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Effects of Ascorbic Acid Application in Strawberry Plants During Heat Stress

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Abstract

Frigo seedlings of 'Redlands Hope' (R. Hope) and 'Festival' strawberry cultivars, which are heat-tolerant and heat-sensitive, respectively were grown in a controlled greenhouse. When the seedlings had 5-6 leaves (11 weeks old); half of the plants were sprayed with 3 mM ascorbic acid (AA) every three days for 3 weeks (7 applications) in order to investigate whether AA is effective on heat stress tolerance of the cultivars, the other half ones were used as control plants. Plants were then transferred to a climate chamber and exposed to gradually increased high temperature up to 44° C. The samples were taken from the plants exposed to 44 °C for six hours. AA content, total chlorophyll content, leaf relative water content (RWC), loss of turgidity and peroxidase (POX) isozyme activity were evaluated in leaf tissues. AA application and heat stress increased AA content in 'R. Hope' while decreased in 'Festival'. Total chlorophyll content of cultivars was increased with high temperature in control plants in contrast to AA applicated plants. RWC content of 'R.Hope' was higher than in 'Festival' at the high temperature, while loss of turgidity of 'Festival' was higher than in 'R. Hope'. In addition, AA application decreased loss of turgidity in both cultivars. A basic POX band was observed in the samples on native PAGE with different intensities. The intensities of the band were generally higher in 'R. Hope' than in 'Festival'.

Keywords: Strawberry plants, heat stress, ascorbic acid, peroxidase.

Askorbik Asit Uygulamasının Çilek Bitkisinin Yüksek Sıcaklığa Toleransına Etkisi

Özet

Redlands Hope (R. Hope) ve Festival çilek çeşitlerinin frigo fideleri 11 hafta (5- 6 yapraklı oluncaya kadar) boyunca serada yetiştirilmişlerdir. Askorbik asitin (AA) çilek bitkilerinin yüksek sıcaklık toleransına etkisini belirlemek amacıyla, bitkilerin yarısına üç günde bir 3 hafta boyunca (7 uygulama) 3mM AA uygulaması yapılmıştır, bitkilerin kalan diğer yarısı da kontrol olarak kullanılmıştır. Daha sonra bitkiler iklim kabinine alınarak, sıcaklık kademeli olarak arttırılmış ve 44^{o}C'de altı saat tutulmuşlardır. Bitkilerden alınan yaprak dokularında AA miktarı, klorofil miktarı, yaprak oransal su kapsamı (YOSK), turgor kaybı ve peroksidaz enzim aktiviteleri incelenmiştir. AA ve sıcaklık stresi uygulaması 'R. Hope'da AA miktarını arttırırken, 'Festival'de azaltmıştır. Kontrol bitkilerinde çeşitlerin toplam klorofil miktarı yüksek sıcaklık uygulamasıyla birlikte artarken, AA uygulamasında tam tersi durum gözlenmiştir. Yüksek sıcaklıkta 'R. Hope'un YOSK'u 'Festival'e göre daha yüksekken, 'Festival'in turgor kaybı 'R.Hope'a göre daha yüksek bulunmuştur. Ayrıca, AA uygulamalarının her iki çeşitte de turgor kaybını azalttığı belirlenmiştir. Native-PAGE'de uygulamalara göre yoğunluğu değişen bazik bir peroksidaz bandı tespit edilmiştir. Belirlenen bandın yoğunluğunun genel olarak 'R. Hope'da 'Festival'e göre daha yüksek olduğu gözlenmiştir.

