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MITIGATION OF WATERLOGGING-INDUCED DAMAGES TO PEPPER BY EXOGENOUS MeJA

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Abstract

In this study, we studied the mitigation effects of exogenous Methyl jasmonate (MeJA) on waterlogging-induced damages to Xinyou No.5 wrinkled skin pepper cultivar by spraying MeJA on leave's surface at different waterlogging periods and investigated its underlying mechanisms. The results showed that administration of MeJA increased antioxidant enzymes' activities, proline and soluble sugar contents and alcohol dehydrogenase (ADH) activity, reduced relative conductivity, malondialdehyde (MDA) and hydroxyl free radical (·OH) accumulation, lactate dehydrogenase (LDH) activity and lactic acid and acetaldehyde accumulation, and maintained high root malate dehydrogenase (MDH) and succinate dehydrogenase (SDH) activities and certain aerobic respiratory metabolism. The study also found that there were significant differences among exogenous MeJA treatments at different waterlogging periods. Peppers treated with exogenous MeJA 1 day and 2 days prior to waterlogging had optimal agronomic traits, higher chlorophyll content, enzymatic activities and osmolytic substances, as well as lower relative conductivity, MDA and ·OH accumulation. Overall, the results suggest that MeJA mitigates waterlogging-induced damages to pepper by adjusting osmolytic substances contents, antioxidant enzymatic activities and root respiration and metabolism and achieves better alleviation effects by spraying prior to waterlogging.

Key words: MeJA; Pepper; Spraying period; Waterlogging stress.

Abbreviations: ADH – alcohol dehydrogenase; Car – carotenoids; CAT – catalase; Chl – chlorophyll; DM – dry mass; FM – fresh mass; GR – glutathione reductase; LDH – lactate dehydrogenase; MeJA – Methyl jasmonate; MDA – malondialdehyde; MDH – malate dehydrogenase;·OH – hydroxyl free radical; POD – peroxidase; SDH – succinate dehydrogenase; SOD–superoxide dismutase; TTC – triphenyl tetrazolium chloride; TTF – trityl hydrazone;

Introduction

Methyl jasmonate (MeJA) is an endogenous signaling molecule naturally occurring in plants. It plays an important role in plant growth and development, stress response and secondary metabolism (Fung *et al*., 2004). As a plant growth regulator, it has important regulatory effects on plant growth and development (Zhang *et al*., 2008). Application of exogenous MeJA to plants not only could induce accumulation of active ingredients such as purple pigment of madder (Fan *et al*., 2013), scopolamine of mandala (Sun *et al*., 2013) and dendrobium polysaccharide in dendrobium (Ying *et al*., 2014), but also acts as a signal molecule to induce cytosolic alkalization, cytosolic Ca^{2+} oscillation (Islam *et al*., 2010) and stomatal closure (Islam *et al*., 2009), accumulate secondary metabolites (Misra *et al*., 2014), inducibility gene (Aziz *et al*., 2017), and mitigate abiotic and biotic stresses (Yan *et al*., 2014; Li *et al*., 2014b; Feng *et al*., 2014). In addition, MeJA also modulates vernalization and flowering time (Diallo *et al*., 2014) and preserve vegetables and fruits fresh (Li *et al*., 2014a, Wen *et al*., 2014), etc.

Pepper belongs to the genus Capsicum of family Solanaceae and is one of the largest vegetable with average annual planting acreage of above 1.3 million hm^2. It is a shallow-rooted plant and has thin, less absorbing roots. Excessive soil moisture will affect its growth (Zou, 2009). Waterlogging occurs frequently in the Yangtze river region, seriously affecting the yield and quality of pepper plants cultivated in open fields during spring and summer. In China, 60%-70% of pepper plants grow in open fields, thus, waterlogging has become one of the major stress factors exposed to pepper production. Pepper plant has strong drought tolerance (Ou *et al*., 2012), but weak waterlogging tolerance (Ou *et al*., 2011). After suffering from waterlogging stress, it is prone to have soaked roots, dead seedlings, yellow and falling leaves as well as shattering flowers and fruits. Therefore, taking external measures to improve pepper plant's tolerance to waterlogging is important to facilitate large-scale and long-seasonal pepper plant cultivation. Previous studies have shown that MeJA can improve the vitality of pepper seeds stored at low temperature (Korkmaz, 2005), induce the expression of certain genes to respond to adverse environments such salt and low temperature stresses (Lee *et al*., 2001; Yi *et al*., 2004; Shin *et al*., 2004). But whether MeJA could improve the tolerance of pepper plant to waterlogging and when to spray MeJA have not been reported. In addition, determining the spraying period is important to maximize the effects of MeJA and alleviate waterlogging-induced damage to pepper production. Therefore, in this paper, we analyzed the impacts of spraying exogenous MeJA at different waterlogging periods on the agronomic characteristics and physiological and biochemical indices of pepper plant and explored the better spraying time to alleviate waterlogging-induced damages to pepper plant, with the hope to provide a basis for establishment of waterlogging tolerant technology in pepper plants.

