution was used for irrigation and pots were maintained in growth chamber of Plant Production Department, Faculty of Food & Agricultural Sciences, King Saud University. The seedlings were grown for more ten weeks with 10 and 14 h daily light-dark cycle, light intensity 200 mmol photons $m^{-2}s^{-1}$. Treatment of MJ was repeated every two weeks and control plants were sprayed with distilled water used as reference. The irrigation and nutrition were supplemented with Hoagland's solution (two times/ week, each 50 ml/pot). At the end of growth chamber experiment, the plants were removed carefully and root system was separated and the nodules were collected for counting and estimation of nodules related attributes.

Estimation of photosynthetic pigments: For estimation of photosynthetic pigments fresh leaves were extracted in 80% acetone and after centrifugation at 3000g for 10 minutes optical densities of supernatant was recorded at 480, 645 and 663 nm (Arnon, 1949).

Nodulation, nodules activity, nitrogen content: Total number of nodules per plant was recorded with help of Leica MZF LIII stereomicroscope. Fresh weight of nodule was taken immediately after harvesting. Estimation of leghemoglobin was done following Keilin & Wang (1945). Fresh nodules were extracted in 50 mM phosphate buffer (pH 7.4) containing 1 mM EDTA. Homogenate was thawed at 2^{o}C and followed by centrifugation at 10,000g for 10 min at 4^{o}C. Thereafter supernatant was mixed with 50 mM phosphate buffer (pH 7.4) containing 1 mM EDTA and optical density was recorded at 710 nm using spectrophotometer. Nitrogenase was extracted from fresh nodules and activity was measured using the method described by Bergersen & Turner (1967). Nitrogen content was estimated in dried plant samples following the method of Lindner (1944).

Estimation of hydrogen peroxide content and lipid peroxidation: Fresh leaf samples were extracted in cold acetone and centrifuged at 6,000 g for 15 min. Optical density was recorded at 415 nm using spectrophotometer and calculations were done from standard curve of hydrogen peroxide (Mukherjee & Choudhuri, 1983).

Lipid peroxidation was determined in accordance with Heath &Packer (1968) by measuring the formation malondialdehyde (MDA) content. After extraction in trichloroacetic acid (TCA) supernatant was reacted with thiobarbituric acid and optical density was recorded at 600 nm and 532 nm An (spectrophotometer, model T80 UV/VIS Spectrophotometer PG Instruments Ltd) extinction coefficient of 155 mM cm^{-1} was used for calculation of MDA using following formula:

$$MDA = \Delta (OD_{532}\text{-}OD_{600})/1.56\times10^{5}$$

Estimation of total phenolics: Method of Slinkard & Singleton (1977) was employed for estimation of total phenolics. After extraction in 80% (v/v) acetone aliquot was reacted with Folin and Ciocalteau's phenol reagent. Absorbance was read at 750 nm and (spectrophotometer, model T80 UV/VIS Spectrophotometer PG Instruments Ltd) standard curve of pyrogallol was used for calculation.

Extraction and estimation of antioxidant enzyme: The fresh leaves (500 mg) was extracted in chilled 50 mM phosphate buffer (pH 7.8). Extract was centrifuged at 15,000g for 15 min at 4°C. Extraction buffer for ascorbate peroxidase (APX) was supplemented by 2.0 mM ascorbate. The supernatant was used as enzyme source. Protein was estimated according to Bradford (1976) using bovine serum albumin as standard. Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by following the photoreduction of nitroblue tetrazolium (NBT) at 560 nm (Van Rossum *et al*., 1997). Amount of protein causing 50% reduction in SOD-inhibitable NBT reduction was considered as one enzyme unit and activity was expressed as Unit mg^{-1} protein. Method of Nakano & Asada (1981) was followed for assaying ascorbate peroxidase (APX).

