Effects of heat stress on physiological index in cultivated strawberries

Preeda Nathewet

ABSTRACT: Heat tolerance of five strawberry cultivars, which have been recently introduced in Northern Thailand, namely “DN 1”, “NO.329”, “Pharajchatan 60”, “Akihime” and “Sachinoka”, were evaluated. Rooted runner plants which had 5-6 leaves (10 weeks old) of all cultivars were grown in the plastic house at Maejo University, Chiangmai, Thailand. Twenty plants of each cultivar were then transferred to either 25/25 °C (control) or 39/29°C (high temperature condition) for 7 days. Heat stress condition caused statistically significant photoinhibition and low level of transpiration rate (E), stomatal conductance (gₛ), CO₂ assimilation rate (A) in all strawberry cultivars as compared with those plants grown in control condition. Among strawberry cultivars grown under heat stress condition, “Parajchatan 60” had markedly the lowest leaf temperature and percentage of electrolyte leakage (EC) than those other remaining cultivars. This study was also revealed that there was a significant relationship between EC and maximum quantum efficiency of PSII photochemistry (Fᵥ/Fₘ), and gₛ and E. Hence, EC together with other indices related to the process of photosynthesis could be highly used as an index for selection of heat tolerant strawberries because EC showed more positive relationship with photosynthesis. Keywords: high temperature, photoinhibition, CO₂ assimilation rate, lipid peroxidation

Introduction

Recently the average global temperatures are expected to increase every year. Heat stress becomes one of the major abiotic stresses affecting agriculture worldwide. An effect of high temperature on crops production and physiology has been reported worldwide. High temperature reduces growth, reproductive and yield of many crops (Hellman and Travis, 1988; Konsi et al., 2001; Leddesma et al, 2004; Mori, 1998). At cellular membrane levels, heat stress results to disturb and injure some physiological activities in plants such as, denaturing of protein, metabolic imbalance and biochemical lesions. There are several studies have been reported that response of plant to high temperature is very complicated. The heat tolerance of plants is related with physiological and morphological reactions (Tian et al., 2015). The mechanism for resistance is depending on the difference of plant species. Strawberry is one of economically important fruit crops over the world as well as Thailand. For strawberry plant, photoperiod and temperature have been defined as primary factor of ensuring quality production. The growth and yield of strawberry plants decline under high temperature (Kadir and Sidhu, 2006). High temperature was negatively affects to vegetative growth, root growth, flower induction, fruit, pollen viability, fruit size, fruit weigh and overall plant growth of cultivated strawberries (Konsi et al., 2001; Leddesma et al, 2004; Mori, 1998). There were reported that the heat tolerance ability among plant genotypes is correlated with the stability of membrane under high temperature (Iba, 2002, Su et al., 2009). In fact, high temperature stress causes change in plant metabolism such as fluidity, permeability and lipid composition.
of the cell membrane resulting in electrolyte leakage (Wahid, et al., 2007). High temperature stress also increases the production of free radical at intracellular level such as hydrogen peroxide and superoxide that can oxidize lipid in the membrane (Xu et al., 2006). During heat stress, lipid peroxidation of membrane is a chain reaction which can seriously damage cell membrane that may contribute to ion leakage (Liu and Huang, 2000). In addition, heat stress negatively affects photosynthesis by altering the activities of some photosynthetic components such as PSII complex, thylakoid membrane and ribulose 1’5 bis phosphate carboxylase (Rubisco) enzyme (De Ronde et al., 2004; Allakhverdiev et al., 2008). Moreover, heat stress causes over-production of reactive oxygen species (ROS) which could induce oxidative damage to lipid membrane and inhibit the repairing process of PSII (Møller et al., 2007). However, information related to evaluate the heat tolerance of cultivated strawberry in Thailand using the physiological indexes remains obscure. Considering the implication of global climate change, in the near future it may become a serious problem for strawberry growing and breeding. Therefore, this study aimed to evaluate heat tolerance of leading strawberry cultivars in Thailand via physiological indexes.

**Materials and Methods**

**Plant materials and treatments.** Five cultivars of cultivated strawberry (Fragaria ×ananassa Duch) namely “DN 1”, “NO.329”, “Pharajchatan 60”, “Akihime” and “Sachinoka” were used in this study. Plug plant of these strawberry cultivars were planted in plastic pot (12.3 × 11.5 × 8.5 cm) containing coconut coir. Plants were grown under natural environment conditions with day/night temperature of 25-32/21-25°C in a plastic-house for 4 weeks (6-8 leaves). The strawberry plants were watered with half-Haogland’s nutrient solutions as need. After that all plants were transferred into a controlled environmental room with day/night temperature of 25/25 °C, relative humidity of 60±10%. Light emission diodes (LED) 3000 K and 5000 K (1:1) lamps provided photosynthesis photon flux density 200 µmol·m⁻²·s⁻² for 16/8 h (light/dark). Then, half of plants were transferred to grow under heat stress at 39/29°C and other half were remained as control at 25/25 °C for 3 weeks. Five replicates represent each treatment with 4 pots per replicate. The experimental design was randomize complete block.