Material and Methods

The tested pepper seeds were placed in the nursery pot after germination. The seedlings with strong and unanimous growth at the five-leaf stage were transplanted in 28x28 cm pots containing soils made of 60% garden soil, 20% compost and 20% river mud with each seedling per pot. Seedlings at the 6-leaf- stage were subjected to waterlogging by keeping water level 2 cm above the soil surface with regular replenishment. Seedlings were assigned into 7 different groups with 90 seedlings in each group and treated with 3 repeats with MeJA at its optimal concentration of 1.0 mmol L^{-1}, which was determined at the preliminary test, by spraying to the whole plant till liquid dripping from the leaves. Seedlings in W1 and W2 groups were subjected to waterlogging 2 days and 1 day after continuously spraying MeJA, respectively; Seedlings in W3 group were subjected to waterlogging at the day of continuously spraying MeJA; Seedlings at W4 and W5 groups were subjected to waterlogging 1 day and 2 days prior to continuously spraying MeJA, respectively. Seedlings in CK group and WCK group were subjected to normal water management (Watering every 2 or 3 days to keep moist) and waterlogging alone, respectively, and same amount of water spraying, and used as negative control and positive control, respectively. During the treatment, the weather conditions were temperature of 34±3.1°C, no wind, no precipitation and no sudden temperature dips. Waterlogging and MeJA spraying treatment were discontinued until the positive control appeared to have severe waterlogging symptoms such as yellow leaves, fallen leaves, and wilt. Samples were collected at the end of the treatment for further examination and the same materials were used for in each determination of physiological aspects.

Agricultural characters: After treatment, 10 plants were randomly selected to measure the height and root length. Others 10 plants were washed with deionized water, dried, and weighted to obtain the fresh weight. Afterward, they were dried at 105°C for 30 min and then at 75°C to the constant weight, and measured for dry weight.

Pigment analysis: was determined by Arnon (1949) method. All leaves were fully development and selected from the second to the fourth leaves of the plants. 2.0 g flesh leaves were homogenized using quartz sand, $CaCO_3$, and 3 ml acetone, and then extracted with 80% acetone. The supernatant was measured by spectrophotometer *(Ruili UV-2100*, Beijing, China) at 663, 645 and 470 nm after centrifugation for 2 min at 2,500 rpm. Contents of chlorophyll (Chl) *a* ,Chl *b*, and carotenoids (Car) were calculated using the equations of below: Chl a= 13.95 OD_{665}–6.88 OD_{649}; Chl b = 24.96 OD_{649}–7.32 OD_{665}; Car = (1000 OD_{470}–2.05 Chl a–114.8 Chl b)/245.

Membrane permeability: was measured as previously reported (Hao *et al.,* 2007). In brief, after washed twice with deionized water, 0.1 g leaves or roots were cut into l cm long small pieces and placed into a tube containing 10 mL of deionized water. After marking the water level, the tube was incubated at 25°C for 1 h with frequent shaking. At that time, the conductivity C1 was determined by conductivity meter (*HI 98309*, Hanna, Mauritius). Then the tube was boiled at 100°C for 15 min. After cooled to room temperature, distilled water was added into to the tube till water level reached the original mark, then the conductivity C2 was measured. Thus, the membrane permeability was expressed as the following:

$$\text{Relative electrolyte leakage} = \frac{(C1\text{-}C0)}{(C2\text{-}C0)} \times 100\%$$

where C0 is the conductivity of distilled water.

Proline determination: The fresh leaves were washed and dried with paper towel. 0.5 g of leaves were cut into pieces, mixed with 5 mL of 3% sulfosalicylic acid solution in a large tube, and boiled for 10 min under shaking. The extract was filtered with a funnel into a clean tube after cooling down, 2 mL of the filtered extract was mixed with 2 mL of acetic acid and 2 mL of acid ninhydrin, and boiled for 30 min after sealed with plastic wrap. After cooling down, 4 mL of toluene was added into the tube and fully oscillated. The upper red solution was collected and its absorption at 520 nm (*Ruili UV-2100*, Beijing, China) was measured using toluene as reference. Proline content [x (μg/mL)] in the 2 mL tested samples was obtained from the standard curve and the proline concentration in fresh leaves was calculated based on the following formula:

$$\text{Proline content } (\mu g/g) = (x \times 5/2) / \text{ fresh leaf weight (g)}$$

Soluble sugars: They were measured with anthrone colorimetry. 0.1 g leaves were placed in a ground glass weighing bottle, mixed with 20 mL distilled water, and extracted twice in boiling water for 30 min. The extract was filtered into a 50 mL volumetric flask, and the weighing bottle and residues were washed repeatedly before being filled to constant volume. 0.5 mL sample extract was mixed thoroughly with 1.5 mL distilled water, 0.5 mL anthrone ethyl acetate solution and 5 mL concentrated sulfuric acid, before being immediately placed in boiling water bath for 1 min. The heated sample extract was cooled to room temperature to determine the absorbance at 630 nm (*Ruili UV-2100*, Beijing, China). The final soluble sugar contents were calculated from the standard curve:

Enzyme activity: The activities of superoxide dismutase (SOD) (hydroxylamine method), peroxidase (POD) (colorimetric method), catalase (CAT) (ammonium molybdate method), glutathione reductase (GR) (colorimetric method), lactate dehydrogenase (LDH) (colorimetric method) and alcohol dehydrogenase activity (ADH) (colorimetric method) were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing China). 0.1 g leaves without midrib were thoroughly with a cold mortar and pestle in an ice bath. The grinding medium was 4 ml of saline, plus homogenizing glass beads. The homogenate was centrifuged for 10 min at 2,500 rmp and 4°C. The supernatant referred was crude enzyme extract and used to determination. The absorbance of the reaction mixture was determined by using a spectrophotometer (*Ruili UV-2100*, Beijing, China).

$$\text{Soluble sugar } (\mu g \cdot g^{-1} \text{ FW}) = \frac{[(\text{Corresponding sucrose content from standard curve } (\mu g) \times \text{Total extract volume (mL)}]}{[(\text{Measurement volume (mL)} \times \text{Fresh weight of the sample (g)}]}$$

Hydroxyl free radical (OH) and malondialdehyde (MDA): were measured using colorimetric method or thiobarbituric acid (TBA method) by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing China). 0.1 g leaves were thoroughly with a cold mortar and pestle in an ice bath. The grinding medium was 4 ml of saline, plus homogenizing glass beads. The homogenate was centrifuged for 10 min at 2,500 rmp and 25°C. The supernatant referred was crude enzyme extract and used to determination. The absorbance of the reaction mixture was determined by using a spectrophotometer (*Ruili UV-2100*, Beijing, China).

Data analysis: The experimental results were expressed as mean ± standard error and analyzed using Excel 2003 and SPSS 17.0. Significance of differences among different data sets was analyzed using Duncan's multiple comparison test method. Meanwhile, the subordinate function value, that is, the ratio of the measured value subtracting its minimal value to difference of its maximal and its minimal values of a certain trait, can be expressed as $R(X_i)=(X_i-X_{min}) / (X_{max}-X_{min})$ and calculated. if a trait has a negative correlation with its waterlogging tolerance, the anti-subordinate function value, that is, $R(X_i)=1-(X_i-X_{min}) / (X_{max}-X_{min})$, was calculated.

Results

Effects of MeJA treatment at different periods on the agronomic traits of pepper: After waterlogging, there were significant differences in agronomic traits of pepper seedlings subjected to different MeJA treatments (Table 1). Seedlings subjected to waterlogging alone had significantly lowered root length, plant height, fresh weight and dry weight than normal seedlings. But spraying exogenous MeJA reversed the declining trend of these indices and there were significantly differences among different exogenous MeJA treatments. The improvements in agronomic traits of pepper seedlings in W1 group were the most significant, followed by W2, W3, W4 and W5, in turn. In detail, the root length, plant height, fresh and dry weight of seedlings in W1 group were 70.14%, 17.99%, 75.89% and 93.33% higher than those of seedlings in WCK group, respectively, while those of seedlings in W5 group were only 36.08%, 27.22%, 33.50% and 37.93% higher than those in WCK group, respectively.

Effects of MeJA treatment at different periods on the chlorophyll content of pepper: After waterlogging, total chlorophyll content was lower in seedlings of all treatment groups compared to that of normal seedlings. But exogenous MeJA treatment alleviated the decrease in chlorophyll content induced by waterlogging and the alleviation degree was significantly different among different exogenous MeJA treatment groups. In detail, the seedlings in W1 group had the smallest decline of chlorophyll a and b contents, which was 11.70% and 8.09%, respectively; the seedlings in W5 group had the highest decline in chlorophyll a and b contents, which was 25.66% and 20.49%, respectively; and the decline in chlorophyll a and b contents in the seedlings of other treatment groups fell somewhere in between (Table 2).

Effects of MeJA treatment at different periods on the membrane permeability of pepper: The relative conductivity of pepper seedlings after waterlogging increased significantly. However, after spraying exogenous MeJA, this increase in relative conductivity was attenuated and there were significant differences among seedlings in different exogenous MeJA treatment groups. In detail, seedlings in W1 and W5 groups had minimum and maximum increases in relative conductivity, which was 59.76% and 112.34% higher than CK, respectively. Compared with seedlings in WCK, the relative conductivity in W1 and W5 groups decreased by 30.73% and 7.93%, respectively. All other MeJA treatments had effects between these two treatments (Table 2).

Table 1. Effect of MeJA on agronomic traits of pepper with different spraying period. Each value is the mean ± standard deviation (SD, *n* = 6). Different letters indicate significant differences between treatments (p<0.05).

Treatment	heigh [cm]	Root length [cm]	Fresh mass [g]	Dry mass [g]
CK	17.40 ± 0.49a	10.03 ± 0.33a	2.65 ± 0.14a	0.34 ± 0.005a
WCK	12.67 ± 0.28e	4.47 ± 0.15d	1.12 ± 0.03d	0.15 ± 0.001d
W1	14.95 ± 0.78b	7.65 ± 0.45b	1.97 ± 0.12b	0.29 ± 0.002b
W2	14.35 ± 0.67b	6.88 ± 0.12db	1.85 ± 0.18b	0.27 ± 0.003b
W3	13.84 ± 0.27c	6.01 ± 0.54c	1.73 ± 0.15b	0.26 ± 0.001b
W4	13.53 ± 0.87c	5.42 ± 0.32c	1.47 ± 0.16c	0.21 ± 0.002c
W5	10.88 ± 0.86d	4.89 ± 0.65d	1.31 ± 0.14c	0.18 ± 0.002c

Note: W1-W5 represent different spraying period, WCK representative simple waterlogging, CK represents the normal controls

Table 2. Effect of MeJA on chlorophyll content and relative conductivity of pepper with different spraying period. Each value is the mean ± standard deviation (SD, *n* = 6).

