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Research Article

Physiological and Molecular Responses of Maize to High Temperature Stress During Summer in the Southern Region of Korea

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ABSTRACT

Environmental stresses caused by climate change, such as high temperature, drought and salinity severely impact plant growth and productivity. Among these factors, high temperature stress will become more severe during summer. In this study, we examined physiological and molecular responses of maize plants to high temperature stress during summer. Highest level of H_2O_2 was observed in maize leaves collected July 26 compared with June 25 and July 12. Results indicated that high temperature stress triggers production of reactive oxygen species (ROS) in maize leaves. In addition, photosynthetic efficiency (Fv/Fm) sharply decreased in leaves with increasing air temperatures during the day in the field. RT-PCR analysis of maize plants exposed to high temperatures of during the day in field revealed increased accumulation of mitochondrial and chloroplastic small heat shock protein (HSP) transcripts. Results demonstrate that Fv/Fm values and organelle-localized small HSP gene could be used as physiological and molecular indicators of plants impacted by environmental stresses.

(**Key words** : Climate change, Heat stress, High temperature, Maize, small HSP)

Ⅰ. INTRODUCTION

Climate change, also called global warming, is an important issue that negatively impact on plant growth due to damaging effect of environmental stresses including high temperature (Bita and Gerats, 2013). Although there are a number of impacts expected from climate change, one of the largest impacts is expected to be on agriculture (Mendelsohn, 2009; Nordhaus, 1991) including forage production. It has been reported that an affect of climate change is also observed in the Korean Peninsula, of which the average annual air temperature has increased by about +1.5ºC over the 100 years. Even this small increase in air temperature caused by climate change could have serious effect on crop production in Korea.

High temperature stress (also called as heat stress) is one of main abiotic stresses constrain crop yield (Vierling, 1991). Heat stress has also become an important factor in limiting forage yields including maize, which is one of major summer forage crop in Korea. Now a day, more extreme high temperatures in summer occur more frequently and persist for longer period in Korean Peninsula, particularly in the southern region. Previous studies reported that high temperature stress damages mainly on photosynthetic apparatus (Berry and Björkman, 1980), which severely reduces crop productivity. Plants also respond to elevated temperature stresses by expressing five conserved classes of heat shock proteins (HSP) based on their molecular weight; HSP100, HSP90, HSP70, HSP60 and small HSPs (Wang et al., 2004). Among HSPs, small HSPs range in size from 15 to 40 kDa, abundant in higher plants, and are divided into six classes based on their sequence and cellular compartment (Vierling 1991, Hu et al., 2010). In addition, previous studies have shown that mitochondria and chloroplast-localized small HSPs are expressed rapidly after exposure to abiotic stresses, particularly to high temperatures (Vierling, 1991). These results suggest that organelle-localized small HSPs play an important role in the thermotolerance of plants (Vierling, 1991). In this study, to evaluate responses of maize plants to climate change, we compared physiological response including photosynthetic efficiency (Fv/Fm) and molecular response of mitochondrial and chloroplastic small HSPs of maize plants against extreme high temperatures grown in summer in the southern region of Korea.

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Ⅱ. MATERIALS AND METHODS

1. Plant Material and Growth Condition

Maize (*Zea mays*, cv. Gwangpyeongok) seeds were planted in plastic pots containing Horticulture Nursery Medium (Biomedia) and grown in a growth chamber maintained at 25°C with light intensity of 450 μmol m^{-2} s^{-1} and 16/8 h light/dark cycle. Four-week-old seedlings were exposed to heat stress treatment. For field experiment, maize was cultivated in 12 m^2 (3 m ′ 4 m) plots in the two different experimental sites located in the southern region of Korea, Jinju (35°12'28"N 128°08'20"E) and Jangheung (34°33'12"N 126°53'37"E). Maize was planted on 14th of May for Jinju and 16th of May in 2018 for Jangheung. Maize plants were grown under natural climate condition of each farm region.

2. Heat Stress Treatment

For heat stress treatment, 4-week-old entire plants grown at 25°C in the growth chamber were placed at 37°C or 42°C in a temperature-controlled incubator for 2 h. Leaves from treated plants (three independent repeats) were collected and immediately frozen with liquid nitrogen and stored at -80°C until use.