Activity was determined by measuring decrease in absorbance at 290 nm and was expressed as EU mg^{-1} protein. Catalase (CAT, EC 1.11.1.6) activity was determined by following the change in absorbance at 240 nm for 2 minutes (Luck, 1974). Reaction mixture contained 50 mM phosphate buffer (pH 7.0) and 5.9 mM $H_2O_2$and 100 µL of the enzyme extract in a final volume of 3 mL. Activity was expressed as EU mg^{-1} protein.Glutathione reductase (GR, EC 1.6.4.2) was assayed in accordance of Carlberg & Mannervik (1985) and decrease in absorbance was recorded at 340 nm for 2 min. An extinction coefficient of 6.2 mM^{-1} cm^{-1} for NADPH was used for calculation of GR activity and expressed as EU mg^{-1} protein.Peroxidase (POD, EC 1.11.1.7) activity was determined by measuring change in absorbance at 480 nm for 2 minutes (Urbanek *et al*., 1991). Assay mixture contained 50 mM potassium phosphate buffer (pH 6.8), 20 mMguaiacol, 20 mM hydrogen peroxide and enzyme extract in a final volume of 2 mL. POD activity was expressed as EUmg^{-1} protein.

Estimation of ascorbic acid: Ascorbic acid from fresh leaves was extracted in 6% TCA and homogenate was mixed with 2% dinitrophenyl-hydrazine in 50% H_2SO_4 and 10% thiourea in 70% ethanol followed by incubation for 15 min in a water bath. Thereafter samples were cooled and centrifuged at 1000 g for 10 min and the resulting pellet was dissolved in 80% H_2SO_4 and was read at 530 nm using spectrophotometer (Mukherjee & Choudhuri, 1983). A standard calibration curve of ascorbic acid was used for calculating ascorbic acid and results were expressed as nmol g^{-1} FW.

Extraction and quantification of plant growth regulators: Endogenous levels of plant growth regulators in leaves were determined by extracting leaf tissue in 80% acetone containing butylated hydroxytoluene (10 mg/l) and purification was done using EtOAc and $NaHCO_3$ (Kusaba *et al*., 1998). Abscissic acid (ABA) was estimated according to the method of Qi *et al*. (1998) and described by Kamboj *et al.* (1999). For salicylic acid estimation extracts were vacuum dried at room temperature and the concentration of SA were estimated adopting Siegrist *et al*. (2000) using HPLC equipped with fluorescence detector (LC-2010 AHT, SHIMADZU, Japan). Standards of ABA and SA were used for calculation.

Statistical analysis: Duncan's multiple range test was performed using one way ANOVA for a completely randomized design by SPSS-21 software and the differences in means were determined by the least significant differences (LSD) at p=0.05.

Results

Low temperature reduced the growth of faba beansignificantly with percent reduction in shoot and root length being 66.1% and 69.5% respectively however, application of MJ (25 µM) improved shoot and root length by 16.7% and 13.9% respectively (Table 1). Lower temperature reduced dry weight of shoot and root by 66.2% and 55.3 % which was however observed to be reduced by only 28.3% and 28.5% in plants treated with MJ. Relative to control, alone MJ was observed to increase shoot and root dry weight by 59.2% and 43.9% respectively (Table 1).

Application of MJ increased chlorophyll a, chlorophyll b, carotenoids and total pigments by 10.0%, 29.7%, 33.1% and 14.9% respectively (Table 2). Faba beangrown under lower temperatures exhibited a reduction of 60.4%, 63.4%, 64.8% and 46.4% in chlorophyll a, chlorophyll b, carotenoids and total pigments respectively. However MJ supplemented chilling stressed (25µM MJ + 10°C) plants showed only 21.1%, 29.2%, 33.1% and 26.1% decrease in chlorophyll a, chlorophyll b, carotenoids and total pigments respectively (Table 2).

Faba beanexposed to lower temperature exhibited reduction in number of nodules and nodule weight (Table 3). Number of nodules per plant, weight of nodules, nitrogenase activity and leghemoglobin content was observed to reduce by 67.7%, 55.7%, 83.3% and 70.7% respectively in low temperature exposed plants, however, relative to chilling stressed plants, MJ application to chilling stressed (10°C + 25 µM) plants improved number of nodules per plant, weight of nodules, nitrogenase activity and leghemoglobin content by 47.3%, 37.0%, 76.2% and 56.6% (Table 3). MJ alone caused significant increase in these attributes.

Effect of chilling and application of methyl jasmonate on nitrogen content and the crude protein is depicted in table 4. Due to application of MJ, nitrogen was observed to increase by 33.8% and 26.9% in shoot and root respectively while it was reduced by 42.3% and 52.4% due to chilling stress. In plants exposed to chilling stress and supplemented with MJ percent reduction was only 26.5% and 29.3%. Similarly chilling reduced crude protein by 42.3% and 52.4% in shoot and root while as application of MJ alone increased it by 33.9% and 26.8% in shoot and root respectively (Table 4).

