Pak. J. Bot., 44(3): 853-860, 2012.

DIFFERENTIAL SENSITIVITY OF FOUR HIGHBUSH BLUEBERRY (*VACCINIUM CORYMBOSUM* L.) CULTIVARS TO HEAT STRESS

WENRONG CHEN, WEIYA CEN, LI CHEN, LULU DI, YONGQIANG LI AND WEIDONG GUO*

College of Chemistry and Life Science, Zhejiang Normal University, Jinhua, 321004, P. R. China
**Corresponding author's e-mail: plant@zjnu.edu.cn; Tel: +86 579-82282641, Fax: +86 579-82282641*

Abstract

Highbush blueberry (*Vaccinium corymbosum* L.) has recently been introduced into Southeast China, a typical subtropical area with a high temperature climate in summer. Understanding of the thermal tolerance among different genotypes of highbush blueberry is needed to select appropriate cultivars. Four highbush blueberry cultivars ('Duck', 'Brigitta', 'Sharpblue' and 'Misty') were exposed for 6 h to different temperatures (25, 30, 35, 40 and 45°C), and then various physiological parameters and ultrastructure of chloroplast were assessed. Exposure to high temperature significantly increased the level of relative electrolyte leakage (REL), contents of malondialdehyde (MDA), proline content, hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\cdot-}$) rate, and the initial chlorophyll fluorescence yield (Fo). Whereas the maximum photochemical efficiency of PSII (*Fv/Fm*) and the quantum efficiency of PSII photochemistry ($\Phi_{PS\ II}$) decreased under high temperature conditions. The most pronounced effect by high temperature stress was obtained in 'Brigitta', while the least effect existed in 'Sharpblue'. Furthermore, the chloroplast ultrastructure in 'Brigitta' was severely damaged under heat temperature stress, whereas that of 'Sharpblue' was almost intact. The results indicated that the 'Sharpblue' was the most heat-tolerant blueberry cultivar while 'Brigitta' was the most sensitive to heat temperature stress, followed by 'Duck' and 'Misty'. These were consistent with results observed from the field trial. This study proved that photosynthetic parameters stressed by short-term heat stress were fairly reliable to evaluate the thermal endurance in highbush blueberry.

Introduction

Blueberry (*Vaccinium corymbosum* L.) belonging to Ericaceae, *Vaccinium* spp. is a perennial evergreen or deciduous shrub native to North America. It is one of the most important recently developing fruit trees, and is mainly cultivated in the Northern area of China. The highbush blueberry (*Vaccinium corymbosum* L.), the lowbush blueberry (*Vaccinium angustifolium* Ait.) and the rabbiteye blueberry (*Vaccinium ashei* Reade; syn. *Vaccinium virgatum* Ait.) are the major economic species (Kole, 2007). Highbush blueberries are further separated into northern highbush blueberry, southern highbush blueberry and halfhigh blueberry depending on their chilling requirements and winter hardiness (Hancock *et al*., 2008). The northern highbush blueberry was suggested to be only planted in North China, as the relatively warm weather in winter in South China cannot meet its chilling requirements. In recent years, the introduction trials have shown that some northern highbush cultivars can blossom and bear fruits normally in Zhejiang area, a province in Southeast China. However, temperatures in this area often approach 40°C or even higher in summer. Moreover, the global mean temperature continues to rise at a rapid rate, and our climate is likely to warm by 1.1-6.4°C within the next century (Jin *et al*., 2011). High temperature has become the most significant abiotic stresses limiting the growth and productivity of highbush blueberry in this area. However, the heat endurance ability related to the different highbush blueberry cultivars is still unclear. Moreover, there is no consistent and concise practice for its cultivar selections, especially in Zhejiang Province (South China). Meanwhile, the establishment of a reliable and efficient method to screen the thermal endurable cultivars is important for the cultivations of this woody plant species.

The plant injury under heat stress primarily includes inhibition of photosynthesis, damage to cellular membrane, senescence and cellular death (Xu *et al*., 2006). One mechanism of injury under heat stress is the overproduction of reactive oxygen species (ROS). This includes superoxide radical ($O_2^{\cdot-}$), hydroxyl radical (.OH) and hydrogen peroxide (H_2O_2). The accumulation of ROS can cause peroxidation of membrane lipids, denaturation of protein and damage of nucleic acids, ultimately upsetting homeostasis (Mittler, 2002). Lipid peroxidation (POL) is considered an appropriate criterion for damage caused by increasing ROS production (Halliwell, 1991; Rao *et al*., 1995). It occurs when (.OH) radicals are generated, close to cellular membranes, and attack the unsaturated fatty acid side chains of membrane lipids resulting in the formation of lipid hydro-peroxides (Bestwick *et al*., 2001). Accumulation of lipid hydroperoxides in membranes disrupts their function and thus, can cause them to collapse, leading to their leakage and loss of selective permeability (Saelim & Zwiazek, 2000).

