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Heat tolerance of highbush blueberry is related to the antioxidative enzymes and oxidative protein-repairing enzymes

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ABSTRACT

Improving the understanding of the heat-tolerance mechanisms in blueberries (*Vaccinium* spp.) and screening high heat-tolerant germplasms are critical for the development of this fruit. In the present study, the heat tolerances of the 'Jersey' and 'Diana' cultivars were compared, which represent the northern and southern highbush blueberry cultivars, respectively. The results showed that exposure to high temperature significantly increased the level of oxidative damage in both highbush blueberries. However, oxidative stress induced by high temperature is significantly lower in 'Jersey' than that observed in 'Diana'. The lower level of oxidative stress in 'Jersey' was related to the significantly higher transcript levels of the antioxidative genes and oxidative protein-repairing genes, which may have resulted in the relatively higher semi-lethal high temperature of 'Jersey' (48.30 °C) than that of the heat-sensitive 'Diana'(47.32 °C). The heat susceptibility of 'Diana' was also confirmed by the 3-year field trial, the total death rates of 'Jersey' and 'Diana' were 7.78 and 12.22%, respectively. Therefore, it can be suggested that some northern highbush blueberry cultivars such as 'Jersey' are more heat tolerant than some southern highbush cultivars, and thus suitable for cultivation in lower latitude areas with hot summers.

1. Introduction

Over the past 10 years, the area planted with blueberry (*Vaccinium* spp.) has extended rapidly to about 1300 ha in North China. Meanwhile, the scale of cultivation has also dramatically increased in South China because of its advantages of a longer growing season and high economic revenue. However, cultivation in South China faces the critical challenge of heat stress during the summer. In Jinhua, for example, the daytime ground temperatures can be above 48 °C for more than 6 h on a typical summer's day. This is considered to be the biggest obstacle for the cultivation of the highbush blueberry in South China. It is therefore important to understand the heat-tolerance mechanisms in blueberry plants and to screen the cultivars for a higher thermal tolerance. However, the ability of different highbush blueberry cultivars to endure heat is still unclear, and a reliable and efficient method to screen cultivars for thermal endurance is still lacking.

Northern highbush, rabbiteye, and southern highbush blueberry are the three most commercially important species cultivated in the USA (Vashisth et al., 2011). In North America, the northern highbush cultivars are cultivated mainly in the Mid-West area owing to their good cold resistance, whereas the southern highbush cultivars and rabbiteye cultivars, which are heat tolerant, are planted in the South and South–West areas (Strik and Yarborough, 2005). As the hybrid of the northern highbush blueberry and *Vaccinium darrowi*, a native blueberry species in the hot and dry areas of Florida, the southern highbush blueberry, is supposed to be better adapted to heat stress than the northern highbush blueberry (Ballington, 1990). However, some northern highbush cultivars, such as 'Jersey' have shown a higher heat tolerance than the southern highbush cultivars in the field. However, the experimental data on this phenomenon are still lacking and the mechanism involved in the heat tolerance of the blueberry also needs to be clarified.

A well-known consequence of elevated temperatures in plants is the damage caused by heat-induced oxidative stress, resulting from the accumulations of reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^\bullet$ OH) (Choudhury et al., 2013). ROS can attack unsaturated fatty acids in the membrane and

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generate lipid peroxidation products, the accumulation of which will lead to the exudation of cell contents and loss of membrane permeability, thereby increasing the relative conductivity (Yeh and Lin, 2003). These harmful ROS are scavenged mainly by both non-enzymatic and enzymatic detoxification systems including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) (Choudhury et al., 2013; Kar, 2011; Mittler, 2002). Abiotic stress such as high temperature can cause a high level of ROS generation in plants, which, if not eliminated promptly, will lead to protein oxidation. The proteins rich in methionine (Met) or cysteine (Cys) residues are the most susceptible to oxidative damage. Cysteine can be involved in disulfide bridges or oxidized to the sulfenic or sulfinic forms, and methionine can be oxidized to methionine sulfoxide (MetSO) (Tarrago et al., 2009). Most altered proteins are eliminated through degradation and only a few repair mechanisms have been reported (Chondrogianni et al., 2014). The oxidized products of cysteine are enzymatically reversed by glutaredoxin (GRX), thioredoxin (TRX), and sulfiredoxin (SRX), while methionine sulfoxide reductases (MSRs) are mainly involved in reducing methionine sulfoxide back to methionine through redox-active cysteines (Davies, 2005). The oxidative protein-repairing systems are well described in animals, but literature on the related mechanism in plants is quite limited.

As the blueberry is a woody plant species, it is a time- and labor-consuming task to determine heat tolerance using field experiments. Using logistic equations combined with conductivity measurement is thus a relatively common and easy alternative (Cheng et al., 2010; Deng et al., 2011). Therefore the aim of the present study was to compare the heat tolerance of the northern highbush blueberry 'Jersey' and the southern highbush blueberry 'Diana', by determining the blueberry's lethal temperature (LT_{50}) based on electrolyte leakage (REC) under heat stress with the logistic equation. Then, their heat tolerances would be verified by the survival rates 3 years after introduction. To elucidate the heat tolerance in the highbush blueberry, the oxidative damage in the two cultivars under heat stress will be analyzed and compared. The gene expression patterns of antioxidative enzymes and oxidative protein-repairing enzymes will also be determined.

