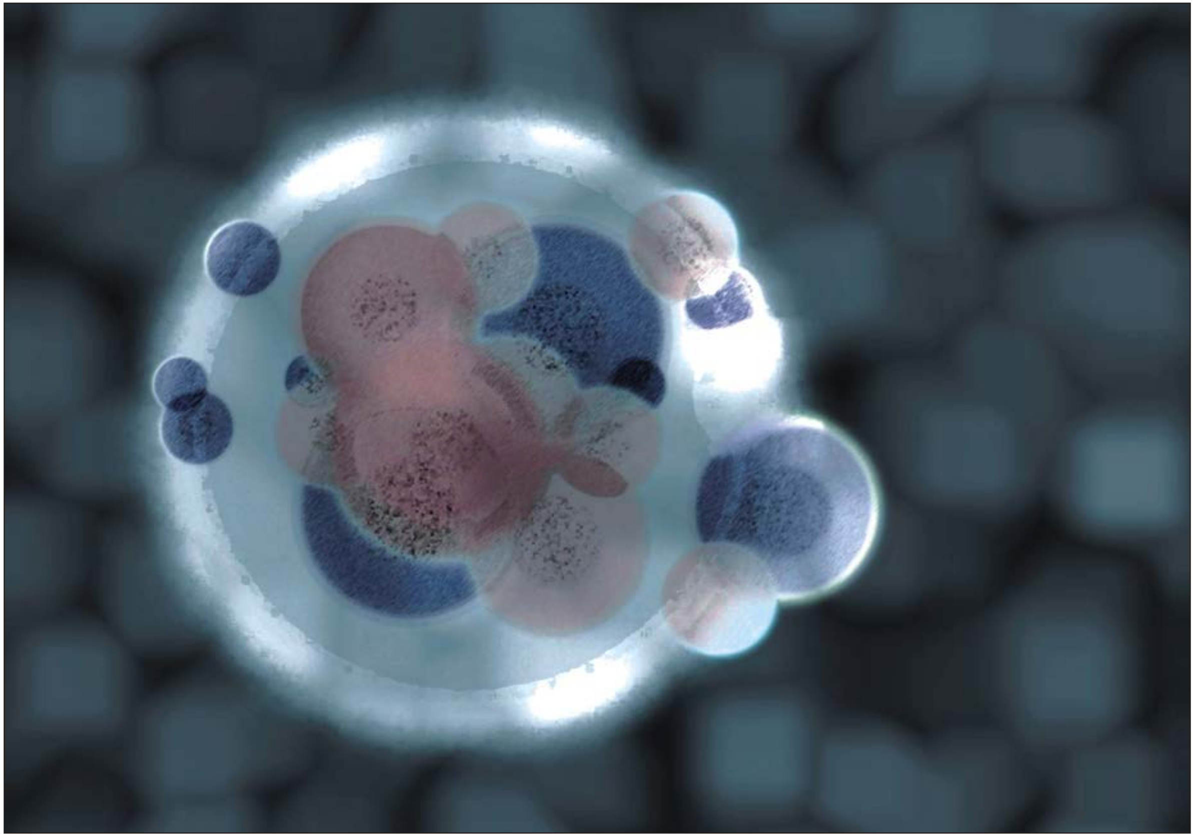

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A Possible Role for Methyl Jasmonate in Effecting Superoxide Dismutase and Catalase Activities under PQ-induced Oxidative Stress in Maize Seedlings

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Abstract: In the present study the effects of different concentrations of MeJA on the activities of SOD and CAT in roots and shoots of corn seedlings were studied in two parts. In the first part, the plants were pretreated with 0, 5, 10, 20, 50 and 100 µmol methyl jasmonate 24 h before PQ application as an inducer of oxidative stress agent. In the second part, the 0, 20, 50 and 100 µmol concentrations of MeJA were used after PQ imposed oxidative stress. Subsequently, the intensity of enzymatic activities in roots and shoots were separately measured. The certainty of oxidative stress imposition by PQ was investigated by measuring the MDA levels as a product of lipid peroxidation and indicator of oxidative stress. Reducing the levels of lipid peroxidation to the control level was accomplished by increasing the activity of SOD and CAT through changes in concentration and time of application of MeJA in roots and shoots. In this relation, the 50 and 100 µmol concentrations of MeJA could decrease the lipid peroxidation with highest efficiency and meaningfully in roots and shoots as compared with controls.

