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Methyl Jasmonate Reduces Water Stress in Strawberry

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Abstract. The effect of methyl jasmonate (MJ) on changes of oxygen-scavenging enzyme activities and membrane lipid composition was studied in strawberry leaves under water stress. Under water stress, MJ treatment reduced the increase of peroxidase (EC 1.11.1.7; POD) activity, maintained higher catalase (EC 1.11.1.6; CAT) and superoxide dismutase (EC 1.15.1.1; SOD) activities, and ascorbic acid content. In addition, MJ treatment reduced transpiration and membrane-lipid peroxidation as expressed by malondialdehyde (MDA) content, lessened the reduction of membrane lipids, glycolipids [monogalactosyl diglyceride (MGDG), digalactosyl diglyceride (DGDG)], and phospholipids [phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylinositol (PI)]. In water-deficit conditions, MJ treatment also alleviated the decline in the degree of fatty acid unsaturation and the ratio of linolenic (18:3) to linoleic acid (18:2). These results indicate that MJ treatment appears to alter the metabolism of strawberry plants rendering the tissue better able to withstand water stress.

Key Words. *Fragaria vesca*—Methyl jasmonate—Membrane lipids—Oxygen scavenging enzymes—Water stress

Jasmonic acid (JA), 3-oxo-2-(2-cis-pentenyl cyclopentane-1-acetic acid), and its methyl ester (methyl jasmonate, MJ) are naturally occurring plant growth regulators, affecting a great diversity of physiological and biochemical processes. In general, JA/MJ inhibit stomatal opening, cell division, plant growth, photosynthetic activities, flower bud formation, seed germination, and embryogenesis. In contrast, JA/MJ also has enhanced induction/promotion of leaf senescence and petiole abscission, fruit ripening, chlorophyll degradation, carotenoid biosynthesis, tuber formation, and protein synthesis (Creelman and Mullet 1997, Davies et al. 1986, Koda 1992, Sembdner and Parthier 1993, Yamane et al. 1981). Amelioration of chilling injury and osmotic stresses by JA/MJ have also been reported on rice, peanut seedlings, and cucumbers (Lee et al. 1996, Pan and Gu, 1995, Wang and Buta 1994).

Water deficit is one of the main environmental factors limiting plant growth and crop production. Drought causes visible injury to leaves and induces stomatal closure, leaf rolling, and osmotic adjustment. Water stress also accelerates the decline in chlorophyll and protein content, negatively alters both the structure and function of membranes, and increases the activities of active oxygen scavenging enzymes (Baisak et al. 1994, Navari-Izzo et al. 1989). A substantial number of drought effects on plants can be mimicked by external application of abscisic acid (Davies et al. 1986). Jasmonates are biologically similar to abscisic acid and, when exogenously applied to plants, elicit a great variety of morphological and physiological responses to stress (Creelman and Mullet 1997, Davies et al. 1986, Koda, 1992, Lee et al. 1996, Pan and Gu 1995, Wang and Buta 1994, Yamane et al. 1981). This study was undertaken to examine the effect of MJ on changes in transpiration, oxygen scavenging enzyme activity, ascorbic acid content, and membrane lipids in strawberry leaves subjected to water stress.

Materials and Methods

Plant Material and Jasmonate Treatments

Plants of *Fragaria vesca* cv. "EMb" were propagated by runner cuttings and were 3 months old with five expanded leaves when the experiments were started. The plants were grown in plastic pots (6.5 ×

Abbreviations: CAT, catalase; DGDG, digalactosyl diglyceride; MDA, malonaldehyde; MGDG, monogalactosyl diglyceride; MJ, methyl jasmonate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; POD, peroxidase; SOD, superoxide dismutase.

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8.25 cm; E. C. Geiger, Inc., Harleysville, PA) containing Pro-Mix BX (Premier Brands, Inc., Stamford, CT) in a greenhouse. Radiation sources in the greenhouse consisted of natural daylight and incandescent lamps that provided a PAR level of about 400 to 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 14 h/day (6AM–8PM). Day/night temperatures were about 25°C/20°C. All plants were watered daily and fertilized biweekly with a Peters nutrient solution (20-20-20, N-P-K). Methyl (±) jasmonate (Aldrich, Milwaukee, WI) was applied as a foliage spray to runoff.

Measuring the Effect of MJ on Transpiration of Strawberry Plants

MJ at 0.01 or 0.1 mM plus 0.05% Tween-20 was applied to the foliage at the beginning (day 0), the second, the fourth, and the sixth days of the experiments. On the first, third, fifth, and seventh days, a subset of 10 MJ-treated plants was removed from the pots, and the roots were cleaned of soil. The plants were then placed in 20-mL deionized H_2O in test tubes (18 × 150 mm) (one plant per tube). To reduce water loss, the test tube openings were sealed with parafilm. The amount of water loss (mL) from plants during 24 h was measured. On termination of the experiment, the fresh weight of foliage was determined and transpiration was expressed as a function of foliage fresh weight.