Treatment	*Chl* a [mg g^{-1} FW]	*Chl* b [mg g^{-1} FW]	Relative conductivity [%]
CK	2.67 ± 0.21a	0.93 ± 0.07a	16.13 ± 2.11e
WCK	1.92 ± 0.03e	0.71 ± 0.02c	37.20 ± 2.89a
W1	2.36 ± 0.11b	0.85 ± 0.05b	25.77 ± 1.34b
W2	2.28 ± 0.09b	0.81 ± 0.05b	28.13 ± 3.01c
W3	2.22 ± 0.10c	0.79 ± 0.04b	30.78 ± 2.78c
W4	2.18 ± 0.05c	0.77 ± 0.06b	32.76 ± 2.77d
W5	1.99 ± 0.06d	0.74 ± 0.03c	34.25 ± 1.89d

Different letters indicate significant differences between treatments (p<0.05)

Effects of MeJA treatment at different periods on the antioxidant enzymes' activities of pepper: Antioxidant system is a set of defense mechanism responsible for the removal of reactive oxygen species and against adverse environments in plants. Induced increase of antioxidant enzymes' activities can relieve stress-induced damages to plants and enhance their tolerance to waterlogging. After waterlogging, the activities of antioxidant enzymes decreased, but MeJA treatment significantly increased the activities of antioxidant enzymes and these increases were different among different MeJA treatments. Among them, W1 treatment showed the greatest increases of 166.82%, 166.88%, 63.61% and 166.67% for SOD, POD, CAT and GR, respectively, compared with WCK, followed by W2, W3, W4 and W5, in turn, in a descending order (Fig. 1).

Effects of MeJA treatment at different periods on the osmolytes of pepper: Under adversity stress conditions, plants will accumulate a variety of organic substances such as proline and soluble sugar to increase cell sap concentration, lower osmotic potential, improve cell water-absorption or water-holding capacity, thus adapting to waterlogging stress conditions. Under waterlogging, proline and soluble sugar contents dramatically increased in pepper seedlings in all treatment groups. Proline content of each treatment group was significantly higher than that of control. Among them, proline content increased by 89.77%, 75.18% and 57.00% in W1, W2 and W3, respectively; Similarly, soluble sugar content was significantly higher in each treatment group than control, the biggest increase was seen in W1 and W2, by 194.66% and 200.42%, respectively, and the smallest increase was seen in W5, by 93.60% (Fig. 2).

Effects of MeJA treatment at different periods on the MDA and hydroxyl radical of pepper: MDA is a membrane lipid peroxidation product under stress. Excessive MDA accumulation will damage cell membrane. Hydroxyl radical is one of reactive oxygen species. Its content also reflects the degree of damages to plants. Under waterlogging, MDA and hydroxyl radical levels were significantly increased, but these increases were smaller in MeJA treatment groups than in WCK group. Among them, both in W1 group were only 61.20% and 41.27% of those in WCK group (Fig. 2).

Effects of MeJA treatment at different periods on the respiratory metabolism of pepper: LDH and ADH are key enzymes in anaerobic respiration. Their activity level to some extent reflects the strength of anaerobic respiration. Under waterlogging, LDH activity in all treatment groups showed a rising trend, but that in WCK group was significantly higher than those in any MeJA treatment group. Of them, the highest LDH activity was seen in W4 group among all MeJA treatment groups, which was 73.21% of that in WCK (Fig. 3). Root ADH activity in control group was maintained at a low level. But after waterlogging, its activity increased significantly. In addition, ADH activity in all MeJA treatment groups was higher than that of waterlogging treatment alone.

MDH and SDH are key enzymes in aerobic respiration. Their activities can be used as general indices to evaluate the degree of Krebs cycle. After waterlogging, MDH and SDH activities decreased significantly compared with that of the CK control, but the magnitudes of reduction were smaller in MeJA treatment groups, for example, those of W1 were 1.71- and 1.68-fold of those of WCK, respectively (Fig. 3).

