Improvement of Tolerance to Chilling in Watermelon Seedlings with Methyl Jasmonate and Methyl Salicylate

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ABSTRACT

Tropical plants usually are sensitive to chilling and easily damaged. Tolerance of watermelon (*Citrullus lanatus* L.), cv. Charleston gray, seedlings to chilling was investigated through exogenous application of methyl jasmonate (MeJA; 15 and 30 μM) and methyl salicylate (MeSA; 50 and 100 μM). Four days after receiving MeJA and MeSA seedlings at the 2–true leaf stage were subjected to chilling at 3°C for 6 h daily for 6 days. The MeJA and MeSA treatments enhanced seedling chilling tolerance and improved plant height and shoot and root fresh and dry weights. The MeJA and MeSA increased leaf chlorophyll and relative water contents (RWC) and reduced rate of malondialdehyde (MDA) buildup and electrolyte leakage (EL), alleviating chilling injury to seedlings. Under greenhouse conditions use of MeJA and MeSA appears to be able to protect watermelon seedlings against chilling at the early stage of their growth.

Keywords: Cold stress, electrolyte leakage, malondialdehyde, reactive oxygen species, relative water content

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INTRODUCTION

In temperate regions, chilling is a limiting environmental factor influencing crop production. Cold stress includes chilling injury (temperature below 15°C) and freezing (temperature below 0°C). About two-hirds (due to freezing) and half (due to chilling) of land areas in the world annually exposed to cold conditions (Ahmad and Rasool, 2014). Depending on the plant species and their sensitivity, emergence of chilling injury symptoms fluctuates from 48 to 72 hr after exposure. Symptoms of plants in response to stress inducing cold can manifest as leaf wilting, chlorosis, necrosis (death of tissues) and reduced leaf expansion. Chilling stress can damage plants at all growth stage (Mahajan and Tuteja, 2005). Chilling stress directly disrupts plant metabolic reactions, indirectly prohibits water absorption and creates oxidative stress, it can change plant vigor and potency (Krasensky and Jonak, 2012). Although Iran's climate may be acceptable for watermelon production there is a danger temperatures can reach below 10°C limiting, or terminating, growth (Baninasab, 2009).

A derivative volatile active methyl ester of jasmonic acid as MeJA and MeSA are naturally-occurring compounds in plants. The MeJA is an endogenous plant growth regulator (PGR) that regulates many aspects of plant growth and development as well as plant responses to abiotic stresses (Cao *et al*., 2009). The MeSA is synthesized in plants from salicylic acid (SA), has a role in the plant defense-mechanism and can be converted back to SA in plants (Sayyari *et al*., 2011; Li *et al*., 2012). Employing SA (Sayyari *et al*., 2012), acetyl salicylic acid (ASA) (Korkmaz *et al*., 2007), paclobutrazol (Baninasab, 2009) and 5–aminolevulinic acid (Korkmaz *et al*., 2010) may alleviate harmful effects of chilling. The MeJA, affects antioxidant enzymes activity to induce chilling tolerance. This compound can protect mitochondria and chloroplast structures from chilling injury, improving plants tolerance to chilling stress (Li *et al*., 2012). Drenching soil or seed priming with SA or ASA induced chilling tolerance (Senaratna *et al*., 2000). The efficacy of these chemicals on improving chilling tolerance under storage has been reported (Sayyari *et al*., 2011; Wang *et al*., 2015). Effects of these chemicals on chilling injury to watermelon seedlings is not complete. The project was undertaken to evaluate effects of MeSA and MeJA on improving chilling tolerance of watermelon seedlings at the early stage of growth.

