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EFFECTS OF EXOGENOUS 24-EPIBRASSINOLIDE ON PHOTOSYNTHESIS AND ATP SYNTHASE B SUBUNIT OF TOMATO UNDER LOW TEMPERATURE / POOR LIGHT

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Abstract

We monitored the effects of exogenous 24-epibrassinolide (EBR) on the photosynthesis and ATP synthase β subunit of tomato (*Solanum lycopersicum* L.). Seedlings of 'Zhongza9' and 'Zhongshu4' cultivars were pre-treated with foliar sprays of 0.1 μM or 0.0 μM (control) EBR and then grown in an environmental chamber simulating conditions of low temperature (12°C/6°C, day/night) and poor light (80 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Stressed seedlings that had received EBR showed increases in stomatal conductance and rates of photosynthesis and transpiration over the untreated (0 EBR) control, but had reduced concentration of intercellular CO_2. Exposure to EBR was also linked with smaller, stress-related declines in the maximum photochemical efficiency of Photosystem II, the actual photochemical efficiency of Photosystem II, the photochemical quenching coefficient, and the efficiency of light energy capture by open PSII reaction centers under our combined stresses. We also found that EBR improved plant stress tolerance, and the mechanism was that ATP synthase β subunit was synthesized in a large amount.

Key words: ATP synthase β subunit, Chlorophyll fluorescence, 24-Epibrassinolide, Photosynthesis, Tomato.

Abbreviations: Ci, intercellular CO_2 concentration; EBR, 24-epibrassinolide; F_0, initial chlorophyll fluorescence; Fm, maximum chlorophyll fluorescence; Fs, steady-state fluorescence level; Fm′, light-adapted maximum fluorescence; Fv/Fm, maximum photochemical efficiency of Photosystem II; Fv′/Fm′, efficiency of light energy capture by open PSII reaction centers; Gs, stomatal conductance; Φ_{PSII}, actual photochemical efficiency of PSII; Pn, net rate of photosynthesis; PSI, Photosystem I; PSII, Photosystem II; qP, photochemical quenching coefficient; Tr, transpiration rate.

Introduction

When plants of tomato (*Solanum lycopersicum* L.) are cultivated in a greenhouse during Winter and Spring, they are often affected by stresses associated with low temperatures and poor light levels. These conditions lead to declines in photosynthesis rates, growth, and crop yields (Ren *et al*., 2002). Photosynthetic capacity, a major determinant of plant development, is the first metabolic process inhibited by chilling and reduced illumination (Hu *et al*., 2010). This complex phenomenon is involved in light energy absorption, energy conversion, electron transfer, ATP synthesis, and CO_2 fixation. Stress conditions interrupt the balance between the production of reducing equivalents and the consumption capacity of photosynthesis. This can potentially cause excess generation of reactive oxygen species such as $^1O_2$ and O_2^-, and ultimately lead to photoinhibition and photo-oxidative damage of Photosystem II (PSII) and Photosystem I (PSI) (Ivanov *et al*., 2012). These disruptions can influence thylakoid electron transport, the carbon reduction cycle, and stomatal control of the CO_2 supply (Ogweno *et al*., 2010), and can also reduce ATP synthesis. In the chloroplasts, ATP synthase is necessary for electron transport and phosphorylation during photosynthesis (Cao *et al*., 2012). In the mitochondria, this enzyme can provide energy for respiration by catalyzing ATP hydrolysis. ATP synthase is composed of an integral membrane CF_0 portion and an extrinsic CF_1 portion. The latter complex comprises five subunits (Ni and Wei 2003). Of those, the β subunit has a catalytic and ADP-binding unit that either catalyzes ATP formation from ADP and Pi or else catalyzes ATP hydrolysis in the presence of a transmembrane proton gradient (Ni and Wei 2003).

