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PROTECTIVE EFFECT OF ENDOGENOUS ABSCISIC ACID ON TOMATO PHOTOSYNTHESIS UNDER SUB-HIGH TEMPERATURE AND HIGH LIGHT STRESS

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Abstract

In northern China, greenhouse tomato production often suffers double stress of sub-high temperature (35ºC) and high light stress simultaneously. The experiment proves that endogenous asscisic acid (ABA) takes protective effect on photosystem I, photosystem II and RuBPCase activity of tomato leaves under sub-high temperature and high light stress. Net photosynthetic rate (Pn) and transpiration rate (E) values of wild type (RR) maintain certain level after 11 hrs stress, however, Pn and E values of ABA deletion mutant (*sit*) are almost 0. Under sub-high temperature and high light stress, endogenous ABA slows down the chlorophyll degradation, while ABA deletion will accelerate chlorophyll to degrade, the mutant cannot protect photosystems I and II due to ABA deletion thus causing greater damage to plant.

Introduction

In the next few decades, abiotic stress will be the main limiting factor of crop yield and quality. Plants surviving under stress will have mechanism confronting and enduring stress to protect themselves, of which ABA is the main research aspect (Saroj *et al*. 2016). Abscisic acid (ABA) is the important regulator inside plant coordinating growth and development and responding to stress (Natalia and Joanna 2016). As an important endogenous hormone inside plant, ABA takes part in regulating photosynthetic process of leaves development (Tholen *et al.* 2007) and all other processes of the whole plant growth and development (Tardieu *et al*. 2010, Thiago *et al*. 2013). As a stress hormone, ABA is the center signal substance of plant responding to many multiple environmental stress (Finkelstein 2013, Waniand Kumar 2015, Yang *et al*. 2016).

Hormone property research involves in two approaches such as exogenous spraying and endogenous deletion. The effect of exogenous ABA on photosystem under sub-high temperature and high light stress has been noted, but researching exogenous hormone treatment cannot completely repeat function of endogenous hormone. Therefore, we adopt some mutants which have been used to find out the effect of endogenous hormone.

There are many reports on ABA deletion mutant, including seed germination, ABA composition and decomposition (Goggin *et al.* 2009, Rodriguez-Gacio *et al.* 2009, Ferguson and Mathesius 2014), but less on photosynthetic aspect, even less such research under stress. Therefore, the experiment takes ABA deletion mutant *sittens* (*sit*) and wild-type Rheinlands Ruhm (RR) as experimental materials to ascertain changes of photosynthetic rate, PS II and PS I photochemical activity, RuBP case activity of mutant and wild-type leaves under sub-high temperature and high light stress treatment to study the protective action of endogenous ABA on photosynthetic apparatus of tomato leaves for making clear the mechanism of ABA affecting tomato photosynthesis under sub-high temperature and high light stress.

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Materials and Methods

The experiment was carried out in the sunlight greenhouse of vegetables Horticulture College of Shenyang Agricultural University. The tomato (*Lycopersicon esculentum* Mill.) varieties are abscisic acid (ABA) deletion mutant *sittens* (*sit*) and wild-type Rheinlands Ruhm (RR). *Sit* were obtained from RR mutation by means of X-ray by Stubbe in 1959.

Seeds provided by Zhejiang University were soaked in 2.7% sodium hypochlorite solution for 30 min, and then directly sown after washing thoroughly with de-ionized water and transplanted in 15 cm × 15 cm plastic pots when the seedlings produced two leaves. The plastic pots containing a 3: 2: 2 (v/v/v) mixture of garden soil: peat: chicken manure. *Sit* growing environment: preferred temperature was 22ºC, natural lighting did not exceed 120 μmol/m^2/s, and the relative humidity was 80%. RR growing environment: preferred temperature was 20 - 30 C, natural lighting did not exceed 500 μmol/m^2/s. A fixed quantity of watering was maintained.

Sit and RR (at six leaves stage) were transferred to phytotron and then treated with sub-high temperature and high light for 3 days. Set phytotron conditions: day/night: 12 hrs/12 hrs, temperature: 35/15ºC, lighting intensity: 800 μmol/m^2/s, constant humidity: 65%. Samples were taken for measurement of indicator when disposing for 0, 1, 3, 7, 11 hrs, repeated three times in each disposal and 3 plants were measured in each repetition.

