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Amelioration of Chilling Injuries in Watermelon Seedlings by Abscisic Acid

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Abstract: A greenhouse study, designed in a randomized complete block design with five replications, was carried out at Clemson University, Clemson, SC, USA, in the spring of 1997. The objective of the study was to investigate whether abscisic acid (ABA) would mitigate chilling damages in the watermelon, a chilling-sensitive plant. 'Crimson Sweet' [*Citrullus lanatus* (Thumb) Matsum. & Nakai.] watermelon seedlings were grown in a greenhouse with a temperature regime of 25ºC (day) and 20ºC (night). Five-day-old seedlings were sprayed with 10^{-4} M cis, trans-abscisic acid 15 hours prior to being exposed to 1.5 ± 0.5ºC for 24 hours in a dark refrigerator. One and two weeks after the chilling exposure, seedlings were visually inspected and rated in order to estimate the extent of chilling injury, and their shoot and root fresh and dry weights were determined. As indicated by higher injury rating values, chilling caused significant visual damage on the plants that were not sprayed with ABA. Plants that were not treated with ABA had significantly lower shoot and root fresh and dry weights compared to plants that were sprayed with ABA prior to chilling exposure. Although the application of ABA did not protect the seedlings completely against chilling injury, chilling tolerance in cold-sensitive plants can be increased with the application of ABA or its analogs.

Key Words: watermelon, *Citrullus lanatus*, chilling injury and abscisic acid

Düşük Sıcaklıkların Karpuz Fideleri Üzerindeki Zararlarının Absisik Asit ile İyileştirilmesi

Özet: Bu çalısmanın amacı absisik asitin düşük sıcaklıklara karşı çok duyarlı bir bitki olan karpuz üzerinde meydana gelebilecek olan soğuk zararlarını önleyip önleyemiyeceğini ortaya çıkarmaktır. Sera çalışmaları Amerikan Clemson Üniversitesi'nde 1997 yılının bahar aylarında yapılmıştır. Deneme "Tesadüf Blokları" deneme desenine göre ve beş tekerrürlü olarak kurulmuştur. 'Crimson Sweet' karpuz çeşidi fideleri serada gündüz 25ºC ve gece 20ºC'de yetiştirilmiştir. Beş günlük fideler üzerine 10^{-4} M konsantrasyonunda olan absisik asit püskürtülmüş ve 15 saat sonra da fideler 24 saat süre ile sıcaklığı 1.5 ± 0.5ºC'deki bir buzdolabında karanlıkta tutulmuşlardır. Fideler soğuğa maruz kaldıktan bir ve iki hafta sonra görsel olarak kontrol edildiler ve soğuk zararını ortaya çıkarmak için sınıflandırıldılar ve toprak üstü kısımlarının ve köklerinin yaş ve kuru ağırlıkları tespit edildi. Absisik asit ile muamele edilmeyen bitkiler üzerinde şiddetli görsel soğuk zararları tespit edildi ve bu bitkilerin toprak üstü kısımlarının ve köklerinin yaş ve kuru ağırlıkları absisik asit ile muamele edilen bitkilerle kıyaslandığında daha düşük bulundu. Absisik asit ile yapılan muameleler bitkileri soğuğa karşı tamamen koruyamamasına rağmen, bitkilerin soğuğa olan toleransları absisik asit ve onun benzerleri ile artırılabilir.

Anahtar Sözcükler: karpuz, fide, üşüme ve absisik asit

Introduction

The watermelon is classified as a very tender warm season crop and is native to the Old World tropics and subtropics (tropical and southern Africa) (Bates and Robinson, 1995). Watermelon seeds germinate at a minimum soil temperature of 15ºC and the optimum air temperature for plant growth ranges from 20 to 32ºC.

Although conditions in the south and western parts of Turkey are ideal for growing watermelons, targeting early harvests requires field planting in early spring before temperatures reach optimum ranges. Once the seedlings have been planted in the field, they may be exposed to temperatures cycling between chilling and optimal for weeks before temperatures stabilize. Exposure of seedlings to low temperatures retards growth, delays flowering, reduces total yields and quality, and even kills the plants (Korkmaz and Dufault, 2001).

