Heat-tolerant pepper cultivar exhibits high rates of chlorophyll, photosynthesis, stomatal conductance and transpiration in heat stress regime at fruit developing stage

Abstract

Relevance. Abiotic stress, as heat, significantly affect plant and floral organs growth and development, fruit set, productivity, the quality, and survival of crops. Heat injury occurs when plants are exposed to these temperatures for a long period of time. Depending on the intensity and duration of exposure to the high temperatures, photosynthesis, respiration, membrane integrity, water relations and the hormone balance of the plants may affected. Material and methods. In this study used the commercial pepper cultivar “NW Bigarim” (HT37) released in South Korea and accessions “Kobra” (HT1) and “Samchukjaere” (HT7) selected as heat tolerant and susceptible, respectively. Total chlorophyll index and photosynthetic activities measured using a SPAD meter (Konica, Japan) and portable photosynthesis measurement system (LI-6400, LI-COR Bioscience, Lincoln, NE, USA), respectively. Results. To evaluate the positive effects of high temperature regime (40/28°C day/night, 14/10-h light/dark cycle) on the response of photosynthetic parameters in pepper plants with different heat susceptibility, we measured the total chlorophyll content (CHL) and photosynthetic activity (Pn) at fruit development stage. Heat-tolerant cultivars showed higher and more stable index of the CHL, Pn, Gs and Tr than those in heat-sensitive cultivars for 14 days of heat treatment (HT) period. However, the initial index of Pn, Gs and Tr showed significant alteration among pepper plants regardless of thermotolerance rate before HT on day 0 and day 7 after recovery at normal treatment condition (NT) except for CHL, meaning that plants response to high temperature regime is different from that in normal condition. These results suggest that constant high rates of Pn, Gs and Tr as well as of CHL in heat stress condition periods confer to avoid from heat injury during reproductive growth stages.

Keywords: pepper, cultivar tolerance, susceptible, high temperature, chlorophyll content, photosynthesis, stomatal conductance to H2O, transpiration

Жаростойкий сорт перца демонстрирует высокие показатели хлорофилла, фотосинтеза, устойчивой проводимости и транспирации в режиме теплового стресса на стадии развития плодов

Abstract

Актуальность. Абиотический стресс, такой как высокая температура, существенно влияет на рост и развитие репродуктивных органов растений, заставляя плоды, продуктивность качества и визуализацию. При длительном воздействии высоких температур у растений наблюдается повреждение, и в зависимости от продолжительности и интенсивности высоких температур нарушается фотосинтез, транспирация, целостность мембраны, водный и гормональный баланс.

Материал и методика. В данной работе использовали районированный в Южной Корее сорт перца «NW Bigarim» (HT37), а также сортобразцы «Kobra» (HT1) и «Samchukjaere» (HT7) выделенные как устойчивый и восприимчивый к высоким температурам, соответственно. Фотосинтез и общее содержание хлорофилла в листьях определяли при помощи портативной системы (LI-6400, LI-COR Bioscience, Lincoln, NE, USA) и спадеметра (Konica Japan), соответственно.

Результаты. Изучено и выявлено положительное влияние высокотемпературного режима (40/28°C день/ночь, 14/10-часовой цикл свет/темнота) на реакцию фотосинтетических параметров у растений под влиянием различной тепловой восприимчивостью, измерено общее содержание хлорофилла (CHL), а также фотосинтетической активности, таких параметров, как фотосинтез (Pn), устойчивая проводимость в H2O (Gs) и скорость транспирации (Tr) у листьев термоустойчивого (HT1) и чувствительного сортов (HT7) в сравнении с районированным сортом (HT37) на стадии развития плода. Термостойкие сорта проявили более высокие и более стабильные показатели CHL, Pn, Gs и Tr, чем температурный сорт HT7 в течение 14 дней термической обработки (NT). Однако исходные показатели Pn, Gs и Tr оказались значительнень различимыми между различными условиями выращивания в NT, за исключением CHL, что означает, что растения реагируют на высокотемпературный режим, отличаясь от условий роста в NT. Эти результаты предполагают, что постоянное высокое снижение Pn, Gs и Tr, а также CHL в периодах теплового стресса позволяет избежать теплового повреждения на стадии репродуктивного роста растения.