Gas exchange, chlorophyll fluorescence and P700 redox states were obtained simultaneously using GFS-3000 and DUAL-PAM-100 measuring systems (Heinz Walz, Effeltrich, Germany). All measurements were made at a CO_2 density of about 400 ± 10 μmol/m^2/s.

According to Zhang (2014), the following Chl fluorescence parameters were calculated: Fv'/Fm' = (Fm' − Fo')/Fm', qP = (Fm' − Fs)/(Fm' −Fo'), Y(II) = (Fm' − Fs)/Fm' , Y(NO) = Fs/Fm, Y(NPQ) = 1− Y(II) − Y(NO), Y(I) = 1 – (ND) – Y(NA), Y(ND) = 1 – P700 red and Y NA) = (Pm – PM')/Pm. Fo' is the minimum fluorescence in the light-adapted state.Fm and Fm' are the dark-adapted and light-adapted maximum fluorescence upon illumination with a pulse (300 ms) of saturating light (10,000 μmol/m^2/s). Fs is the light-adapted steady-state fluorescence; Fv'/Fm' is the light-adapted maximum quantum yield of PSII. qP is the photochemical quenching coefficient . Y(II) is the effective quantum yield of PSII. Y(NO) is the quantum yield of non-regulated energy dissipation of PSII. Y(NPQ) is the quantum yield of regulated energy dissipation of PSII.The quantum yield of PSI [Y(I)] is defined by the proportion of overall P700, which is reduced at a given state and not limited by the acceptor side. It is calculated from the complementary PSI quantum yields of non-photochemical energy dissipation Y(ND) and Y(NA), i.e., Y(I) = 1 − Y(ND) - Y(NA). Y(ND) = 1 − P700red and Y(NA) = (Pm − Pm′)/ Pm.

RuBP carboxylase (EC 4.1.1.39) is extracted from leaves using 0.5 g of sample with extract solution of 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM $MgCl_2$, 12.5% (v) glycerol, 10% PVP, and 10 mMβ-mercaptoethanol. The extract is subsequently centrifuged at 15,000 g at 4°C for 15 min. The supernatant was the RuBisCO crude extract. RuBisCO activity quantification is conducted by means of enzyme immunoassay kits (RuBisCO Enzyme Immunoassay Test Kit, Shanghai MiaoYan).

Results and Discussion

Chlorophyll content (Chl) and Chl a/b of ABA deletion mutant (*sit*) is obviously lower than that of wild type (RR) (Fig. 1), net photosynthetic rate (Pn) of *sit* is prominently lower than that of RR (Fig. 2a), which indicates that ABA deletion inside tomato plant will affect the synthesis of chlorophyll thus affecting net photosynthetic rate, which is in accordance with the results of predecessor (Bengtson *et al.*1977, Thiago 2013). It is because that ABA deletion causes stoma open, and burn point on leaves, which affects chlorophyll content and directly affects efficiency

for solar energy utilization.

Chl of RR and *sit* all present the trend of rising first then falling, Chl within 1 hr stress rises, which is possibly attributed to sudden increase of light intensity. In order to adapt to sharp increase of high light pigment molecule, the plant decreases sharply after 1 h, and RR has a larger extent of descent (Fig. 1a). However, Chl a/b value presents the trend of decreasing after sub-high temperature and high light stress, RR and *sit* decreasing degree difference is not obvious (Fig. 1b). It indicates that ABA slows down Chl degradation, while deletion ABA will accelerate Chl degradation.

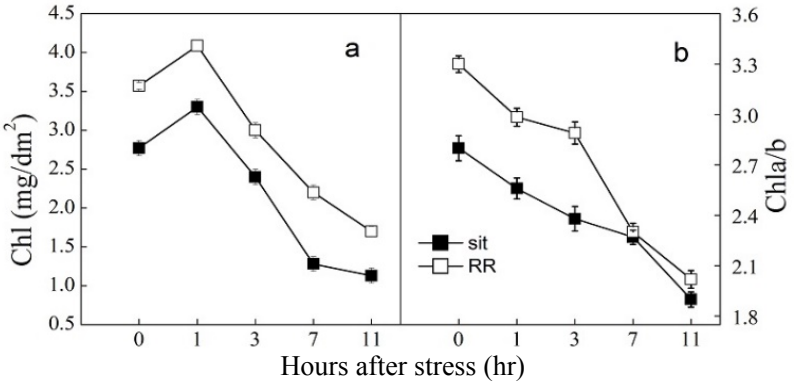

Fig. 1. Effect of endogenous ABA on chlorophyll content of leaves of tomato seedling under (1a) sub-high temperature and (1b) high light stress.