Determining the Effect of MJ on Changes in Oxygen Scavenging Enzyme Activity, Ascorbic Acid, MDA, and Membrane Lipid Content in Relation to Water Stress in Strawberry Leaves

MJ at 0.1 mM plus 0.05% Tween-20 was applied to the foliage at 2-day intervals for 8 days. Leaves were collected after 8 days. The technique used for inducing water stress was described by Apelbaum and Yang (1981). Batches of excised fully expanded leaves from control and MJ-treated plants were spread out on sheets of filter paper and allowed to wilt in a growth chamber (22°C) at 60% RH and about 100 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photosynthetic photon flux (PPF) provided by 20-watt cool white fluorescent lamps. When the fresh weight loss in the leaves reached 15% of their original weight, the leaves were collected and used for the measurement of enzyme activity, ascorbic acid, lipid peroxidation (MDA content), and membrane lipid content.

Catalase (CAT). Strawberry leaf tissue (1.0 g original fresh weight) was homogenized in 10 mL Tris-HCl buffer (pH 8.5) containing 5 mM dithiothreitol, 10% w/v polyvinylpolypyrrolidone (PVP), and 0.5 mM phenylmethylsulfonyl fluoride (PMSF) (PMSF added just before extraction) in chilled mortars and pestles. The homogenate was passed through four layers of miracloth and centrifuged at 20,000 × *g* for 30 min at 4°C, and CAT activity of the supernatant was determined by the floating disk method (Nir et al. 1986). Ten microliters of each crude enzyme extract was placed into 6-mm disks excised from Whatman 3-mm chromatographic paper, which was then placed in a vial containing 5 mL of 30 mM H_2O_2 at 25°C. The elapsed time for the disks to float from the bottom to the top of the solution was determined with a stopwatch. Ten to 20 replicates of individual disks were used for each crude extract. The relative CAT activity was compared with a bovine-liver CAT standard. One unit of CAT activity was equal to 1 μmol of H_2O_2 degradation per min at 25°C.

Peroxidase (POD). Strawberry leaf tissue (1.0 g original fresh weight) was homogenized using chilled mortars and pestles in 5-mL extraction medium consisting of 0.1 M phosphate buffer (pH 6.1), 0.1 mM EDTA-Na_2, and 10% (w/v) insoluble polyvinylpyrrolidone. The homogenate was filtered through four layers of miracloth, adjusted to 10 mL with extraction medium, and centrifuged at 12,000 × *g* for 10 min at 4°C. The extracted supernatant was used for the POD assay. The assay mixture contained 0.1 M phosphate buffer (pH 6.1), 4 mM guaiacol as donor, 3 mM H_2O_2 as substrate, and 0.1 mL crude enzyme extract. The total reaction volume was 3.0 mL. The rate of change in absorbance at 420 nm was measured with a Shimadzu UV-160A spectrophotometer (Columbia, MD), and the level of enzyme activity was expressed as the difference in absorbance (OD) per milligram protein per minute.

Superoxide Dismutase (SOD). Strawberry leaf tissue was immediately pulverized with a cold mortar and pestle with 0.1 M Na-phosphate buffer (pH 7.8) containing 0.1 mM EDTA-Na_2, and 0.5% (w/v) insoluble polyvinyl pyrrolidone. The ratio of the leaf tissue to the homogenizing medium was 1:10 (w/v). The homogenate was strained through four layers of miracloth and centrifuged at 15,000 × *g* for 20 min at 0°C. The supernatant was purified by gel filtration using Sephadex G-25 (Pharmacia, Piscataway, NJ) that had been equilibrated with extraction buffer. Naturally occurring reductants or antioxidants with low molecular weight (Lee et al. 1986) were depleted by passing the extract through a Centricon 10 molecular filter (Amicon, Danvers, MA) before assay of the SOD enzyme activity.

Total SOD activity was assayed photochemically (Giannopolitis and Reis 1977, Monk et al. 1987) on the basis of the photoreduction of nitro blue tetrazolium by light in the presence of riboflavin and methionine. Nitro blue tetrazolium is reduced to blue diformazan, which has a strong absorbance at 560 nm. Under aerobic assay conditions, SOD inhibits the formation of blue diformazan. Dicoumarol was included in the reaction to inhibit reduction by pyridine nucleotide (Thayer 1990) and to obtain a completely O^-_2-dependent reduction of nitro blue tetrazolium. The reaction mixture (1 mL) contained 100 μM dicoumarol, 1.3 μM riboflavin, 13 mM methionine, 0.05 M Na_2CO_3, 0.01 M Na-phosphate buffer (pH 7.8), and 0.1 mL of the enzyme extract; after 3 min, 68 μM nitro blue tetrazolium was added. The mixtures were illuminated by a fluorescent lamp (PPF was about 170 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 3 min. Identical solutions kept in darkness served as blanks. One unit of SOD was defined as the amount of enzyme required to produce a 50% inhibition of nitro blue tetrazolium reduction under assay conditions (Beauchamp and Fridovich 1973). Because inhibition is not linearly correlated with SOD concentration, a V/v transformation was used to obtain linearity (V = basic reaction rate without strawberry leaf extract, v = reaction rate with extract). Linear correlation gave the equation: SOD units/mL = 0.532 V/v−0.197) × dilution factor. The correlation coefficient for the line was 0.988.

Protein. Protein was determined according to Bradford (1976) with bovine serum albumin as a standard.

Ascorbic Acid. One gram of leaf tissue was homogenized in 4 mL of cold 5% (w/v) trichloroacetic acid containing 80 mg polyclar AT and 400 mg sea sand. The homogenate was filtered using four layers of miracloth and centrifuged at 16,000 × *g* for 10 min at 4°C. The supernatant was used for the ascorbic acid assay after the procedure described by Arakawa et al. (1981). This assay is based on the reduction of ferric ion to ferrous ion with ascorbic acid in acid solution followed

by the formation of red chelate between ferrous ion and 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) that absorbs at 534 nm.