Photochemical reactions in thylakoid lamellae and carbon metabolism in the stroma of chloroplast have been suggested as the primary sites of injury at high temperatures (Wise *et al*., 2004). PSII is highly thermoliable, and its activity is greatly reduced or even partially stopped under high temperatures (Bukhov *et al*., 1999; Camejo *et al*., 2005). This is probably due to the properties of thylakoid membranes where PSII is located (Mcdonald & Paulsen, 1997). The inhibition of PSII usually leads to a decrease in the variable chlorophyll fluorescence. Thus, in vivo chlorophyll fluorescence has been shown to be a sensitive and reliable method for detection and quantification of temperature-induced changes in the photosynthetic apparatus (Liu & Huang, 2000; Xu & Huang, 2001; Camejo *et al*., 2005). It is well known that chloroplast is one of the important intracellular generators of ROS responsible for the damage to cell membranes under abiotic stresses (Sairam & Srivastava, 2002; Perez *et al*., 2002; Meloni *et al*., 2003). The abnormal chloroplast ultrastructure is a direct evidence for the damages caused by heat stress. Despite the many studies that have investigated plant responses to heat stress at different scales; but there are few reports on chloroplast fine structure responses to high temperature stress.

In this study, 2 northern highbush cultivars ('Duke' and 'Brijitta') and 2 southern highbush cultivars ('Sharpblue' and 'Misty'), with high yield and fruit quality, were selected to examine the effects of short-term heat stress on the chlorophyll fluorescence, chloroplast ultrastructure, and oxidative stress of the highbush blueberry. Their adaptability to long-term heat stress was also observed through field experiments. The objectives of this study were to evaluate the effects of the heat stress on the photosynthetic physiologies of blueberry and increase our biological knowledge of this plant species, then prove the photosynthetic physiological response to the short-term heat stress that could be used as criteria for screening the heat-endurable cultivars for the field cultivation, and finally to screen the most heat tolerant cultivars of blueberry which might be suitable for growth in a North subtropical area.

Materials and Methods

Plant material and high temperature treatment: Healthy and uniform growth plants of 3-year-old highbush blueberry ('Duke', 'Brigitta', 'Sharpblue' and 'Misty') were selected and grown in a green house with a 14-h photoperiod, a photosynthetic photon flux density of 200μ mol m^{-2} s^{-1}, a relative humidity of 75% and a temperature of 25/20°C (day/night). The plants with uniform size were exposed to five temperature treatments: 25 (control), 30, 35, 40 and 45°C for 6h, with triplicate each treatment. As the high temperature in summer always appears at 10:00 AM and cools down at 16:00 PM in Zhejiang area, this study imitated the short-term high temperature regularly experienced in the summer and set the treatment duration as 6h. The newly matured leaves of the plants were sampled or used for assays of physiological and photosynthetic parameters.

Determination of relative electrolyte leakage (REL): Cellular membrane stability was estimated by measuring relative electrolyte leakage (REL) from leaf tissues. Samples were washed three times with deionized water to remove surface-adhered electrolytes. Leaf samples (0.1g) were cut into discs of uniform size and taken in test tubes containing 10mL deionized water. The conductivity of the solution (Cinitial) was measured after the leaves were shaken for 3 h using a conductivity meter (YSI- 3100, Guangzhou, China). Afterwards tubes were heated in a boiling water bath for 30 min and then cooled to 25°C in the shaker. The conductance was determined and referred to as Cmax. The relative electrolyte leakage (REL) was defined as follows: REL (%) = (Cinitial / Cmax) × 100.

Determination of membrane lipid peroxidation: Membrane lipid peroxidation was estimated by the level of MDA production followed the thiobarbituric acid (TBA) method with some modification (Li, 2000). Fresh leaves (0.2 g) were homogenized in 10 mL of 10% trichloroacetic acid (TCA). The homogenate was centrifuged at 4,000 × g for 10 min and 2 mL of the supernatant obtained was added to 2mL of 0.6% TBA. The mixture was heated in a water bath at 100°C for 15min. After cooled to room temperature and centrifuged at 4,000 x g for 10min, the supernatant was read for absorbance at 450, 532 and 600nm, respectively. The MDA concentration in reaction mixture was calculated according to the following formula: MDA (μmol/L) = 6.45 x (A532-A600)-0.56xA450.

