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Effects of Methyl Jasmonate on the Enzymatic Antioxidant Defense System in Maize Seedlings Subjected to Paraquat

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Abstract: In the present study the effects of different concentrations of methyl jasmonate on the enzymatic activity of antioxidant defense system in roots and shoots of a maize cultivar (SC 704) seedlings were investigated in two parts. In the first part, the plants were pretreated with 0, 5, 10, 20, 50 and 100 μM methyl jasmonate 24 hours before PQ application as an inducer of oxidative stress agent. In the second part, the 0, 5, 10, 20, 50 and 100 μM concentrations of methyl jasmonate were used after PQ imposed oxidative stress. Subsequently, the intensity of enzymatic activities in roots and shoots were separately measured. The data showed that induction of oxidative stress by PQ with respect to increased malondialdehyde levels has been completely executed. Reducing the levels of lipid peroxidation to the control level was accomplished by increasing the activities of glutathion reductase, guaiacol peroxidase and ascorbate peroxidase through changes in concentration and time of application of methyl jasmonate in roots and shoots. In this relation, the 50 and 100 μM concentrations of methyl jasmonate could decrease the lipid peroxidation with highest efficiency and meaningfully in roots and shoots as compared with controls.

Key words: Ascorbate peroxidase, glutathion reductase, guaiacol peroxidase, jasmonates, oxidative stress, paraquat

INTRODUCTION

Oxidative stress is a major damaging mediator in plants exposed to a variety of stresses such as drought[1,2], low temperature[3,4] and air pollutants such as ozone[5,6] or chemicals including paraquat[2,5]. Under these conditions, some Reactive Oxygen Species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen are generated[7]. A number of enzymes participate in protecting plants from oxidative damage[8,9]. Members of the enzymatic antioxidant defense system include superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11), phenolic peroxidases such as guaiacol peroxidase (GPX; EC 1.11.1.7) and the ascorbate/glutathione cycle that includes glutathione reductase (GR; EC 1.6.4.2). The superoxide radical (O_2^-) is dismutated to H_2O_2 by SOD and CAT, APX and GPX metabolize H_2O_2 to H_2O. APX requires reduced ascorbate and GPX requires a phenolic compound like guaiacol to function. GR functions in the regeneration of reduced ascorbate after it is converted to monodehydroascorbate by APX[10].

Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage[11,12]. There are many reports that bipyridylium herbicides such as paraquat, which are electron deficient, attract electrons from photosynthetic and respiratory electron transport chains[13,14] and generate superoxide radicals[8]. So paraquat can be used to induce oxidative lipid peroxidation[15]. Thus lipid peroxidation can be taken as an indicator of oxidative stress. Lipid peroxides are quantified by TBA test, which is easy to perform and allows the results to be conveniently expressed as TBARS[16].

The role of MeJA in protecting plants from various stresses has been reported for several plants[17-20]. These reports lead to the suggestion that Jasmonates could mediate the defense response to various environmental stresses. The aim of the present study was to examine the role of MeJA on oxidative stress resistance and on antioxidant enzyme activities in seedlings of maize subjected to oxidative stress. In addition, the effects of MeJA as a pre-treatment and post-treatment with different concentrations were investigated.

MATERIALS AND METHODS

Plant material: Maize (*Zea mays* L. genotype single cross 704) seeds were obtained from Iranian Agricultural Research Center in 2004. Seeds were washed with