**Measurement of cell membrane thermostability.** Cell membrane thermostability was determined by modified procedures of Bajji et al. (2001). Six leaf disks with 0.5 cm diameter from youngest fully expanded leaves of each cultivar (two leaf disks from each plant) were collected. Leaf disks for each cultivar were put into test tube, added with 10 ml of distill water to remove electrolyte of injury at cut edge and any surface and kept in dark for 16 h. Sample from each cultivar was represent by six test tubes. After that electrical conductivity was measured (first electrolyte leakage) in solutions by using conductivity meter (Suntex Conductivity meter SC-170). Then all samples were autoclaved for 15 min and let them stand at 25 °C for 1 h. The second electrolyte leakages were measured. Percentage of ion leakage was calculated by the following equation:

\[ \text{%EC} = \left( \frac{\text{first electrolyte leakage}}{\text{second electrolyte leakage}} \right) \times 100. \]
Leaf chlorophyll fluorescence measurement. Leaf chlorophyll fluorescence was measured by using Fluorescence Monitoring System FMS2 (Hansatech Instruments Ltd., King’s Lynn, Norfolk, UK). Darked - adapted parameter were recorded in all plants after kept in the dark for 30 min. PSII operating efficiency (ΦPSII) and maximum quantum efficiency of PSII photochemistry (Fv/Fm) were calculated according to Baker (2008).

Leaf gas exchange and leaf surface temperature measurement. Leaf gas exchange parameters and leaf temperature were measured after exposure to high temperature for three weeks using, LCI-SD (BioScientific Ltd., Hertfordshire, UK). In each cultivar, six of the youngest fully expanded leaves of 3 independent plants were measured.

Results

Effect of high temperature on cell membrane thermostability

Effect of high temperature on percentage of electrolyte leakage (EC) of five strawberry cultivars were shown in Figure 1. After 3 weeks of exposure to high temperature condition (39°C/29°C) the percentage of ion leakage of all strawberry cultivars were significantly higher than those plants grow under 25 °C/25 °C conditions. High values of EC were observed in all strawberry cultivars grown under high temperature condition. The value was found to be lowest in “Phrarajchatan 60” plant.

Effect of high temperature on PSII efficiency

The efficiency of PSII (ΦPSII) in leaves of strawberry plants was measured by using chlorophyll florescence parameters. The operating efficiency of PSII values of all strawberry cultivars grown under high temperature were significantly two fold lower than that grown under 25 °C/25 °C . Among all cultivars, “Akihime” and Phrarajchatan 60" had the highest ΦPSII while “NO.329" had the lowest value of ΦPSII at 39°C/29 °C (Figure 2). In all strawberry cultivars that exposure to high temperature at 39°C/29 °C for three weeks had significantly lower value maximum quantum efficiency of PSII in a dark-adapted state (Fv/Fm) than plant grown under 25 °C/25 °C. The lowest value of Fv/Fm was observed in “DN 1” while the highest was “Akihime”.

Effect of high temperature on leaf gas exchange parameters

Heat stress significantly reduced stomatal conductance rate (A), stomatal conductance (gs), and transpiration rate (E) of all strawberry cultivars (Figure 2, 3, and 4). High temperature resulted in lower in operating efficiency of PSII than those of plants grown under 25°C/25°C. Stomatal conductance rates (A) of all strawberry cultivars grown under heat stress were decreased approximately 5 to 9 folds as compared with grown under 25/25°C. Similar to the results of A, all strawberry cultivars exhibited lower in gs value under the heat stress. Under heat stress, the lowest value of gs was found in “DN 1” while “Phrarajchatan 60” was the highest (Figure 3.). Result in this study also shown that there was significantly difference in all strawberry cultivars for leaf temperature. The leaf temperature of plants under high temperature was 4-5 °C higher than plants grown under 25/25°C (Figure 7).
Correlation coefficient analysis among physiological indexes.

Simple linear correlation analysis of heat tolerance coefficients of physiological indexes showed in Table 1 during heat stress period revealed that the change of cell membrane thermostability (%EC) was significantly negative correlated among Fv/Fm, Gs, and E. Result also showed that A value was significant positive correlated with GS while E was positive correlated with Fv/Fm and Gs.