Determination of proline: The proline content was determined by Ninhydrin colorimetry with pure proline as a standard (Zhang, 1992). Leaf samples (0.3 g) were homogenised in 10mL of 80% (v/v) ethanol. The homogenate was decanted and heated in 80°C water bath for 20min. Activated charcoals were added to the extraction solution and then shocked strongly for 5 minutes. The mixture was filtered and 2mL of filtrate was transferred to another tube with a plastic cover, then 2mL glacial acetic acid and 2mL of 2.5% acidic ninhydrin were added. After the mixture was boiled for 15 min and then cooled, the absorbance was read at 520nm.

Contents of hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$): The hydrogen peroxide (H_2O_2) concentration was measured by monitoring the absorbance of titanium-peroxide complex at 410nm according to (Patterson *et al*., 1984) with some modifications. 0.5g of fresh leaves was ground in 5mL cold acetone, and centrifuged for 10min at 1,500 x g. The supernatant was used for the assay of H_2O_2. The supernatant (0.1mL) was mixed with 0.1mL of 20% $TiCl_4$-HCl and 0.2mL concentrated ammonia. After 10 min of reaction at 25°C, the reaction mixture was centrifuged for 10min at 3,000 x g. Precipitate was dissolved in 3mL 1M H_2SO_4 and then re-centrifuged. Supernatant was read at 410nm against reagent blank. Content of H_2O_2 was determined using standard curve plotted with known content of H_2O_2.

The content of $O_2^{\cdot-}$ was assayed according to (Ke *et al*., 2002) with minor modifications. Fresh leaves (0.5g) was ground in 5mL 50mM potassium phosphate buffer (pH 7.8), and centrifuged for 10min at 4,000 x g. The supernatant was used for the assay of $O_2^{\cdot-}$. Supernatant (0.1mL) was mixed with 0.9mL of 100mM phosphate buffer (pH 7.8), 0.1mL of 10mM hydroxylammonium chloride, then incubated for 20 min at 25°C, and finally 0.1mL of 17mM sulfanilic amide and 0.1mL of 7mM α-naphthylamine were added before maintaining the mixture for 20min at 25°C. The reaction mixture was centrifuged for 3min at 10,000 x g and the absorption at 530nm was determined. Sodium nitrite was used as standard solution to calculate the content of $O_2^{\cdot-}$.

Determination of chlorophyll fluorescence: Chlorophyll fluorescence was measured with a portable fluorometer (Li-Cor-6400, USA) at 25°C. Stressed and control leaves were pre-darkened for 20 min before starting the experiment. The initial chlorophyll fluorescence yield (*Fo*), the maximum chlorophyll fluorescence yield (*F*m), and the maximum photochemical efficiency of PSII (*Fv/Fm*) in dark-adapted leaves were read in the fluorometer. After the same leaf was photoactivated for 20min, the photochemical quenching coefficient (qP), the non-photochemical quenching coefficient (qN), and the quantum efficiency of PSII photochemistry (Φ_{PSII}) were calculated.

Electron microscopic analysis: Samples were prepared according to (Peng & Zhang, 2000), with some modifications. Rectangular segments (2×4 mm) were cut from the leaf between the second and third principal vein, 5mm from the midvein. Segments were fixed in 2.5% glutaraldehyde solution overnight at 4ºC. After the leaf samples were washed 3 times with PBS, they were re-fixed in 1% (v/v) OsO_4 solution for 2 h and washed with 0.1M phosphate buffer as before. The fixed leaf was dehydrated in a graded series of ethanol (50, 70, 80, 90, 95 and 100%). The segments were embedded in the intermixture of acetone and Epon-812 (1:1) for 4 h at room temperature. Polymerization was conducted at 70°C for 24 h. Ultrathin sections cut on a Reichert Ultratome were stained with uranyl acetate and lead citrate according to Reynolds's method (Reynolds, 1963) and photographed under the JEM-1230 transmission electron microscope.

Field experiment design: The study site was located at Shangwan village in Jinhua City (latitude 29°08′N, longitude 119°70′E). The red soil was sandy and with pH around 5.8. Three plots of 50x10m were established for the four highbush blueberry cultivars. In the early spring of 2009, the two-year-old seedlings (around 20cm high) were transplanted by randomized block design, at 2x2m spacing. There were 20 seedlings for each cultivar. The plants were well irrigated, fertilized and grown in summer season. In April of 2011, the death rate, plant height and crown diameter were recorded.

Statistical analysis: Each reported data are the mean ± standard error (SE) of three replicates. Statistical analysis was performed by analysis of variance (ANOVA) using SPSS software (SPSS Institute Inc., 2008). Significance of between treatment means was tested at the 0.05 level of probability.

