Signaling Necessities and Function of Polyamines/ Jasmonate - Dependent Induced Resistance in Sugar Beet Against Beet Mosaic Virus (BtMV) Infection

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Abstract: Induced systemic resistance (ISR) in plants against pathogens is a pervasive phenomenon that has been intensively investigated with respect to the essential signaling pathways as well as to its potential use in plant protection. In the present study, sugar beet plants treated with methyl jasmonate (MJ) exhibited augmentation resistance to Beet Mosaic Virus (BtMV), associated with increased polyamines (PAs) and salicylic acid (SA) buildup. BtMV-inoculated plants showed symptoms resembling severe mosaic, mottling and deformations. Spraying the leaves of sugar beet with MJ helped to preclude the twisted destructive possessions. Conspicuously the double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) values were decreased in the extracts of MJ-treated plants. In addition, the lesion numbers and concentration of BtMV- were abridged by polyamines i.e. L-Ornithine; L-Ornithine.Hydro; L-Ornithine.monohyd; Pentamidine and Diminidine treatment. Polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting of -coat proteins accumulation in leaf tissue revealed that MJ at 3.0 µg/ml concentration entirely subdued BtMV protein accumulation. The changes in some biochemical and molecular parameters in sugar beet leaves allied to BtMV infection and the effect of exogenous application of MJ were showed, 15 days following treatment. Significant increases in levels of free and conjugated putrescine, spermidin and spermine were noticed after treatment of the leaves with MJ. These changes were accompanied by increasing the activity of soluble ornithine decarboxylase (ODC) and polyamine oxidize (PAO), in leaves following treatment with MJ. Analysis of soluble protein, salicylic acid (SA), peroxidase, chitinase, polyphenoloxidase and phenols in protected plants revealed conspicuous accumulation of such substances. In addition, protein patterns symbolized some newly synthesized polypeptides which mirror formation of certain pathogenesis associated proteins in MJ treatment. All results show significant changes in metabolism affected by either viral infection or MJ treatments and also indicated that exogenous MJ plays an important role in the induction of defense mechanism against BtMV infection. [New York Science Journal 2010;3(8):95-103]. (ISSN: 1554-0200).

Key Words*:* BtMV, induced resistance, methyl jasmonate, polyamines, sugar beet

1. Introduction

Sugar beet (*Beta vulgaris* L.) lines the second crucial sugar crop after sugar cane, producing annually about 40 % of sugar production all over the world (Mirvat Gobarah and Mekki, 2005). Needless to say that countless viruses are well thought-out as disparaging plant pathogens, and substantial types of them have been again and again isolated from sugar beet plants just about the world (Sutic et al., 1999). Examples isolated in Egypt, might be represented by cucumber mosaic virus (CMV) (Omar et al.,1995); BtMV (Abdel-Ghaffar et al.,2003 and Beet curly-top virus (BCTV) (Mahmoud et al., 2004) . BtMV causes mosaic in sugar beet, red beet and spinach (Juretic, 1999). It is easily transmitted by aphids, filament in shape and with single-stranded RNA viruses belong to potyviridae (Russell, 1971). This virus usually occurs in the form of mild strains that don't cause a significant economic damage to sugar beet or spinach. However, some sever strains of BtMV that cause significant yield losses in sugar beet have been initiated (Abde-Ghaffar, et al., 2003). Plants shield themselves against the pathogen invasion through the action of specific resistance (R) genes together with various nonspecific host responses (Li, et al., 1999). Most of the plants dominate defense mechanisms against pathogen attack triggered by a stimulus preceding the pathogen attack that reduces the disease. The stimulus can boost the concentration of accessible defense compounds that induce the assembly of new defensive structures and chemicals (Baileya et al., 2005). Jasmonates have been reported to persuade systemic protection against plant fungal diseases such as rust and powdery mildew in wheat and barley, respectively (Haggag, Wafaa and Abd-Kreem , 2009 and Walters et al., 2002). The key forms of polyamines are putrescence, spermidine and spermine which are ingredient of eukaryotic and prokanytic cells that perform as crucial regulators for growth and differentiation and are engaged in plant responses to