MATERIALS AND METHODS

This experiment was done in a greenhouse of the Agriculture College, Ilam University, Ilam, Iran, in 2014. Seed of watermelon were sown in plastic pots filled with a mix of fine sand, cow manure and clay loam soil in the ratio of 1:1:1. The greenhouse average temperature was 25/18°C (day/night) and exposure was to natural light. Seedlings were watered twice weekly using a 1/1000 v:v complete Bio Fertilizer (FosamcoBio™, Mashhad, Iran) containing: 100 $g{\cdot}L^{-1}$ N, 40 $g{\cdot}L^{-1}$ P, 70 $g{\cdot}L^{-1}$ K, 1.8 $g{\cdot}L^{-1}$ Mg, 1.3 $g{\cdot}L^{-1}$ Mn, 1 $g{\cdot}L^{-1}$ Cu, 0.7 $g{\cdot}L^{-1}$ Zn, 0.2 $g{\cdot}L^{-1}$ B, 0.07 $g{\cdot}L^{-1}$ Fe and 0.03 $g{\cdot}L^{-1}$ Mo and an extract of sea alga (*Ascophyllum nodosum*). Seedlings at 2–true leaf stage were treated (15 minutes) with 15 or 30 μM MeJA and 50 or 100 μM MeSA, so both sides of leaves were completely drenched under natural light and at 20 ± 2°C. Control plants were treated with distilled water. All materials were purchased from Sigma-Aldrich at the highest available purity (above 95%) and used as treatments. Four days after treatment, seedlings were transferred to a growth chamber at 3°C for 6 hr and this activity was repeated for 6 days. Afterward, all seedlings were returned to the greenhouse. To determine effects of treatment all seedlings were assessed 72 hr after chilling treatment (Baninasab, 2009; Sayyari *et al*., 2012).

To determine shoot fresh weight (SFW), aerial parts of seedlings were collected and weighed. To determine root fresh weight (RFW), plants were gently removed from the growing medium and roots washed with distilled water, dried with paper towels and RFW measured. Plant tissues were placed in paper bags and transferred to an electric oven and dried at 75°C for 72 hr, after which dry weights were recoded.

To determine chlorophyll (Chl) content the protocol of Strain and Svec (1996) was used. Samples (0.1 g) of fresh leaf tissue was homogenized in 5 mL of 80% acetone. The crude extract was centrifuged for 15 min at 1008 g. Then, 1 mL of supernatant and 4 mL 80% acetone was used for assessing absorbance at 663 and 645 nm with a spectrophotometer.

Wilting and necrosis of shoots were considered as the indictors of chilling injury index and classified by using the following scale: 1: no visible symptoms; 2: less than 5% of leaf area necrotic on shoots but without growth restrictions; 3: less than 15% of leaf necrotic area on shoots; 4: well–defined necrotic areas on shoots (less than 30% of leaf area necrotic); and 5: extensive necrotic areas (more than 50%) and severe growth restrictions but plant still alive (Baninasab, 2009).

Equal number of leaves in each sample were randomly taken from seedlings of each replicate. Leaves were weighed (FW) and then iμ Mediately floated on distilled water for 5 hr in the dark. Turgid weights (TW) of leaves were obtained after drying excess surface water with paper towels. Dry weights (DW) of leaves were measured after drying at 75°C for 48 hr. Relative water content (RWC) was calculated using the following formula (Korkmaz *et al*., 2010):

$$RWC = (FW-DW/TW-DW) \times 100$$

To evaluate EL, a protocol described by Korkmaz *et al*. (2010) was used. To remove surface contamination, leaf discs were cut with a cork borer and rinsed 3 times with distilled water. They were placed in test tubes containing 10 mL of distilled water. Afterward, samples were placed on a shaker at 150 rpm for 24 hr at room temperature and the first electrical conductivity (EC_1) determined. The solution containing samples were then placed in a hot water bath for 20 min and after cooling their secondary electrical conductivity (EC_2) measured. The EL was calculated as EC_1/EC_2 and expressed as a percentage.

To determine MDA content, which indicates rate of peroxidation of cellular membrane lipids, the method of Stewart and Bewley (1980), with slight modification, was used. Samples (0.25 g) of fresh leaf tissues were homogenized in 5 mL of 0.1% trichloroacetic acid. After centrifuging at 11,200 g for 20 min, 1 mL of supernatant was mixed with 4 mL of 20% trichloroacetic acid and 0.5 g thiobarbituric acid, was incubated in a water bath at 95°C for 30 min followed by transferring to an ice bath to stop the reaction. The absorbance of the supernatant measured at 532 and 600 nm.

The experiment was arranged in a completely randomized design (CRD) with 5 treatments and 3 replications containing 5 samples (5 × 3 × 5 = 75 samples). The data were subjected to ANOVA in SAS (ver. 9.1, SAS, Inc., Cary, NC). Means were separated using Duncan's range test at $P < 0.05$.