Results

Relative electrolyte leakage (REL): Relative electrolyte leakage (REL) value showed a trend of increase in response to increasing temperature in all four blueberry cultivars (Fig. 1). As compared with the temperature of 25°C (control), increases of REL in the leaves of four cultivars were not significant at 30 and 35°C, but were significant at 40 and 45°C. In 'Duke' leaves, REL values significantly increased by 66.67% and 83.33% at 40 and 45°C, respectively, in comparison with the controls. REL values of 'Brigitta' were 127.27% and 209.09% higher than the control (at 25°C) at 40 and 45°C, respectively. In contrast, both 'Sharpblue' and 'Misty' showed relatively lower increases in REL values. The REL values in leaves of 'Sharpblue' were only increased by 15.38% and 46.15%, respectively, at 40 and 45°C, as compared with the control, with the lowest increase level among the 4 cultivars.

Membrane lipid peroxidation level: Lipid peroxidation level in the leaves of all cultivars, measured as the content of MDA, is shown in Fig. 2. The response of MDA concentration in leaves of 4 blueberry cultivars paralleled to the change of the REL value. At 45°C, MDA concentrations of 'Duke', 'Brigitta', 'Sharpblue' and 'Misty' were 2.62, 4.02, 1.17 and 1.97 times higher than that of the controls, respectively.

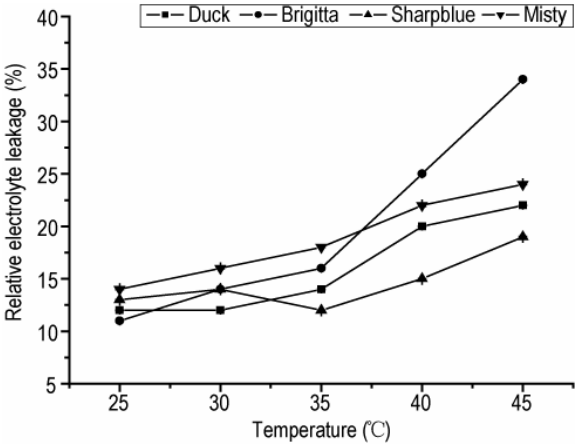

Fig. 1 Relative electrolyte leakage in leaves of four blueberry cultivars after different temperature treatments. Error bars represent the standard error (S.E.) (n=3).

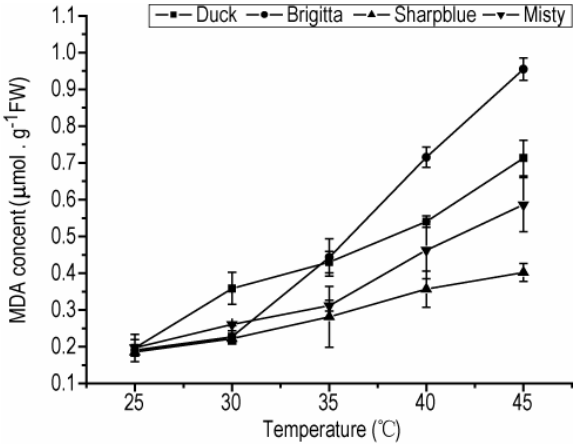

Fig. 2 Concentrations of MDA in leaves of four blueberry cultivars after different temperature treatments. Error bars represent the standard error (S.E.) (n=3).

Contents of proline: The proline accumulated continuously in all 4 cultivars with increasing temperature (Fig. 3). Significantly higher content of proline was accumulated in 'Sharpblue' ($p<0.05$) than the other 3 cultivars at 30 and 35°C. The proline concentration increased rapidly as the temperature rose from 40 to 45°C for all cultivars, but it differed among the cultivars. The maximum and minimum increases were observed in 'Sharpblue' and 'Brigitta', with the values of 4.09 and 2.61 fold higher than the control, respectively.

Contents of hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$): Hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$) increased significantly in leaves of each cultivar at 40 and 45°C when compared with the control (Fig. 4). At 45°C, the concentration of H_2O_2 and $O_2^{\cdot-}$ in 'Brigitta' were 1.34 and 3.12 times higher than control levels, whereas the corresponding increasing levels in 'Sharpblue' was only 0.77 and 2.29 times, respectively.

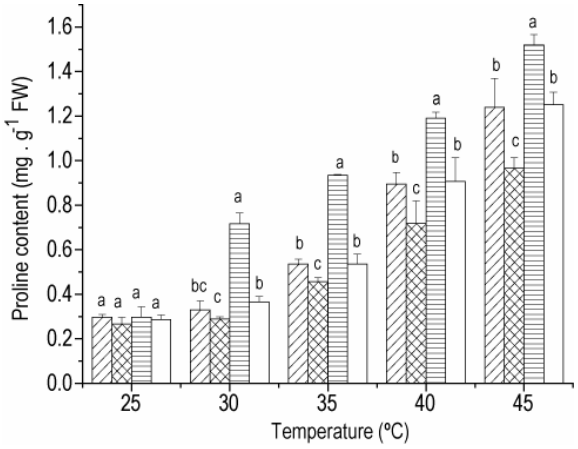

Fig. 3 Proline contents in leaves of four blueberry cultivars under high temperature stress. Error bars represent the standard error (S.E.) (n=3). Different lowercase letters represents significant differences among cultivars at $p<0.05$.