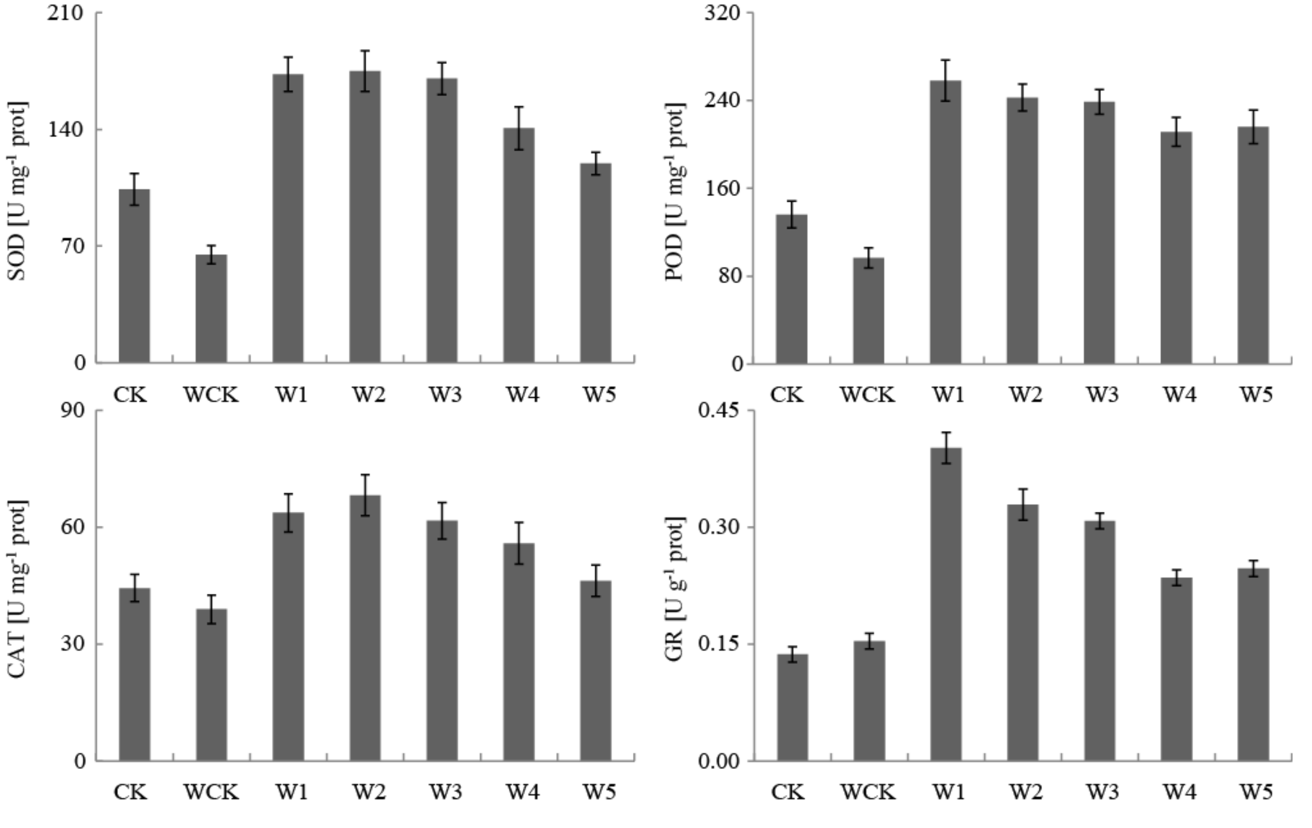

Fig. 1. Effect of MeJA on antioxidant enzyme system of pepper with different spraying period.