The effects of exogenous substances have been examined in alleviating developmental defects with various plants, e.g., eggplant (*Solanum melongena*; Wu *et al*., 2014), cucumber (*Cucumis sativus*; Fariduddin *et al*., 2011; Yuan *et al*., 2012), melon (*C. melo*; Zhang *et al*., 2013), and wheat (*Triticum aestivum*; Ali *et al*., 2008). Brassinosteroid hormones can improve the photosynthetic efficiency of seedling leaves (Serna *et al*., 2012), and increase plant tolerances to several abiotic stresses, including low or high temperatures (Singh *et al*., 2012; Chandrakala *et al*., 2013), poor light (Wang *et al*., 2010), salinity (Alyemeni *et al*., 2013; Wu *et al*., 2012), hypoxia (Ma, 2014) and drought (Yuan *et al*., 2010).

To investigate whether exogenous 24-epibrassinolide (EBR) can increase the tolerance of tomato seedlings to the combined stresses of low temperature and poor light, we measured parameters for gas exchange and chlorophyll fluorescence, and analyzed the expression of photosynthesis related protein. Such information might be applied in future efforts toward improving plant tolerance under these stress conditions.

Materials and Methods

Plant materials and treatments: We used two genotypes of tomato that respond differently to low temperature and poor light: the tolerant 'Zhongza9' and the sensitive 'Zhongshu4'. Seeds of both were sown in plastic pots containing a 1:1 (v:v) mixture of soil and perlite, and then placed in a greenhouse of Yangling, Shaanxi Province, China. Growing conditions included 25°C/18°C (day/night) under a 12-h photoperiod (350 $\mu mol \cdot m^{-2} \cdot s^{-1}$). When they reached the five-leaf stage, half of the seedlings were sprayed with 0.1 μM of EBR (+EBR), while the remaining half were treated with distilled water as the control (–EBR). This pretreatment period continued

for 7 d. Afterward, the +EBR and –EBR plants (40 each) were placed in a controlled environment chamber where they were exposed to the combined stress conditions of low temperature (12°C/6°C, day/night) plus poor light (80 $\mu mol \cdot m^{-2} \cdot s^{-1}$), again with a 12-h photoperiod. For each cultivar, three plants per treatment were randomly selected for analyzing gas exchange and chlorophyll fluorescence. The second fully expanded leaf was harvested on Day 12 of treatment as experiment material.

Evaluation of gas exchange parameters: Gas exchange parameters were monitored on the second fully expanded leaf from the top of each selected seedling, using a Li-6400 Photosynthesis System (Li-Cor, Lincoln, NE, USA) at 25°C. The net rate of photosynthesis (Pn), stomatal conductance (Gs), transpiration rate (Tr), and intercellular CO_2 concentration (Ci) were determined from these measurements. While the data were being recorded, the air temperature, relative humidity, ambient CO_2 concentration, and photosynthetic photon flux density (PPFD) were maintained at 25°C, 80 to 90%, 360 $\mu mol \cdot mol^{-1}$, and 1000 $\mu mol\ m^{-2} \cdot s^{-1}$, respectively.

Assessment of chlorophyll fluorescence parameters: Chlorophyll fluorescence parameters were examined with a Li-6400 Photosynthesis System (Li-Cor) in a fluorescence chamber, using the same leaves as those for gas exchange parameters. The initial chlorophyll fluorescence (F_0), maximum chlorophyll fluorescence (Fm), steady-state fluorescence level (Fs), light-adapted maximum fluorescence (Fm′), and light-adapted minimum fluorescence (F_0') were determined. From these data, we calculated the following values: for maximum quantum efficiency of PSII, Fv/Fm = (Fm – F_0) / Fm; efficiency of open PSII centers, Φ_{PSII} = (Fm′ – F_s) / Fm′; quantum yield of open PSII centers under irradiation, Fv′/Fm′= (Fm′ – F_0') / Fm′; and photochemical quenching, qP = (Fm′ – Fs) / (Fm′ – F_0').

Preparation of extracts of total proteins: 0.2 g tomato seedling leaves were weighed and homogenized in extraction buffer containing 20 mM Tris-HCl (pH 7.0) , 5 mM EDTA-Na_2 and PMSF on the ice bath (Dai *et al.*, 2009). Then the homogenate was centrifuged at 10000 rpm for 20 min at 4°C and the supernatant was protein crude extract. It was added the sample buffer containing 1.0 mM Tris-HCl (pH 6.8), 10% glycerinum, 2% SDS, 0.5% β-mercaptoethanol and 0.1% bromophenol blue, then was in boiling water bath for 5 minutes (Masouleh, 2005).