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distilled water and sterilized with 5% sodium hypochlorite for 10 min. After washing with distilled water, seeds were incubated in 15 mL distilled H_2O in 15 cm Petri dishes at 25°C in darkness. After 4 days seedlings with 10 mm roots were used for all experiments. A part of them were used for PQ and MeJA treatments and enzyme assay in roots. The seedlings were planted in 15 cm Petri dishes (30 seedlings per dish). Half of them (Group 1) were incubated in 15 mL PQ solution (20 μM for 6 h) as an inducer of chemical stress and were maintained in this solution for 6 h. After rinsing with distilled water they were treated with MeJA solution of 0 (as a PQ control), 5, 10, 20, 50 and 100 μM concentrations for 24 h. The second group was first pre-treated with different concentration of MeJA as described above. After 24 h the roots were rinsed with H_2O and exposed to PQ (20 μM for 6 h). All treated seedlings (Group 1 and 2) were incubated in darkness, at 25°C. A water control (with 3 replications) was also used without application of PQ and/or MeJA. The second part of 4 days old seedlings were transferred to small vessels containing 500ml one-half-strength of Hogland's nutrient solution in a greenhouse with a controlled atmospheric temperature (35/25) and photoperiod (15 h light, 9 h dark) with a light intensity 150 μE m^{-2} s^{-1} PAR. After 2 weeks half of them were pre-treated in vessels with different concentrations of MeJA solution (0, 5, 10, 20, 50 and 100 μM) for 24 h and then rinsed and transferred to vessels containing 500 mL PQ (20 μM for 6 h). The other half of 2 weeks old seedlings, were first exposed to PQ (20 μM, 6 h) and then were treated with 0, 5, 10, 20, 50 and 100 μM concentrations of MeJA. One treatment with 3 replications was used as a water control without application of PQ and/or MeJA under the same conditions. Finally, samples (roots and leaves) were harvested, weighed and stored in liquid N_2 for analysis.

PQ induced lipid peroxidation: The extent of PQ damage was measured as lipid peroxidation by determination of MDA, which were measured using a TBARS reaction[21,22]. About 0.5 to 1.0 g of tissue was homogenized in 5 mL of 5% (w v^{-1}) trichloroacetic acid and the homogenate was centrifuged at 12,000 g for 15 min at room temperature. The supernatant was mixed with an equal volume of thiobarbitoric acid (0.5% in 20% (w v^{-1}) trichloroacetic acid) and the mixture was boiled for 25 min at 100°C followed by centrifugation for 5 min at 7,500 g to clarify the solution. Absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the A_{600}. MDA content was calculated using an extinction coefficient of 155 M^{-1} cm^{-1}. Values of MDA content were taken from measurement of three independent samples and SEs of means were calculated.

Preparation of enzyme extract: Root tips or leaf fragments were excised from the seedlings before any treatment (i.e. controls) or after treatment with PQ and MeJA. The 0.5 g F.W. was homogenized at 4°C in 3 mL of extraction buffer (0.05 M Tris-HCl buffer, PH 7.5, 3 mM $MgCl_2$, 1 mM EDTA and 1.5% w v^{-1} PVPP) with mortar and pestle. The extraction buffer used for the APX assay contained 0.2 mM ascorbate. The homogenate was centrifuged at 25000 g for 20 min and the supernatant was used as the crude extract for assay of antioxidant enzyme activity[10].

Enzyme assay: GR activity was assayed by measuring the decrease in absorbance at 334 nm due to the oxidation of NADPH[23]. The 1 mL reaction mixture contained 0.1 M Tris-HCl, pH 8.0, 1 mM EDTA, 0.1 mM NADPH, 1 mM GSSG and 50 μL enzyme extract at 30 °C.

GPX activity was determined according to Upadhyaya *et al.*[24]. The reaction mixture contained 2.5 mL of 50 mM phosphate buffer (pH 6.1), 1 mL of 1% hydrogen peroxide, 1 mL of 1% guaiacol and 20 μL enzyme extract. The increase in absorbance at 420 nm was followed for 1 min.

APX activity was determined according to the method of Chen and Asada[25] with minor modification. The 1 mL reaction mixture was composed of 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 0.5 mM ascorbate. 1.54 mM hydrogen peroxide and 50 μL enzyme extract. The oxidation of ascorbate was followed by the decrease in the absorbance at 240 nm.

Protein content was determined using method of Lowry[26]. The activity of all enzymes in treated samples (roots and leaves) was calculated per milligram of protein per minute and expressed as a percentage of the control. Relative specific activity was calculated as the mean of triplicate independent replications with ± SE of means.