3. Measurement of Climate Parameters

Mean of daily air temperature and precipitation were measured and recorded according to data-loggers (WatchDog whether station, WatchDog, England) established at each field of experimental site.

4. Measurement of Photosynthetic Efficiency

Photosynthetic activity was evaluated by measuring the maximum quantum yield (Fv/Fm) of photosystem II (PSII), using a Handy PEA chlorophyll fluorometer (Hansatech Instruments, King's Lynn, UK). After 30 min dark adaptation, fluorescence was measured using a saturating red light (3000 μ mol m^{-2} s^{-1}). All measurements were conducted in the apparently healthy leaves.

5. Determination of H_2O_2 Content

The H_2O_2 content in maize leave was measured spectrophotometrically as described previously (Jana and Choudhuri, 1981). In brief, maize leaf tissue was homogenized with phosphate buffer (50 mM, pH 6.8) containing 1 mM hydroxylamine. The homogenate was centrifuged at 6,000 ×g for 25 min, and the supernatant was mixed with 0.1% titanium sulfate in 20% (v/v) H_2SO_4 followed by centrifugation at 6,000 ×g for 15 min. The absorbance was measured at 410 nm, and H_2O_2 content was calculated using the extinction co-efficient 0.28 $\mu mol^{-1}cm^{-1}$.

6. Total RNA extraction and RT-PCR

Total RNA was extracted from maize leaves by the RNeasy Plant Mini Kit (Quiagen), and subjected to RNase-free DNase treatment (Quiagen). Total RNA was quantified by using spectrophotometer. For the reverse transcription (RT)-PCR analysis, cDNA synthesis was performed using the reverse transcriptase kit (Maxiprime RT PreMix, iNtRON). One microgram of total RNA was used for a template with oligo (dT) as a primer. Reversed transcribed cDNA was then diluted 1:10 and 1 ml was used for PCR amplification together with specific primers for mitochondrial and chloroplastic small HSP genes (Table 1). Amplified PCR products were visualized by gel electrophoresis on1.2% agarose gel.

7. Statistical analysis

Physiological data were statistically analyzed using SPSS program (ver 16.0). All the results are presented as mean±SE of at least three independent replications.

Table 1. Primers used for RT-PCR amplification from maize

Genes	Forward primers	Reverse primers
Mitochondrial small HSP, *Zmhsp22*	5'-TCGCGCCAGAGGTTTACA-3'	5'-ACCGGCCTTGACACGAAA-3'
Chloroplastic small HSP, *Zmhsp26*	5'-CAGGAGAACAGGGACAACAGT-3'	5'-GCATCTTCACCTCCTTCTCG-3'

Ⅲ. RESULTS AND DISCUSSION

1. Effect of High Temperature on Reactive Oxygen Species

To investigate high temperature stress response in maize at physiological level, we analyzed H_2O_2 content in leaves that had been subjected to heat treatment with plants grown in growth chamber at 25ºC. The H_2O_2 content was increased with applied high temperatures in both 35ºC and 42ºC treatments. In 42ºC for 2 h treatment, H_2O_2 content was increased up to 1.5 fold higher compared to 25ºC control plants (Fig. 1A). This result suggests that high temperature stress triggers production of reactive oxygen species (ROS) in the cells.

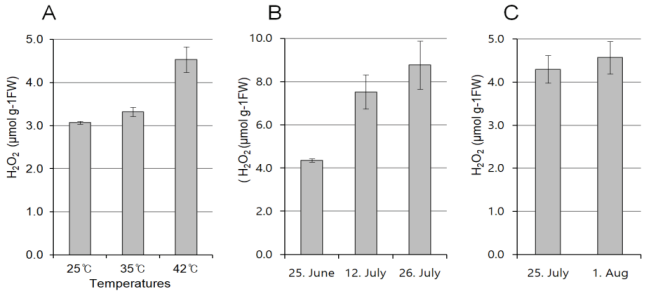

Fig. 1. Changes in hydrogen peroxide (H_2O_2) levels in leaves of maize after exposure to high temperatures (A) or grown in summer in the experimental field located in Jinju (B) and Jangheung (C).