Measurement of protein contents and SDS-PAGE: Protein content measurement used Commasie brilliant blue staining method (Gao, 2006). 0.1 mL protein crude extract was added 0.9 mL distilled water and 0.5 mL coomassie brilliant blue G-250, and was placed for 2 min after fully mixing. Then absorption value was measured at 595 nm using spectrophotometer (Shimadzu, UV 1800, Japan). Equal amounts of protein were subjected to SDS-PAGE, using 15% resolving gels and 4% stacking gels (Laemmli, 1970).

Mass spectrometry analysis and prediction of binding sites and active sites of the target protein: The target proteins were cut down and their amino acid fragments were gained using matrix assisted laser desorption ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) (Lei *et al*., 2007). Through alignment with NCBI database and Mascot program, amino acid sequence of the target protein was determined. Amino acid sequence was used to predict the binding sites and active sites of the target protein using I-TASSER server and COFACTOR server (Roy *et al.*, 2010, 2012).

Statistical analysis: All physiological parameters were measured twice, with three replicates. Statistical analysis was performed with Microsoft Excel 2013 and SPSS16.0.

Results

Effects of EBR on gas-exchange parameters: As the stress period became prolonged, values for Pn, Gs, and Tr were gradually reduced in the leaves from both tomato cultivars, whereas Ci increased (Fig. 1). However, when compared with the –EBR controls, plants of both cultivars pre-treated with 0.1 μM EBR prior to stress imposition showed significant increases in Pn, Gs, and Tr, and a significant decrease in Ci. For example, on Day 12, net rates of photosynthesis were 20.52% and 16.38% more rapid in +EBR ‘Zhongza9’ and ‘Zhongshu4’, respectively, than in –EBR plants (Fig. 1a). Stomatal conductance values rose by 0.72% and 0.45% in +EBR ‘Zhongza 9’ and ‘Zhongshu4’, respectively, after plants were exposed to the combined low temperature/poor light stress (Fig. 1b). Transpiration rates were 4.85% and 2.64% higher in +EBR ‘Zhongza 9’ and ‘Zhongshu 4’, respectively, when compared with the –EBR plants (Fig. 1c). Finally, the intercellular CO_2 concentrations were 19.32% and 11.94 % lower in + EBR ‘Zhongza 9’ and ‘Zhongshu 4’, respectively, after stresses were introduced (Fig. 1d).

Effects of EBR on chlorophyll fluorescence parameters: Values for Fv/Fm, Fv′/Fm′, qP, and Φ_{PSII} tended to decrease in tomato seedling leaves when plants were grown under our combined stress conditions (Fig. 2). Over time, the maximum photochemical efficiency of PSII continuously declined (Fig. 2a). Nevertheless, on Days 3, 6, 9, and 12, Fv/Fm was 5.85%, 7.96%, 5.84%, and 15.32% higher, respectively, in + EBR ‘Zhongza9’ than in the untreated controls of that cultivar, and was 7.86%, 3.38%, 7.45%, and 10.95% higher, respectively, in +EBR ‘Zhongshu 4’ than in the –EBR counterpart.

In stressed –EBR plants, photochemical quenching decreased over time (Fig. 2b). However, for +EBR plants, qP values were 7.78%, 5.32%, 9.48%, and 15.20% higher for ‘Zhongza 9’ and 6.43%, 10.52%, 15.43%, and 12.92% higher for ‘Zhongshu4’ after Days 3, 6, 9, and 12, respectively, of induced stresses.

In contrast to the steady decline calculated for –EBR plants, values for Fv′/Fm′ were higher in leaves that had been pre-treated prior to the stress period (Fig. 2c). For example, readings were 3.81%, 4.00%, 2.34%, and 8.23% greater for + EBR ‘Zhongza 9’ and 4.30%, 6.73%, 1.74%, and 6.15% greater for +EBR ‘Zhongshu 4’ on Days 3, 6, 9, and 12, respectively.