Key words: Jasmonates, oxidative stress, paraquat, superoxide dismutase, catalase

INTRODUCTION

When plants are under oxidative stress, reactive oxygen species (ROS) such as superoxide ($O_2^{\circ -}$) and hydroxyl (OH^-) radicals are produced in chloroplasts in the light[1-3] and in other subcellular compartments such as peroxisomes and mitochondria[4,5]. The oxidative stress damages the biomolecules as a result of the imbalance between the production of ROS and antioxidant defense systems[6]. Some enzymes such as superoxide dismutase (SOD; EC 1.15.1.1) and catalase (CAT; EC 1.11.1.6) participate in protecting plants from oxidative damage[7]. SODs are metaloenzymes that convert $O_2^{\circ -}$ to H_2O_2 in all aerobic as well as anaerobic organisms[8] Plants contain distinct SOD isozymes in the cytosol, mitochondria and chloroplasts[9,10], peroxisomes[11] and glyoxisomes[12]. Later, catalases use H_2O_2 as a substrate and convert it to H_2O and O_2. In leaf tissue, catalase is localized in peroxisomes[13] and a mitochondrial isozyme was found in maize[14].

There are many reports that bipyridylium herbicides such as paraquat (PQ), which are electron deficient, attract electrons from photosynthetic and respiratory electron transport chains[15,16] and generate superoxide radicals[17]. So paraquat can be used to induce chemical and oxidative stress[18]. The toxic superoxide radical causes extensive lipid peroxidation[19]. Thus lipid peroxidation can be taken as an indicator of oxidative stress.

Jasmonic Acid (JA) and it's methyl ester (MeJA) occur in many plant species and are involved in various physiological processes[20]. It has been reported that jasmonate functions as a signaling molecule[21] and is an inducer of defense reactions[22]. In recent years jasmonates have been the focus of much attention because of their ability to provide protection to salinity stress[23], UV irradiation[24], to increased freezing tolerance in bromegrass[25], or PQ-induced oxidative stress[26], leading to the suggestion that jasmonates can mediate the defense response to various environmental stresses. This study was undertaken to determine the response of SOD and CAT, which are considered two primary defense systems, to PQ-induced oxidative stress in maize plants; to evaluate the effects of MeJA treatment in reducing oxidative damage caused by PQ in maize.

MATERIALS AND METHODS

Plant material: Maize (*Zea mays* L. genotype single cross 704) seeds were obtained from Iranian agricultural research center (2004). Seeds were washed with 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

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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 $dish^{-1}$). Half of them (group 1) were incubated in 15 mL PQ solution (20 μmol 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 μmol 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 μmol for 6 h). All treated seedlings (groups 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 500 mL one-half-strength of Hogland's nutrient solution in a greenhouse with a controlled atmospheric day/night temperature of 35/25°C and photoperiod (15 h light, 9 h dark) with a light intensity of 150 μE m^{-2} s^{-1} PAR. After 2 weeks, half of them were pre-treated in vessels with different concentrations of MeJA (0, 20, 50 and 100 μmol) for 24 h and then rinsed and transferred to vessels containing 500 mL PQ (20 μmol for 6 h). The other half of 2 weeks old seedlings, were first exposed to PQ (20 μmol, 6 h) and then were treated with 0, 20, 50 and 100 μmol 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[27,28]. About 0.5 to 1.0 g of tissue was homogenized in 5 mL of 5% (w/v) 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] 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 PVPP) with mortar and pestle. 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[29].