Extraction, Fraction, and Analysis of Polar Lipids. Methods for the extraction, purification, fractionation and analysis of lipids were described previously (Wang and Faust 1989). After purification, lipids were concentrated under N_2 and then dissolved in 1 mL $CHCl_3$. One tenth of this solution was used for determining lipid peroxidation by measuring the formation of malonaldehyde (MDA)(Ottolenghi 1959). The other parts of purified lipid were separated into neutral and polar lipid (glyco-and phospholipid) fractions by silicic acid chromatography on 100–200 mesh Bio Sil A (Bio Rad Laboratories, Richmond, CA).

Total glycolipids and phospholipids in the polar lipid fraction were determined by the spectrophotometric assays of Roughan and Batt (1968) and Ames (1966), respectively. The glycolipid and phospholipid fractions were further separated by thin-layer chromatography on 20 × 20-cm glass plates precoated with 250-μm-thick silica gel (EM Reagents, Darmstadt, Germany) using acetone: acetic acid: water (100:2:1, v/v/v). Individual glycolipids and phospholipids were identified by co-chromatography with authentic standards (Sigma Chemical Co., St. Louis, MO; Supelco, Bellefonte, PA) and by detection with spray reagents specific for hexose sugars or phosphate. Individual lipid bands were scraped and eluted in chloroform: methanol (2:1, v/v). Total fatty acids were derivatized for flame ionization detection-gas chromatography analysis (Wang and Faust 1989). *n*-Heptadecanoic acid was included in all samples as an internal standard, and methyl heptadecanoate was used as an external standard. Individual fatty acid methyl esters were identified by comparison of retention times with those of authentic standards (Supelco). This tentative identification of major polar lipid fatty acids was corroborated by further analysis of the fatty acid methyl esters by gas chromatography-mass spectrometry (Wang et al. 1988).

Statistical Analysis

The variables were analyzed as two-factor general linear models using Proc Mixed (SAS Institute Inc, 1997) with Treatment × Day as the factors for Fig. 1 and with Stress × MJ for Figs. 2 through 8 and Tables 1 and 2. The means were compared using pair-wise contrasts. In Fig. 6, treatments were grouped into similar variance groups before analysis to correct variance heterogeneity. Treatments with different letters were significantly different at $p \leq 0.01$ or 0.05.

Results

Reduction of Transpiration

Foliar application of MJ reduced transpiration in strawberry plants (Fig. 1). The day × MJ treatment interaction for reduced transpiration in strawberry plants (expressed as a function of foliage fresh weight) was statistically significant ($p \leq 0.001$). Control plants lost about 8 mL of H_2O per gram fresh weight per day, whereas plants treated with 0.01 mM MJ lost only 6.48, 5.17, 3.70, and 2.98 mL H_2O per gram fresh weight per day after 1, 3, 5, and 7 days of MJ treatment, respectively. Higher MJ (0.1 mM) treatments further reduced water loss in plants (Fig. 1). Expressed on the basis of leaf area, MJ-treated plants also showed reduced transpiration (data not shown).

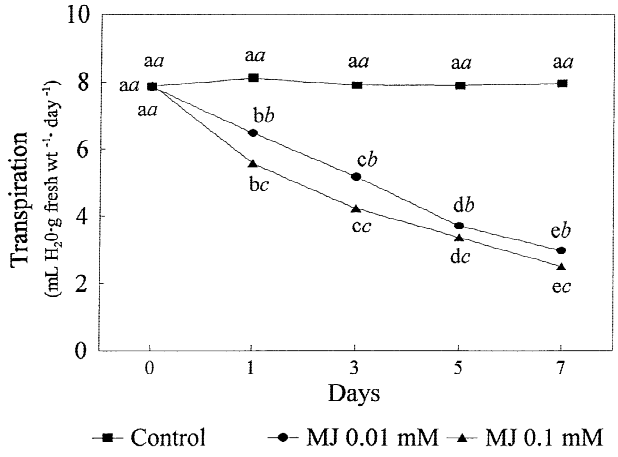

Fig. 1. Effect of MJ on transpiration of strawberry (*Fragaria vesca* cv. EMb) plants. Expressed as mL $H_20 \cdot$ g fresh wt $^{-1} \cdot$ day $^{-1}$. MJ at 0.01 or 0.1 mM plus 0.05% Tween-20 was applied to the foliage at the beginning (day 0), the second, the fourth, and the sixth days. On the first, third, fifth, and seventh days, a subset of 10 MJ-treated plants were removed from pots, and roots were cleaned of soil. The plants were then placed in 20-mL deionized H_2O in test tubes (one plant per tube). To prevent water loss the mouths of the test tubes were sealed around the plants with parafilm. The amount of water loss (mL) during 24 h was measured. On termination of the experiment, fresh weight of the foliage was determined and transpiration was expressed as a function of foliage fresh weight. Within each treatment, transpiration values among various days with different letters are significantly different at $p \leq 0.05$. Within each sampling day, transpiration values among various treatments with different italicized letters are significantly different at $p \leq 0.05$.