RESULTS

The activation of GR, GPX and APX in root and shoot tissues in genotype 704 of corn under PQ-induced oxidative stress was investigated in two stages: treatment with MeJA before and after the induction of oxidative stress by PQ.

An increased level of MDA is an indication of the increased levels of reactive oxygen radicals such as superoxide and hydroxyl radicals. Increase in these

Table 1: Measurement of MDA in root samples which were treated with MeJA (0-100 μM, for 24 h) before or after application of PQ (20 μM, for 6 h)

Treatments	MDA (ng g^{-1} f.w.)
Control	0.735±0.056*a
PQ	0.967±0.067b
MeJA (5 μM)+PQ	0.974±0.011b
MeJA (10 μM)+PQ	0.964±0.022b
MeJA (20 μM)+PQ	0.930±0.021b
MeJA (50 μM)+PQ	0.952±0.049b
MeJA (100 μM)+PQ	0.911±0.032bc
PQ+MeJA (5 μM)	0.953±0.084b
PQ+MeJA (10 μM)	0.958±0.083b
PQ+MeJA (20 μM)	0.787±0.041c
PQ+MeJA (50 μM)	0.783±0.081c
PQ+MeJA (100 μM)	0.868±0.029b

*±SE of means

Table 2: Measurement of MDA in leaf samples which were treated with MeJA (0-100 μM, for 24 h) before or after application of PQ (20 μM, for 6 h)

Treatments	MDA (ng g^{-1} f.w.)
Control	1.218±0.067*a
PQ	1.402±0.049b
MeJA (5 μM)+PQ	1.389±0.051b
MeJA (10 μM)+PQ	1.389±0.028b
MeJA (20 μM)+PQ	1.368±0.033b
MeJA (50 μM)+PQ	1.358±0.050b
MeJA (100 μM)+PQ	1.192±0.018c
PQ+MeJA (5 μM)	1.338±0.035b
PQ+MeJA (10 μM)	1.321±0.062b
PQ+MeJA (20 μM)	1.210±0.025c
PQ+MeJA (50 μM)	1.171±0.087cd
PQ+MeJA (100 μM)	1.068±0.034 d

*±SE of means

Table 3: GR activity in root samples which were treated with MeJA (0-100 μM, for 24 h) before or after application of PQ (20 μM, for 6 h). Activity in treated samples was expressed as a percentage of activity from control

Treatments	Relative activity of GR (%)
Control	100.417±6.097*a
PQ	85.00±6.232b
MeJA (5 μM)+PQ	82.083±7.196b
MeJA (10 μM)+PQ	93.75±5.662b
MeJA (20 μM)+PQ	88.75±8.177b
MeJA (50 μM)+PQ	102.083±3.560bc
MeJA (100 μM)+PQ	106.667±5.033c
PQ+MeJA (5 μM)	85.168±6.409b
PQ+MeJA (10 μM)	89.557±5.481b
PQ+MeJA (20 μM)	87.026±6.171b
PQ+MeJA (50 μM)	105.853±4.149c
PQ+MeJA (100 μM)	98.251±5.062bc

*±SE of means

species, at the first stage, causes oxidation of membrane lipids. Therefore, in order to measure the intensity of oxidative stress MDA levels were measured (Table 1 and 2). As is shown the amounts of MDA in PQ treated plants increased meaningfully as compared with untreated controls.