We further analyzed whether the temperatures during the three hottest months June, July and August affects accumulation of H_2O_2 in maize plants. Leaf samples were collected from each maize plant grown in two different fields of experimental site, which are located in the southern region of Korea, Jinju and Janheung, and analyzed H_2O_2 contents. In case of plants grown in Jinju site, highest level of H_2O_2 was observed in leaves from 26th July compared with 12th July or 25th June (Fig. 1B and 1C). Similar pattern in H_2O_2 accumulation was found in samples from Jangheung. These results demonstrate that exposure to higher temperatures in field also triggers production of ROS in the cells. A number of previous studies reported that exposure of plants to increased temperature (heat) stress resulted in elevation of cellular H_2O_2 levels (Volkov et al., 2006).

2. Effect of High Temperature on Photosynthesis Efficiency

Chlorophyll fluorescence was measured to determine changes in the maximum quantum yield (Fv/Fm) of photosystem II (PSII) after high temperature stress treatment. We first compared the effects of high temperature stress treatment on Fv/Fm using maize plants grown in the growth chamber.

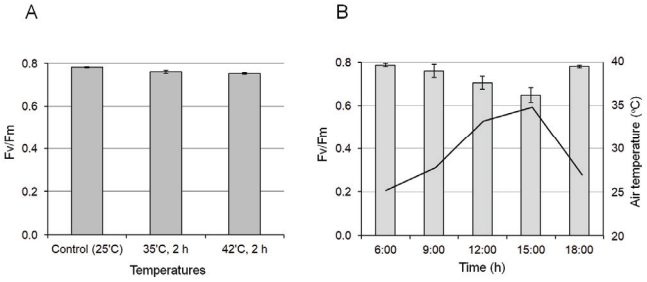

Fig. 2. Changes in photosynthesis efficiency (Fv/Fm) of maize plants after treated with high temperature stress. A. Fv/Fm after 35 or 42ºC, 2 h treatment in the growth chamber. B. Fv/Fm was measured through the daytime of 30th July in the experimental field of Jinju.

As shown in Fig. 2A, the Fv/Fm values under high temperature stress treatment with 35ºC and 42ºC for 2 h was slightly decreased to 0.760±0.008 and 0.752±0.003, respectively, compared with the control (25ºC). Therefore, photosynthetic efficiency of PSII was decreased in maize leaves when subjected to artificial heat stress treatment. We further analyzed daytime (6 A.M. to 6 P.M.) change of PSII activity in the field grown maize plants (Fig. 2B). Fv/Fm value at 6 A.M. showed similar value compare to the control (25ºC) of growth chamber grown plants. However, Fv/Fm was sharply decreased by increasing air temperatures in the field, showed maximum decrease at 3 P.M., and then gradually recovered along with time up 6 P.M. These results demonstrated that exposure of maize plants to high temperature stress by either abruptly or gradually damages on photosynthetic apparatus of PSII. In this study, results show that increased temperatures were well correlated with declines in Fv/Fm. Berry and Bjorkman (1980) reported that the cause of Fv/Fm decline in high temperature stress is related to decline in the function of photochemical reaction of PSII. Previous studies have reported that photosynthesis is one of the most sensitive physiological processes in response to elevated temperature

(Berry and Björkman, 1980; Srivastava et al., 1997; Mathur et al., 2014). In addition, Fv/Fm is decreased by a number of abiotic stresses (Grover et al., 1986; Zhang et al., 2008), including heat stress, which is consistent with our results. Therefore, our data suggest the possibility that Fv/Fm measurement can be used to reliable monitoring instrument for the high temperature stress responses of field-grown maize plants.

3. Expression of Heat-responsive Genes in the Field-grown Maize Plants

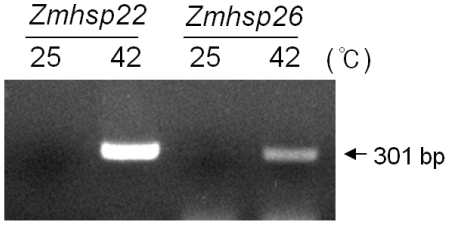

Fig. 3. Expression of organelle-localized small HSP genes in leaves of maize after high temperature stress treatment. A. Mitochondrial small HSP (*Zmhsp22*). B. Chloroplastic small HSP (*Zmhsp26*). Four-week-old maize plant was exposed to 42ºC for 2 h, and subjected to RT-PCR with specific primers.