stress (Walters, 2000). Polyamines occur in plants in free form, bound electrostatically to a negatively charge molecular conjugated to small molecules and proteins (Walters et al., 2002). Consequently, they amend DNA-protein (Shah et al., 1999), and protein-protein interactions (Thomas et al., 1999). In general, polyamine metabolism has long been known to distort in plant cells responding to insightful changes in plants interacting with fungal and viral pathogens (Walters, 2003). Accumulation of polyamines has been observed in tobacco cultivars resistant to TMV, but not in TMV-susceptible counterparts (Marini et al., 2001). Polyamines conjugated to phenolic compounds, hydroxycinnamic acid amides (HCAs), have been shown to accumulate through incompatible interactions between plants and a variety of pathogens, while changes in the diamine catabolic enzyme diamine oxidase signifies a role for this enzyme in the production of hydrogen peroxidase during plant defense responses (Walters, 2003).

Another procession verifying the role of jasmonates in disease resistance arises from their stimulatory effect on secondary metabolite production including ribosome-inactivating protein, serine proteinase inhibitors, phenylalanine ammonia lyase, alkaloids, thionin, terpenes, phenolics and including hydroxycinnamic acid amides (HCAs) (Biondi et al., 2000 and Martin et al., 2002). HCAs are formed from the covalent binding of polyamines (putrescine, spermidine and spermine) to hydroxycinnamic acids like caffeic acid and coumaric acid (Martin et al., 2002). This form of induced resistance is generally referred to as systemic acquired resistance (SAR). SAR is an inducible plant defense response involving a cascade of transcriptional events induced by salicylic acid (SA) (Naylor et al., 1998; Madhusudhan et al., 2008). This paper reports, induce systemic protection against sever isolate of BtMV in sugar beet. Also show that components of the MJ/ PAs–mediated resistance pathway are required for plant resistance.

2. Materials and Methods

Virus strains and plant material. A previously isolated strain of BtMV (Abdel-Ghaffar *et al.* , 2003) was used in this study. Symptoms caused by this isolate were small chlorotic lesions, mosaic, apical necrosis and mottle mosaic. For inoculums preparation, young BtMV-infected leaves of greenhouse- grown plants of the cultivar Kawemira were harvested about 25 days after inoculation. Leaves were ground in 0.1 M phosphate buffer pH 7.4 (1:10, w/ v) and the sap was filtered through two layers of cheesecloth and mixed with Carborundum (600-mesh) at 2% (w/v). Healthy sugar beet (*Beta vulgaris* L. cv Kawemira) and *Chenopodium amaranticolor* plants were maintained under greenhouse conditions at 23 ±2°C and sprayed with solutions of Methyl jasmonate and polyamines ***i.e.*** L-Ornithine.monohyd; L-Ornithine.Hydro; L-Ornithine. monohyd; Pentamidine and Diminidine (1.5 µg/ml), (Sigma chemicals) as described by Wafaa and Abd-El-Kareem, (2009). The sprayed plants were viral inoculated 2 dayes after treatment. Up to 25 days post inoculation (DPI), disease incidences were observed every day in sugar beet as the number of plants showing symptoms increased. Also, samples of leaves were taken for DAS-ELISA test. On the other hand, local lesion numbers were counted in *C. amaranticolor* plant leaves.