Chilling (10°C) induced peroxidation of lipids and an increase of 41.2 % was observed which was however reduced by application of MJ (10°C + 25 µM) by 23.1% (Fig. 1A). Similarly hydrogen peroxide was observed to increase by 59.5% in chilling stressed plants as compared to the control, however, MJ application resulted in reduced production of hydrogen peroxide (Fig. 1B).

MJ (25 µM) application was observed to increase total phenol content by 7.9% which was however reduced by chilling stress by 51.7%. In MJ treated chilling (10°C + 25 µM) stressed plantdecrease in total phenol content was only 18.8% and relative to chilling stressed MJ treatment caused an increase of 40.5% (Fig. 2).

Application of MJ to normal grown faba bean increased abscissic acid (ABA) and salicylic acid (SA) production by 18.3% and 13.4% respectively (Fig. 3A, B). Chilling caused an increase of 62.1% and36.6% in ABA and SA which was however reduced by MJ under such conditions (Fig. 3A, B).

Relative to control ascorbic acid was increased by 8.4% due to application of MJ and by 39.4% due to chilling stress (Fig. 4). An increase of 35.3%, 14.0%, 7.2%, 14.8% and 17.7% was observed in the activity of SOD, APX, CAT, POD and GR due to MJ application (Fig. 5A-E). As a result of chilling, activity of SOD, APX, CAT, POD and GR was observed to get up-regulated by 45.9%, 30.0%, 24.1%, 42.5% and 50.6% respectively and application of MJ causing further enhancement of 12.1%, 31.6%, 5.2%, 26.3% and 21.8% (Fig. 5A-E).

Table 1. Effect of chilling (10°C) on germination (percent), shoot and root length (cm), shoot and root dry weight (mg) in *Vicia faba* treated with methyl jasmonate (MJ, 25 µM).

Treatment	Morphological criteria				
	Germination %	Shoot length (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
Control	86.3b	36.3b	26.5b	72.0b	213.6b
MJ	90.6a	43.7a	30.8a	176.6a	381.3a
Chilling stressed	39.0d	12.3d	8.06d	24.3d	95.3d
Chilling stressed + MJ	74.3c	27.8c	15.2c	51.6c	152.6c

Data presented are the means ± SE (n = 5). Different letters indicate significant difference at p<0.05

Table 2. Effect of chilling (10°C) on chlorophyll a, chlorophyll b, total pigments and carotenoids in *Vicia faba* treated with methyl jasmonate (MJ, 25 µM).

Treatment	Photosynthetic pigments (mg/ g fresh weight)				
	Chl. a	Chl. b	Chl. a/b	Carotenoids	Total pigments
Control	1.13b	0.49b	2.29c	0.29b	1.82b
MJ	1.26a	0.70a	1.79d	0.34a	2.25a
Chilling stressed	0.45d	0.18d	2.49ab	0.10d	0.97d
Chilling stressed + MJ	0.89c	0.34c	2.57a	0.19c	1.34c

Data presented are the means ± SE (n = 5). Different letters indicate significant difference at p<0.05

Table 3. Effect of chilling (10°C) on number of nodules per plant, fresh weight of nodule, nitrogense (Unit mg^{-1} protein) activity and leghemoglobin (mM gm^{-1} FW) content in *Vicia faba* treated with methyl jasmonate (MJ, 25 µM).

Treatment	Nodulations and nodule activities			
	Nodule number (nodules/ plant)	Fresh weight of nodules (g/ plant)	Nitrogenase activity (Unit mg^{-1} protein)	Leghemoglobin content (mM gm^{-1} FW)
Control	10.3b	38.4b	1.38b	0.123b
MJ	21.6a	111.9a	1.85a	0.290a
Chilling stressed	3.3d	17.0d	0.23d	0.036d
Chilling stressed + MJ	6.3c	27.6c	0.97c	0.083c

Data presented are the means ± SE (n = 5). Different letters indicate significant difference at p<0.05.

Table 4. Effect of chilling (10 °C) on total nitrogen content (mg g^{-1} DW) and crude protein (mg g^{-1} DW) content in shoot and root of *Vicia faba* treated with methyl jasmonate (MJ, 25 µM).