To investigate the specifically expressed genes of maize leaves response to high temperature stress, total RNA was extracted from the third leaf and amplified by RT-PCR with specific primers for mitochondrial small HSP or chloroplastic small HSP after 4-week-old maize plants were subjected to high temperature stress treatment at 42ºC for 2 h. Both organelle specific small HSP genes were strongly expressed in the leaves of maize plants treated with high temperature stress treatment, but extremely very low levels in the control (Fig. 3). This result demonstrates that maize mitochondria (*Zmhsp22*) and chloroplast-localized small HSP genes (*Zmhsp26*) show high temperature-specific expression at temperatures higher than normal growth temperature of 25ºC.

We compared changes in expression level of *Zmhsp22* and *Zmhsp26* in leaves of maize plants grown under natural climate conditions of summer period (from June to August) in the experimental fields, which are located in the southern region of Korea and show usually higher mean air temperatures than other regions. Data and samples were taken at 2 P.M. of specified days except 25 June for Jinju, which was taken at 11 A.M., and detail air temperature and daily mean temperatures are shown in Table 2. Transcripts for both mitochondrial and chloroplastic small HSP genes were accumulated in leaf samples of maize plants harvested from June or July, and increased by increasing air temperatures up to 1 August in both experimental fields in Jinju and Jangheung (Fig. 4; Table 2). Mitochondrial small HSP (*Zmhsp22*) showed higher level of expression compared with chloroplastic HSP (*Zmhsp26*) gene. These results suggest that the field-grown maize plants might have been exposed to the high temperature stress in the onsite air temperatures higher than 30ºC in the experimental sites. Previous studies reported that plant small HSP genes are specifically induced not only by heat stress but also many other abiotic stresses (Waters et al., 1996). In organelle-localized small HSP, it has been demonstrated that mitochondrial HSP22 protects mitochondrial complex I from heat stress (Downs and Heckathorn, 1998), and chloroplastic HSP26 protects photosystem II (PSII) during heat stress (Heckathorn et al., 1998). Therefore, maize mitochondrial and chloroplastic small HSPs likely have an important function during high temperature stress condition, and could be use as good indicators whether maize plants grown in the field are exposed to high temperature stress.

Table 2. Temperature conditions in the field of experimental sites.

Region	Date of measurement	Air temperature (ºC)	Daily mean temperature (ºC)
Jinju	25 June	30.4	25.5
	12 July	32.0	27.5
	26 July	36.4	30.8
Jangheung	25 July	32.5	27.5
	1 August	34.6	27.4

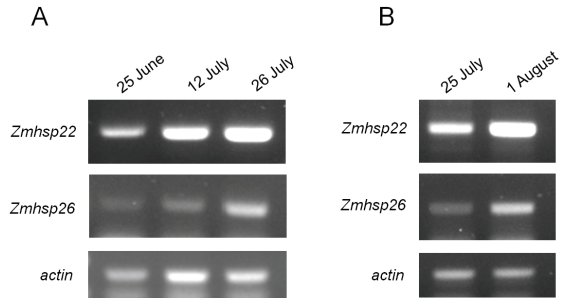

Fig. 4. Expression of mitochondral and chloroplastic small HSP genes in leaves of maize plants grown in the fields of experimental sites of Jinu (A) and Janheung (B).

Ⅳ. CONCLUSIONS

Extreme climate including extreme high temperatures in summer adversely effects on crop productivity in the field. Recently, extreme high temperatures in summer season observed more frequently even in Korea Peninsula. We have analyzed physiological and molecular responses of maize plants grown in the hottest months of summer in the southern region of Korea. Physiological parameters, such as H2O2 content and photosynthetic efficiency of PSII (Fv/Fm), were responded to the degree of changes in air temperatures. In addition, expression level of mitochondrial and chloroplastic small HSP genes were also increased by the degree and duration of exposure to high temperatures in the fields located in the southern region of Korea. Data of physiological and molecular responses of maize to high air temperatures grown in summer could be use as indicators, and would be helpful to understanding maize response to climate change.

Ⅴ. ACKNOWLEDGEMENTS

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