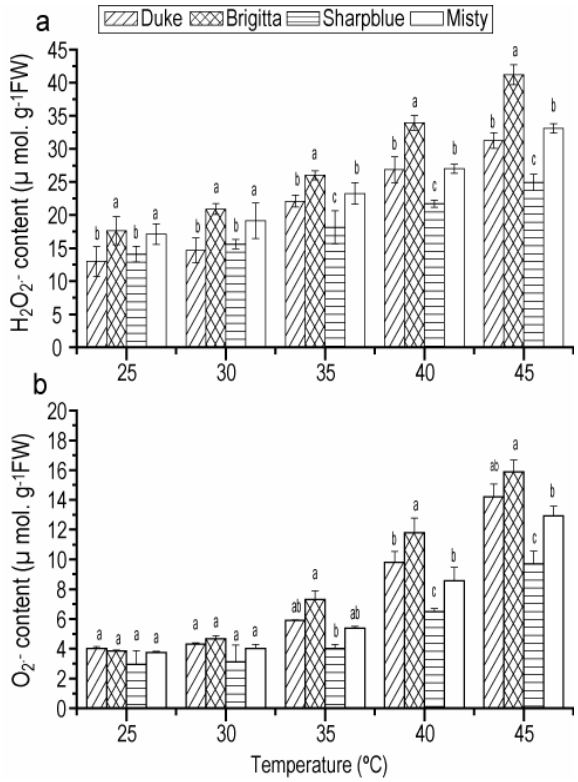

Fig. 4 Concentrations of H_2O_2 (a) and $O_2^{\cdot-}$ (b) in leaves of four Blueberry cultivars under high temperature stress. Error bars represent the standard error (S.E.) (n=3). Different lowercase letters represents significant differences among cultivars at $p<0.05$.

Chlorophyll fluorescence: As shown in Fig. 5a and 5c, a progressive increase of Fo and a gradually reduction of ΦPSII under high temperature stress were observed in leaves of all four cultivars. Fo value increased by 24.2, 26.24, 13.18 and 16.81% at 45°C, respectively, for 'Duke', 'Brigitta', 'Sharpblue', and 'Misty'. At 45°C, ΦPSII value, in leaves of 'Duke', 'Brigitta', 'Sharpblue' and 'Misty' decreased to 46.94, 32.73, 58.91 and 49.41% of the control level, respectively. Leaf Fv/Fm ratios of all cultivars were lower than the controls (25°C) when exposed to high temperatures (Fig. 5b). In contrast, 'Sharpblue' had relatively higher Fv/Fm ratio than the other three cultivars at 35, 40 and 45°C while 'Brigitta' had the lowest Fv/Fm ratio ($p<0.05$). The Fv/Fm ratio of 'Misty' was slightly higher than 'Duke'.

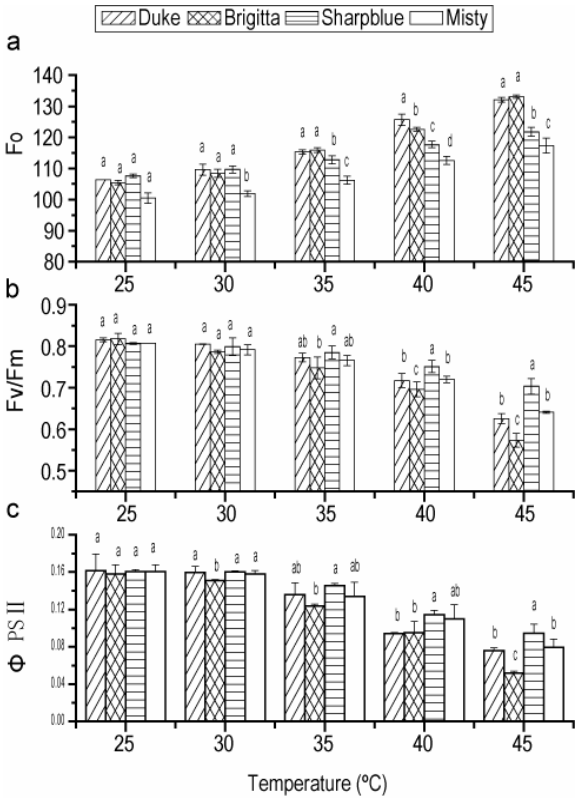

Fig. 5 Changes of Fo (a), Fv/Fm (b), ΦPSII (c) in leaves of four Blueberry cultivars under high temperature stress. Error bars represent the standard error (S.E.) of mean (n=3). Different lowercase letters represents significant differences among cultivars at $p<0.05$.