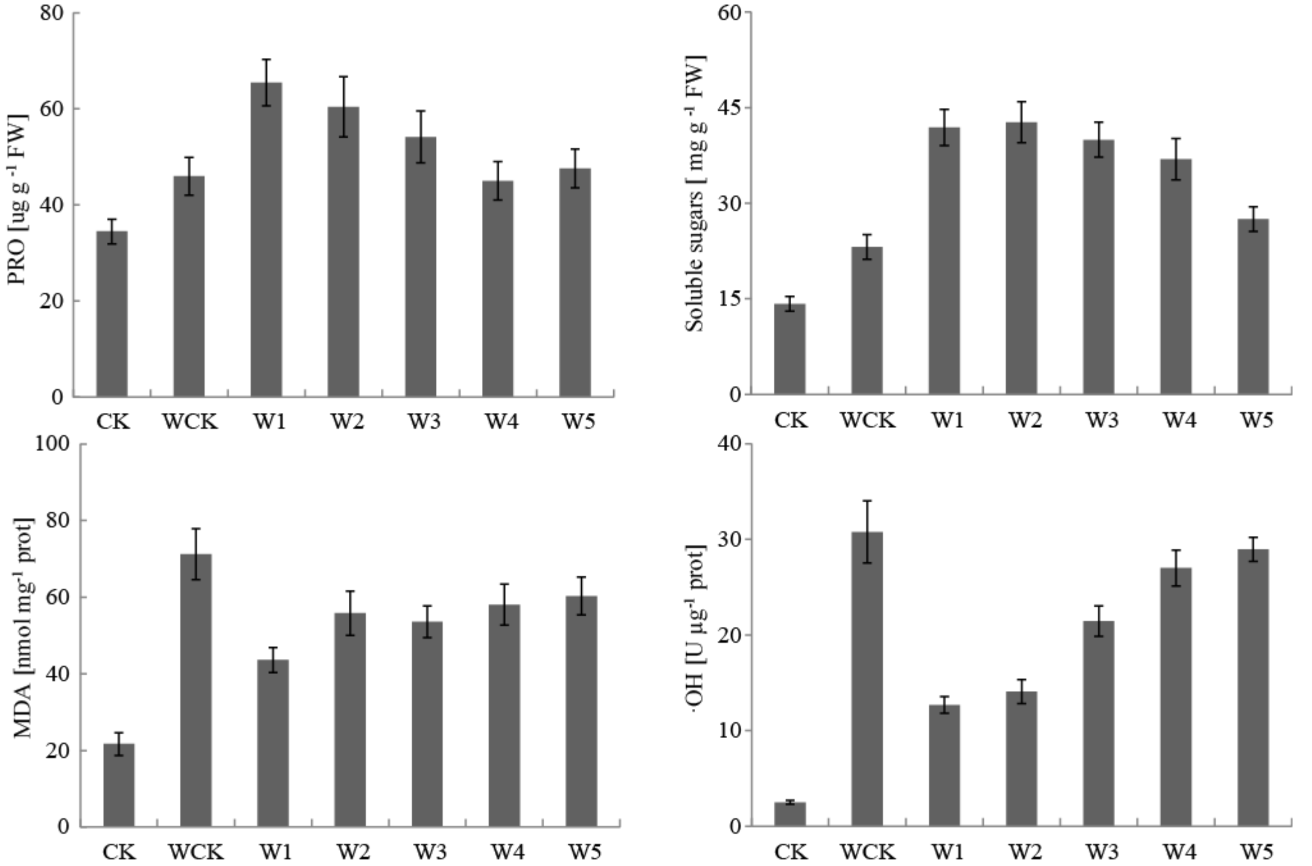

Fig. 2. Effect of MeJA on osmolytes and lipid peroxidation products of pepper with different spraying period.

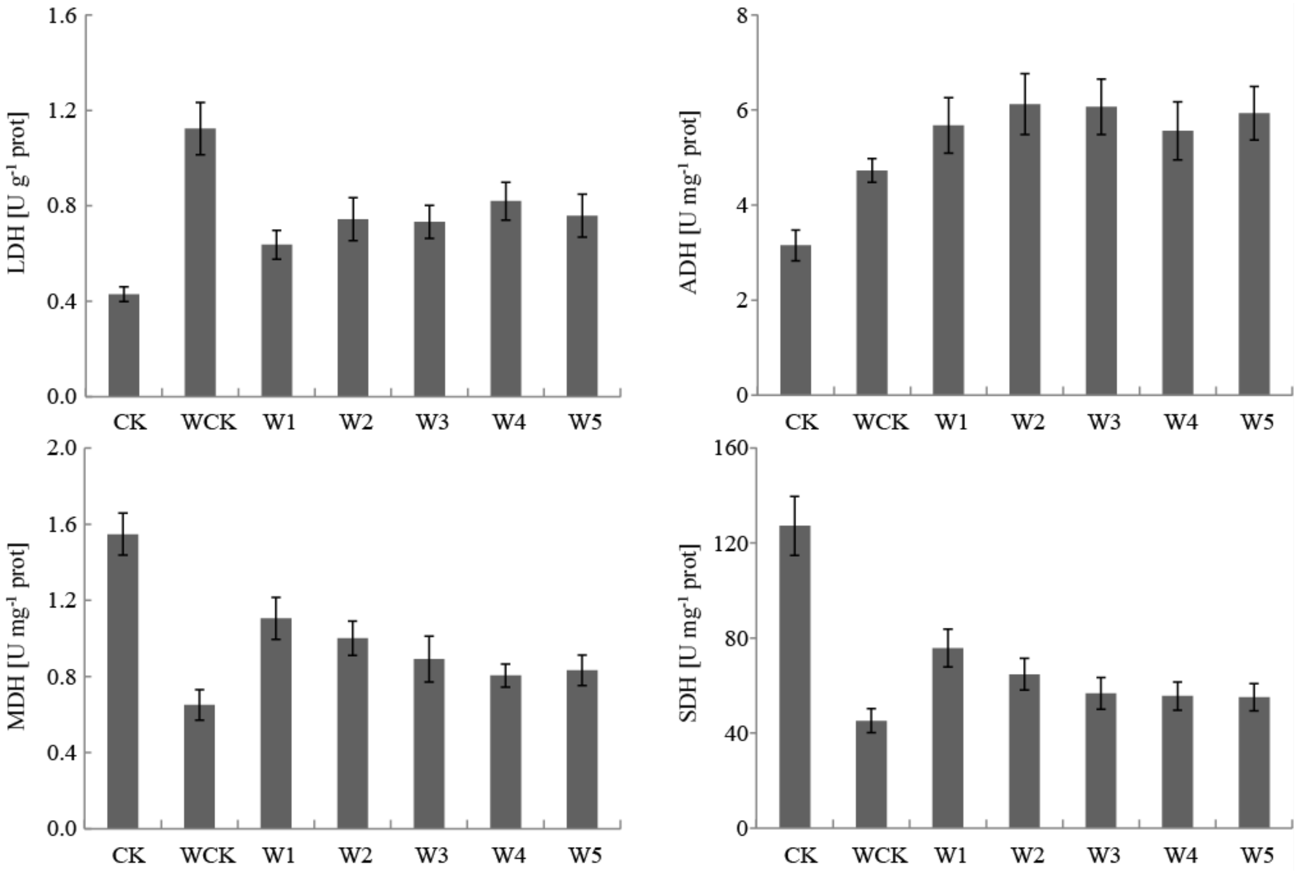

Fig. 3. Effect of MeJA on enzyme activity of respiratory of pepper with different spraying period.

