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Amelioration of chilling stress by paclobutrazol in watermelon seedlings

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ABSTRACT

Paclobutrazol (PBZ) is a member of the triazole plant-growth inhibitor group that is responsible for inducing tolerance to number of biotic and abiotic stresses. An experiment was, therefore, conducted to test whether PBZ application at various concentrations (0, 25, 50 and 75 mg L^{-1}) through seed soaking or foliar spray would protect watermelon (*Citrullus lanatus*) seedlings, subjected to chilling stress. Thirty-five-day old plants were exposed to chilling 5 h/day at 4 °C for 5 days. PBZ improved growth rate of watermelon seedling subjected to chilling stress and increased relative leaf chlorophyll content (RLCC) and chlorophyll fluorescence ratio (Fv/Fm) compared with the control at the end of chilling stress. PBZ ameliorated the injury caused by chilling stress by inhibiting increases in proline and leaf electrolyte leakage, which suggested that PBZ ameliorated the negative effect of chilling stress. PBZ was most effective in increased chilling tolerance of watermelon seedling when applied using the seed soak method than as a foliar spray. The best protection appeared to be obtained from seedlings seed soaked with PBZ at 50 and 75 mg L^{-1}.

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1. Introduction

Chilling sensitivity has been looked upon as an important issue in plant physiological and biophysical studies, particularly in tropical and sub-tropical species, which show characteristic damage symptoms when subjected to low, above zero temperatures (Prasad et al., 1994). Plants native to warm regions such as maize, tomato, cucumber and watermelon are generally injured at temperatures below 10 °C (Saltveit, 2000). Symptoms of chilling injury include wilting, reduced growth and photosynthetic capacity, chlorosis, necrosis, discoloration, abnormal ripening, increased disease susceptibility, leakage of ions from cell membranes, and changes in respiration and ethylene production (Rab and Saltveit, 1996; Sato et al., 2001).

Although conditions in the many parts of Iran are ideal for growing watermelons, targeting early harvests required field planting in early spring before temperatures reach optimum ranges (20–32 °C). Once the seedlings have been planted in the field, in some years due to temperature fluctuations they may be exposed to temperatures cycling between chilling and optimal for some days before temperatures stabilize. This condition may retard growth, delays flowering, reduc total yields and quality, and even kills the plants (Korkmaz and Dufault, 2001).

Numerous attempts such as breeding for increased chilling tolerance, genetic engineering, modifying crop management practices and application of chemicals have been used to increase chilling tolerance and avoid chilling injury (Lee et al., 1985; Zhang et al., 1987; Kang et al., 2002). Paclobutrazol (PBZ) [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol], a member of the triazole plant-growth inhibitor group, is a broad spectrum gibberellin biosynthesis inhibitor (Davis and Curry, 1991). Triazoles have both fungitoxic and plant-growth regulatory effects. They also increase tolerance of various plant species to biotic and abiotic stresses including fungal pathogens, drought, air pollutants, and low and high temperature stress (Fletcher et al., 2000). Therefore, the triazoles have been characterized as plant multi-protectants (Fletcher et al., 2000). PBZ has been reported to confer protection to plants experiencing stress by reducing oxidative damage via elevation of antioxidants or reducing the activity of oxidative enzymes (Lin et al., 2006). PBZ normally is applied as a foliar spray or growth medium drench (Still and Pill, 2004).

The purpose of this experiment was to test the possibility that application of PBZ would protect watermelon plants from damaging effects of chilling. Our specific objectives were: (1) to determine which application method (seed treatment and foliar spray) of PBZ would be more effective, and (2) to determine the optimum PBZ concentration that would provide the best protection against chilling stress.

2. Materials and methods

2.1. Plant material and cultural practice

Watermelon seeds (*Citrullus lanatus*) cultivar Charleston Grey, which is one of the most important cultivar grown in Iran, were

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used in this experiment. Seeds were disinfected in 1% (active ingredient) sodium hypochlorite solution for 10 min to eliminate possible seed-borne microorganisms, rinsed for 1 min under running water prior to drying for 30 min at room temperature.

Seeds were soaked with PBZ (CULTAR®, Syngenta Crop Protection, Basel, Switzerland) at 0 (control), 25, 50 and 75 mg L^{-1} for 24 h (Berova et al., 2002; Still and Pill, 2004) at room temperature (23 ± 2 °C) after which the seeds were washed and planted immediately. Seeds were planted into 1.5 L plastic pots filled with a 1:1:1 mixture of fine sand, leafmould and soil. The pots were then transferred to the greenhouse with average temperature of 25.5/19.5 °C (day/night) and natural light (November and December, 2007).

A second batch of seeds was also soaked in distilled water under the same conditions prior to sowing to obtain seedlings for foliar application of PBZ, and these seedlings were also raised in greenhouse under the same conditions. When the seedlings had two true leaves (28 days after sowing), the seedlings were sprayed with 0 (control), 25, 50 and 75 mg L^{-1} PBZ solution until both sides of the leaves were completely wet. Irrigation was done two times in a week to keep the optimum moisture level in the growth medium.