2. Materials and methods

2.1. Plant materials and temperature treatments

In July 2012, healthy and uniformly grown plants of 2-year-old northern highbush blueberry 'Jersey' and southern highbush blueberry 'Diana' were individually transplanted into pots (18 × 18 cm) filled with mixed medium (sphagnum peat: sandy soil = 1:1; pH 5.5; organic matter 10.5%; P-Olsen 18 $\mu g\,g^{-1}$). The seedlings were well irrigated and regularly managed. After being pre-cultured in a greenhouse for 21 days (day/night temperature 25/20 °C, photoperiod 14 h, light intensity 200 $\mu mol\,m^{-2}\,s^{-1}$, relative humidity 75%), the plants were exposed to different temperature treatments, with the other conditions kept the same as for the pre-culture. Each treatment had four replications and five plants per replication. After heat treatments, the second to fifth fully expanded leaves were sampled and used for assays of physiological parameters and RNA extractions.

Based on the pre-experiments, the duration of the heat stress treatment was set as 12 h for the semi-lethal high temperature experiments. As the high temperature in summer always appears between 10:00 and 16:00 in the Zhejiang area, this study imitated the short-term high temperatures regularly experienced in the summer. The temperature and the treatment duration were set as 48 °C and 6 h for the physiological and molecular analysis.

2.2. Determination of relative electrolyte leakage and assay of semi-lethal high temperature

The method used to determine the semi-lethal high temperature followed that described by Deng et al. (2011). The treatment temperatures were set at 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, and 53 °C for 12 h. The electrical conductivity (EC) both before and after boiling was measured for each temperature treatment using a dedicated meter (Model DDS-320, Shanghai Kangyi, Shanghai, China). Each measurement was the mean of three replicates. The relative conductivity (REC%) was defined as follows: REC% = (electrical conductivity before boiling/electrical conductivity after boiling) × 100, Using the REC% of blueberry leaves at 25 °C as the control. Cell damage rate (%) = [1 − (1 − REC_t%)/(1 − REC_c%)] × 100, where REC_t% = REC% at different temperature treatments and REC_c% = REC% of the control.

The cell damage rates at different temperatures were fitted using the Logistic Equation: $y = K/(1 + ae^{-bx})$, where 'x', 'y', and K represent the treatment temperature, the cell damage rate, and the maximum cell damage rate, respectively. The semi-lethal high temperature (LT_{50}) of blueberries was given by the inflection point of the curve.

2.3. Field trials design

The field trials, lasting 3 years, were designed to confirm the heat tolerance data derived from the semi-lethal high temperature calculations. Three plots (60 plants per plot, 180 plants in total) were established using a randomized block design for each highbush blueberry cultivar. In the early spring of 2010, the 2-year-old seedlings (around 20 cm high) were planted in sandy soil 2.0 m apart in rows, with the rows spaced 2.0 m apart. Organic fertilizer (45% organic contents, total N 1.85%, P_2O_5 2.34%, K_2O 2.28%) was applied to the soil at 10,000 kg ha^{-1} with the pH being adjusted to 5.5 using 1500 kg ha^{-1} flotation sulfur. The soil in the rows was mixed with sawdust (10%) and peat (15%). After planting, the shrubs were fertilized with 15 and 30 g of 'Stanley mixed fertilizer' (Stanley, China) per plant in 2011 and 2012, respectively. The field was located at Shangwan village in Jinhua (latitude 29°08′N, longitude 119°70′E). The plants were well irrigated and regularly cultivated under open conditions, and the weeds were manually controlled. In April of each year, the number of dead seedlings was recorded on each plot and the death rates calculated based on the average of the three replicate plots. The daily air temperatures were recorded from data provided by the local bureau of meteorology.

2.4. Assay on ROS contents and oxidative damages

2.4.1. Determinations of H_2O_2 and $O_2^{\bullet -}$ contents

Highbush blueberries of both genotypes were subjected to two different temperature regimes in the growth chambers for 15 h: 25 °C as the control and 48 °C as the heat stress treatment. The H_2O_2 content was determined following the method of Patterson et al. (1984) with slightly modification. Briefly, blueberry leaves (0.3 g) were homogenized in 10 mL of cold acetone. The extract was centrifuged for 10 min at 1500 × g and the supernatant (1 mL) mixed with 0.1 mL of 20% $TiCl_4$–HCl. The Ti–H_2O_2 complexes, together with the unreacted Ti, were then precipitated by adding 0.2 mL of concentrated ammonia. The precipitates were dissolved in 2N H_2SO_4 (5 mL) and measured by monitoring the absorbance at 410 nm of this titanium-peroxidase complex using a spectrophotometer (UV2550, Shimadzu, Japan). Each result was plotted against a H_2O_2-standard curve (from 0 to 100 $\mu mol\,L^{-1}$).