Enzyme assay: SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of NBT using the method of Dhindsa *et al.*[30]. The 3 mL reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μmol NBT, 2 μmol riboflavin, 0.1 mM EDTA and 0 or 50 μL-enzyme extract. Riboflavin was added last and the tubes were shaken and placed 30 cm below a light bank consisting of two 15 W fluorescent lamps for 10 min. The absorbance of the reaction mixture was read at 560 nm.

CAT activity was assayed by measuring the rate of disappearance of hydrogen peroxide using the method of Maehly and Chance[31]. The reaction mixture contained 2.5 mL of 50 mM phosphate buffer (pH 7.4), 0.1 mL of 1% hydrogen peroxide and 50 mM enzyme extract diluted to keep measurement within the linear range of the analysis. The decrease in hydrogen peroxide was followed as a decline in absorbance at 240 nm.

Activity in treated samples (roots and leaves) was expressed as a percentage of the activity of untreated controls. Relative activity was calculated as the means of triplicate independent replications with±SE of means.

RESULTS

The activation of SOD and CAT in root and shoot tissues in genotype 704 of corn under PQ induced oxidative stress was investigated in two stages: treatment with MeJA before or after the induction of oxidative stress with PQ.

An increased level of MDA is an indication of the increased levels of reactive oxygen radicals such as superoxide ($O_2^{\circ -}$) and hydroxyl (OH°) radicals. Increase in these 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.

Table 1: Mean±SE for content of MDA in root samples which were treated with MeJA (0-100 μmol, for 24 h) after or before application of PQ (20 μmol, for 6 h).

Treatments	MDA (ng/g f.w.)
Control	0.73±0.06a
PQ	0.97±0.07b
PQ + MeJA (5 μmol)	0.95±0.08b
PQ + MeJA (10 μmol)	0.96±0.12b
PQ + MeJA (20 μmol)	0.78±0.04c
PQ + MeJA (50 μmol)	0.78±0.02c
PQ + MeJA (100 μmol)	0.87±0.03b
MeJA (5 μmol) + PQ	0.97±0.01b
MeJA (10 μmol) + PQ	0.96±0.02b
MeJA (20 μmol) + PQ	0.93±0.02b
MeJA (50 μmol) + PQ	0.95±0.05b
MeJA (100 μmol) + PQ	0.91±0.03b

Table 2: Mean±SE for content of MDA in leaf samples which were treated with MeJA (0-100 μmol, for 24 h) after or before application of PQ (20 μmol, for 6 h).

Treatments	MDA (ng/g f.w.)
Control	1.21±0.06a
PQ	1.40±0.05b
PQ + MeJA (20 μmol)	1.36±0.02b
PQ + MeJA (50 μmol)	1.35±0.08ab
PQ + MeJA (100 μmol)	1.19±0.03a
MeJA (20 μmol) + PQ	1.21±0.03a
MeJA (50 μmol) + PQ	1.17±0.05a
MeJA (100 μmol) + PQ	1.06±0.02c

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

Treatments	Relative activity of CAT (%)
Control	100.00±5.21a
PQ	75.63±5.92b
PQ + MeJA (5 μmol)	77.66±2.43b
PQ + MeJA (10 μmol)	75.62±2.99b
PQ + MeJA (20 μmol)	67.81±5.47b
PQ + MeJA (50 μmol)	92.73±2.59a
PQ + MeJA (100 μmol)	59.35±2.53c
MeJA (5 μmol) + PQ	70.43±10.05b
MeJA (10 μmol) + PQ	68.50±11.84b
MeJA (20 μmol) + PQ	84.39±5.99b
MeJA (50 μmol) + PQ	84.84±7.53b
MeJA (100 μmol) + PQ	111.29±1.80c

Table 4: Mean±SE for SOD activity in root samples which were treated with MeJA (0-100 μmol for 24 h) after or before application of PQ (20 μmol for 6 h). Activity in treated samples was expressed as a percentage of activity from control.