Anahtar Kelimeler: Çilek, sıcaklık stresi, askorbik asit, peroksidaz

Introduction

Heat stress is generally defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to growth and development of plants (Wahid et al., 2007). High temperatures lead to the decrease of photosynthetic activity due to inhibiting chlorophyll biosynthesis and over-producing ROS of the cells (Mittler, 2002; Ristic et al., 2007). The elevated ROS poses great damage to nucleic acid, proteins, lipids and to the normal functioning of the cell (Mittler, 2002). However, plants are able to

cope with ROS by enzymatic and non enzymatic antioxidants. AA is the one of the most important non enzymatic antioxidants and exists in various cell organelles and in the apoplast (Smirnoff, 2000). AA is able to scavenge O_2^-, OH^-, $^1O_2$ directly and is also capable of reduction of H_2O_2 to H_2O via the ascorbate peroxidise (APX) reaction. AA is also responsible for regenaration of tocopherol from the tocoperoxyl radical (Smirnoff, 2000; Foyer and Noctor 2011). Peroxidases (POX) which are belong to enzymatic antioxidants, have many physiological functions in plants such as removal of H_2O_2, oxidation of toxic reductants, biosynthesis and degradation of lignin cell walls, auxin catabolism, defensive responses to wounding, defence against pathogen or insect attack and some respiratory processes (Gaspar et al., 1982). The H_2O_2 in chloroplasts is scavenged by a POX which triggers the conversion of H_2O_2 to water and oxygen and also the changes in antioxidant enzymes contribute to the plants resistance to high temperature (Nakano and Asada, 1981; Jeffrey, 2002; Yin et al., 2008; He and Huang, 2010).

Previous studies are reported that, exogenous applications of AA decreased adverse effects of various stress conditions including heat stress in rice, sunflower, bean, mungbean and wheat (Dolatabadian and Jouneghani, 2009; Kumar et al., 2011; Shah et al., 2011; Ebrahimian and Bybordi, 2012; Dwivedi et al., 2012; Malik and Ashraf 2012). The physiological and molecular effects of heat stress in strawberry cultivars have been reported (Gulen and Eris, 2003; 2004; Ledesma et al., 2004; 2008; Wang and Lin, 2006; Ergin, 2012; Ergin et al., 2012; Kesici et al., 2013). However, to our knowledge, no previous studies have been published about the effects of exogenous applications of AA on thermotolerance on strawberry cultivars.

In this study, the potential role of AA has been investigated for the alleviation of heat stress related problems by improving the physiological and antioxidant performance of strawberry. In this regard, the current research was carried out with predetermined heat sensitive (Festival) and heat tolerant (R. Hope) strawberry cultivars (Kesici ,et.al 2013) to study the interactive effect of foliar application of AA and heat stress on physiological traits of strawberry plants under controlled environment.

Materials and Methods

Frigo seedlings of 'Redlands Hope' (R. Hope) and 'Festival' strawberry cultivars were grown in a controlled greenhouse (26/10°C day/night temperature, 60% relative humidity and 16/8 h (light/dark) photoperiod regime. When the seedlings had 5-6 leaves (11 weeks old); half of the plants were sprayed with 3 mM ascorbic acid (AA) (Elwan and El-Hamed, 2012) every three days for 3 weeks (7 applications) in order to investigate whether AA is effective on heat stress tolerance of the cultivars, the other half ones were used as control plants. AA solutions were applied during late afternoon using a hand-held sprayer. Plants were then transferred to a climate chamber and temperature was stepwise increased (2°C/h) started from 30°C to 44°C. The samples were taken from the plants exposed to 44 °C for six hours.

Leaf relative water content (RWC) and loss of turgidity: Leaf RWC (%) and loss of turgidity were measured using the methods of Barr and Weatherley (1962). Leaf discs of 1 cm diameter were cut from the fully expanded and uniform leaves of each of the three plants (replicates) per treatment. First, the fresh weight was recorded, and then samples were placed in a petri dish of distilled water for 4 h. After gently blotting the leaf surface with paper, turgid weights were recorded. At the end of this period, leaf samples were placed in an incubator at 70°C for 24 h, to determine the dry weight. Leaf RWC and loss of turgidity were measured as fallow; RWC (%)=[(fresh weight-dry weight) / (turgid weight-dry weight)] × 100, loss of turgidity (%)=[(turgid weight- fresh weight) / turgid weight] × 100.