Enzyme-linked Immunosorbent Assay (ELISA). Double-antibody sandwich (DAS)-ELISA test according to Clark *et al.*, 1977 with polyclonal antisera was used to determine BtMV presence in plants treated with MJ (from 0.08 to 3.0 µg/ml) or PAs (1.5 µg/ml*)*. The uppermost expanded leaves of sugar beet plants were collected at 15 DPI, and sap was expressed using phosphate buffer saline (PBS) containing 0.05% Tween-20 at a ratio of 1:10 (w/v). Plates were coated with anti-BtMV obtained from previous study (Abde-Ghaffar, et al., 2003) then diluted at 1:200 in phosphate buffer. Plates were incubated for 4 h at room temperature (RT).Plant samples were incubated in the coated plate at RT for 2 h before adding alkaline phosphatase-conjugated anti-BtMV diluted at 1:200 in PBS-T. After 2-h incubation at RT, substrate (*p*-nitrophenylphosphate at 1 mg/ml in diethanolamine, pH 9.8) was added and incubated at room temperature for 1 h. Absorbance values were determined at 405 nm.

Western blotting for BtMV-coat protein analysis . Leaf samples of sugar beet were collected 15 days after viral inoculation and then extracted according to the method of Donald et al., 1993. Samples were centrifuged at 15.000 rpm for 3 min and the supernatant were separated using polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred onto nitrocellulose membrane using transfer buffer. The membranes were blocked overnight in 3% (w/v) milk powder in TBS buffer at 4 °C, followed by washing in TBS three times, 5 min each. The membranes were probed with polyclonal antibody diluted (1:1000) raised in rabbits against BtMV coat protein then washed 3 times, 5 min each. The membranes were probed using a secondary antibody, goat antrabbits conjugate with horseradish peroxidase (1:5000), and then developed using West Dura extracted (Pierce) and photographed by x0Ray film.

Chemical analysis.

After three days of inoculation, three leaves/ plant treated with MJ were separately collected, frozen for 36 hrs, dried and powdered. Generally, 100 mg of dried samples was employed for analysis.

uantification of polyamines (PAs)

Determination of free polyamines and polyamine conjugates: Free and conjugated PAs in sugar beet leaves were quantified. Free polyamines were extracted and hydrolyzed using the method described by Slocum and Galston (1985). This yielded a non – hydrolyzed perchloric acid (used at 10%) supernatant, containing the free polyamines, and the hydrolyzed supernatant and pellet fractions, containing polyamines liberated from various types of conjugates. Polyamines were extracted with 2ml of 0.5*M* $HClO_4$ overnight at room temperature, derivatized with benzoyl chloride and quantitated with high performance liquid chromatography (HPLC) using standard chemicals (Sigma chemicals). Separation and quantification of derivatized polyamines were performed with a Shimadzn Lc-6A HPLC equipped with a UV detector. The analytical condition was as follows: 6×150 mm in column size; 45 ^{0}C column temperature; 64% methanol mobile phase and detection on 254 nm.

Activities of polyamine biosynthetic enzymes: Ornithine decarboxylase (ODC) and polyamine oxidase (PAO) activityies were determined according to as described previously (Zarb and Walters, 1993).

Evaluation of MJ-Induced Resistance against BtMV Infection

Measurement of Protein: Protein in leaves treated with MJ was extracted by the method of Bollag and Eldelstein (1992). Fifty µg protein of each treatment was analyzed by 12% sodium dodecyl sulfate (SDS-PAGE) according to the method described by Laemmli, (1970) using 10% acrylamide in the separating gel and 3% in the stacking gel. Molecular weights of polypeptide bands (KDa) were calculated from a calibration curve of low molecular weight marker kit of Phramacia (Uppsala, Sweden).

Measurement of Salicylic Acid (SA). Changes in the level of free SA were determined in the leaves by using a modified spectrophotometric method (Li et al.,1999). Leaves were ground in liquid nitrogen with a mortar and pestle then extracted with 2 ml of 50% ethanol. The supernatant was centrifuged (3,000 rpm for 15 min), filtered through four layers of cheesecloth, and then 0.5 ml of 6MHCl was added for SA hydrolysis. To extract SA, 10 ml of tetrachloride was added to each sample, and the extract was mixed with 5 ml of ferric nitrate solution for 2 min. After centrifugation, the aqueous phase was analyzed by spectrophotometry (530 nm). For quantitative analysis, a standard curve was established with commercial SA (Sigma, St. Louis, MO) suspended in 50% ethanol.