ABA deletion seriously affects Pn of leaves (Fig. 2a). Because stoma cannot close, the stomatal conductance (gs) and transpiration rate (E) of *sit* are obviously higher than those of RR (Fig. 2c, d). Sub-high temperature and high light stress markedly reduces net photosynthetic rate

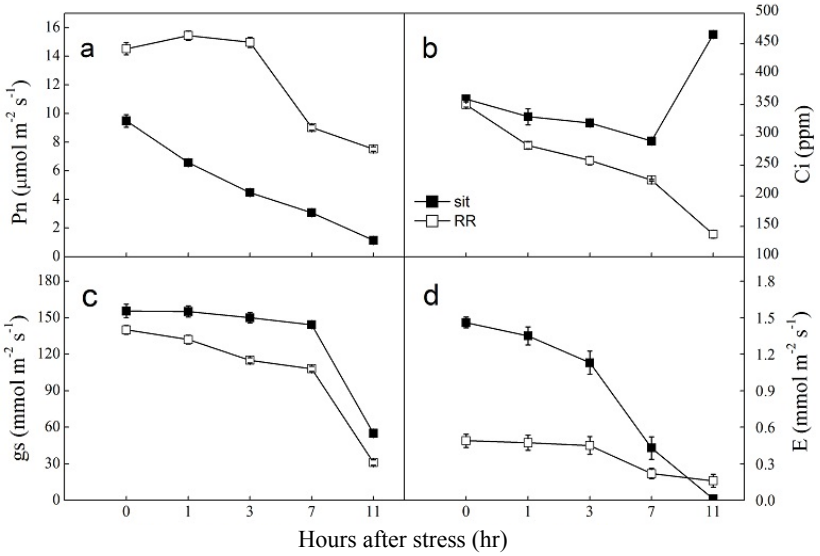

Fig. 2. Effect of endogenous ABA on gas exchange (gs) of tomato seedling leaves under sub-high temperature and high light stress.

(Pn) of RR and *sit*, and Pn of RR has no obvious change within 3 hrs stress, but decreases rapidly after 3 hrs. Pn of *sit* is always presenting the trend of decreasing after stress, and RR is obviously higher than *sit*. It shows that endogenous ABA slows down decrease of photosynthetic rate after stress, which is coincident with result of photosynthetic rate decreased under exogenous spraying ABA relieving stress.

Within 7 hrs sub-high temperature and high light stress, Ci values of RR and *sit* notably decrease; after 7 hrs, Ci of *sit* increases, and Ci of RR continuously decreases, which may be due to the damage of *sit* cellular structure, while RR is not (Fig. 2b). It can be considered that endogenous ABA can protect cellular structure from being destroyed by sub-high temperature and high light stress within certain time.

Sub-high temperature and high light stress obviously reduces gs and E values of wild type and mutant (Fig. 2c, d). Francesca (2013) reported that gs of *sit* did not change under moderate water stress, however gs decreased under severe stress. The results indicate that gs value of *sit* within 7hrs stress did not change obviously, while gs of RR decrease slowly. After 7 hrs, gs of mutant and wild type decrease (Fig. 2d), which is consistent with the results of Francesca *et al.* (2013).

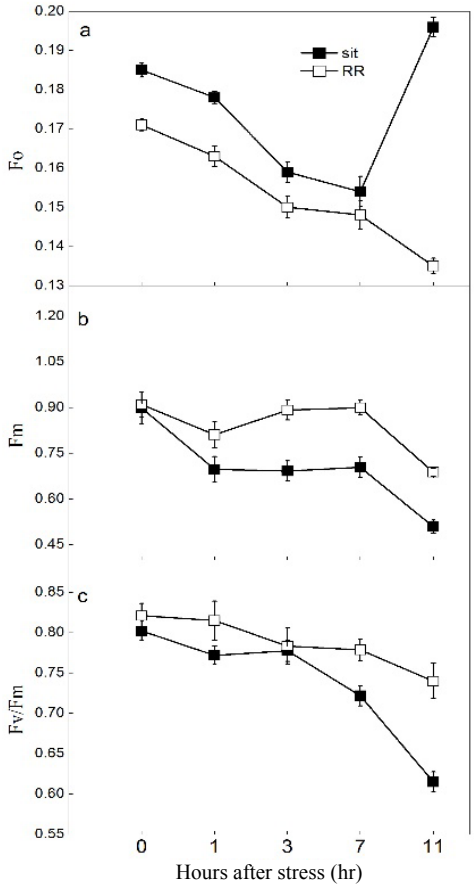

Fig. 3. Effect of endogenous ABA on photosystem, photoinhibition of leaves of tomato seedlings under sub-high temperature and high light stress.