RESULTS AND DISCUSSION

The MeSA and MeJA affected plant height, numbers of leaves, SFW, SDW, RFW and RDW of watermelon seedlings exposed to chilling (Table 1). Seedlings treated with 100 µM MeSA, 30 µM MeJA, 30 µM MeJA, 30 µM MeJA, 50 µM MeSA and 50 µM MeSA produced the tallest plants, most leaves and highest SFW, SDW, RFW and RDW respectively (Figures 1 and Figure 2). Cold stress can reduce chlorophyll content, photosynthesis electron translocation, photosynthesis enzyme activities, stomatal conductance and nutrition absorption (Berova *et al*., 2002) and this condition can reduce its growth and development. In this study, when seedlings were pretreated with PGRs followed by exposing them to chilling, this condition caused to improve their growth. In similar findings, Seydpour and Sayyari (2016) reported that treating cucumber seedlings with lower concentrations of MeSA caused an increase in seedling growth under chilling condition. In this respect, Li *et al*. (2012) also reported similar results in growth of cucumber seedlings in response to MeJA application. Our results are consistent with others who reported positive effects of PGRs including ASA (Senaranta *et al*., 2000), paclobutrazole (Baninasab, 2009) and 5–aminolonic acid (Korkmaz *et al*., 2010) on increasing plant growth under chilling stress. The ability of these chemical to increase plant growth, by ameliorating adverse effects of chilling, may have significant implications in improving plant growth and overcoming barriers arising from chilling stress.

Table 1 ANOVA results for measured parameters of watermelon seedling subjected to treatment under chilling

Source	Plant height	No. of leaves	Shoot		Root		Chlorophyll			Chilling index	RWC[d]	EL[e]	MDA[f]
			FW[b]	DW[c]	FW	DW	A	b	a+b				
Treatment[a]	0.939*	0.436*	2.356**	0.017**	0.162**	0.0007**	0.171**	0.026**	0.313**	0.369**	2.248*	11.838*	0.0002**
Error	0.162	0.122	0.166	0.001	0.006	0.0000	0.004	0.000	0.010	0.047	2.156	2.138	0.0000
CV	4.118	6.960	7.919	6.608	5.073	6.240	5.312	4.121	5.174	9.720	1.510	4.902	6.8570

Significant at $P < 0.05$ or $P < 0.01$, ANOVA. *, **
[a] The treatments tested were distilled water (control), 15µM MeJA, 30µM MeJA, 50µM MeSA and 100µM MeSA.
[b]FW = Fresh weight, [c] DW = Dry weight, [d] RWC = Relative water content, [e] EL = Electrolyte leakage and [f] MDA = Malondialdehyde

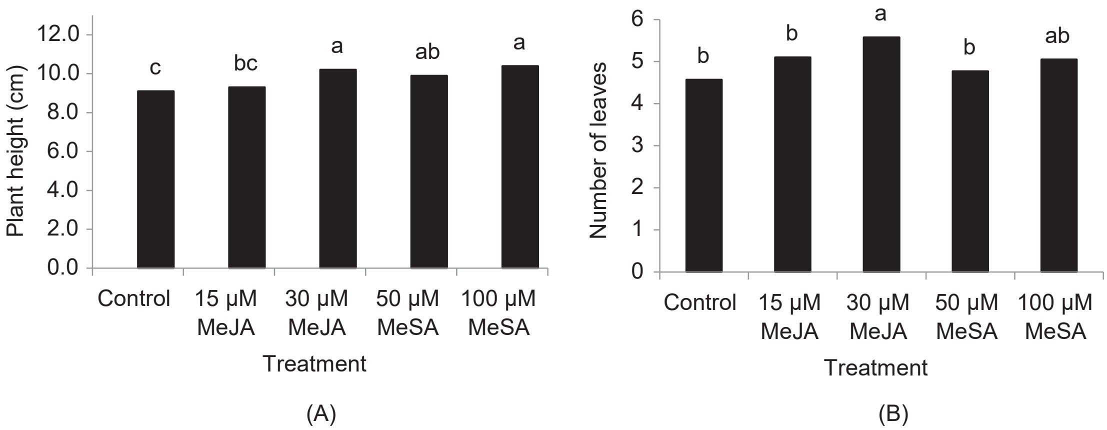


Figure 1 Effect of methyl jasmonate (MeJA) and methyl salicylate (MeSA) on plant height (A) and number of leaves (B) in watermelon seedlings subjected to chilling at 3°C for 6 hr/day for 6 days