Ultrastructure of chloroplast: Under 25°C treatment, the chloroplasts of four cultivars was oblong and the chloroplast membrane were intact, with the thylakoid lamellas arranged orderly and tightly, large starch grains observed in the chloroplasts (Fig. 6, A, B, C and D). At 45°C, no obvious change of chloroplast was observed for 'Sharpblue' except for a few of osmiophilic globuli (Fig. 6, G). The majority of chloroplasts of 'Duke' (Fig. 6, E) and 'Misty' (Fig. 6, H) were similar to the controls, but some chloroplasts became swollen, with disorderly and loosely arranged thylakoid lamellas and some osmiophilic globuli. In contrast, the chloroplast of 'Brigitta' was evidently seriously damaged, with swollen and the deformed structures, and the thylakoid were in a seriously disorganized form and most thylakoid were ruptured. In the seriously damaged cells, the chloroplast envelope was broken, and much infiltration in the chloroplast was exuded (Fig. 6, F).

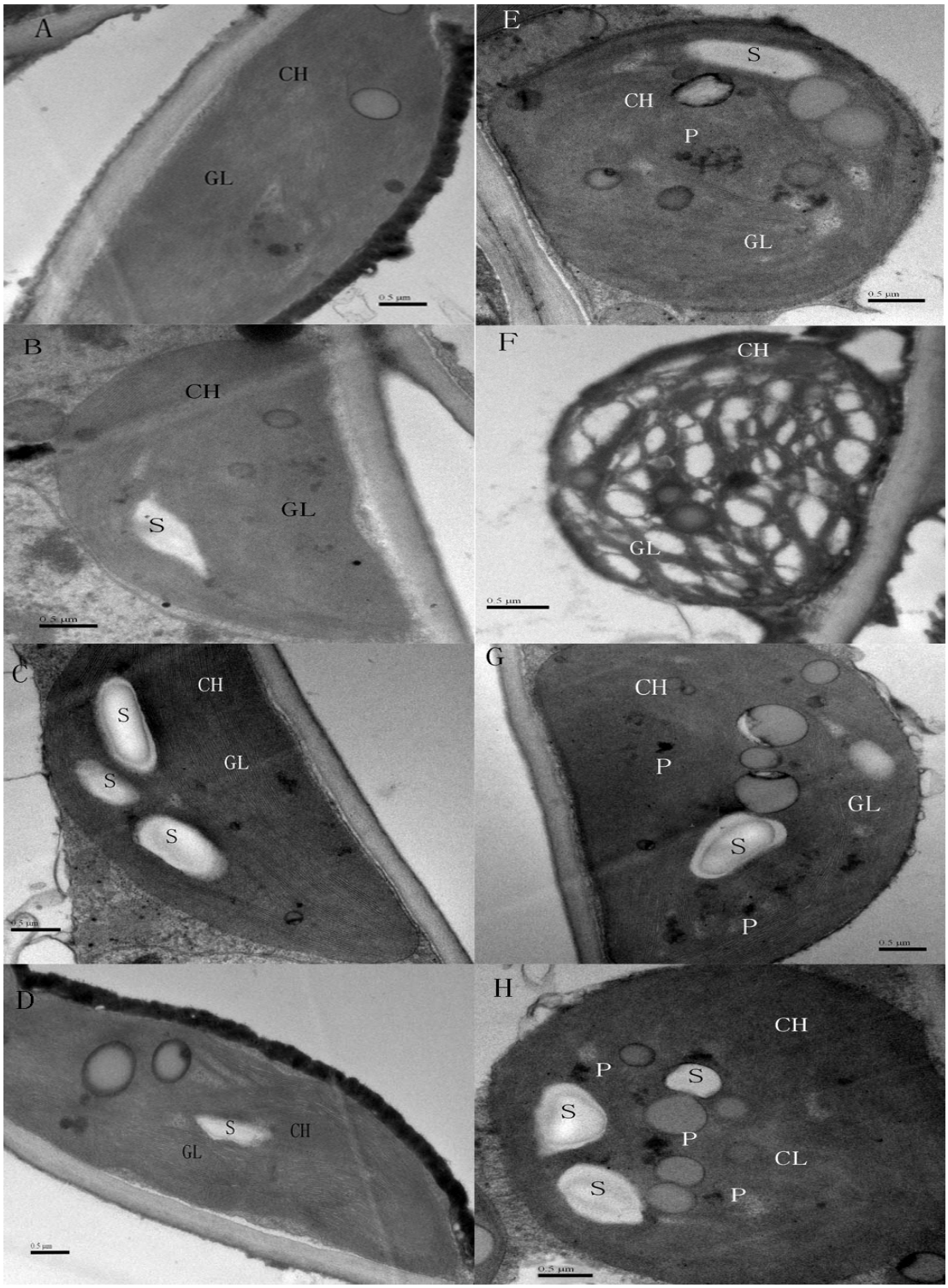

Fig. 6. Effects of heat stress on chloroplast ultrastructures in leaves of four Blueberry cultivars.
A, B, C and D represent the chloroplast ultrastructure of 'Duke', 'Brigitta', 'Sharpblue', 'Misty' under normal conditions, showing standard chloroplast untrastructure with distinct thylakoid lamella, A, Bx25000, C, D x30000, G represents the chloroplast ultrastructure of 'Sharpblue' under 45°C treatment, no obvious destruction occurred, Gx25000; E, H represent the chloroplast ultrastructure of 'Duke' and 'Misty' under 45°C treatment, the chloroplast were round, the thylakoid lamellas arranged disorderly and loosely, E x30000, H x25000; F represent the chloroplast ultrastructure of 'Brigitta' under 45°C treatment, most thylakoid were ruptured much infiltration in the chloroplast was exuded, F×30000. CH: chloroplast; GL: grana lamella, S: starch granule; P: osmiophilic glonules.

The field experiments: Four highbush blueberry cultivars differed significantly in the thermal adaptability in two-year field experiment (Table 1). The 2 southern highbush blueberry cultivars, 'Sharpblue' and 'Misty', had death rates below 20%. Meanwhile Brijitta had the highest death rate of 43.46% and followed by 'Duke'. Although no difference was observed in the plant heights of these 4 cultivars, the crown diameters increased following the sequence as 'Brijitta', 'Duke', 'Misty' and 'Sharpblue'.

Table 1. The death rates, plant heights and crown diameters of the field experiment.