Lipid peroxidation in roots: When MeJA is used for reduction of stress after PQ application, the levels of MDA clearly decreased in roots. The reduction of MDA

Table 4: GR activity in leaf samples which were treated with MeJA (0-100 μM, for 24 h) before or after application of PQ (20 μM, for 6 h). Activity in treated samples was expressed as a percentage of activity from control

Treatments	Relative activity of GR (%)
Control	100.001±3.847*a
PQ	93.734±5.209ab
MeJA (5 μM)+PQ	89.145±4.556b
MeJA (10 μM)+PQ	92.625±4.986b
MeJA (20 μM)+PQ	108.219±5.973c
MeJA (50 μM)+PQ	112.815±4.429c
MeJA (100 μM)+PQ	115.461±2.878c
PQ+MeJA (5 μM)	94.651±5.000b
PQ+MeJA (10 μM)	98.784±5.321b
PQ+MeJA (20 μM)	109.333±3.427c
PQ+MeJA (50 μM)	107.801±6.954bc
PQ+MeJA (100 μM)	125.907±5.428d

*±SE of means

Table 5: GPX activity in root samples which were treated with MeJA (0-100 μM, for 24 h) before or after application of PQ (20 μM, for 6 h). Activity in treated samples was expressed as a percentage of activity from control

Treatments	Relative activity of GPX (%)
Control	100.00±5.616*a
PQ	91.983±1.688b
MeJA (5 μM)+PQ	85.26±7.495bc
MeJA (10 μM)+PQ	91.69±9.106b
MeJA (20 μM)+PQ	98.03±5.374b
MeJA (50 μM)+PQ	124.88±10.893d
MeJA (100 μM)+PQ	81.83±6.491c
PQ+MeJA (5 μM)	98.734±6.972b
PQ+MeJA (10 μM)	116.878±7.173c
PQ+MeJA (20 μM)	126.582±6.424c
PQ+MeJA (50 μM)	107.173±8.330bc
PQ+MeJA (100 μM)	90.717±6.101b

*±SE of means

levels at 5 and 10 μM MeJA is not considerable but at higher concentrations (20 and 50 μM) is meaningful and brings the MDA levels to PQ- control levels. Thus, it can be deduced that MeJA reduces the stress induced lipid peroxidation. But it appears that MeJA at 100 μM is not effective in reducing stress level. Pretreatment of roots with MeJA, 24 h before PQ application did not cause considerable changes in PQ-induced lipid peroxidation and MDA levels (Table 1).

Lipid peroxidation in shoots: As shown in Table 2 the use of 20, 50 and 100 μM concentrations of MeJA after application of PQ caused a meaningful reduction of MDA levels in leaves. Whereas the reduction of MDA levels at 5 and 10 μM concentration of MeJA was not significant, the 100 μM concentration was most effective. In cases of MeJA treatment prior to PQ application, the results were different. Significant reduction of MDA levels by MeJA was shown only at 100 μM concentration and other concentrations did not have meaningful effects on MDA levels.

GR, GPX and APX activity in roots: The addition of PQ at 20 μM for 6 h caused a significant decrease in GR

Table 6: GPX activity in leaf samples which were treated with MeJA (0-100 μM, for 24 h) before or after application of PQ (20 μM, for 6 h). Activity in treated samples was expressed as a percentage of activity from control

Treatments	Relative activity of GPX (%)
Control	99.996±5.998*a
PQ	113.844±9.839ab
MeJA (5 μM)+PQ	125.355±6.546b
MeJA (10 μM)+PQ	118.565±5.770b
MeJA (20 μM)+PQ	123.049±8.538b
MeJA (50 μM)+PQ	126.743±7.766b
MeJA (100 μM)+PQ	117.190±4.121b
PQ+MeJA (5 μM)	111.000±7.986ab
PQ+MeJA (10 μM)	114.954±4.330b
PQ+MeJA (20 μM)	110.497±7.269ab
PQ+MeJA (50 μM)	128.488±4.145c
PQ+MeJA (100 μM)	129.651±6.003c

*±SE of means

Table 7: APX activity in root samples which were treated with MeJA (0-100 μM, for 24 h) before or after application of PQ (20 μM, for 6 h). Activity in treated samples was expressed as a percentage of activity from control