It is known today that low temperatures increase free abscisic acid (ABA) content in many plants such as tomatoes (King et al., 1982), cotton (Rikin et al., 1980), and cucumbers (Rikin and Richmond, 1976). Increasing evidence indicates that high levels of endogenous ABA are related to increased chilling tolerance, and an increase in ABA content before low temperature exposure might be an essential step in activating a protection mechanism against chilling (Zhang et al., 1986). Applications of exogenous ABA at warm temperatures have been reported to reduce injury from subsequent low temperature exposure in tomatoes (King et al., 1982), cucumbers (Yamazaki et al., 1995), and beans (Wilson, 1976). Therefore, the purpose of this experiment was to test the possibility that exogenous application of ABA would alleviate chilling damages in the watermelon, a chilling-sensitive plant.

Materials and Methods

The experiment was carried out at Clemson University, Clemson, SC, USA, with the seedlings of the watermelon (*Citrullus lanatus* L. cv. Crimson Sweet) in the spring of 1997. The seeds were obtained from the Burpee Seed Company (Warminister, PA, USA). Watermelon seeds were planted in 8 x 8 cm plastic containers filled with an artificial growth media—Metro Mix-300 (Grace Sierra Co, Milpitas, CA, USA), which is a combination of peat, vermiculite, and composted pine bark. The plants were kept in a greenhouse under natural photoperiod and a temperature regime of 25ºC (day) and 20ºC (night). After all seedlings had emerged, they were thinned and two seedlings were left in each container. The plants were watered with half-strength Hoagland nutrient solution (Hoagland and Arnon, 1950).

Each replication had two plants and all treatments were arranged in a randomized complete block design. There was a total of four treatments and each treatment was replicated five times. Since the same measurements were taken one week and two weeks after the treatments and the measurements were destructive to the plant material, additional sets of plants were also grown side by side with these plants under the same conditions and also treated as mentioned below.

Five-day-old seedlings, the shoots of which consisted of two cotyledons with no other leaves yet visible, were transferred to a dark refrigerator with a temperature of 1.5 ± 0.5ºC ("chilled") and kept there for 24 hours. A comparable group of seedlings was left in darkness in the greenhouse ("not chilled"). Fifteen hours before the exposure to chilling, half of the chilled and half of the non-chilled seedlings were sprayed with a solution of 10^{-4} M cis, trans-abscisic acid (ABA) (Rikin and Richmond, 1976) and the other half with water until the leaves were completely wet. At the end of the chilling period, the plants were returned to the pre-experimental conditions in the greenhouse.

All of the plants were visually examined to determine the extent of chilling injury and classified by using the following scale: none, no visible symptoms; slight, small necrotic areas on shoots but without growth restrictions (less than 5% of leaf area necrotic); moderate, well-defined necrotic areas on shoots (less than 25% of leaf area necrotic); severe, extensive necrotic areas and severe growth restrictions (more than 50% of leaf area necrotic but plant still alive); and killed, entire plant necrotic and collapsed. By assigning values of 1, 2, 3, 4, and 5 respectively to each group, the average injury for each treatment was calculated.

After the determination of the extent of chilling injury, shoots of the seedlings were cut at the growth medium line and their fresh weights were recorded. The roots of the seedlings were 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 65ºC for one day and their dry weights were recorded.

All of the data for each plant set were analyzed separately and subjected to analysis of variance using SAS statistical software (SAS Inst., 1997). The entire experiment was repeated twice and, since there was no significant difference between the results of two experiments, data from both experiments were combined.

Results and Discussion

After exposure to chilling for 24 h, the seedlings not sprayed with ABA showed typical chilling injury symptoms. First, the leaves of chilled and non-ABA-treated seedlings were completely wilted and lost a large portion of their foliage as indicated by higher injury rating values compared to chilled and ABA-treated seedlings both one and two weeks after chilling exposure (Table 1). Especially two weeks after the exposure, most non-ABA-treated seedlings continued to suffer heavily and therefore injury rating values increased from 3.80 to 4.60, which resulted from seedling deaths. In contrast, only a few ABA-treated seedlings died within two weeks

Table 1. Effect of application of ABA and chilling on chilling sensitivity of watermelon seedlings.