Treatment	Nitrogen assimilation			
	Total nitrogen content		Crude protein	
	Shoot	Root	Shoot	Root
Control	46.3b	36.4b	289.7b	227.5b
MJ	70.1a	49.8a	438.3a	311.2a
Chilling stressed	26.7d	17.3c	167.0d	108.1d
Chilling stressed + MJ	34.0c	25.7c	212.9c	160.8c

Data presented are the means ± SE (n = 5). Different letters indicate significant difference at p<0.05

Discussion

Low temperatures are having severe effects on the growth of plants and hence cause significant reductions in yield. As per the results of present study growth attributes including germination percentage, length and dry weight of faba bean was considerably reduced by the chilling and application of MJ not only improved the growth characteristics but also ameliorated the stress effects significantly. Jasmonates have active role in different developmental events including germination and root growth, leaf development and in addition impart tolerance to biotic and abiotic stresses (Wasternack, 2014; Dar *et al*., 2015). In the present study application of MJ to chilled stressed faba bean resulted in enhanced growth and hence biomass accumulation thereby depicting the active involvement of MJ in cell division and hence organ differentiation. Earlier cold induced reduction in growth and biomass accumulation has been reported in *Zea mays* (Abdelgawad *et al*., 2014) *Fagopyrum tataricum* (Gumerova *et al*., 2015) and *Brassica oleracea* (Rodríguez *et al*., 2015). Exposure of plants to low temperature hinders stomatal movements causing more often restriction in its closure in spite of root leaf water status. Such a failure in control of stomatal functioning further aggravates the chilling induced water stress. In addition of this low temperatures have a prominent effect on the root hydraulic conductivity (Aroca *et al*., 2005; Murai-Hatano *et al*., 2008).

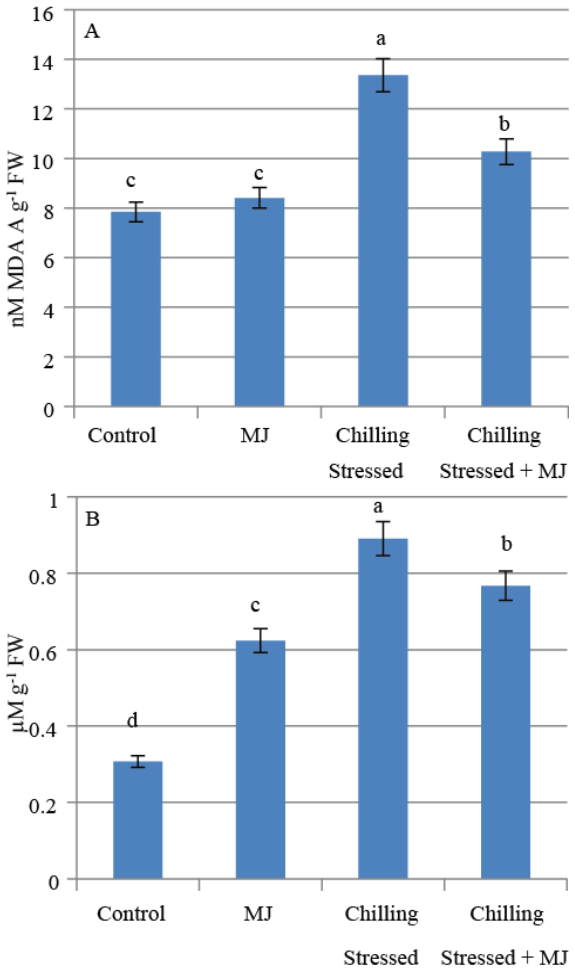


Fig. 1. Effect of chilling (10°C) on lipid peroxidation [MDA] (A) and hydrogen peroxide [H_2O_2] (B) in *Vicia faba* treated with methyl jasmonate (MJ, 25 µM). Data presented are the means ± SE (n = 5). Different letters indicate significant difference at $p<0.05$.

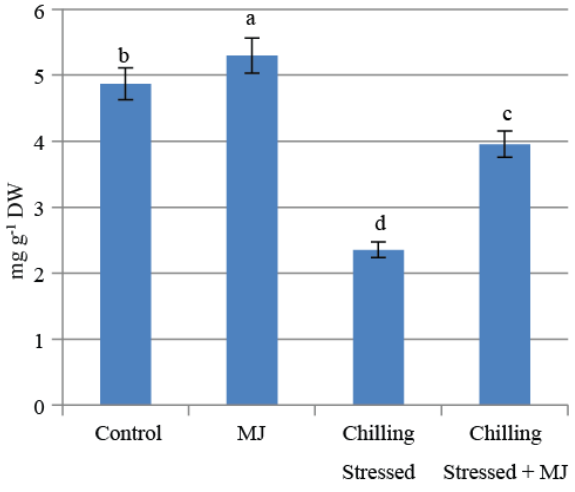


Fig. 2. Effect of chilling (10°C) on total phenol content in *Vicia faba* treated with methyl jasmonate (MJ, 25 µM). Data presented are the means ± SE (n = 5). Different letters indicate significant difference at $p<0.05$.