Enzyme Activity, Ascorbic Acid, and MDA Content

Water stress decreased CAT and SOD activities and increased POD activity (Fig. 2). The stress × MJ treatment interactions for POD and SOD activities were statistically significant ($p \leq 0.001$). MJ treatment significantly ($p \leq 0.001$) increased CAT and SOD activities whether the leaves were from water-stressed plants or not. In contrast, MJ treatment reduced POD activity under water-deficit conditions but had no effect on POD activity in unstressed plants (Fig. 2). Water stress and MJ treatment also exhibited an interaction for MDA and ascorbic acid content (Fig. 3). Water-stressed strawberry leaves had increased membrane-lipid peroxidation as expressed by MDA content and decreased ascorbic acid. MJ treatment was effective in reducing membrane-lipid peroxidation and preventing ascorbic acid loss (Fig. 3).

Membrane Lipids

Water stress reduced both glycolipid and phospholipid content of strawberry leaves compared with nonstressed controls (Figs. 4 and 5). Application of MJ was effective

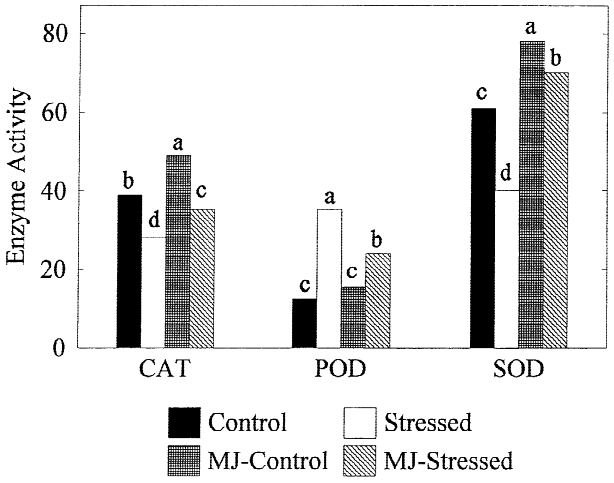

Fig. 2. Effect of MJ treatment on enzyme CAT, POD, and SOD activity in stressed and nonstressed strawberry leaves. MJ at 0.1 mM plus 0.05% Tween-20 was applied to the foliage at 2-day intervals for 8 days. The enzyme activity was determined when 15% fresh weight losswas reached. The CAT or SOD activity was expressed as units · mg protein^{-1}, and POD activity was expressed as ΔA · mg protein^{-1} · min^{-1}. Different letters indicate significant difference within treatments at $p \leq 0.05$.

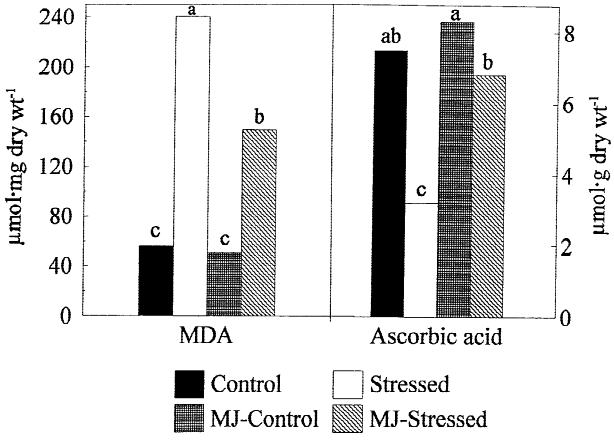

Fig. 3. Effect of MJ treatment on MDA and ascorbic acid content in stressed and nonstressed strawberry. For details see Fig. 2.

in lessening the total amount of glycolipid and phospholipid loss when water stressed. However, under normal (nonstressed) conditions, MJ treatment had no effect on glycolipid or phospholipid content compared with controls (Figs. 4 and 5). Polar lipid fatty acids included mainly palmitate (C16:0), stearate (C18:0), oleate (C18:1), linoleate (C18:2), and linolenate (C18:3). Linolenic acid (C18:3) was the dominant fatty acid found in the glycolipids MGDG and DGDG (Table 1). Palmitate (C16:0), linoleate (C18:2), and linolenate (C18:3) were the major fatty acids found in the phospholipids PG, PC,

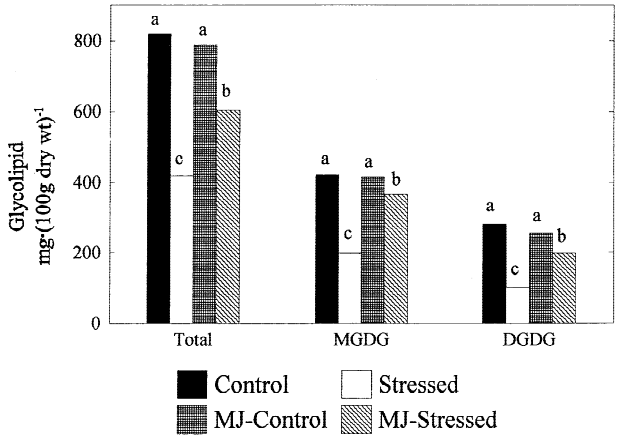

Fig. 4. Effect of MJ treatment on glycolipid content in relation to water stress in strawberry leaves. For details see Fig. 2.