Treatment	Injury Rating Values[z]	
	one week after chilling	two weeks after chilling
Chilled – ABA	3.80 a	4.60 a
Chilled + ABA	2.70 b	3.10 b
Not Chilled – ABA	1.00 c	1.00 c
Not Chilled + ABA	1.00 c	1.20 c
$LSD_{0.05}$	0.27	0.70

[z] Injury rating values: 1, none; 2, slight; 3, moderate; 4, severe; 5, killed.

of the chilling treatment. These results are in an agreement with those of Rikin and Richmond (1976), who reported that the leaf area of chilled and ABA-treated cucumber seedlings was two and a half times higher than those of non-ABA-treated seedlings.

In addition, shoot and root fresh and dry weights of ABA-treated seedlings were always higher than those of non-ABA-treated seedlings (Tables 2 and 3). It was observed during the two-week growth period after the exposure to chilling that the morphological differences between ABA-treated and non-ABA-treated plants became larger. Thus, chilling may have damaged the growing points of watermelon seedlings and this damage was noticeably prevented by ABA application prior to chilling. However, even the application of ABA was not enough to protect seedlings completely, since there were significant differences between chilled and non-chilled plants in terms of every measurement taken during the experiment.

Certain researchers have suggested that the cell membranes are the primary site of chilling injury in plant cells. Lyons (1973) proposed that the immediate and direct response to low temperatures in chilling-sensitive plants is a phase change— a transition from a liquid state to a semi-crystalline gel state— that occurred in the lipid portion of cellular membranes at chilling temperatures. As membranes become less fluid, their protein components can no longer function normally, causing water and soluble materials to leak out into the intercellular spaces where the water is lost through evaporation, which is the primary cause of wilting (Wright, 1974). Since chilling injuries were found to be far less severe under conditions of high relative humidity in which water loss from the cell is minimized (Wright and Simon, 1973), perhaps the effect of ABA on decreasing chilling injury may result from its effect in

Table 2. Effect of pre-treatment with ABA and chilling on shoot fresh and dry weights of watermelon seedlings one and two weeks after exposure to chilling.

Treatment	Shoot Fresh Weight (g)		Shoot Dry Weight (g)	
	one week after chilling	two weeks after chilling	one week after chilling	two weeks after chilling
Chilled – ABA	0.20 b	0.24 c	0.021 c	0.025 c
Chilled + ABA	0.42 b	0.88 b	0.040 b	0.065 b
Not Chilled – ABA	1.25 a	2.65 a	0.082 a	0.163 a
Not Chilled + ABA	1.09 a	2.30 a	0.071 a	0.145 a
$LSD_{0.05}$	0.22	0.37	0.015	0.025

Table 3. Effect of pre-treatment with ABA and chilling on root fresh and dry weights of watermelon seedlings one and two weeks after exposure to chilling.

Treatment	Root Fresh Weight (g)		Root Dry Weight (g)	
	one week after chilling	two weeks after chilling	one week after chilling	two weeks after chilling
Chilled – ABA	0.230 a	0.285 a	0.010 c	0.013 c
Chilled + ABA	0.290 b	0.475 b	0.032 b	0.042 b
Not Chilled – ABA	0.400 c	0.720 c	0.050 a	0.079 a
Not Chilled + ABA	0.515 c	0.770 c	0.056 a	0.076 a
$LSD_{0.05}$	0.045	0.150	0.012	0.010

improving the water balance in plants. ABA may improve the water balance of chilled plants by decreasing the transpiration via promoting stomatal closure (Jones and Mansfield, 1970), as well as by increasing root resistance to water efflux (Glinka, 1973).

Besides the direct effect of ABA on plant water status, the experiments that employed ABA and its terpenoid analogs revealed that these compounds protect the cell membrane system against chilling-induced degradation. Membrane permeability as measured by electrolyte efflux was greatly stabilized by the application of ABA and its analogs before chilling (Flores et al., 1988).

Certain pre-treatments such as withholding water from plants and salinization of root medium can be used to increase plant ABA content (Rikin et al., 1976). These treatments induce water deficiency in plants, elevating the ABA content, which, in turn, help protect plants from subsequent chilling temperature exposures.

In conclusion, chilling tolerance in plants can be augmented by applying ABA or ABA analogs. Alternatively, selecting and breeding plants with high endogenous ABA content or pre-treatment of plants to increase endogenous level of ABA may also help protect plants against chilling injury.

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