Cultivars	Death rate (%)	Plant height (cm)	Crown diameter (cm)
Sharpblue	07.78 ± 2.94 a	74.34 ± 8.66 a	79.07 ± 1.98 a
Misty	16.70 ± 0.93 ab	71.75 ± 5.04 a	61.77 ± 11.29 b
Duke	33.61± 7.43 b	60.59 ± 6.24 a	55.47 ± 1.26 b
Brijitta	43.46 ± 4.17 c	64.36 ± 7.97 a	54.96 ± 5.60 b

Discussion

Heat stress is an important abiotic stress causing various physiological and biochemical changes associated with plant growth and development (Liu & Huang, 2000). 'Duke', 'Brigitta', 'Sharpblue' and 'Misty' were 4 cultivars widely introduced in Zhejiang Province of China, where its typical subtropical weather largely prevents their extensions. In the present study, we analyzed the membrane integrity, chlorophyll fluorescence, reactive oxygen species contents and chloroplast ultrastructure in the leaves of the above 4 blueberry cultivars in response to the high temperature stress. Great differences were observed among these four cultivars after exposure to short-term and high temperature stress. The thermal endurances were sequenced as follows: 'Sharpblue', 'Misty', 'Duke' and 'Brijitta'. The sensitivities to short-term heat stress were very consistent with the death rates and the bio-growths obtained in the two-year field experiments (Table 1). This indicates the photosynthetic response to short-term heat stress can be used as reliable criteria to screen the thermal endurable highbush blueberry cultivars for the field cultivation.

Malondialdehyde (MDA) is the final product of peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage, this assay has often been used as an indicator of the level of lipid peroxidation (Halliwel & Gutteridge, 1989; Scandalios, 1993). The increased solute leakage, as an indication of decreased cell membrane thermostability, has long been used as an indirect measure of heat-stress tolerance in diverse plant species (Ashraf *et al*., 1994; Marcum, 1998; Ismail & Hall, 1999; Blum *et al*., 2001; Wahid & Shabbir, 2005). In the present study, REL value and MDA content increased in the leaves of four cultivars under heat stress (Figs. 1, 2). The maximum and minimum increases were observed in 'Brigitta' and in 'Sharpblue', respectively. Meanwhile, the increase extent of 'Duke' is slightly higher than 'Misty'. This indicated that 'Sharpblue' and 'Misty', as two southern highbush cultivars, were more capable to minimize lipid peroxidation and cell membrane damage than the other two northern highbush cultivars at high temperatures.