Treatments	Relative activity of APX (%)
Control	100.000±5.213*a
PQ	62.132±5.927b
MeJA (5 μM)+PQ	65.077±2.438b
MeJA (10 μM)+PQ	65.077±2.995b
MeJA (20 μM)+PQ	72.651±5.479bc
MeJA (50 μM)+PQ	75.175±2.598c
MeJA (100 μM)+PQ	76.016±2.535c
PQ+MeJA (5 μM)	72.089±6.056bc
PQ+MeJA (10 μM)	72.931±3.843bc
PQ+MeJA (20 μM)	80.785±5.994c
PQ+MeJA (50 μM)	84.712±4.031c
PQ+MeJA (100 μM)	72.230±6.080bc

*±SE of means

Table 8: APX activity in leaf samples which were treated with MeJA (0-100 μM, for 24 h) before or after application of PQ (20 μM, for 6 h). Activity in treated samples was expressed as a percentage of activity from control

Treatments	Relative activity of APX (%)
Control	100.000±5.752*a
PQ	62.545±4.780b
MeJA (5 μM)+PQ	58.929±5.495b
MeJA (10 μM)+PQ	60.159±4.106b
MeJA (20 μM)+PQ	60.014±6.374b
MeJA (50 μM)+PQ	68.908±2.893bc
MeJA (100 μM)+PQ	72.668±3.491c
PQ+MeJA (5 μM)	68.329±6.972b
PQ+MeJA (10 μM)	64.148±7.173b
PQ+MeJA (20 μM)	67.462±2.424b
PQ+MeJA (50 μM)	69.848±7.330b
PQ+MeJA (100 μM)	83.441±3.101c

*±SE of means

activity in the roots (Table 3). When the effects of MeJA (as a mediator for exhibition of enzymatic defense system) were examined at different concentrations before or after PQ treatment, low concentrations (5, 10 and 20 μM) did not have any effect. But high concentrations of MeJA (50 and 100 μM) caused a significant increase in GR activity when applied before or after PQ stress.

GPX activity was also decreased significantly by PQ induced oxidative stress, similar to GR activity. MeJA increased GPX activity when used after treatment with PQ at 10 and 20 μM concentrations. At other concentrations it did not have a meaningful effect. Using 50 μM concentration of MeJA prior to PQ application was effective in increasing the GPX activity, whereas other concentrations were not effective (Table 5).

APX activity was affected dramatically by PQ-induced oxidative stress and its activity decreased to 60% of control. All concentrations of MeJA before or after PQ treatment did not increase APX activity to control level (Table 7), except the 50 μM concentration, which significantly increased the activity of APX when it was used as a post-treatment.

GR, GPX and APX activity in shoots: When maize seedlings were subjected to PQ-induced oxidative stress, GR activity in leaves decreased slightly, which was not significant. The results were similar to activity of GR in MeJA treated leaves at 5 and 10 μM concentrations before or after PQ application. But MeJA treatments at 20, 50 and 100 μM-concentrations increased the activity of GR significantly at pre or post-treatments (Table 4).

In leaves, unlike the activities of other antioxidant enzymes, GPX activity increased when seedlings were subjected to PQ. MeJA treatments either pre- or post-PQ treatment did not have a meaningful effect, except at 50 and 100 μM concentrations post-PQ treatment, where it increased GPX activity meaningfully (Table 6).

In leaves PQ caused a meaningful decrease in APX activity and APX activity reached control level with increasing MeJA concentration when used as a post-treatment. This increase was significant when MeJA was used at 100 μM concentration. MeJA was not significantly effective at all when used as a pre-treatment (Table 8).