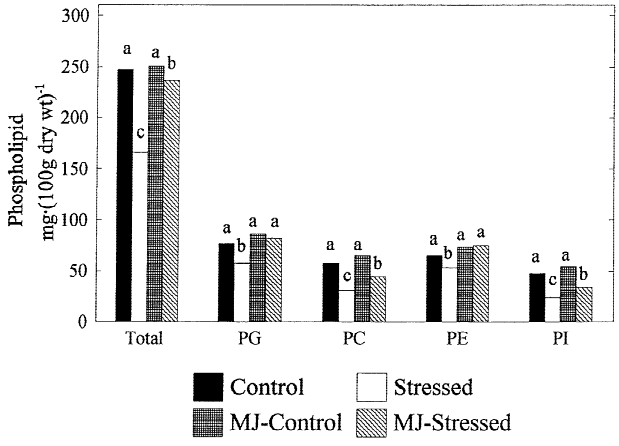

Fig. 5. Effect of MJ treatment on phospholipid content in relation to water stress in strawberry leaves. For details see Fig. 2.

PE, PI (Table 2). Water stress also affected the fatty acid composition of strawberry leaves. In particular, water stress decreased linolenic acid content, increased the linoleic and oleic percentages, and reduced the ratio of unsaturated:saturated fatty acids in the leaves (Figs. 6 and 7). MJ was effective in lessening the decline in the unsaturated:saturated fatty acid ratio [(18:1+18:2+18:3)/(16:0+18:0)] and in maintaining a higher percentage of unsaturated membrane lipids under water stress conditions in both glycolipids and phospholipids (Figs. 6 and 7).

Under nonstressed conditions, MJ treatment increased 18:3 and decreased 18:2 lipids, resulting in an increased fatty acid ratio of 18:3/18:2 in glycolipids (MGDG and DGDG) and phospholipids (PG, PC, PE, PI) (Figs. 6 and 8). Under water stress conditions, strawberry leaves had a decline in the 18:3/18:2 fatty acid ratio. However, MJ treatment effectively alleviated this decline (Figs. 6 and 8).

Table 1. Effect of 0.1 mM MJ treatment on fatty acid composition (weight percentage of total) of glycolipids in water-stressed strawberry leaves. Means of different fatty acids separated by different letters within a column for each individual glycolipid are significantly different at the 5% level.

	Fatty acid (wt %)				
Treatment	16:0	18:0	18:1	18:2	18:3
Monogalactosyl diglyceride					
Control	8.28 d	3.44 b	2.76 b	13.45 b	72.07 b
Stressed	20.51 a	6.76 a	3.18 a	15.21 a	54.34 d
MJ control	10.13 c	3.10 b	2.35 c	8.56 c	75.86 a
MJ stressed	17.74 b	6.11 a	3.07 ab	12.53 b	60.55 c
Digalactosyl diglyceride					
Control	12.67 b	3.12 b	4.49 a	12.14 b	67.58 b
Stressed	17.19 a	4.68 a	4.69 a	14.54 a	58.90 c
MJ control	11.50 c	2.19 c	2.57 c	9.95 c	73.79 a
MJ stressed	13.01 b	4.17 a	3.36 b	12.95 b	66.51 b

Table 2. Effect of 0.1 mM MJ treatment on fatty acid composition (weight % of total) of phospholipids in water-stressed strawberry leaves. Means of different fatty acids separated by different letters within a column for each individual phospholipid is significantly different at the 5% level.

	Fatty acid (wt %)				
Treatment	16:0	18:0	18:1	18:2	18:3
Phosphatidylglyceride					
Control	27.40 c	5.65 a	4.14 c	26.13 b	36.68 b
Stressed	38.54 a	5.71 a	5.13 b	27.74 a	22.88 d
MJ control	26.87 c	3.78 c	6.46 a	24.04 c	38.85 a
MJ stressed	30.35 b	4.65 b	4.86 b	25.71 b	34.43 c
Phosphatidylcholine					
Control	23.68 c	5.44 b	2.30 a	12.00 c	56.59 b
Stressed	29.66 a	7.83 a	2.92 a	19.14 a	40.45 d
MJ control	21.95 c	5.56 b	1.51 b	9.98 d	61.00 a
MJ stressed	27.05 b	5.73 b	2.68 a	15.00 b	49.54 c
Phosphatidylethanolamine					
Control	24.06 c	6.28 b	1.86 b	27.48 c	40.32 b
Stressed	29.89 a	7.47 a	3.34 a	36.59 a	22.78 d
MJ control	22.52 c	5.74 b	2.15 b	27.15 c	42.44 a
MJ stressed	27.00 b	7.18 a	2.30 b	31.08 b	32.44 c
Phosphatidylinositol					
Control	40.75 c	4.71 c	0.52 b	13.49 c	40.44 b
Stressed	44.94 a	6.86 a	0.63 b	21.43 a	26.14 d
MJ control	38.46 d	4.24 c	0.48 b	11.56 c	45.26 a
MJ stressed	42.47 b	5.14 b	0.98 a	18.43 b	32.98 c

Discussion

Transpiration in strawberry plants was significantly reduced by foliar application of MJ. MJ appears to alter the metabolism of strawberry plants so that they can better withstand water stress. MJ has been shown to inhibit stomata opening in barley (Horton 1991). Our data indicate that MJ has an advantage in transpiration control and this may be due to biophysical and structural modifications such as changes in stomatal guard cell membrane properties (Leshem et al. 1994). It has been reported that unlike abscisic acid, MJ is photostable and does not undergo ultraviolet-induced photoinactivation (Leshem et al. 1994, Satler and Thimann 1981). Therefore, MJ produces a wider variety of stress-associated manifestations than abscisic acid, including reduction of transpiration (Leshem et al. 1994).