Comparison of waterlogging tolerance of pepper treated with MeJA at different waterlogging periods: Because the mechanisms of waterlogging tolerance in plants are very complex, only measuring single or two indices to determine the tolerant strength of plants to waterlogging is incomplete. Therefore, using a comprehensive evaluation system composed of subordinate function values of multi-traits such agronomic traits, chlorophyll content and relative conductivity to determine the tolerant strength of plants to waterlogging can eliminate the sidedness of using individual index and is more scientific. The physiological indices such as antioxidant activities were affected in seedlings in CK, i.e. The normal group, which were not subjected to waterlogging. Therefore, when using comprehensive evaluation system to evaluate the tolerant strength of plants to waterlogging, comparison of seedlings in W1-W5 groups with WCK was more accurate.

Comparison of subordinate function values of agronomic traits, chlorophyll content and relative conductivity: Table 3 shows the weighted values and the total weighted index values of the 7 different treatment groups. Except that the relative conductivity is adverse to waterlogging tolerance and calculated using anti-subordinate function, the other six indices of root length, plant height, fresh weight, dry weight, chlorophyll a and chlorophyll b content, are favorable to waterlogging tolerance and calculated using subordinate function. The strength of waterlogging tolerance of peppers evaluated using agronomic traits, chlorophyll contents and relative conductivity is in the order of W1> W2> W3> W4> W5> WCK.

Comparison of subordinate function values of antioxidant enzymes, proline and soluble sugars: Table 4 shows the weighted values and total weighted index values of 6 different indices of different treatment groups. All the six indices are favorable for waterlogging tolerance and calculated using subordinate function. The strength of waterlogging tolerance of peppers evaluated using the 6 indices is in the order of W1> W2> W3> W4> W5> WCK.

Comparison of subordinate function values of respiratory enzymes, MDA and OH: Table 5 shows the weighted values and total weighted index values of the 6 indices. LDH, MDA and ·OH are unfavorable for waterlogging and their subordinate function values were calculated using anti-subordinate function. By contrast, ADH, SDH and MDH are favorable for waterlogging and their subordinate function values were calculated using subordinate function. The physiological indices indicated that the order of waterlogging tolerance strength was W1> W2> W3> W4> W5> WCK.

Table 3. Subordinate function value of agronomic traits, chlorophyll and relative conductivity.

Treatment	R(X1)	R(X2)	R(X3)	R(X4)	R(X5)	R(X6)	R(X7)	R(S1)
W1	1	1	1	1	1	1	1	7
W2	0.7579	0.8526	0.8588	0.8571	0.8208	0.7586	0.7935	5.6993
W3	0.4843	0.7273	0.7176	0.7857	0.6763	0.5517	0.5617	4.5046
W4	0.2987	0.6511	0.4118	0.4286	0.5954	0.4138	0.3885	3.1877
W5	0.1321	0	0.2235	0.2143	0.1387	0.2069	0.2581	1.1736
WCK	0	0.4398	0	0	0	0	0	0.4398

Note: The X1-X7 representing root length, height, fresh mass, dry mass, *chl* a, *chl* b and relative conductivity

Table 4. Subordinate function value of physiological indicators.

Treatment	R(X8)	R(X9)	R(X10)	R(X11)	R(X12)	R(X13)	R(S2)
W1	0.8453	1	0.9382	1	1	0.9581	5.7865
W2	1	0.9047	1	0.7200	0.7538	1	5.3785
W3	0.7769	0.8800	0.9599	0.6400	0.4469	0.8588	4.5626
W4	0.5786	0.7112	0.6883	0.3600	0	0.7038	3.0419
W5	0.2510	0.7387	0.4966	0.4000	0.1263	0.2230	2.2356
WCK	0	0	0	0	0.0455	0	0.0455

Note: The X8 -X13 representing CAT, POD, SOD, GR, PRO and soluble sugar

Table 5. Subordinate function value of respiratory enzymes, MDA and OH.

Treatment	R(X14)	R(X15)	R(X16)	R(X17)	R(X18)	R(X19)	R(S3)
W1	1	0.6835	1	1	1	1	5.6835
W2	0.7917	1	0.7609	0.6403	0.5568	0.9230	4.6727
W3	0.8125	0.9640	0.5217	0.3751	0.6389	0.5149	3.8271
W4	0.625	0.5971	0.3261	0.3401	0.4772	0.2082	2.5737
W5	0.75	0.8633	0.3913	0.3221	0.3944	0.1008	2.8219
WCK	0	0	0	0	0	0	0

Note: The X14 -X19 representing ADH, LDH, SDH, MDH, MDA and OH

Table 6. Weighted Subordinate function value of integrated indicators.