Ключевые слова: перец, сорт, устойчивость, температура, хлорофилл, фотосинтез, устойчивые, транспирация
Introduction

Global climate change including very high temperatures, which has increased by 0.6°C over the past 100 years [1] and the average increase is expected to be 0.5–2.8°C by the end of the 21st century [2,3], which predict to have a general negative effect on plant growth and productivity among agricultural crops.

Pepper (Capsicum annuum L.) is considered as an agriculturally important vegetable of global significance. However, high sub-optimal temperatures over 30 °C frequently influence physiological traits and reduce the productivity of Capsicum species [4-6]. Meanwhile, the reproductive traits of pepper cultivars have been changed by the high temperature, since the mechanisms controlling heat stress response in plants are complex and the response of cultivars as well as their physiological and biochemical parameters on the high temperature is different and may vary in growth stages [4,7,8].

Recently, it has been found that different heat damage levels of tomato leaf [9] and pepper plants [6] significantly affect the vegetative parameters at recovery, where faster recovery was identified in a heat-tolerant cultivar than susceptible one. And, this is possibly due to the higher rates of CHL and photosynthetic activity such as Pn, Gs and Tr in a heat-tolerant cultivars at seedling growth stages through the entire days of HT than those in heat-susceptible cultivars [9]. It has been also reported that constant photosynthetic rate via increased transpiration rate as well as high proline content in heat stress condition confers fast recovery from heat damage of heat-tolerant cultivars at seedling stages of pepper [6]. Moreover, it has been confirmed that grafting a pepper cultivar onto appropriate rootstocks can overcome the negative effects of heat stress conditions with a higher relative growth rate, leaf area and Fv/Fm, lower electrolyte leakage under the controlled conditions, and higher marketable yields under the greenhouse conditions [10].

Heat-tolerant plants may show properly functioning of the photosystem with normal CHL and chlorophyll fluorescence in stable PSII under high temperature conditions as compared to that of heat-sensitive cultivars [9,11,12]. Therefore, the investigation of the effect of high temperature regime on physiological activity and the understanding some mechanisms of heat tolerance in pepper cultivars is actual.

In this study, an attempt has been made to evaluate the response of physiological traits of pepper cultivars with a different susceptibility on high temperature condition.

Materials and Methods

2.1. Plant Material and Growth Conditions

Planting material used in this study is the commercial pepper cultivar HT37 (NW Bigarim) released in South Korea and accessions HT1 (Kobra) and HT7 (Samchukjaere) selected as heat tolerant and susceptible, respectively from National Institute of Horticultural and Herbal Science (South Korea, Wanju, 35°83′ N, 127°03′ E). Seeds of pepper accessions were sown in plastic trays (2021.02.23) and transplanted to plastic pots (2021.05.13) and maintained in normal treatment (NT) condition in a glasshouse (28/18 °C in day/night with relative humidity within 60–70%). All plants were grown in the soil medium containing 1:1 sand and commercial bed soil (Bio Sangto, Seoul, Korea) containing coco peat (47.2%), peat moss (35%), zeolite (7%), vermiculite (10.0%), dolomite (0.6%), humectant (0.006%), and fertilizers (0.194%) containing 270 mg kg⁻¹ of N, P, and K, respectively.

The plants of pepper on 140 days after sowing at the fruit developmental stage were transferred to a growth chamber for HT, where plants were maintained under high temperature (40/28 °C day/night, 14/10-h light/dark cycle) and light intensity of 800 μmol m⁻² s⁻¹ within 60% relative humidity. For each cultivar, a four biological replications were heat-treated in the growth chamber for 14 days and watered twice a day with total two-liter to avoid drought stress. After HT, the pepper plants were transferred to NT.

2.2. Measurement of chlorophyll contents and photosynthetic rate in seedlings under heat treatment

Total chlorophyll index (CHL) was estimated from fully expanded 3rd or 4th leaves from the top of the stem axis from each cultivar. Measurement of CHL was done before HT (0 day), on 2nd, 7th, 14th days of HT and after 7 days of recovery at normal condition. Measured of CHL was done in four biological replications by SPAD meter (Konica, Japan).