Measurement of Chitinase, Peroxidase and Polyphenoloxidase. Chitinase activity was evaluated according to the methods described by Boller and Mauch (1988). Colloidal chitin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars. Chitinase activity was expressed as mM N-acetylglucose amine equivalent released / gram fresh weight tissue / 60 minutes. Peroxidase activity was evaluated according to the methods described by Allam and Hollis (1972) as one unit of perioxidase activity was expressed for the change in absorbance at 425 nm/minute /g fresh weight. Polyphenoloxidase activity was quantitatively determined according to the method described by Matta and Dimond (1963). One unit of polyphenoloxidase was expressed as the change in absorbance at 420 nm for 30 min at 25 ^{0}C/ g fresh weight.

***Determination of free and conjugated** phenol contents* . Free and conjugated phenols were determined in treated leaves after 15 days of plant spraying, according to the A.O.A.C. (1975). In this study the Folin–Danis reagent phenols identified by High Performance Liquid Chromatography (HPLC) was used. Also, a reverse phase C8 column was used then compared with a standard (Sigma chemicals).

Statistical analysis: For data analysis the statistical computer application package SPSS 10.0 was employed. The data generated were average of three independent experiments. Data were subjected to analysis of variance (ANOVA) and the means were compared for significance using Duncan's Multiple Range Test (DMRT; $P = 0.05$).

3. Results

The effects of MJ and PAs on BtMV infection in inoculated sugar beet. The experiments were performed to weigh up the effect of MJ and PAs on infection and presence of BtMV in sugar beet leaves through symptoms (Fig. 1); infectivity assay (Fig. 2A) and DAS-ELISA confirmation (Fig. 2B). Twenty five days after inoculation by BtMV, treated plants showed symptoms ranged from severe mosaic (Fig. 1C and D), (wheares L-Ornithine.Hydro and Pentamidine were sprayied) to healthy one (Fig, 1E), (wheares MJ was used). Treated sugar beet leaves with 1.5 µg/ml of MJ as a foliar spray, banned the emergence of

disease symptoms caused by BtMV. Furthermore, the number of local lesions which appeared on *C. amaranticolor* leaves after inoculation with sap extracted from treated-viral inoculated plants, not showed when MJ was used. But greatly decreased with PAs, when L-Ornithine, L-Ornithine.monohyd and Diminidine. On the other hand, there is no dissimilarity on local lesion numbers when sap extracted from L-Ornithine.Hydro and Pentamidine treated and non-treated plants. L-Ornithine at 1.5-µg/ml was the most effective polyamine tested compared with control. BtMV accumulation in MJ-treated and non-treated plants was evaluated using DAS-ELISA analyses. The results of ELISA represent the mean value for 5 samples in each treatment were showed in (Fig. 2B). When the mean ELISA absorbance values for those plants infected with BtMV was compared, MJ treatment showed lower values in treated plants. MJ treatment significantly reduced virus accumulation compared with the non treated plants. In addition, PAs treatment exhibited less effect on virus multiplication with that of the control one that showing mosaic symptom.

Figure 1. Effect of MJ and PAs treatments on symptoms induced by BtMV on sugar beet plants A: Diminidine; B: L-Ornithine; C: L-Ornithine Hydro; D: Pentamidine and E: MJ.

To examine whether MJ-mediated activation of the defense responses, different concentrations from 3 to 0.08 µg/ml concentrations were used and tested by DAS-ELISA (Fig. 3) . The mean values of DAS-ELISA were decreased gradually with increasing concentration of MJ. Greatest reduction and non significant results were achieved by using MJ as foliar spray at 3.0; 1.5; 0.75 and 0.35-µg/ml (Fig. 3).