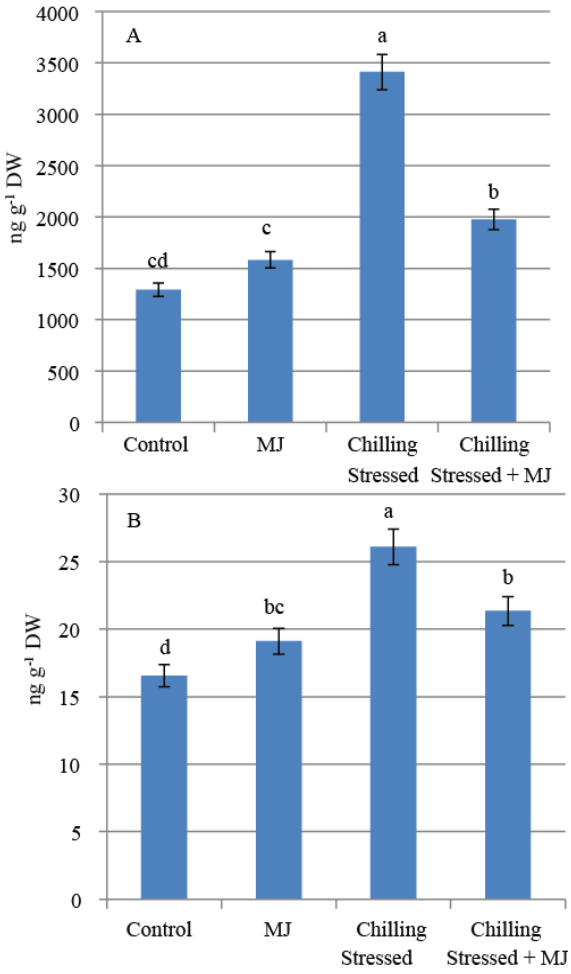


Fig. 3. Effect of chilling (10°C) on endogenous levels of abscissic acid (A) and salicylic acid (B) in *Vicia faba* treated with methyl jasmonate (MJ, 25 µM). Data presented are the means ± SE (n = 5). Different letters indicate significant difference at $p<0.05$.