Total chlorophyll content: Total chlorophyll content was determined using methods developed by Moran and Porath (1980). Three leaf discs of 1 cm diameter were taken from the fully expanded leaves of each treatment and soaked in 5 mL of dimethylformamide (DMF) for 72 h at 4°C (in the dark). The absorbance was read at 652 nm in spectrophotometer to measure chlorophyll content, which was calculated as mg/gFW.

Extraction and determination of AA: AA concentrations were determined according to Schoner and Krause (1990). Samples of leaf material (1 g) were ground in a mortar with 2 ml 10% (v/v) metaphosphoric acid. Homogenized solution was diluted with distilled water and centrifuged at 4000 g for 10 min. An aliquot of 0.5 ml of extract, diluted up to 5-fold with 2% (v/v) metaphosphoric acid, was mixed with 1 ml of Na-citrate buffer, pH 2.6, and 1 ml 100 µM dichlorophenolindophenol (DCPIP). Following incubation for 30 s, the optical density of the solution was determined at 524 nm and the amount of AA was calculated by reference to a standard curve.

Peroxidase (POX) isozyme: The POX was extracted from leaf tissues using the extraction methods described by Gulen et al. (2002). Ground

leaf tissues (0.1 g) were homogenized at 4ºC in 0.6 ml extraction buffer [0.1 M potassium phosphate pH 7.5, 30 mM boric acid, 50 mM L-ascorbic acid, 17 mM sodium metabisulfite, 16 mM dithiocarbamic acid, 1 mM EDTA and 4% (w/v) PVP-40 and final pH was readjusted to 7.5 with NaOH]. Homogenates were centrifuged at 15 000 rpm for 20 min and supernatant was used for electrophoresis. Discontinuous PAGE was performed with a PROTEAN tetra vertical electrophoresis unit (Bio-Rad, Hercules, Calif.) for acidic and basic PRX, respectively, according to Davis (1964) and Reisfeld et al. (1962). In PAGE, 5% stacking gels and 10% separating gels were prepared for both systems. For each sample 20 µl of crude extract was loaded to the gel. Electrophoresis was performed at 20 mA for 30 min, followed by 40 mA for 3h. Gels were stained for POX using the method of Wendel and Weeden (1989). The relative distance (Rf value) of the bands on the gel was calculated as described by Manganaris and Alston (1992) using Rf=1.0, distance to the fastest band and Rf=0.0, the starting point.

Results

Effects of AA application on leaf RWC content of strawberry plants exposed to heat stress were shown in Figure 1. However leaf RWC of cultivars decreased from control to the high temperature in AA non-applied plants, it was almost unchanged in AA applied plants. A two way ANOVA revealed a significant effect of application with regard to percent RWC (Table 1). On the other hand, loss of turgidity was at least in control plants, it was increased with high temperature (Figure 2). AA application was showed alleviate effect on loss of turgidity in both cultivars. Loss of turgidity of 'R. Hope' was less than 'Festival' in all applications. A two way ANOVA revealed a significant effect of application and cultivar with regard to percent loss of turgidity (Table 1).

The amount of total chlorophyll in the leaves after AA and high temperature applications was examined in the cultivars (Figure 3.). High temperature was increased the total chlorophyll content of the cultivars in AA non-applied plants opposite to AA applied plants. Total chlorophyll content of 'R. Hope' was higher than 'Festival' in AA non-applied plants, while it was less in 'R. Hope' than 'Festival' in AA applied plants. A two way ANOVA revealed a significant effect of application and a significant interaction of application and cultivar on total chlorophyll content (Table 1).