Discussion

Electrolyte leakage from the cell membrane is known to be simple, reliable and effective measurement of cell membrane thermostability and a primary indicator of injury of cell membrane under stressful conditions (Arora, 1998; Bajji, et al., 2001; Fan et al., 2012). Thus, an ion leakage measurement is generally used to evaluate the tolerance of various plant species to abiotic stress factors. In this study, high temperature caused significant increases in injury of cell membrane among strawberry cultivars which is indicated by electrolyte leakage from the cell membrane (Figure 1). Significant differences in percentage of ion leakage among strawberry cultivars reported here could be also related to variation in membrane lipid composition among those strawberry cultivars. It may also offer partial explanations for the degree of different tolerance to heat stress observed in these cultivars. Although it cannot be accurately indicated how much membrane capability may contribute to heat tolerance in strawberry plants, this result suggests that it may be an important component.

The low value of leaf chlorophyll fluorescence (ΦPSI and Fv/Fm) parameter under high temperature condition revealed the deterioration of PSII efficiency, which may imply the low of net photosynthesis rate. This was consistent with Yin et al (2010) indicated that net photosynthesis rate has robust relationship with ΦPSI. Decreasing of Fv/Fm during the heat stress suggested a reduction of the rate of energy-trapping by PSII centers, which might be the result of a damaging in PSII reaction center (Havaux, 1993). Significantly negative relationships between Fv/Fm and percentage of ion leakage was observed (Figure 2) for all cultivars. This result was consistent with Xu et al. (2008) found that percentage ion leakage from maize’s leaf under drought stress was significantly negative correlated with Fv/Fm. Moreover, the lowest electrolyte leakage (EC) among strawberry cultivars was observed in “Phrarajchatan 60” plant. Kesici et al (2013) reported that the response to heat stress among strawberry cultivars depend on the origin of those plant. In this study, all strawberry cultivars were introduced from abroad with exception “Phrarajchatan 60” which bred and selected in Northern Thailand by Royal Project. This would be explained that “Phrarajchatan 60” were more adapted to tropical weather and tolerant to high temperature than other the rest cultivars.
Figure 1 Effect of high temperature on percentage of electrolyte leakage (EC) and of newly fully leaflet “DN 1”, “NO.329”, “Pharajchatan 60”, “Akihime” and “Sachinoka” exposed to 39°C for 3 weeks.

Figure 2 Effect of high temperature on operating efficiency of PSII in a light-adapted state (above) and maximum quantum efficiency of PSII in a dark-adapted (below) of newly fully leaflet “DN 1”, “NO.329”, “Pharajchatan 60”, “Akihime” and “Sachinoka” exposed to 42°C for 3 weeks.

Figure 3 Effect of high temperature on net CO₂ assimilation rate (above) and stomatal conductance (gs) (below) of newly fully leaflet “DN 1”, “NO.329”, “Pharajchatan 60”, “Akihime” and “Sachinoka” exposed to 42°C for 3 weeks.
Conclusion

The electrolyte leakage in leaves of five strawberry cultivars in this study can be used as one of parameters by combing with other physiological values such as Fv/Fm ratio and PS II values to evaluate the heat tolerance. Considering the parameter investigated, “DN 1”, would be the most susceptible strawberry cultivar to heat stress (highest value for percentage and lowest decline in Fv/Fm ratio), whereas the highest tolerance to heat stress would be “NO.329”, and “Pharajchatan 60” follow by “Akihime” (highest value in Fv/Fm and lowest value for percentage).

Table 1 Correlative coefficient matrix heat tolerance coefficients of physiological indexes.

<table>
<thead>
<tr>
<th></th>
<th>ΦPSII</th>
<th>Fv/Fm</th>
<th>Gs</th>
<th>A</th>
<th>E</th>
<th>Tl</th>
<th>%EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΦPSII</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fv/Fm</td>
<td>-0.077</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gs</td>
<td>0.244</td>
<td>0.483</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.090</td>
<td>-0.088</td>
<td>0.651*</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.240</td>
<td>0.689*</td>
<td>0.962**</td>
<td>0.462</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tl</td>
<td>0.232</td>
<td>0.235</td>
<td>-0.492</td>
<td>-0.927**</td>
<td>-0.274</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>%EC</td>
<td>0.110</td>
<td>-0.941**</td>
<td>-0.704*</td>
<td>-0.237</td>
<td>-0.838**</td>
<td>0.101</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*’** indicate the significant differences at P<0.05 and 0.01, respectively

References


Figure 4 Effect of high temperature on transpiration rate (above) and leaf temperature (below) of newly fully leaflet “DN 1”, “NO.329”, “Pharajchatan 60”, “Akihime” and “Sachinoka” exposed to 42°C for 3 weeks.