The photosynthetic rate Pn (μmol CO₂ m⁻² s⁻¹), stomatal conductance Gs (mol H₂O m⁻² s⁻¹) and transpiration rate Tr (mmol H₂O m⁻² s⁻¹) measured using a portable photosynthesis measurement system (LI-6400, LI-COR Bioscience, Lincoln, NE, USA) on 2nd, 7th, 14th days of HT and after 7 days of recovery at NT. Light response curves (PAR) was 800 μmol m⁻² s⁻¹. The leaf chamber temperature was 25°C, and the CO₂ concentration was 400 μmol (CO₂) mol⁻¹. The photosynthetic rate was measured automatically after 3-4 min light exposure [6,13]. Data were recorded in four plants from fully expanded 3rd or 4th leaves from the top of the stem axis from each cultivar.

2.3. Statistical analysis

The experimental design of this study was completely randomized. Statistical analysis (ANOVA) was performed using the SAS Enterprise Guide 7.1 (SAS Institute Inc., NC, USA) to identify the significant difference in the parameters of CHL, Pn, Gs and Tr and mean values were compared with a significance level of 5% using Duncan’s multiple range test at the p ≤ 0.05, p ≤ 0.01, and p ≤ 0.001 levels, respectively.

Results and Discussion

In measurement of the total chlorophyll content among all pepper cultivars under high temperature treatment showed degradation of the total CHL in leaves and ranged depending on treatment period and cultivar features (Fig. 1). The present study demonstrated that high temperature induces a reduction in CHL among all studied pepper cultivars and decreased to minimum on day 14 of heat treatment experiment, but the heat tolerant pepper plants persist the higher index of CHL than that of the susceptible cultivar. The significant degradation of CHL in leaves at HT condition was observed more in heat susceptible genotype HT7 than heat tolerant HT1 and HT37. This is in agreement with observations in Solanaceae plants [6,9,12,14].
These results suggest that the cultivar with stay-green leaves is tolerant and the sensitive cultivars cannot stay green due to decrease in chlorophyll a, chlorophyll b and carotenoid content and show early chlorosis and withered leaves [13,15,16]. The same pattern, the highest index of CHL in heat tolerant peppers HT1 and HT37, and the lowest in heat susceptible HT7 was identified on day 7 in pepper leaves after recover at NT condition. However, the index of CHL content slightly increased among all cultivars compared to the last treatment day 14.

Although the stay-green trait could be used as a morphological indicator to screen for heat tolerance in tomato, where the ability of tomatoes to stay green and maintain photosynthesis at different developmental stages, especially at anthesis (vegetative growth) which could be vital for reproductive growth and yield at high temperature [9,16].

Several studies have shown that CHL measured by SPAD increased in tomatoes and it may be an acclimation response of plants to high temperatures and duration of the treatment [6,12,17]. The mechanism by which high temperature may have caused chlorophyll loss is although it is unclear [18]. But, it might be a consequence of heat induced damage to thylakoid membrane, lipid peroxidation of chloroplast and oxygen evolving complex of PS II [19,20]. Also, degradation of chlorophyll in stressed leaves is due to activity of enzyme chlorophyllase [21,22]. By increasing chlorophyllase activity and decreasing the amount of photosynthetic pigments, heat stress ultimately reduces the plant photosynthetic and respiratory activity [23,24].

As photosynthesis and reproductive development are the most sensitive physiological processes to stress [13,25], depending on growth stages, it can be varied [9,16]. Exposure to high temperature of the pepper plants significantly has an effect on photosynthetic parameters. So, the index of Pn was higher in cultivars HT7 and HT37 and the lowest was determined in HT1 before start of HT on day 0, and then on day 2. It was slightly risen in heat tolerant genotypes HT1 and HT37, and reached a maximum point with no significant difference on day 14, whereas high temperature slightly reduced the Pn in heat susceptible HT7 and showed the significant lowest value in comparison with heat tolerant cultivars during all HT period (Fig. 2).

These findings are similar to those obtained from tomato and pepper plants, where a heat-tolerant variety is usually characterized by higher photosynthetic rates reflected in stay-green leaves, increased membrane thermostability and successful fruit set and yield under (over 30°C) high temperature conditions [9,16,17]. Meanwhile, in a literature also presented contrary results, where high temperature (30°C) reduced the photosynthetic rates and poor yield in pepper [26].

In general, heat tolerant pepper plants HT1 and HT37 may show properly functioning of the photosystem with normal CHL and chlorophyll fluorescence in stable PSII under high temperature conditions as compared to heat sensitive as previously reported [9,11,12].