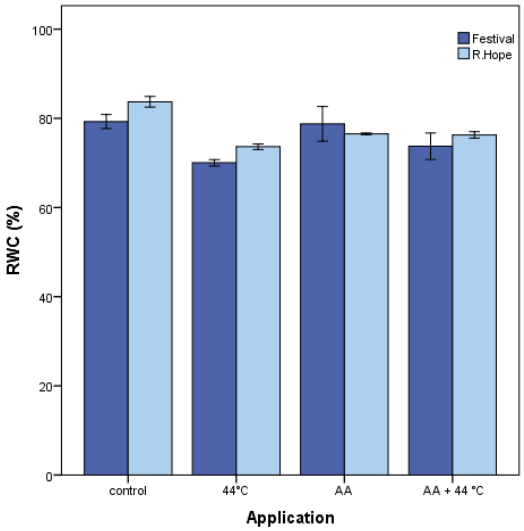

Figure 1. Effects of AA application (3 mM) on relative water content (RWC) of strawberry plants exposed to heat stress (44°C) for 6 h. Values are means from three replications and vertical bars indicate ± S.E.

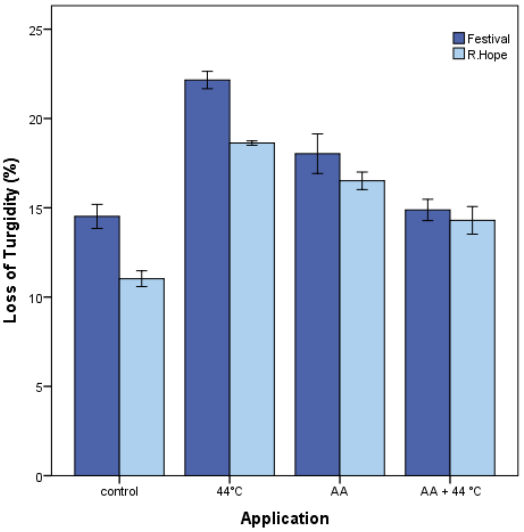

Figure 2. Effects of AA application (3 mM) on loss of turgidity of strawberry plants exposed to heat stress (44°C) for 6 h. Values are means from three replications and vertical bars indicate ± S.E.

AA content of cultivars was decreased with high temperature in AA- applied plants in contrast to AA non-applied plants (Figure 4.). A two way ANOVA revealed a significant effect of application and cultivar on AA content (Table 1).

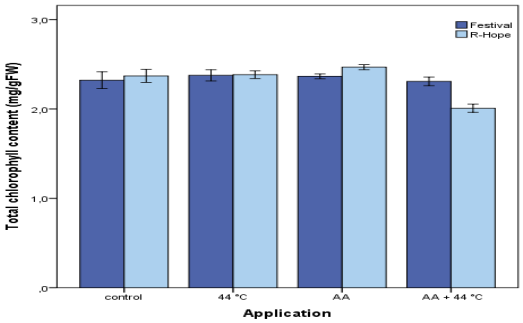

Figure 3. Effects of AA application (3 mM) on total chlorophyll content of strawberry plants exposed to heat stress (44°C) for 6 h. Values are means from three replications and vertical bars indicate ± S.E.

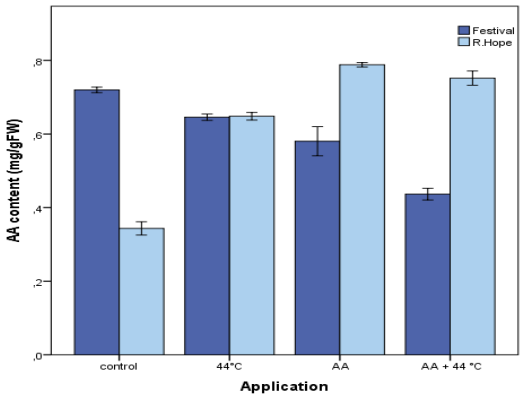

Figure 4. Effects of AA application (3 mM) on ascorbic acid (AA) content of strawberry plants exposed to heat stress (44°C) for 6 h. Values are means from three replications and vertical bars indicate ± S.E.

Native PAGE of POX was performed to obtain acidic and basic isozyme profiles in strawberry leaf tissues. Although no any acidic POX band was observed in both cultivars, a single sharp basic POX band with Rf= 0.49 was obtained (Figure 5). The darkest bands were observed in 'R. Hope' whereas it was light in 'Festival'. While the band intensity of 'R. Hope' was highest in control, it is followed by AA + 44°C, AA and 44°C applications; the intensity of 'Festival' was lowest in control, and is followed by AA, AA + 44°C and 44°C applications.