As an important component of photosynthetic apparatus, PSII is the critical part of being hurt in various stresses (Nishiyama *et al*. 2006). It indicates that endogenous ABA has certain positive control effect on plant tolerance induced by water stress (Nayyar *et al*. 2003, Perales *et al*. 2005). In this experiment, initial fluorescence (Fo) value of *sit* is always higher than that of RR under sub-high temperature and high light stress, the maximal fluorescence (Fm) value of RR is always higher than that of *sit*; with stress time extension, Fo of RR presents the trend of decreasing, while Fo value of *sit* presents the trend of falling first then rising (Fig.3a), however, decrease degree of Fm of mutant and maximal photochemical efficiency (Fv/Fm) is obviously higher than that of wild type (Fig. 3b,c).

Sub-high temperature and high light stress notably reduces PS II electron transfer (ETR II), photochemical quantum yield [Y(II)], non-photochemical dissipation (NPQ) (*sit*), photochemical quenching (qP), PSII potential photochemical efficiency (Fv/Fo) and PSII maximal photochemical efficiency (Fv'/Fm') of wild type and mutant(Fig. 4). *Sit* within 7 hrs stress can protect PS II by means of NPQ, while 7 hrs later, reaction center inactivation occurs, but RR can also protect themselves. One can speculate that endogenous ABA has a protective effect on PSII by means of non-photochemical heat dissipation protection mechanism.

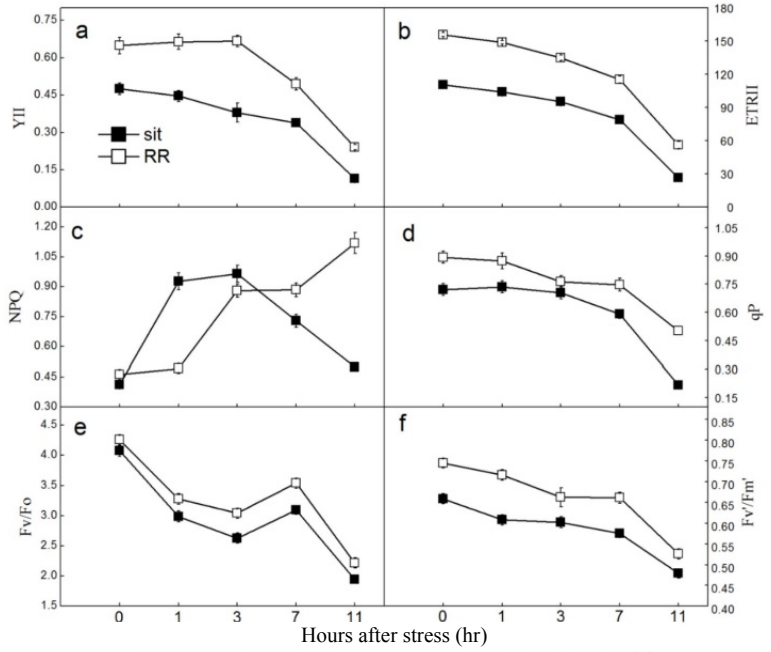

Fig. 4. Effect of endogenous ABA on PS II photochemistry activity of leaves of tomato seedlings under sub-high temperature and high light stress.

Fig. 5 shows PSI non-photochemical yield Y(ND) and Y(NA), respectively reflects the state of electron donor and acceptor. Sub-high temperature and high light stress for 3 hrs obviously reduces PSI photochemical yield [Y(I)] of *sit*, Y(NA) begins to decease, and Y(ND) notably increases, which testifies that PSI suffers photoinhibition. PSI of RR within 11 hrs stress is not affected. It is obvious that ABA deletion will cause tomato leaves PSI hurt after 3 hrs sub-high temperature and high light stress, and endogenous ABA takes protective effect on PSI as well.