The layout was a 2×4 factorial experiment in a complete randomized design with four replications and three plants per replication (plastic pot).

2.2. Stress imposition

One week after the foliar PBZ application or 5 weeks after the seed treatment, all seedlings (seed soaked + foliar spray) were exposed to chilling in a growth chamber at 4 ± 0.5 °C for 5 h and then returned to the greenhouse. Chilling period was repeated for 5 days. All plants were assessed 72 h after the end of chilling stress to determine the extent of chilling injury (Lin et al., 2006; Korkmaz et al., 2007), and data were collected.

2.3. Injury rating valves

Chilling injury in watermelon seedlings was characterized by the wilting, dehydration and necrosis of the leaves and shoots and classified by using the following scale: normal, no visible symptoms; trace, small necrotic areas on shoots but without growth restrictions (less than 5% of leaf area necrotic); slight, small necrotic areas on shoots (less than 15% of leaf area necrotic); moderate, well defined necrotic areas on shoots (less than 30% of leaf area necrotic); and severe, extensive necrotic areas and severe growth restrictions (more than 50% of leaf area necrotic but plant still alive). By assigning values of 1, 2, 3, 4, and 5, respectively to each group (Wang, 1985).

2.4. Relative leaf chlorophyll content (RLCC)

RLCC of the youngest fully expanded leaf of all three plants per replicate was determined by using a chlorophyll content meter (Hansatech Instrument Ltd., King's Lynn, Norfolk, UK). The chlorophyll meter readings were used as relative values for chlorophyll content (Kapotis et al., 2003).

2.5. Electrolyte leakage

Electrolyte leakage was used to assess membrane permeability. This procedure was based on Lutts et al. (1996). Electrolyte leakage was measured using an electrical conductivity meter CC-501 (Elmetron, Zabrze, Poland). Five leaf discs of randomly chosen plant per replicate were taken from the youngest fully expanded leaf. Leaf samples were then placed in test tubes containing 10 mL of distilled water after three washes with distilled water to remove surface contamination. These samples were incubated at room temperature on a shaker for 24 h. Electrical conductivity (EC) of bathing solution (EC_1) was read after incubation. The same samples were then placed in a boiling water bath for 20 min and the second reading (EC_2) was determined after cooling of the solution to room temperature. The electrolyte leakage was calculated as EC_1/EC_2 and expressed as percent.

2.6. Chlorophyll fluorescence

The chlorophyll fluorescence induction parameters of the youngest fully expanded leaves were measured using a Plant Efficiency Analyzer (Hansatech Instrument Ltd., King's Lynn, Norfolk, UK) with saturation light pulse of about 3000 μmol m^{-2} s^{-1} (Kooten and Snel, 1990). Variable (Fv) to maximal (Fm) fluorescence ratio (Fv/Fm) was determined after the leaves were dark adapted for 30 min.

2.7. Proline determination

Proline was determined according to the method described by Bates et al. (1973). Seedlings (0.5 g of fresh plant material) were homogenized with 10 mL of 3% aqueous sulfosalicylic acid and filtered through Whatman's no. 2 filter paper. 2 mL of filtrate was mixed with 2 mL of acid–ninhydrin and 2 mL of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C. The reaction mixture was extracted with 4 mL of toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with a Shimadzu UV 160A spectrometer (Shimadzu Corporation, Kyoto, Japan). Appropriate proline standards (Sigma Chemical Co., USA) were included for calculation of proline in the sample.

2.8. Shoot and root characters

After the determination of chilling injury, shoots of the seedlings were cut at the growth medium line and their fresh weights were recorded. The roots of the seedling were carefully washed under running tap water to remove the growth medium and dried with paper towels to remove the surface water and their fresh weights were recorded. The shoots and roots were dried at 80 °C for 72 h and their dry weights were determined.

2.9. Statistical analysis

Data were analyzed for significant differences using a factorial analysis of variance with PBZ application methods and PBZ concentrations as main factors. Statistical analysis was performed using MSTATC software program and the means compared using the least significant differences (L.S.D.) test at $p = 0.05$.

3. Results and discussion

The results showed that chilling injury valve was not affected by application method (Tables 1 and 2). After exposure to chilling, the seedlings not applied with PBZ (0 mg L^{-1} PBZ) exhibited typical chilling injury symptoms in moderate to severe level, while PBZ-treated seedlings were slightly damaged. However, the least injury was obtained from the application of PBZ at 50 and 75 mg L^{-1}. There was no interaction between application method and PBZ concentration (Table 2). These results correlate well with the findings of Feng et al. (2003) who reported that triazole compounds resulted in increasing seedlings survival following a chilling stress in cucumber plants. Fletcher et al. (2000) also reported that PBZ protected wheat seedlings from injury due to chilling and that PBZ treatment reduced all symptoms of damage at various stages of growth. PBZ, by inhibiting gibberellin (GA)

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