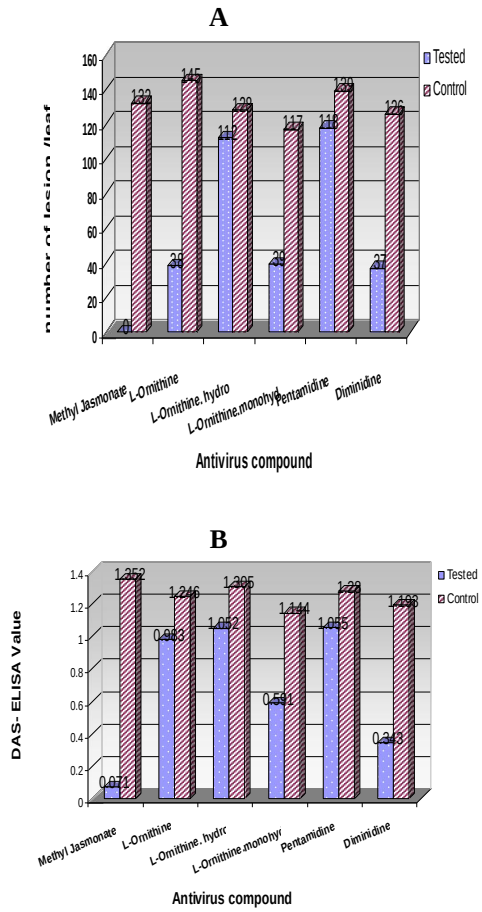

Figure 2. Survey of antiviral activity of MJ and PAs measured biologically in *C. amaranticolor* plant as local lesion numbers (A) or serologically by DAS-ELISA test (B)

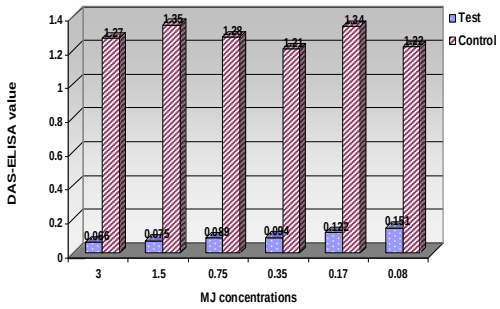

Figure 3. ELISA-vaues in inducer treated host plants challenge inoculated with BtMV and sprayed with methyl jasmonate concentrations

Western blotting for viral coat protein. The coat protein of the BtMV purified by SDS-PAGE was cross reacted specifically to the antiserum raised against the coat protein of the Egyptian isolate of BtMV, using western blotting analysis. One out of three MJ-treated plants negatively reacted (as negative

control) in the presence of the coat protein of BtMV isolate (Figure,4; lane 6). On the other hand, tow plants give faint reaction (Figure, 4; lanes 7 and 8) In this experiment BtMV-infected sugar beet and not treated with MJ was used as a positive control (Figure, 4).

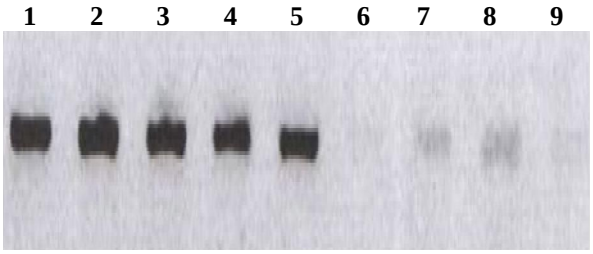

Figure 4 Effect of methyl jasmonate (MJ) treatment on accumulation of BtMV coat proteins in inoculated sugar beet tissue. Lanes 1-5: samples from MJ-untreted and BtMV-infected plants (as a positive control); Lanes 6-8: samples from BtMV-infected and then MJ-treated plants and Lane 9: sample from BtMV-uninfected and MJ-untreated plant (as a negative control).