It has been found that various environmental stresses (including heat stress) cause oxidative stress resulting from accumulation of reactive oxygen species (ROS) (Li & Wang, 2004). Hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$) are two toxic ROS, which damage the membrane by attacking unsaturated fatty acids of lipid to induce lipid peroxidation, in turn damage cell membranes (Sairam *et al*., 2002). Under heat stress, the H_2O_2 and $O_2^{\cdot-}$ concentration in all cultivars leaves increased paralleled to the increased external temperatures (Fig. 4). 'Brigitta' showed a more rapid and greater increase in H_2O_2 and $O_2^{\cdot-}$ concentration than other three cultivars which indicated that the oxidative damage is most serious in 'Brigitta'. In contrast, the lowest contents of H_2O_2 and $O_2^{\cdot-}$ were observed in 'Sharpblue'. Similar findings were reported by Guo *et al*. in citrus cultivars (Guo *et al*., 2006), who reported Navel orange (*Citrus sinensis* Osbeck) was more tolerant to heat stress than Satsuma mandarin (*Citrus unshiu* Marc.). This is because Navel orange (*Citrus sinensis* Osbeck) was able to maintain lower H_2O_2 and $O_2^{\cdot-}$ production than Satsuma mandarin (*Citrus unshiu* Marc.) during elevated temperatures.

Chloroplasts are the main sites for generating ROS under both stressed and unstressed conditions, and it is one of the organelles in leaf tissues most sensitive to high temperature stress (Sun *et al*., 2002). Therefore, it may be inevitable to be affected or even damaged on ultrastructure of chloroplasts by heat stress resulting from much accumulation of ROS. It is well known that chloroplast is center of photosynthetic response and it is characterized under heat stress by membrane damage, such as membrane of thylakoid expanded and ruptured (Xu *et al*., 2006). Our results showed that the chloroplasts of four cultivars were damaged with different severities after heat stress (Fig. 6). The chloroplasts of 'Brigitta' (Fig. 6, F) were most severely damaged under heat stress, as compared with the other three cultivars. However, the chloroplasts of 'Sharpblue' (Fig. 6G) under heat stress was almost completely intact, which may contribute to its lower REL values, lower MDA, hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$) concentrations.

The effects provoked by heat stress on the photosynthetic apparatus of blueberry plants were also evidenced through analysis of chlorophyll fluorescence. Chlorophyll fluorescence parameter Fv/Fm is very sensitive to temperature in plants (Salvucci & Crafts-Brandner, 2004).

Therefore, Fv/Fm could be used as an indicator of thermal tolerance. A sustained decrease in dark-adapted Fv/Fm and increase in Fo indicate the occurrence of photoinhibitory damage in response to high temperature (Gamon & Pearcy, 1989). In the present study, the increased Fo (Fig. 5a) and decreased Fv/Fm (Fig. 5c) in Blueberry leaves suggested that PSII reaction centers were damaged (Heber *et al*., 2001). The Fo (Fig. 5a) was higher and Fv/Fm (Fig. 5b) was lower in the leaves of 'Brigitta' than the other cultivars at high temperature stress ($p<0.05$), indicating that inactivation of part of PSII reaction centers is more severe in 'Brigitta' leaves (Guo *et al*., 2006). This also indicated that 'Brigitta' is the most thermal sensitive cultivar.

The proline is known to occur widely in higher plants and accumulates in large quantities in response to environmental stresses (Ozturk & Demir, 2002; Hsu *et al*., 2003; Kavi Kishor *et al*., 2005). The mechanisms of proline counteract with abiotic stress are not fully understood, but it has been suggested that in addition to its role as an osmolyte for osmotic adjustment. Proline also contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions (Ashraf & Foolad, 2007). Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants (Ashraf & Foolad, 2007). The higher thermal endurability in 'Sharpblue' than the other cultivars may partly be related to their relatively higher proline content (Fig. 3). These results agree with earlier findings with some Freesia seedlings (Yuan *et al*., 2011) and cowpea (Mayer *et al*., 1990) in which a considerable increase in proline content was reported due to thermal shock.

Conclusion

In conclusion, the heat stress induced oxidative injury in 4 blueberry cultivars, as demonstrated by the increase in REL value, MDA concentration, proline concentration, ROS concentrations, Fo value and the reduction in Φ_{PSII} value and Fv/Fm ratio. On the other hand, 'Sharpblue' can maintain a lower level of ROS, lower level of membrane lipid peroxidation and keep intact chloroplast fine structure and a relatively higher photosynthetic capacity. Both the short-term and two-year field experiments indicated thermal tolerance of the four northern highbush blueberry cultivars were sequenced as: 'Sharpblue', 'Misty', 'Duke' and 'Brijitta'. The photosynthetic response to short-term heat stress can be used as reliable criteria to screen the thermal endurable highbush blueberry cultivars for the field cultivation.

Acknowledgements

This study was financially supported by the Zhejiang Science and Technology Project (#2008C12015-1) and Zhejiang Technology Innovation Group Project (#2011R09033-06). The authors also thank Prof. Yueming Jiang from South China Botanical Garden of Chinese Academy of Sciences and Prof. Min Wu from University of Arizora for their critical review and English correction of the manuscripts.

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(Received for publication 15 March 2011)