Oxidative stress leads to the formation of reduced oxygen species such as hydrogen peroxide (H_2O_2) and superoxide radicals (O_2^-). These species can lead to the formation of damaging free radicals that cause lipid peroxidation and denaturing of protein. CAT and POD are oxidative enzymes. Burris (1960) reported that CAT ensures the removal of hydrogen peroxide, thus supplying free O_2 and detoxifying harmful metabolic products. PODs are involved in a large number of biochemical and

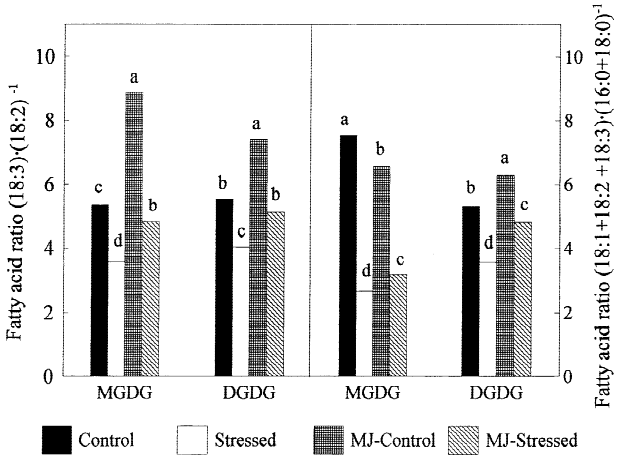

Fig. 6. Effect of MJ on fatty acid ratio, U/S [(18:1+18:2+18:3)/(16:0+18:0)] or (18:3)/(18:2), of MGDG and DGDG in water-stressed and nonstressed strawberry leaves. For details see Fig. 2.

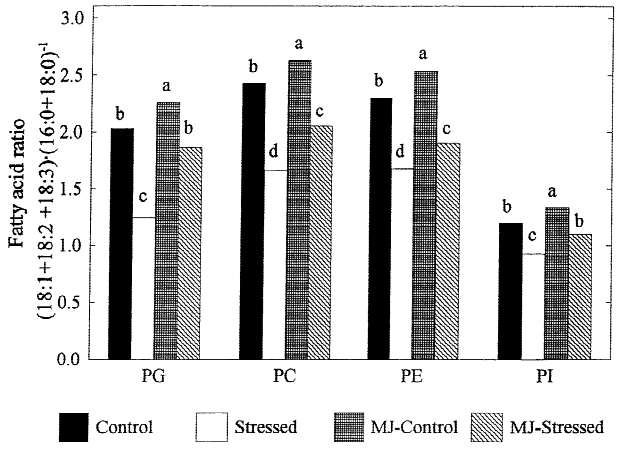

Fig. 7. Effect of MJ treatment on fatty acid ratio, U/S (18:1+18:2+18:3)/ (16:0+18:0)] of PG, PC, PE, and PI in water-stressed and nonstressed strawberry leaves. For details see Fig. 2.

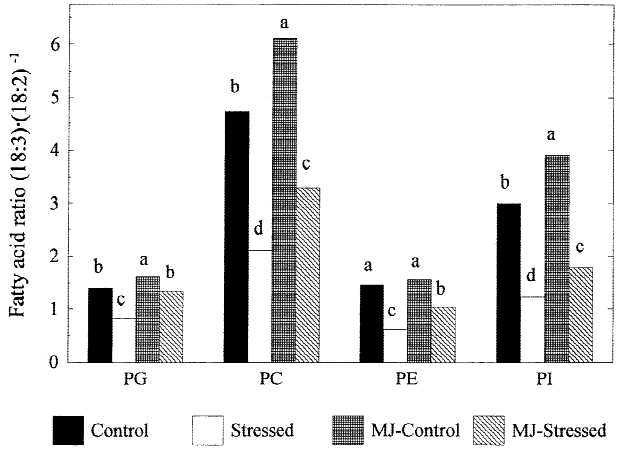

Fig. 8. Effect of MJ treatment on fatty acid ratio, (18:3)/(18:2), of PG, PC, PE, and PI in water-stressed and nonstressed strawberry leaves. For details see Fig. 2.

physiologic processes (Yip 1964) and may change quantitatively and qualitatively during growth and development (Shannon 1969). The mode of action of POD on the H_2O_2 substrate differs from CAT in that POD liberates free radicals rather than oxygen. These free radicals are highly phytotoxic. The H_2O_2 formed by POD may be scavenged by CAT. Water stress decreased CAT and increased POD activity, thus triggering an increased formation of the superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2), both of which can inactivate SH-containing enzymes. MJ treatment significantly increased CAT activity and reduced POD activity.