Free and conjugated polyamines in plants treated with MJ: The relationship between MJ and the polyamines biosynthesis was then examined (Table 1). Data of polyamine levels showed significant changes in polyamine biosynthesis either MJ-treated and/or BtMV-inoculated leaves. Putrescine, spermidine and spermine were greatly decreased in leaves following inoculation with BtMV. On disparity, treated sugar beet leaves with MJ produced moderate significant effect on levels of free polyamines in compared with untreated control. Moreover, levels of conjugates of spermidine and spermine were significantly increased in leaves after exposure to MJ.

Activities of polyamine biosynthetic enzyme: ODC and PAO activities were determined in leaves of sugar beet following exposure of the leaves to MJ and BtMV-inoculated leaves (Table 2). Activity of both enzymes was decreased in leaves following treatment to BtMV. Very large and significant increases in activities of both ODC and PAL were found in leaves after treated with MJ and inoculated with BtMV in compared with untreated control.

Induction of resistance to BtMV in sugar beet by MJ. Protein patterns represent some newly synthesized polypeptides which reflect formation of pathogenesis related proteins in MJ treatment (Fig. 5). Induction of PR proteins by MJ was also confirmed at the protein level. Two bands were observed by MJ treatment. Large and significant increases in soluble protein activity were found in leaves of sugar beet following treatment of the leaves with MJ, with a 5-fold increase. A biochemical assay for Salicylic Acid (SA) revealed accumulation of SA in leaves treated with MJ and inoculated with BtMV (Fig. 6). To reveal the possible involvement of plant defense enzymes in MJ-induced protection against BtMV in sugar beet, the activities of chitinase, peroxidase and polyphenoloxidase were monitored (Table 3). Inoculation of the leaves with BtMV led to a decrease in enzymes activities. Activities of the plant defence-related enzymes chitinase, peroxidase and polyphenoloxidase were increased significantly in leaves following treatment leaves with MJ. Chitinase activity was significantly increased in leaves following treatment with MJ. Activity of peroxidase was greatly increased in treated leaves with MJ. Data also show that the polyphenoloxidase activity was similar to that of peroxidase activity. Polyphenoloxidase activity was greatly decreased in plants inoculated with BYMV in comparison with untreated control. The BtMV inoculated plants showed slightly significant difference in phenol contents compared with the healthy plants (Table 4). MJ at 3.0-µg/ml sprayed on sugar beet plants, resulted in an increase in conjugated phenols content in compared with free phenols. Since, the greatest increase in conjugated phenol contents were 39.1 Catechol /g/ /F.W. compared with free phenol 27.4 Catechol /g/ /F.W, inoculated control 8.9 and 11.4 Catechol /g/ /F.W and untreated control 10.3 and 11.4 Catechol /g/ /F.W., respectively (not clear).

Table 1. Concentrations of free and conjugated forms of polyamines in leaves of sugarbeet plants treated with different concentrations of methyl jasmonate and inoculated with BYMV under greenhouse conditions.

Methyl Jasmonate concentration (µg/ml)	Polyamine concentration[y] (nmol g^{-1} FW)					
	Putrescine		Spermidin		Spermine	
	Free	Conjugated	Free	Conjugated	Free	Conjugated
3.0	97.9a	351.2a	84.5a	253.3a	98.9a	298.5a
0.17	82.9b	319.6b	71.9b	229.4b	71.8b	251.7b
0.0	71.3c	276.5c	60.5c	141.2c	66.5c	201.2c
Inoculated	42.8d	121.7d	32.8d	89.4d	44.7d	121.9d

Every value represents the mean of three replicates with standard error and values with the different letters are significantly different according to Duncan's Multiple Range Test (P = 0.05).

Table 2. Concentrations of ornithine decarboxylase (ODC) and polyamine oxidase (PAO) in leaves of sugarbeet plants treated with different concentrations of methyl jasmonate and inoculated BYMV under greenhouse conditions.