Treatments	Relative activity of SOD (%)
Control	99.99±8.22a
PQ	79.99±7.37b
PQ + MeJA (5 μmol)	99.99±10.99a
PQ + MeJA (10 μmol)	98.94±10.99a
PQ + MeJA (20 μmol)	111.57±9.36a
PQ + MeJA (50 μmol)	116.83±7.29a
PQ + MeJA (100 μmol)	108.94±7.59a
MeJA (5 μmol) + PQ	85.16±8.40b
MeJA (10 μmol) + PQ	78.90±14.54b
MeJA (20 μmol) + PQ	81.25±14.40b
MeJA (50 μmol) + PQ	109.37±16.92ab
MeJA (100 μmol) + PQ	106.64±19.18ab

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

Treatments	Relative activity of CAT (%)
Control	100.00±13.7a
PQ	91.50±6.9a
PQ + MeJA (20 μmol)	101.40±8.8a
PQ + MeJA (50 μmol)	97.00±8.6a
PQ + MeJA (100 μmol)	122.70±7.2b
MeJA (20 μmol) + PQ	103.80±3.8a
MeJA (50 μmol) + PQ	93.00±9.3a
MeJA (100 μmol) +PQ	101.10±11.2a

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

Treatments	Relative activity of SOD (%)
Control	100.000±4.652a
PQ	88.528±4.220b
PQ + MeJA (20 μmol)	91.014±4.757b
PQ + MeJA (50 μmol)	84.895±2.014b
PQ + MeJA (100 μmol)	112.429±5.098c
MeJA (20 μmol) + PQ	84.704±5.711b
MeJA (50 μmol) + PQ	92.161±3.693b
MeJA (100 μmol) +PQ	98.662±2.014ab

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 levels at 5 and 10 μmol MeJA is not considerable but at higher concentrations (20 and 50 μmol) 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 μmol is not effective in reducing stress level and is acting as an inhibitor.

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: Use of 20, 50 and 100 μmol concentrations of MeJA after application of PQ caused a meaningful reduction of MDA levels in leaves and the 100 μmol concentration was most effective (Table 2). 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 μmol-concentration and other concentrations did not have meaningful effects on MDA levels.

CAT and SOD activity in roots: The activity of CAT in roots decreased when the plants were treated only with PQ (Table 3). On the other hand MeJA was not effective at 5-20 μmol concentrations when applied after PQ

induced oxidative stress. But, MeJA at 50 μmol increased the activity of CAT meaningfully just about to control level. Like to MDA results, here also, 100 μmol MeJA decreased CAT activity meaningfully as compared to untreated controls, suggesting the inhibitory effect of MeJA at 100 μmol or above. This reveals the threshold concentrations of MeJA for it's inhibitory action to cellular metabolism (Table 3). Treating the plants with MeJA before PQ application was not effective in increasing the CAT activity at 5 to 10 μmol concentrations. But CAT activity started to increase from 20 μmol and interestingly it reached the highest level at 100 μmol concentration of MeJA (Table 3).

The activity of SOD like CAT decreased when PQ was applied alone and induced oxidative stress (Table 4). MeJA increased the SOD activity significantly when applied 24 h after PQ application. But, when MeJA applied 24 h before PQ, only 50 and 100 μmol concentrations were effective in increasing the SOD activity to PQ control levels.

CAT and SOD activities in shoots: Similar to roots, in leaf tissues also the amount of MDA increased in response to PQ induced lipid peroxidation, which is an indication of oxidative stress (Table 2). MeJA when applied before PQ application, decreased the extent of MDA level meaningfully at 50 and 100 μmol concentrations, but when applied after PQ treatment, it decreased the MDA to PQ control level only at 100 μmol (Table 2). In shoot tissues, MeJA showed greater ability in protecting the plants against PQ oxidative stress. Our data shows that PQ application at 20 μmol concentrations from the root media did not decrease CAT activity significantly in leaves (Table 5). On the other hand, application of MeJA before PQ treatment did not change the pattern of CAT activity at 20 and 50 μmol concentrations, but MeJA at 100 μmol increased it's activity to some extent, when applied after PQ treatment (Table 5).