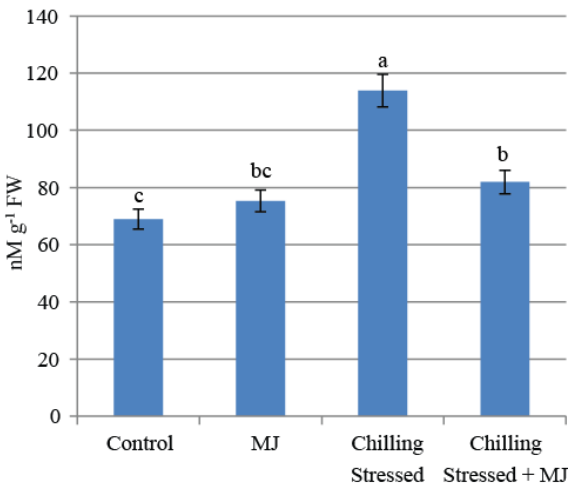
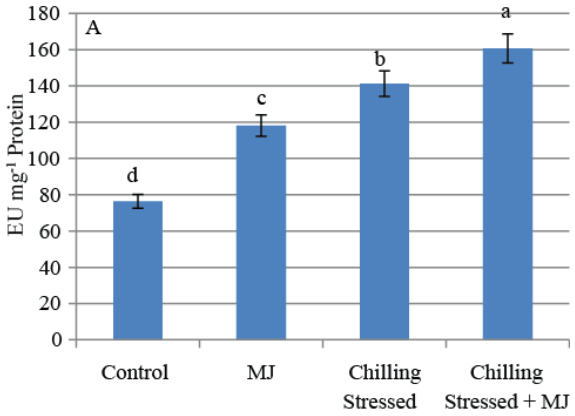
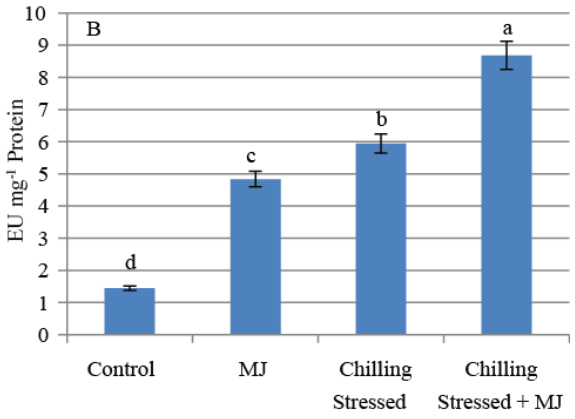
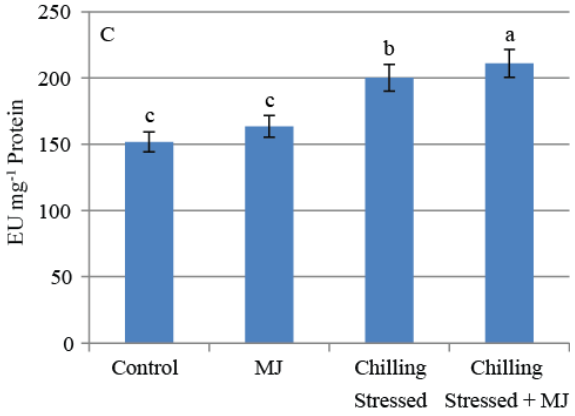
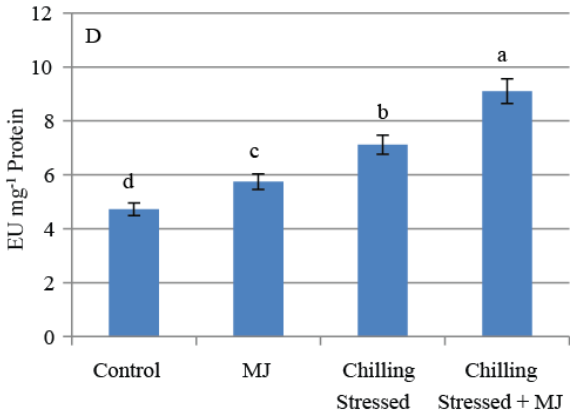


Fig. 4. Effect of chilling (10°C) on ascorbic acid content in *Vicia faba* treated with methyl jasmonate (MJ, 25 µM). Data presented are the means ± SE (n = 5). Different letters indicate significant difference at $p<0.05$.









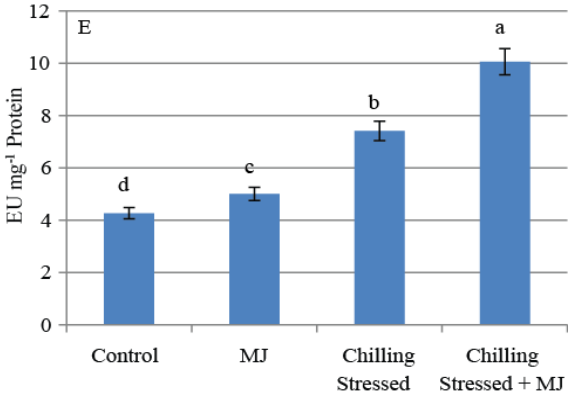


Fig. 5. Effect of chilling (10°C) on activity of superoxide dismutase (A), ascorbate peroxidase (B), Catalase (C), peroxidase (**D**) and glutathione reductase (**E**) total phenol content in *Vicia faba* treated with methyl jasmonate (MJ, 25 µM). Data presented are the means ± SE (n = 5). Different letters indicate significant difference at $p<0.05$.