Treatment	R (S1)	R (S2)	R (S3)	R (S)
W1	7	5.7865	5.6835	18.4700
W2	5.6993	4.3785	4.6727	15.7505
W3	4.5046	4.5626	3.8271	12.8943
W4	3.1877	3.0419	2.5737	8.8033
W5	1.1736	2.2356	2.8219	6.2311
WCK	0.4398	0.0455	0	0.4853

Comprehensive comparison of subordinate function values: The weighted values and total weighted index values of five agronomic traits, chlorophyll content, conductivity and 12 physiological indices of different treatment groups. The results indicated that among the different treatment groups with waterlogging, W1 group showed the beast performance, followed by W2, while WCK had the worst performance. Based on the comprehensive weighted evaluation values, the order of waterlogging tolerance of pepper seedlings is W1> W2> W3> W4 > W5> WCK (Table 6).

Discussion

Environmental stresses could increase the level of ROS such as H_2O_2 and $O_2^{\cdot-}$ in plants, both of which can destroy the phospholipid membranes and increase MDA content (Smirnoff *et al*., 1993). As a protective measurement, plants could accumulate large amounts of compatible osmotic agents including proline and soluble sugars to maintain osmotic balance and protect the ability of antioxidant enzymes to scavenge free radicals (Hoque *et al*., 2007). Ahmed *et al*. (2010) found that exogenous proline could increase the activity of antioxidant enzymes in olive trees under salt stress; Bolouri-Moghaddam *et al*. (2010) found that soluble sugars can eliminate ROS and relieve external stress to plants. Khalid *et al*. (2010) found that plants under drought stress had higher proline and soluble sugar contents. In this paper, we showed that waterlogging significantly decreased pepper’s agronomic indices such as root length, plant height, biomass, etc, as well as chlorophyll content, but significantly increased malondialdehyde level, indicating that pepper plants were damaged by waterlogging. In addition, although waterlogging enhanced proline and soluble sugar contents in pepper, the activities of antioxidant enzymes such as SOD, POD and CAT but except GR were decreased, indicating that waterlogging-induced damages to pepper cannot be mitigated by increasing proline and soluble sugar contents.

MeJA, as a plant signal transduction molecule, could inhibit plant growth, induce anti-stress activity, and promote aging among its many other physiological functions (Sembdner & Parthier 1993; Creelman and Mullet 1997; Wang and Jiang 2002). When plants are subjected to stress-induced damages, MeJA can induce the expression of plants’ defense genes. In addition, MeJA content is significantly increased in rice, wheat, corn and other plants suffering from abiotic stresses-induced damages (Lehmann *et al*., 1995). In many plant species, MeJA is often used to induce plants to produce anti-biotic and abiotic stress mechanisms (Li *et al*., 2002). To some extent, MeJA is able to enhance the activities of SOD and other protective enzymes (Liu *et al*., 2015, Li *et al*., 2012) and the contents of osmolytes (Jin *et al*., 2011). More importantly, MeJA has certain effects on plants’ root system. Application of exogenous MeJA at appropriate concentration can enhance root metabolism (Yang *et al*., 2015), promote lateral root formation (Kiyosh *et al*., 1994) and increase the number of adventitious roots (Li & Pan, 1998), all of which can rise root activity and respiration while alleviate waterlogging-induced damages. In this study, we found that application of exogenous MeJA significantly increased the activities of antioxidant enzymes, contents of osmotic adjustment substances, root length and root metabolism in pepper. These results further confirmed the above conclusions. Meanwhile, we also found that application of exogenous MeJA at different waterlogging periods had different alleviation effects on waterlogging-induced damages to pepper. Pepper seedlings subjected to treatment of exogenous MeJA at 1 or 2 days prior to waterlogging showed highest activities of antioxidant and respiratory metabolic enzymes, highest contents of osmotic adjustment substances, lowest relative conductivity, lowest MDA and ·OH accumulation, and significantly better agronomic traits compared with those subjected treatment of exogenous MeJA at other periods, indicating that application of MeJA prior to waterlogging had better alleviation effects on stress-induced damages to pepper. These results may be related to that application of MeJA in advance could promote MeJA accumulation, accelerate the increase of extracellular MeJA concentration, initiate cells’ pre-stress responses, quickly turn on the early responses mechanisms at the beginning of waterlogging, and induce a series of physiological and biochemical changes in order to adapt to waterlogging.

Conclusions

Comparison of the comprehensive weighted index values indicated that the order of different MeJA treatments to alleviate waterlogging-induced damages is W1 (continuous spraying MeJA 2 days prior to waterlogging) > W2 (continuous spraying MeJA 1 day prior to waterlogging) > W3 (continuous spraying MeJA at the day of waterlogging) > W4 (continuous spraying MeJA 1 day post waterlogging) > W5 (continuous spraying MeJA 2 days post waterlogging) > WCK (waterlogging control without MeJA treatment). At the technology advanced today, it is possible to predict the occurrence of heavy precipitation. Thus, to protect plants in the areas vulnerable to waterlogging such as those with low-lying terrain and poor drainage, it is necessary to continuously spray MeJA days prior to heavy rainfall in order to early start MeJA response mechanisms. In the case of unexpected flooding occurs, spraying MeJA at the waterlogging day could also achieve better results.

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