Methyl Jasmonate concentration (µg/ml)	Enzymes activities	
	ODC activity[y] (nmol CO_2 [mg protein]$^{-1}$ h^{-1})	PAO activity (pmol product [mg protein]$^{-1}$ h^{-1})
3.0	39.2a	178.2a
0.17	21.5b	93.5b
0.00	12.5c	18.65c
Inoculated	5.87d	7.65d

Every value represents the mean of three replicates with standard error and values with the different letters are significantly different according to Duncan's Multiple Range Test (P = 0.05).

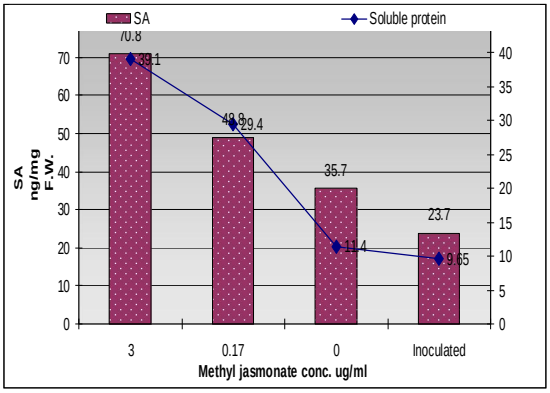

Figure 5.Concentrations of plant defense related protein, salicylic Acid (SA) in leaves of sugarbeet plants treated with different concentrations of methyl jasmonate and inoculated BtMV under greenhouse conditions.

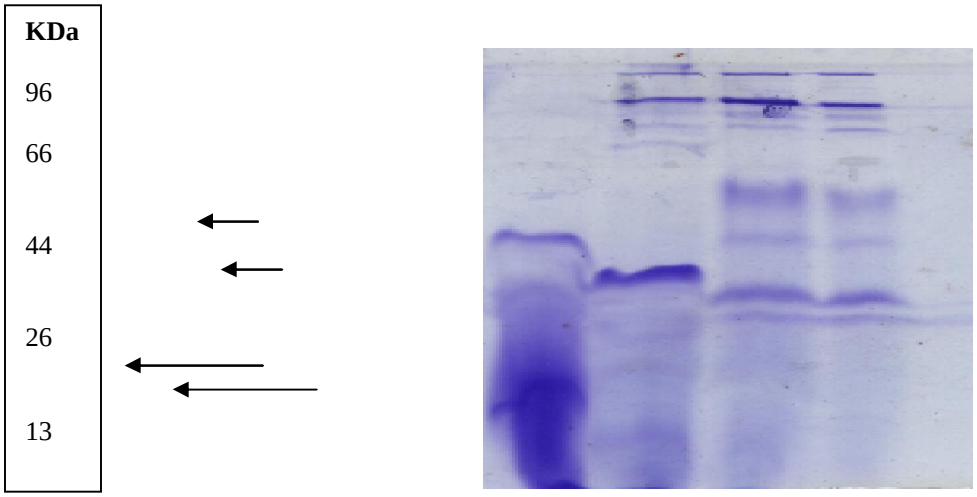

Figure 6. Methyl jasmonate induced virus resistance in sugar beet. Line 1: untreated control plants; Line 2: infected plants with BtMV; Line 3: Leaves treated with MJ at 3.0 µg/ml and inoculated with BtMV; Line 4: Leaves treated with MJ at 3.0 µg/ml only and M: molecular weight marker.

Table 3. Concentrations of chitinase and peroxidase in leaves of sugarbeet plants treated with different concentrations of methyl jasmonate and inoculated BYMV under greenhouse conditions.

Methyl Jasmonate concentration (µg/ml)	Chitinase (unit)	Peroxidase (unit)	Polyphenoloxidase (unit)
3.0	7.0a	31.8a	0.6a
0.17	6.0b	21.1b	0.5b
0.0	4.87c	9.40c	0.3c
Inoculated	3.21d	5.87d	0.2d

Every value Every value represents the mean of three replicates with standard error and values with the different letters are significantly different according to Duncan's Multiple Range Test (P = 0.05).