Measuring the activity of SOD in leaf tissues revealed that it decreased meaningfully in leaves when PQ is applied singly (Table 6). SOD activity increases at 20 and 50 μmol MeJA slightly and is increased very drastically at 100 μmol concentrations, when it is applied after PQ induction of oxidative stress (Table 6). When MeJA is applied before PQ, it gives a very good protection at 50 and 100 μmol and rescues the corn from oxidative damage.

DISCUSSION

Environmental stresses such as PQ are responsible for oxidative stress in roots and shoots, which causes an increase in reactive oxygen species[15] and is followed by changes in activity or concentration of antioxidant enzymes as a part of plant defense mechanism. These changes for all ROS scavenging enzymes and their isoforms in different cell compartments are not equal[18, 32].

Exposure of corn roots to 20 μmol solution of PQ alone was not lethal, but imposed an oxidative stress on it. This is due to the production of PQ free radicals which in turn are autooxidized to hydrogen peroxide (H_2O_2), superoxide ($O_2^{\circ-}$) and finally to hydroxyl radicals (OH°) and singlet oxygen ($^1O_2$)(15). We demonstrated that PQ induced oxidative stress by measuring the increased MDA levels in roots and leaves of corn (Table 1 and 2). Our data coincide with the finding of Choi *et al.*[33] and Babbs *et al.*[19].

In all of our experiments, PQ alone at 20 μmol concentrations caused a reduction in activity of SOD and CAT. Due to reduced activities of these enzymes, lipid peroxidation in PQ treated plants was higher than control plants. The higher production of MDA (a lipid peroxidation product that, has been used widely to assess the levels of free radicals in living cells,[5] is caused by lower protection of antioxidant enzymes[6,18,34], including SOD and CAT due to their lower scavenging activities. In root tissues PQ-induced oxidative damage via lipid peroxidation was two-fold that of shoot tissues (31% against 15%). This finding was probably due to rate of translocation of PQ from root to shoot[15].

In roots CAT activity, which is reduced at single PQ treatments, gradually increases with increased levels of MeJA (Table 3). It seems that MeJA plays a role in signal transduction pathway, at least partially under PQ imposed oxidative stress[26] and other stresses[21,35-37]. Interestingly, when MeJA was used before PQ application, at 100 μmol levels, not only did not increase the CAT activity but also it became inhibitory and decreased its activity. This suggests that, signal transduction by MeJA occurs around 50 μmol and becomes inhibitory at concentrations above 100 μmol.

SOD activity in roots also decreased when PQ alone was given to corn roots at 20 μmol concentration (Table 4). Here also, MeJA treatments before and after PQ increased the SOD activity to control level; suggesting that MeJA is involved in signal transduction and gene expression[38-40].

Investigation of enzyme activities in shoot tissues under two conditions (use of MeJA before and after of PQ application) showed that SOD activity decreased slightly (27 and 12% in optimal concentrations) in comparison to root tissues (46 and 42% in optimal concentrations). But, CAT activity in shoot tissues decreased only when MeJA was used before application of PQ (non significant against 47% increase in optimal concentrations) whereas

it increased when MeJA was used after PQ application (34% against 22% in optimal concentration) in comparison to root tissues. Addition of MeJA to shoot tissue before and after PQ treatment easily increased the SOD and CAT activities relative to PQ control levels (Table 5 and 6). This also suggests the involvement of MeJA in signal transduction and gene expression in leaf tissue under oxidative stress[21,36,41].

Since the treatment of samples with MeJA was done via roots, its optimal concentration for recovery effects in roots was lower than in shoots (50 against 100 μmol). This may be due to metabolization, compartmentation or decreased concentration during translocation from roots to shoots.

On the other hand, 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 about 100 and 50 μmol 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 (Table 1 and 2)[23-25]. The relieving function of MeJA was especially out-standing at 50 and 100 μmol treatments in our investigation.

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