Fig. 6 shows that RuBPCase of RR is obviously higher than that of *sit* without sub-high temperature and high light stress, because the growing environment of *sit* is low light, and the photosynthetic capacity is weak, which cannot directly illustrate that ABA deletion inside plant will restrain RuBP carboxylase activity. Sub-high temperature and high light stress cause RuBPCase

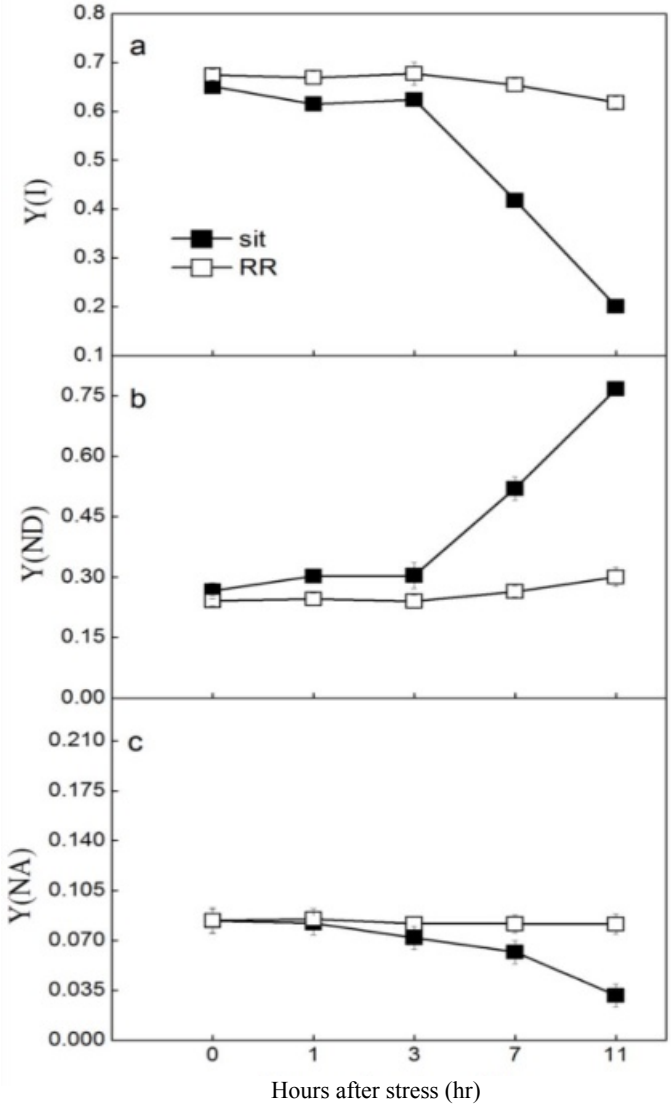

Fig. 5. Effect of endogenous ABA on photosystem, photoinhibition of leaves of tomato seedlings under sub-high temperature and high light stress.

value of wild type and mutant to present the trend of rising first then falling, it rises within 3 hrs, and RuBPCase value difference of wild type and mutant is not notable, it decreases after 3 hrs stress, RuBPCase value of RR is obviously higher than that of *sit*. When being under stress for 11

hrs, RuBPCase value of RR is two times of *sit*'s, RuBPCase activity of *sit* is obviously lower than that before stress, however, RuBPCase activity of RR is higher than that before stress.

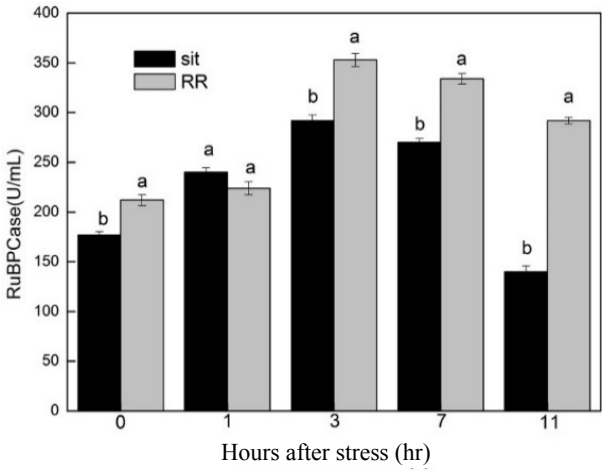

Fig.6. Effect of endogenous ABA on RuBPCase activity of leaves of tomato seedlings under sub-high temperature and high light stress.

The experiment proves that endogenous ABA takes protective effect on photosystem I, photosystem II and RuBPCase activity of tomato leaves under sub-high temperature and high light stress.

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