The effects of chilling stress on growth were observed to be well correlated with the reduction in chlorophyll pigments and application of methyl jasmonate maintaining its positive influence on the chlorophyll biosynthetic capacity under normal conditions, in addition of its ameliorative impact in stressed plants. Our results of reduced chlorophyll pigments due to chilling corroborate with the results of Haghjou & Shariati, (2007); Huang *et al*. (2010) and Hanaka *et al*. (2015). Low temperature exposure in the present study could have affected structure and the stability of pigment protein complex by inducing cholorphyll degradation and in addition it has been reported that stressed plants show up-regulated chlorophyllase activity, a prime cause for chlorophyll degradation (Fang *et al*., 1998). Reduced chlorophyll biosynthesis due to low temperature has been reported to impart alterations in the net photosynthetic rate (Haghjou & Shariati, 2007) by brining changes in the Hills activity and the function of PSI (Huang *et al*., 2010). Recently protective role MJ has been demonstrated by Hanaka *et al*. (2015) in copper treated *Phaseolus coccineus* plants who have reported significant improvement in chlorophyll pigments and the associated attributes of photosynthesis like photosystem functioning. Very few reports are available depicting protective role of MJ in plants exposed to chilling.

Induction of chilling stress caused a drastic decline in number of nodules per plant, nodule weight and the nitrogenase activity in addition of its prominent effect on the leghemoglobin content. Junior *et al*. (2005) have demonstrated that leguminous plants such as bean, lentil and pea grown under cold conditions exhibited hampered nodule growth. Reduced leghemoglobin content in chilling stressed plants may have occurred due to its effect on the nodule bacteroid soluble protein which has been demonstrated to be dependent on the endogenous levels of abscissic acid (Gonalez *et al*., 2001). In the same context, Lloyd *et al*. (2011) have reported that uptake and assimilation of nitrogen content gets declined under cold conditions. In the present investigation application of MJ resulted in enhancement in the uptake of nitrogen by

causing obvious up-regulation of the activity of nitrogenase thereby helping in maintaining the optimal nitrogen pool for various metabolic purposes. Such an improvement in nitrogen content due to MJ application has been reported by Volder *et al*. (2000) in both *Saxifraga caespitosa* and *Cerastium alpinum* and Rossato *et al*. (2002) in *Brassica napus*. Presence of sufficient nitrogen promotes expression of RNA-binding proteins that are low temperature responsive and are involved in bringing integration of environmental and developmental signals (Mori *et al*., 2003). Present study provides a strong evidence for MJ in promoting the uptake of nitrogen and also in bringing regulation of the assimilation.

Faba bean (*Vicia faba*) exposed to chilling exhibited up-regulation of the activities of antioxidant enzymes and application of MJ was observed to further strengthen the activity. Present study is in support of the findings of Milyutina *et al*. (2008) in *Pinus sylvestris*. Similarly, Fan *et al*. (2014) reported that *Cynodon dactylon*exposed to cold treatments exhibited induction of the several isozymes of SOD, APX and POD resulting in quick removal of ROS and providing strength to withstand the low temperatures. SOD is the frontline protector in antioxidant system for counteracting the scavenging of superoxide radicals and up-regulation of SOD activity by MJ application may have benefitted stressed plants by causing modulations in the substrates of Haber-Weis reaction, superoxide and H_2O_2therefore preventing the generation of more toxic hydroxyl radicals. Increased activity of antioxidant enzymes due to MJ application has been reported by several investigators as well (Abdelgawad *et al*., 2014; Sirhindi *et al*., 2016). Elimination of toxic ROS is mediated by the efficient antioxidant mechanism which therefore leads to the stability of cellular metabolism. MJ induced enhancement in the POD activity reported in the present study may have contributed to improvement of cell wall components by improving the formation of lignin so that chances of biotic stress outbreak is declined. Application of JA to nickel stressed *Glycine max* resulted in enhanced expression and the activity of antioxidant enzymes therefore protecting photosynthetic apparatus from the free radical induced damage (Sirhindi *et al*., 2016).

CAT, APX and POD usually act upon a common substrate, H_2O_2, and are indispensable for maintaining growth of plants under stress and up-regulation in their activity provides strength to plants to counter the ROS induced oxidative damage (Abdelgawad *et al*., 2014; Krol *et al*., 2015; Sirhindi *et al*., 2016). In addition MJ also showed a direct influence on the key enzymes of ascorbate-glutathione pathway, APX and GR (Fan*et al*., 2014; Dar *et al*., 2015). Recently, Fan *et al*. (2014) have demonstrated that up-regulation of the enzymes of ascorbate-glutathione cycle increased tolerance of *Cynodon dactylon* to chilling stress. APX and GR mediate redox reactions resulting in scavenging of H_2O_2 in chloroplast and cytosol, and the maintainence of glutathione pool and NADP in the cells and in the present study MJ induced enhancement in APX and GR activity showed its obvious effect on the hydrogen peroxide scavenging and the photosynthetic pigments. Earlier, Fahimirad *et al*. (2013) have also demonstrated that cold stress induces lipid peroxidation by enhancing ROS production. Very few reports are available discussing the role of MJ in stress amelioration through its involvement in antioxidant metabolism. Abdelgawad *et al*. (2014) have also reported increased APX and GR activity in *Zea mays* due to water stress and similar to our findings application of MJ further enhanced the activity resulting in reduced lipid peroxidation. In present study application of MJ further strengthened the antioxidant potential of *Vicia faba.* Enhanced activity of antioxidant enzymes is correlated to efficient stress mitigation (Abdelgawad *et al*., 2014). In the present study, reduced lipid peroxidation concomitant with increased GR activity in MJ supplemented plants may be due to its protective role for chloroplast membranes thereby maintaining photosynthetic electron transport (Hayat *et al*., 2010).