SOD is one of the metal-containing proteins that scavenge superoxide radicals. The mechanism of SOD action involves an alternating reduction and oxidation of the metal associated with the enzyme. SOD catalyzes the breakdown of O_2^- to O_2 and H_2O_2, removes singlet oxygen as well as O_2^-, prevents formation of OH^- (Fridovich 1973), and has been implicated as an essential defense mechanism against the potential toxicity of oxygen (McCord 1979). Reduction in SOD activity induced by water stress also favors accumulation of O_2^- and H_2O_2. Thus, the elevated levels of both O_2^- and H_2O_2 could impair the O_2^- scavenging system of cells and lead to the production of highly active hydroxyl radicals (OH^-). The hydroxyl radical can in turn initiate the peroxidative destruction of lipids with consequent membrane damage. The accumulation of H_2O_2 could also lower SOD activity. MJ treatment significantly increased SOD activity in the leaves from water-stressed plants.

Ascorbic acid is an antioxidant and is present in high amounts in strawberry leaves. A high level of ascorbic acid is necessary for a plant's defense against water-deficit conditions because ascorbic acid, in addition to its role in the H_2O_2 detoxification cycle, can directly act as a scavenger of hydroxyl radicals (Halliwell and Gutteridge, 1985). Strawberry leaves under water stress reduced the amount of ascorbic acid and exhibited an increase in membrane-lipid peroxidation as expressed by MDA content. MJ treatment reduced MDA content and maintained a higher amount of ascorbic acid under water stress. These results suggest that under water stress, MJ can induce antioxidant defense activity in plants to remove the possible toxic effects of free radicals, making the plants more resistant to water stress.

Cell membranes play an important role in the regulation of plant metabolism (Hellergren et al. 1984). The structure and composition of membranes vary with various physiological and environmental conditions. In strawberry leaves, MGDG and DGDG were the major

galactolipids, and PG, PE, PC, and PI were the major phospholipids. Water stress caused a significant reduction in both glycolipid and phospholipid content in strawberry leaves. Reduction of glycolipids and phospholipids in other crops under water stress has also been reported (Chetal et al. 1981, Douglas and Paleg 1981, Ferrari-Iliou et al. 1984). These reductions in stressed strawberry leaves may directly alter membrane functionality and could have deleterious effects on fundamental metabolic processes such as carbohydrate transport (Benson 1964), fatty acid synthesis (Selldén and Selstam 1976), and photoreduction of cytochrome (Chang and Lundin 1965). Application of MJ significantly lessened the amount of glycolipid and phospholipid loss under water stress. Water stress also caused a significant decrease in linolenic acid, an increase in the linoleic and oleic percentage, and reduced the ratio of unsaturated:saturated fatty acids in the leaves. The decrease in long-chain polyunsaturated fatty acid content may result in decreased fluidity and increased permeability of membranes that in turn would adversely affect the compartmentation of cells (Ferrari-Iliou et al. 1984). MJ effectively reduced the decline of the unsaturated:saturated fatty acid ratio and maintained a higher percentage of unsaturated membrane lipids in water stressed strawberry leaves.

MJ increased the ratio of 18:3 to 18:2 fatty acids in strawberry leaves in this study. Czapski et al. (1992) showed that MJ applied to mature green tomato fruit greatly increased the concentration of free linolenic acid and decreased the amount of free linoleic acid. The ratio of linolenic acid to linoleic acid content increased 4.5 to 7.7 times in MJ-treated tomatoes. The significant increase of linolenic acid in tomato fruit treated with MJ was found to be due to the inhibitory effect of MJ on lipoxygenase activity (feedback) and/or through stimulation of synthesis of linolenic acid from linoleic acid (Czapski et al. 1992). In this study, water-stressed strawberry leaves showed a significant decline in the 18:3/18:2 fatty acid ratio. However, MJ treatments of 0.1 mM partially blocked this decline. This may be due to MJ's apparent ability to induce proteins (jasmonate-induced proteins) and to protect cell membranes against physical stresses (Creelman and Mullet 1997, Koda 1992). The high unsaturation level of fatty acids maintained by treatment with MJ could also affect the overall physiology of strawberry plants and help to overcome damage in the lipid membrane bilayer caused by water deficit, thus making MJ-treated plants more capable of withstanding water stress.

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References

Ames BN (1966) Assay of inorganic phosphate, total phosphate and phosphatases. Methods Enzymol 8:115–118

Apelbaum A, Yang SF (1981) Biosynthesis of stress ethylene induced by water deficit. Plant Physiol 68:594–596

Arakawa N, Tsutsumi K, Sanceda NG, Kurata T, Inagaki C (1981) A rapid and sensitive method for the determination of ascorbic acid using 4,7-diphenyl-1,10 phenanthroline. Agric Biol Chem 45:1289–1290

Baisak R, Rana D, Acharya PBB, Kar M (1994) Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. Plant Cell Physiol 35:489–495

Beauchamp CO, Fridovich I (1973) Isoenzymes of superoxidase dismutase from wheat germ. Biochem Biophys Acta 317:50–64

Benson AA (1964) Plant membrane lipids. Annu Rev Plant Physiol 15:1–16

Bradford MM (1976) A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Burris RH (1960) Hydroperoxidase (peroxidases and catalases). In: Ruhland W (ed) Encyclopedia of plant physiology, vol.12. Springer-Verlag, Berlin, pp 365–400

Chang SB, Lundin K (1965) Specificity of galactolipids in photochemical reactions coupled with cytochrome *c* reduction. Biochem Biophys Res Commun 21:424–431

Chetal S, Wagle DS, Nainawatee HS (1981) Glycolipid changes in wheat and barley chloroplast under water stress. Plant Sci Lett 20:225–230