The actual photochemical efficiency of PSII in the control plants was decreased under combined stress conditions (Fig. 2d). By contrast, Φ_{PSII} values were increased by 5.26%, 3.24%, 6.21%, and 13.11% in +EBR ‘Zhongza 9’, and by 4.72%, 5.58%, 9.53%, and 10.82% in +EBR ‘Zhongshu4’ on Days 3, 6, 9, and 12, respectively, of imposed stresses.

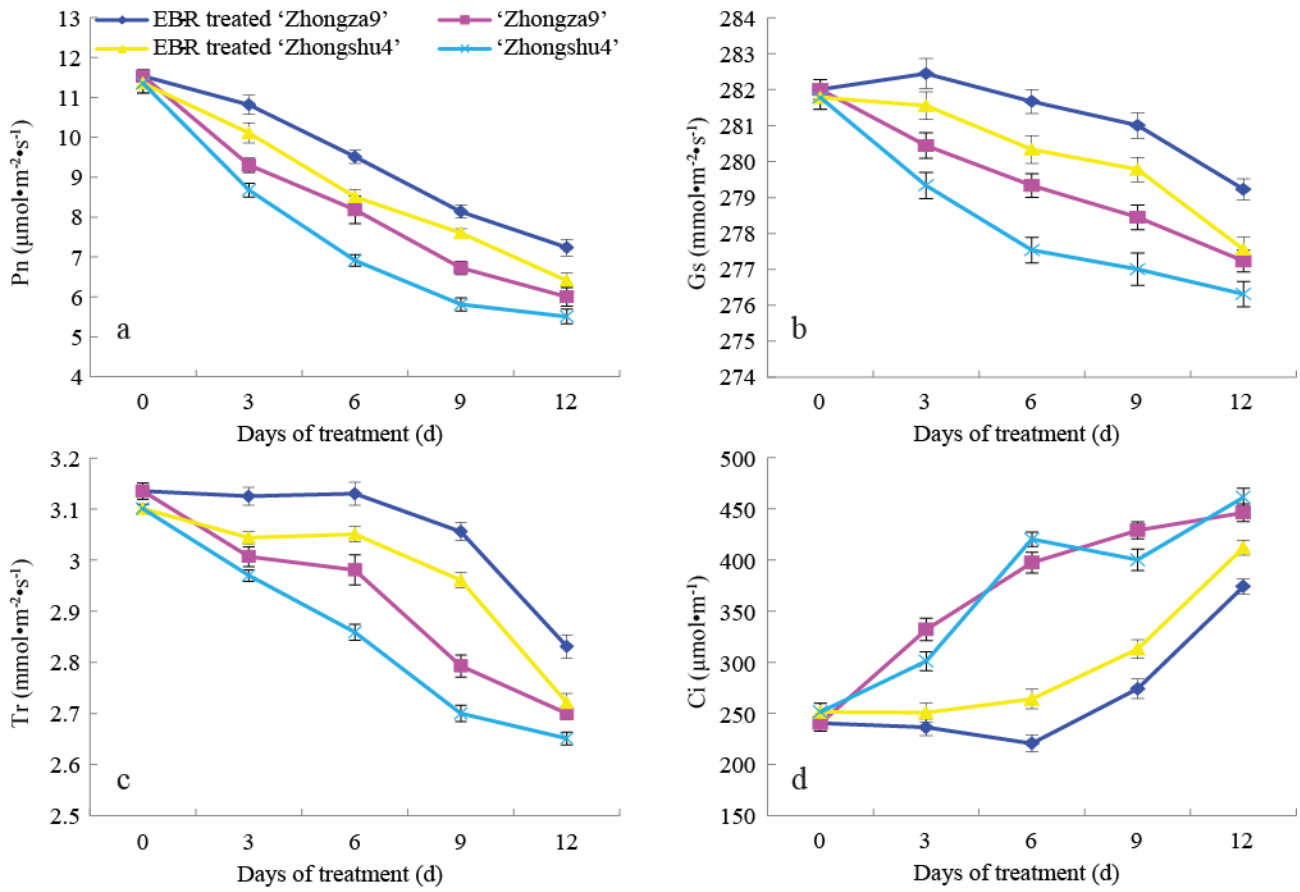

Fig. 1. Effect of EBR on photosynthesis parameters in tomato seedling leaves under low temperature and poor light.