Table 4. Concentrations of Phenols and SA in leaves of sugarbeet plants treated with different concentrations of methyl jasmonate and inoculated BYMV under greenhouse conditions.

Methyl Jasmonate concentration (µg/ml)	Free Phenols (Catechol /g/ /F.W.)	(Catechol /g/ Conjugated phenols /F.W.)
3.0	27.4a	33.1a
0.17	25.2b	17.2b
0.00	14.2c	10.3c
Inoculated	11.4cd	8.9cd

Every value represents the mean of three replicates with standard error and values with the different letters are significantly different according to Duncan's Multiple Range Test (P = 0.05).

4. Discussion

Gained results accentuate that treatment with the MJ induces the resistance in the sugar beet plants against BtMV. Sprayed leaves of sugar beet with 1.5 µg/ml of MJ disallowed the appearance of disease symptoms caused by BtMV and reduced its concentration compared with control as apparent in the indirect ELISA and indicator plant tests. This conclusion was confirmed by diminution in the lesions numbers and DAS-ELISA test. The typical dominant resistance response is allied with several defense-related dealings, including rapid activation of polyamines, PR protein, and biosynthesis of SA, oxidative enzymes, and phenols contents. There is some degree of evidence that PAs boast a role in plant self-defense. When leaves were sprayed with MJ or PAs, and then inoculated with BtMV, lesions became much fewer in comparison with those of controls. This result might designate that treatment of host plants with MJ and PAs reduce viral commonness in comparison with controls. Results gained from independent pharmacological experiments resiliently indicate that polyamines donate to plant resistance. It is worthy to state that sugar beet leaves treated with MJ showed discernible increases in free and conjugated putrescine spermidine and spermine. The increase in soluble polyamine free and conjugates found in treated leaves confirm with our previous reports in wheat plants treated with MJ (Haggag, Wafaa and Abd-El-Kareem, 2009). Moreover, in the present work, MJ treatment of leaves led to increased activities of the polyamine biosynthetic enzymes ODC and of PAL activity. Both substrates enhancing for the formation of soluble polyamine free and conjugates were likely to have been increased in these tissues. The noticed increase in activities of the three polyamine biosynthetic enzymes support the conclusion of Biondi et al., 2000 reached in MJ-treated tobacco. All concentrations of MJ treatments increased PR protein and SA. Accumulations of PR-proteins and SA have been correlated with systemic resistance in plants. Previous findings show that SA treatment inhibit the replication, cell-to-cell movement, and long distance movement of plant viruses (Shekara et al., 2004; Madhusudhan et al., 2008). Moreover, results in this study indicated that all treatments motivated the enzymes activities. Several studies have verified that over-expression of chitinases, ß-1,3- glucanase , peroxidase and polyphenoloxidase in transgenic plants is always associated with superior resistance to various viral pathogens (Thomas et al., 1999). The results of our experiments showed a higher level of free and conjugated phenols was induced by the treatment with MJ, indicating their possible role in viral resistance. Interestingly, jasmonates are known to increase the formation of phenolic compounds by stimulating the phenylpropanoid pathway. In many cases, resistance is associated with increased expression of defense genes, including the pathogenesis related (PR) genes and the accumulation of SA in the inoculated leaf; localized host cell death at the site of pathogen entry, a

phenomenon known as the hypersensitive response (HR), also occurs (Shekara et al., 2004). Salicylic acid is an important module in the signal transduction pathway leading to systemic acquired resistance (SAR) to the entire spectrum of plant pathogens: bacteria, fungi, and viruses (Naylor et al., 1998). Results showed that MJ could inhibit the development of virus disease in plants in two ways: by inhibiting replication of the virus at the initial point of infection, or by stimulating PR protein, PAs and SA.

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