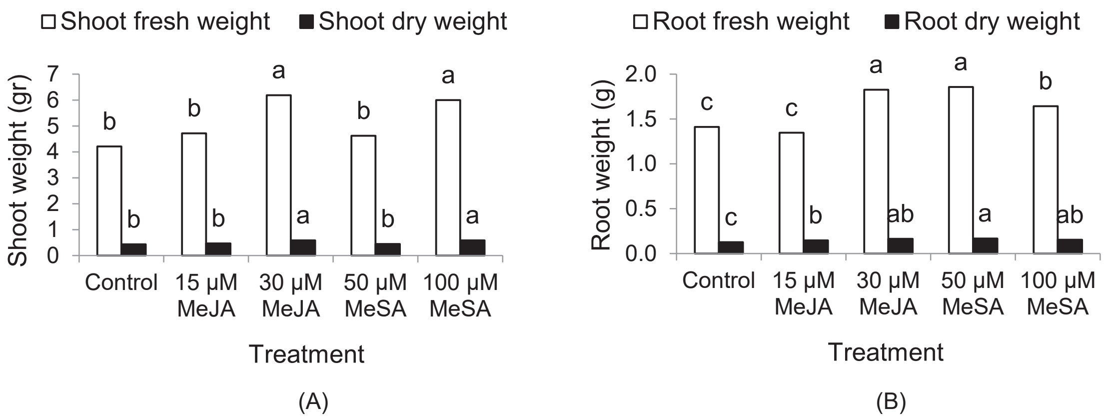


Figure 2 Effect of methyl jasmonate (MeJA) and methyl salicylate (MeSA) on shoot (A) and root (B) fresh and dry weights in watermelon seedlings subjected to chilling at 3°C for 6 hr/day for 6 days

Treatment affected photosynthetic pigments chlorophyll a (Chl a), chlorophyll b (Chl b) and chlorophyll a+b (Chl a+b) (Table 1). The application of MeSA and MeJA increased the photosynthetic pigments chlorophyll a, b and total chlorophyll. The concentration of 15 μM MeJA was most effective treatment on increasing all photosynthesis pigments compared to controls (Figure 3). By lowering temperature, all processes of chlorophyll production will be inhibited, since temperature plays a significant role on chlorophyll production (Mohanty *et al*., 2006). At the end of chilling period, the chlorophyll content denotes a plants' tolerance, or sensitivity, to chilling (Krasensky and Jonak, 2012).

Based on intensity and duration of the stress and stage of plant growth, the impact of cold on chlorophyll a, b and total chlorophyll will be different. Mohanty *et al*. (2006) demonstrated a relationship between reduced chlorophyll synthesis and its destruction by chlorophylase enzyme and a relationship between photosynthesis reduction, chlorophyll destruction and chloroplast structure. As lower temperatures less chlorophyll production occurs chlorosis is manifest indicating stress in plants (Bohnert *et al*., 1995; Ahmad and Rasool, 2014). Our findings are in agreement with those reported by Sayyari *et al*. (2012), Li *et al*. (2012) and Seydpour and Sayyari (2016) who found SA, MeJA and MeSA treatments induced chlorophyll content in Cucurbitaceae plants under chilling. The impact of MeJA and MeSA on maintaining pigments under chilling stress indicate that MeJA and MeSA pretreatment, by alteration in some tolerant responses, could be effectively used to protect cucumber from the adverse effects of low temperatures stress.