In several genotypes of tomatoes differing in their capacity for thermotolerance, an increased chlorophyll a/b ratio is observed in the tolerant genotypes under high temperatures [16,20], indicating that these changes are related to thermostolerance [20,27]. Heat tolerant tomato plants maintained more available carbohydrates and accumulated more biomass as a result of higher Pn under heat stress, which benefits pollen development, fruit set and yield [13].

Interesting results were taken in measurement of the Pn on day 7 after heat treatment at recovery condition, where all pepper plants were found significantly more declining the index of Pn than those displayed during heat treatment periods. However, heat tolerant pepper cultivars HT1 and HT37 persist pattern with higher values of Pn than susceptible HT7. It means that heat treated plants need a period to recovery the photosynthetic activity.

Transpiration rate was significantly higher in heat susceptible HT7 plants than heat tolerant HT1 and HT37 on day 0 before...
start the exposure to high temperature of pepper plants (Fig. 3).

However, as mentioned above, the measurement of Pn pepper heat sensitive HT7 showed almost the same pattern in study of Tr in heat treatment condition, where the index of Tr significantly reduced on day 2 and persist trend for 14 days of heat treatment more than heat tolerant peppers HT1 and HT37. And, index of Tr showed significantly ranging among pepper plants regardless of cultivar thermotolerance capacity, where it is significantly increased in HT1 and showed the higher index than in initial measurement on day 0 compared to HT7 and HT37.

While Tr can be significantly varied depending on genotypes in plants at high temperature conditions, and the heat-tolerant plants had an increase in transpiration [6,9,12,13], or decreased in pepper plants as previously reported [17,26].

The loss of heat from the leaf surface due to enhanced transpiration led to decreased leaf temperature [12,28] and the PSII functionality was not affected at low leaf temperature, which was evident by higher chlorophyll fluorescence at HT [29,30]. It was reported that membrane thermo-stability has correlation with photosynthetic activity and respiratory activity under temperature stress condition in tomato [25], on the contrary, there is no the link between cell membrane thermo-stability to photosynthetic and transpiration rates, as shown in previous studies [9,27].

The evaluation of the effect high temperature regime on pepper plants stomatal conductance to H2O (Gs) was observed almost with the same pattern as mentioned above in measurement of Pn and Tr, where the initial Gs index was significantly higher in heat susceptible HT7 than other on day 0 before start of heat treatment (Fig. 4). However, Gs values significantly declined on day 2 in HT7 and was persisted during all HT periods, whereas heat tolerant cultivars showed steady index of Gs. And, as mentioned in the study of Tr, the Gs index was significantly increased more in heat susceptible HT7 than that in tolerant cultivars.

An increase in transpiration likely facilitated an increase in the heat tolerant tomato plants stomatal conductance than sensitive [9,12,13,25,29], subsequently leading to an increase in CO2 diffusion into the leaves and slightly increasing intercellular CO2 concentration (Ci) has been reported [9,13,28]. Also, stomatal closure (decreasing at HT) may be occurred as a main limiting factor of photosynthesis in Capsicum annuum plants [31].

However, a literature presented contrary results, where Pn, Gs and Tr in pepper with increasing temperature from 15 to 30 °C were decreased [26], while Pn was increased at temperature 40 °C, but Gs and Tr values decreased in tolerant cultivar at seedling stage of pepper [17]. Additionally, at heat stress regime (within 36-40 °C) main photosynthetic parameters such as Pn, Gs, Ci and Tr values were increased more in heat tolerant tomato seedlings than those in sensitive [9,13], while in other study, Pn and Gs were not significantly ranged more in heat tolerant tomato seedlings than those in sensitive one under HT (40 °C), where Pn and Ci decreased but Gs increased [11].

**Conclusion**

High temperature makes to reduce of CHL in all cultivars, but the response of cultivars is different. Pepper cultivar HT7 was identified as heat sensitive, which showed the lower index of CHL than heat tolerant HT1 and HT37 under HT regime. Heat tolerant cultivars HT1 and HT37 showed the higher values of CHL, Pn, Gs and Tr than heat sensitive HT7, in which the lowest values for all heat treatment periods were observed. It should be noted that the index of Pn, Gs and Tr showed the significant alteration before HT on day 0 and day 7 after recovery at normal condition, it means that plants response to high temperature regime is different from that in normal condition.
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