DISCUSSION

In this study the results showed that in the presence of different concentrations of MeJA there was an increase in the activities of the various antioxidant enzymes examined. These enzymes play key roles in the resistance of the maize seedlings, to PQ-induced oxidative stress. The capacity to maintain a high-level of activity of GR, GPX and APX in roots and shoots of maize seedlings treated by different concentrations of MeJA as a pre or post-treatment under PQ-induced stress conditions resulted in lower levels of H_2O_2 and MDA production. The present results show that the higher activities of GR, GPX and APX under PQ stress had a greater protective effect in high concentrations of MeJA (mostly, 50 and 100 μM) than in low concentrations of MeJA (mostly, 5, 10 and 20 μM), similar to previous suggestions in the case of hordeum plants[18] and cotton callus[7] that had been subjected to PQ and water stresses. Decrease in activity of APX could result in an increase in levels of H_2O_2 in leaf cells[27]. GR participates in the scavenging of hydrogen peroxide in chloroplasts and together with APX[8,28], may act to suppress hydrogen peroxide build up under stressful conditions.

In all of our experiments PQ alone at 20 μM concentration caused a reduction in activity of GR and APX. In contrast, although PQ decreased GPX activity in roots, it stimulated GPX activity in leaves. In roots GR activity, which is reduced by PQ treatment, gradually increased with high levels of MeJA (Table 3). This can be seen when MeJA is applied at 50 and 100 μM, before or after PQ treatment. It seems that MeJA plays a role in signal transduction pathway, at least in part under PQ-imposed oxidative stress[18] and other stresses[29-32].

In the case of leaves, GR activity increased only in seedlings that were subjected to 100 μM concentration of MeJA after application of PQ. The use of MeJA before exposure to oxidative stress will not increase the activity of GR significantly. This suggests that signal transduction efficiency by MeJA decreases when it moves to the leaves.

GPX activity in roots also decreased when PQ alone was given to corn roots at 20 μM concentration (Table 5). Here also, treatment of the samples with MeJA before PQ at 20 and 50 μM increased the GPX activity as compared to PQ control. But this activity decreased with higher concentration of MeJA (100 μM). Treatment of the seedlings with MeJA after exposure to PQ caused an increase of GPX activity at 10 and 20 μM concentrations (Table 5), whereas it decreased at 50 and 100 μM concentration. Therefore optimum concentration of MeJA for maximum GPX activity in roots not only is different from optimum concentration of MeJA for maximum GR activity, but it is also different from its optimum concentration for maximum GPX activity in leaves. Table 6 shows that increase of GPX activity occurred at 50 and 100 μM concentration under both conditions (pre and post-treatment of MeJA) significantly. It should be noted that PQ increased GPX activity only in leaves, in contrast to the other treatments. These results with corn leaves were in agreement with those observed in a study with hordeum leaves[18].

APX activity in roots decreased about 40% when samples were subjected to PQ (Table 7). Different concentrations of MeJA could restore the level of APX activity to higher than PQ-treated samples. But the only concentration, at which increase in APX activity was meaningful, was 50 μM when applied as a post-treatment. The same pattern was observed in leaves, but MeJA concentration which caused a meaningful increase was 100 μM, when applied as a post-treatment (Table 8). This result is in agreement with findings of Li and Staden[7] with maize callus. Finally, since treatment of samples with MeJA was done via roots, its optimal concentration for recovery effects in roots was lower than in shoots (generally 20 and 50 against 100 μM). This may be due to metabolization, compartmentation or decreased concentration of MeJA during translocation from roots to shoots.

Comparison of effects of MeJA on the enzymatic activities between treated samples before and after PQ application showed that there is a clear difference in optimal concentration of MeJA for reaching this effective concentration. It caused meaningful differences at concentrations of about 100 and 50 μM for treated samples with MeJA before and after PQ application respectively. This may be because the defense system is not activated prior to stress-inducing conditions. Finally, it is clear that the addition of MeJA at different concentrations before and after PQ treatment relieves the corn plants from oxidative stress[33-35] (Table 1 and 2). The relieving function of MeJA was especially out-standing at 50 and 100 μM treatments in our investigation.

The reason for the increase in antioxidant enzymes activity affected by this regulator is unknown. It is possible that the regulator-induced increases in the antioxidant enzyme activities observed in this study, may be due to an up-regulation of the genes controlling the synthesis of these enzymes or an increased activation of constitutive enzyme pools[7].

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