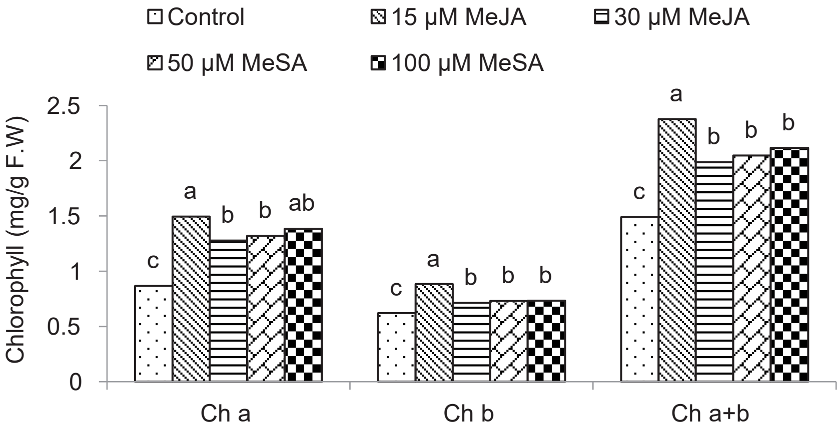


Figure 3 Effect of methyl jasmonate (MeJA) and methyl salicylate (MeSA) on chlorophyll content in watermelon seedlings subjected to chilling at 3°C for 6 hr/day for 6 days

The RWC was significantly affected by treatments (Table 1). The 15 μM MeJA and 50 μM of MeSA increased leaf RWC of chilled seedlings; the other treatments did not affect RWC (Figure 4). In another study, Seydpour and Sayyari (2016) found that using exogenous MeSA pretreatment may pave the way for elevating RWC and consequently induce cucumber's tolerance to chilling stress and their results are in agreement with our findings. The RWC is a good indicator of water status in plants and is an appropriate criterion for selecting plant tolerance to environmental stresses. A positive, high, correlation between water potential and RWC indicates an ability better tolerate stress. A reduction in RWC generally causes reduced stomatal conductance, photosynthesis and CO_2 processes (Hassanpour *et al*., 2008). During chilling, plants suffer from water deficit which starts with reduction in root hydraulic conductivity and intensifying reduction in leaf water. Plant stomata will close, and the rate of root hydraulic conductivity reduced due to reduced leaf transpiration (Joshi *et al*., 2007). Exogenous application of MeJA and MeSA reduces damage due to chilling by maintaining leaf RWC at suitable levels when plants are stressed.

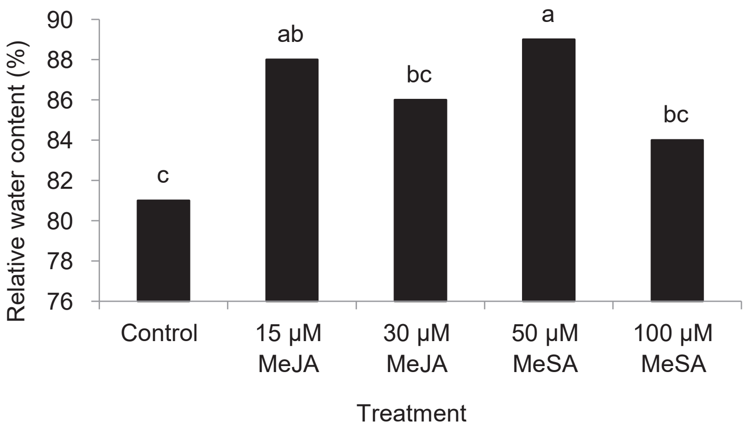
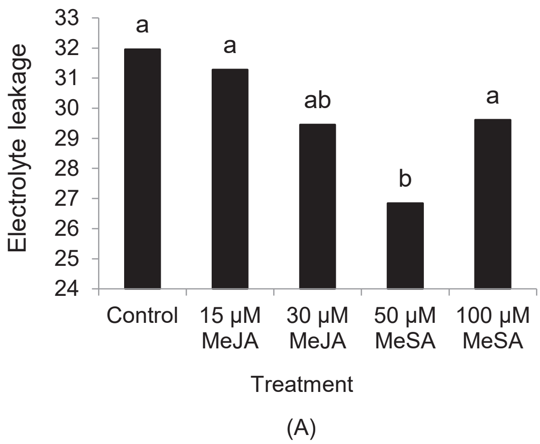


Figure 4 Effect of methyl jasmonate (MeJA) and methyl salicylate (MeSA) on relative water content in watermelon seedlings subjected to chilling at 3°C for 6 hr/day for 6 days