Phenols increased in plants supplemented with MJ however were observed to decrease due to chilling stress. Results of enhanced phenol production due to MJ treatment observed in present study support the findings of Horbowicz *et al*. (2011) for *Fagopyrum esculentum* and Gumerova *et al*. (2015) for *Fagopyrum tataricum.* Phenolic compounds are reported to improve the antioxidant potential significantly thereby contributing to quick radical scavenging and hence protectingcellular metabolism (Dumont *et al*., 2011; Fahimirad *et al*., 2013). Environmental stresses may eitherincrease or decrease the synthesis of phenolic compounds in cells.In the present study exposure to chilling temperaturesresulted in drastic decline in phenolic compound synthesis and MJ application helped to recover this loss to some extent. Improved synthesis of phenolic compounds in MJ treated plants provided additional protection to *Vicia faba* to chilling by mediatingscavenging of ROS. Polyphenols form complexes with stress intermediates and prevent oxidative damage. In contentious context, Konan *et al*. (2014) have demonstrated that application of MJ to *Gossypium hirsutum*induced accumulation of phenolic compounds like ferulic acid, gossypetin, gossypol, 3-p-coumaroylquinic acid and piceatannol thereby protecting it from the *Fusarium oxysporum* wilt. MJ shares crosstalk with several protective molecules to mediate the stress tolerance. Ren & Dai (2012) observed that in *Atractylodeslancea*,MJ application mitigates the fungal damage by inducing volatile oil production through interactions with nitric oxide. In the present study MJ enhanced ascorbate production and it can be suggested that MJ may have interacted with other important molecules to assuage chilling effects on *Vicia faba*.

Continuous and controlled cell growth results from regulated cell division and expansion. Low temperature exposure reduces growth rate and affects cell cycle progression (Gimenez-Abian *et al*., 2004).In the present study chilling temperature altered the synthesis of phytohormones including abscissic acid (ABA) and salicylic acid (SA). Plant exhibiting higher accumulation of SA showed enhanced cellular expansion and the tissue growth under low temperatures (Scott *et al*., 2004). In addition to this ABA contents were observed to increase

due to chilling. Xiong *et al*. (2001) have suggested that enhancing synthesis of ABA in response to cold stress leads to induction of stress tolerant genes and in the present study MJ induced ABA accumulation may have contributed to cold stress acclimation by improving the transcript levels of important stress genes. Both ABA and SA have been reported time and again to regulate the signalling events in response to different stresses and in the present study MJ induced enhancement in their synthesis advocates the exogenous application of MJ for stress mitigation in plants. MJ may have showed crosstalk with these phytohormones for eliciting the appropriate stress response. In addition it can be proposed from the present study that MJ induced ABA and SA synthesis may have helped in cell cycle regulation for controlling the meristem growth under cold conditions and such an observation has been demonstrated by Xia *et al*. (2009) in *Arabidopsis thaliana* using SA and cytokinin.

Conclusion

In conclusion, the growth of *Vicia faba* is drastically declined due to exposure to temperature of 10 ^{o}C. Growth, photosynthetic pigments and nitrogen assimilation by plants were negatively affected by low temperature. However application of MJ mitigated the effects of chilling by bringing up-regulation of the antioxidant system. Mediating protection to the membranes and the nitrogen assimilation, application of MJ resulted in enhanced growth by improving the synthesis of growth hormones. MJ treated plants hold potential to counteract the chilling more efficiently than untreated plants. From the present study we recommend using application of MJ to bring stability in growth and yield of *Vicia faba* under low temperature conditions.

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