Creelman R A, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 48:355–381

Czapski J, Horbowicz M, Saniewski M (1992) The effect of methyl jasmonate on free fatty acids content in ripening tomato fruits. Biol Plant 34:71–76

Davies WJ, Metcalfe J, Lodge TA, Rosa de Costa A (1986) Plant growth substances and the regulation of growth under drought. Aust J Plant Physiol 13:105–125

Douglas TJ, Paleg LG (1981) Lipid composition of *Zea mays* seedlings and water stress-induced changes. J Exp Bot 32:499–501

Ferrari-Iliou R, Pham Thi AT, Vieira da Silva J (1984) Effect of water stress on the lipid and fatty acid composition of cotton (*Gossypium hirsutum*) chloroplasts. Physiol Plant 62:219–224

Fridovich I (1973) Superoxide radical and superoxide dismutase. Biochem Soc Trans 1:48–50

Giannopolitis CN, Reis SK (1977) Superoxide dismutases I. Occurrence in higher plants. Plant Physiol 59:309–314

Halliwell B, Gutteridge JMC (1985) Free radicals in biology and medicine. Clarendon, Oxford, 340 pp

Hellergren J, Lundborg T, Widell S (1984) Cold acclimation of *Pinus sylvestris*: Phospholipids in purified plasma membranes from needles of pine. Physiol Plant 62:162–166

Horton RF (1991) Methyl jasmonate acid and transpiration in barley. Plant Physiol 96:1376–1378

Koda Y (1992) The role of jasmonic acid and related compounds in the regulation of plant development. Int Rev Cytol 135:155–199

Lee TM, Lur HS, Lin YH, Chu C (1996) Physiological and biochemical changes related to methyl jasmonate-induced chilling tolerance of rice *Oryza sativa* L. Plant Cell Environ 19:65–74

Lee YB, Kim YS, Ashmore CR (1986) Antioxidant property in ginger rhizome and its application to meat product. J Food Sci 51:20–23

Leshem YY, Gottlieb H E, Bach D (1994) Surface tension-related mechanical effects of methyl jasmonate on membrane phospholipid. J Plant Physiol 144:691–695

McCord JM (1979) Superoxide dismutases: occurrence, structure,

function and evolution. In: Rattazzi M, Scandalios J, Whitt GS (eds) Isozymes: Current topics in biological and medical research, vol. 3. Liss, New York, pp 1–21

Monk LS, Fagerstedt KV, Crawford RMM (1987) Superoxide dismutase as an anaerobic polypeptide. Plant Physiol 85:1016–1020

Navari-Izzo F, Quartacci M F, Izzo R (1989) Lipid changes in maize seedlings in response to field water deficits. J Exp Bot 40:675–680

Nir G, ShulmanY, Fanberstein L, Lavee S (1986) Changes in the activity of catalase (EC 1.11.1.6) in relation to the dormancy of grapevine (*Vitis vinifera* L.) buds. Plant Physiol 81:1140–1142

Ottolenghi A (1959) Interaction of ascorbic acid and mitochondrial lipids. Arch Biochem Biophys 79:355

Pan RC, Gu H (1995) Effect of methyl jasmonate in the growth and drought resistance in peanut seedlings. Acta Phytophysiologica Sinica 21(3):215–220

Roughan PG, Batt RD (1968) Quantitative analysis of sulfolipid (sulfoquinovosyl diglyceride) and galactolipids(monogalactosyl and digalatosyl diglycerides) in plant tissues. Anal Biochem 22:74–88

SAS Institute Inc (1997) SAS/STAT Software: Changes and enhancements through release 6.12. Cary, NC: SAS Institute Inc., pp 1167

Satler SO, Thimann KV (1981) Le jasmonate se méthyle: nouveau et puissant promoteur de la sénescence des feuilles. Compt Rend Acad Sci Paris Ser III 293:735–740

Selldén G, Selstam E (1976) Changes in chloroplast lipids during the development of photosynthetic activity in barley etio-chloroplasts. Physiol Plant 37:35–41

Sembdner G, Parthier B (1993) The biochemistry and physiological and molecular actions of jasmonates. Annu Rev Plant Physiol Plant Mol Biol 44:569-–589

Shannon LM (1969) Plant isoenzymes. Annu Rev Biochem 38:189–210

Thayer WS (1990) Superoxide-dependent and superoxide-independent pathways for the reduction of nitroblue tetrazolium in isolated rat cardiac myocytes. Arch Biochim Biophys 276:139–145

Wang CY, Buta JG (1994) Methyl jasmonate reduces chilling injury in *Cucurbita pepo* through its regulation of abscisic acid and polyamine levels. Environ Exp Bot 34:427–432

Wang SY, Faust M (1989) Changes in membrane polar lipids associated with bud break in apple induced by nitroguanidines. J Plant Growth Regul 8:153–161

Wang SY, Sun T, Whitaker B D, Faust M (1988) Effect of paclobutrazol on membrane lipids in apple seedling. Physiol Plant 73: 560–564

Yamane H, Sugawara J, Suzuki Y, Shimamura E, Takahashi N (1981) Synthesis of jasmonic acid and related compounds and their structure-activity relationships on the growth of rice seedlings. Agric Biol Chem 44:2857–2864

Yip CC (1964) The hydroxylation of proline by horseradish peroxidase. Biochem Acta 92:395–396