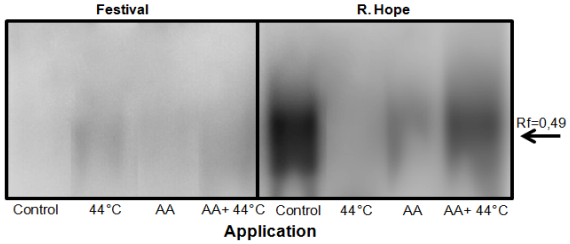

Figure 5. Effects of AA application (3 mM) on basic PRX profiles of 'Festival' and 'R. Hope'. Basic peroxidase activity (arrow on the left indicates the Rf=0.49 isoperoxidase). Equal volumes of the crude extracts, 20µl, were loaded in each lane.

Table 1. Results of variance analysis (ANOVA) of application (A), cultivar (Cv.) and their interactions for relative water content (RWC), loss of turgidity, total chlorophyll content and ascorbic acid content (AA).

Dependent variable	Independent variable		
	A	Cv.	A x Cv.
RWC	9.11 *	2.36 ^{ns}	1.21 ^{ns}
Loss of turgidity	52.99*	25.01*	2.60 ^{ns}
Total chlorophyll content	7.24*	0,64^{ns}	4.58*
AA	25.19*	7.95 *	132.96 ^{ns}

F-values at the 0.05 level
*, ^{ns} Significant and non significant at P ≤ 0.05

Discussion

Primarily, high temperatures cause increases in transpiration, and this change leads to a reduction in the leaf RWC and increment of the loss of turgidity (Cansev, 2012). The loss of water in the cells is triggered cellular signal transduction and a series of genes are activated thus stress response mechanism is started to work (Gonzalez and Gonzalez-Vilar, 2001). Gulen and Eris (2003) reported that high temperature treatments were decreased the leaf RWC and conversely increased loss of turgidity in strawberry cv. 'Camarosa'. Similarly, it was determined that decreases in leaf RWC and increases in loss of turgidity with heat stress treatments in small reddish bean cv. Keklik (Aydoğan and Turhan, 2013). Increasing heat stress was also reduced of leaf RWC in tall fescue and perennial rye-grass (Xu et al., 2006). In this study, the leaf RWC and loss of turgidity were varied in response to high temperatures. Also, it is determined that, positive effects of AA application on leaf RWC and loss of turgidity in strawberry plants under high temperature.

Previous studies reported that an increase in chlorophyll content in strawberry under heat stress (Gulen and Eris 2003; Kesici et al., 2013). Similarly, in the present study, the chlorophyll content of cultivars was increased with high temperature in AA non-applied plants in contrast to AA applied plants. This situation could explain that AA may protects the plants to heat stress as an antioxidant.

High endogenous AA in plants is necessary to counteract oxidative stress in addition to regulating other processes of plant metabolism. Endogenous AA can be increased by exogenous application of AA through the rooting medium, as a foliar spray and as seed priming (Malik and Ashraf, 2012; Ebrahimian and Bybordi, 2012). In this study

exogenous AA application was also increased AA content of 'R. Hope' opposite to 'Festival'.

POXs are a large group of isoenzymes with an extreme range of isoelectric points, serving a multitude of functions and each group is thought to have a different function in the cell (Huystee, 1987; Walter, 1992). Similar with this study, one basic isoperoxidase band was determined with Rf = 0.22 and Rf= 0.55 in strawberry plants under heat stress (Gulen and Eris, 2004, Ergin 2012; Ergin et al., 2012).

Conclusion

Our data indicated a correlation between AA application and high temperature. AA applied plants showed less loss of turgidity and higher RWC and AA content and POX activity was higher especially in heat tolerant cultivar (R. Hope) compared to non-applied ones under heat stress. Thus, it may be concluded that exogenously applied AA is effective in ameliorating the adverse effect of heat stress in strawberry.

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