The EL was affected by treatment (Table 1). The 50 µM MeSA treatment reduced EL compared to controls; the other treatments did not affect EL (Figure 5). Treating seedlings with 30 µM MeSA and 50 µM MeJA reduced MDA accumulation (Figure 5). Temperature influences fluidity, stability and flexibility of cell membranes which act as a biosensor of chilling stress (Taiz and Zeiger, 2002). All reactive oxygen species (ROS), i.e., anion peroxide, peroxide hydrogen and radical hydroxyl, are able to attack unsaturated fatty acid, proteins and nucleic acids in cells. The ROS decreases membrane fluidity, ionic translocation, enzyme activities and protein synthesis (Mahajan and Tuteja, 2005). The ROS destroy nuclear DNA and mitochondria leading to cell death. Peroxidation of membrane lipids is a reaction stimulated in the presence of ROS, producing the MDA and ethanol. The effect of the active oxygen radical on lipids and their peroxidation is due to its influence on double bonding located on unsaturated fatty acids. This active radical stimulates peroxidation of chain reactions leading to deactivated fatty acids (Qiujie *et al.*, 1996).



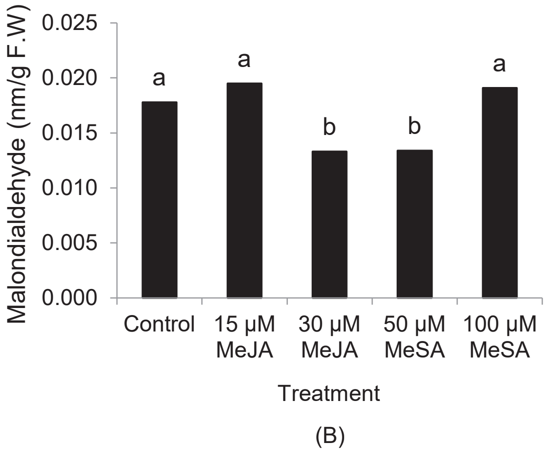


Figure 5 Effect of methyl jasmonate (MeJA) and methyl salicylate (MeSA) on electrolyte leakage (A) and malondialdehyde content (B) in watermelon seedlings subjected to chilling at 3°C for 6 hr/day for 6 days

Cellular EL can be used to determine the degree of damage to structure and functions of cell membranes due to chilling stress. Plant acclimation to stress depends on lowering ROS activities through stimulating antioxidant activities. It seemed that released radicals led to increased MDA and EL in chilled tissues. Due to their regulating effects on plant physiological process, MeJA and MeSA are able to reduce damage to cell membranes caused by chilling, decreasing MDA accumulation. Our findings with MeJA and MeSA agree with Sayyari *et al*. (2012) and Li *et al.* (2012) in that chilled seedlings treated with these chemicals enhanced their tolerance to stress through reducing EL. Treated seedlings exhibited few chilling injury symptoms, while non–treated seedlings were severely damaged, or died. Only the 30 μM MeJA treatment reduced chilling–injury symptoms in stressed seedlings (Figure 6). This agrees with a report indicating the efficiency of MeJA on reduction of damage inflicted by chilling through increasing antioxidant activities (Cao *et al*., 2009).

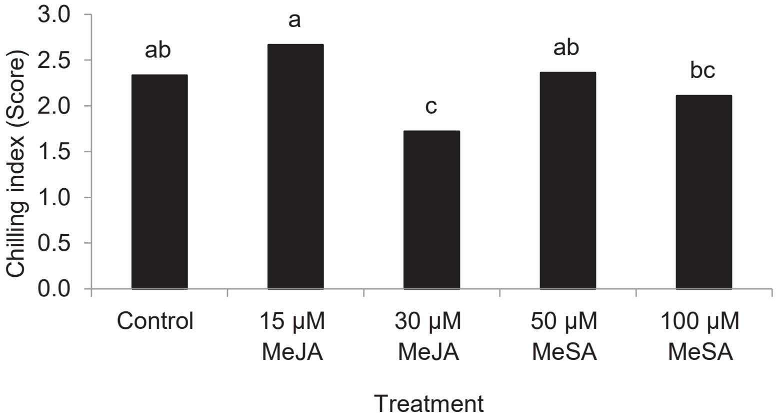


Figure 6 Effect of methyl jasmonate (MeJA) and methyl salicylate (MeSA) on chilling index injury in watermelon seedlings subjected to chilling at 3°C for 6 hr/day for 6 days

CONCLUSION

In conclusion, under greenhouse conditions it appears that MeJA and MeSA could be used as PGR to prevent chilling damages in watermelon seedling, but additional work on plants in the field is needed.

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