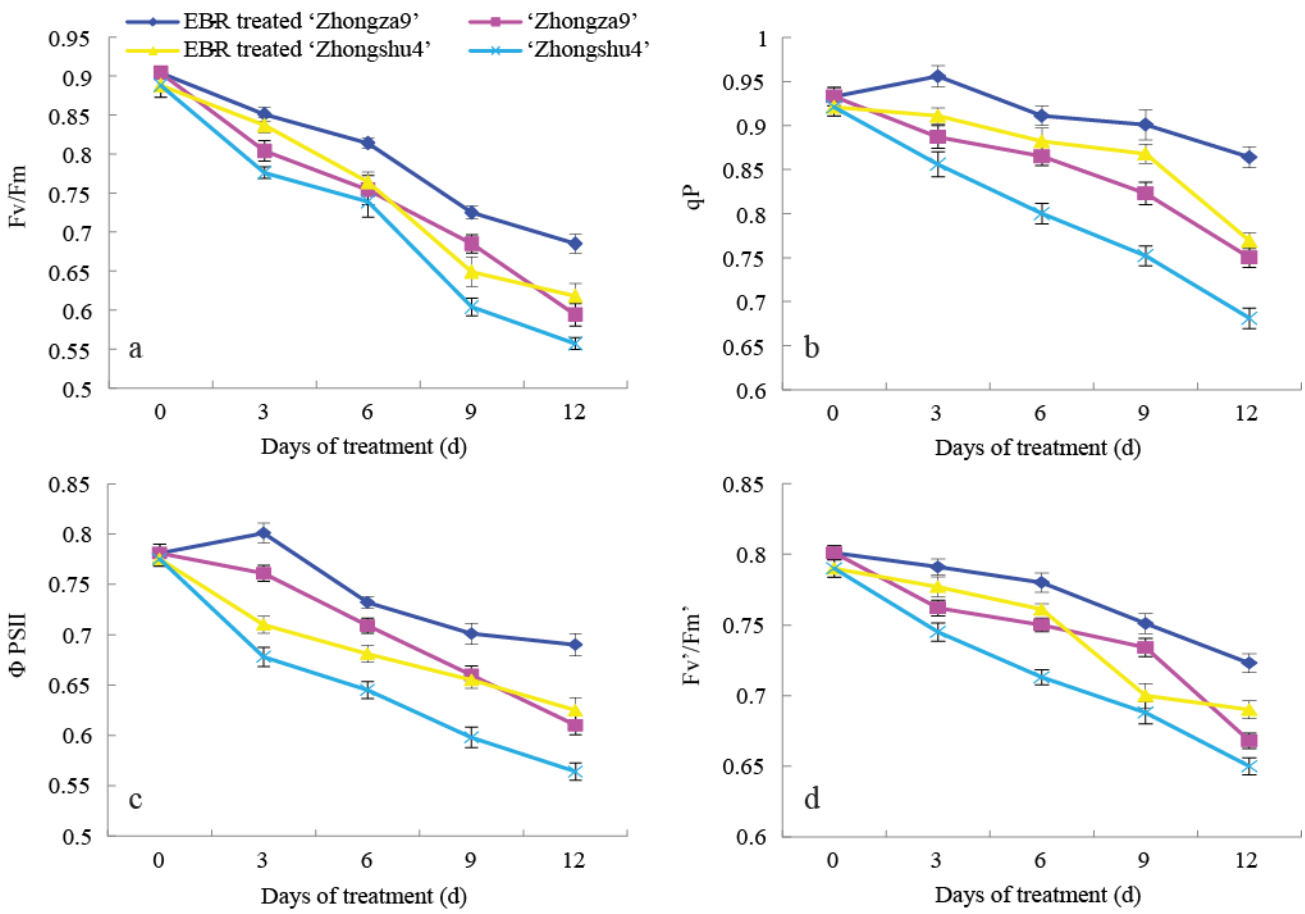

Fig. 2. Effect of EBR on chlorophyll fluorescence in tomato seedling leaves under low temperature and poor light.

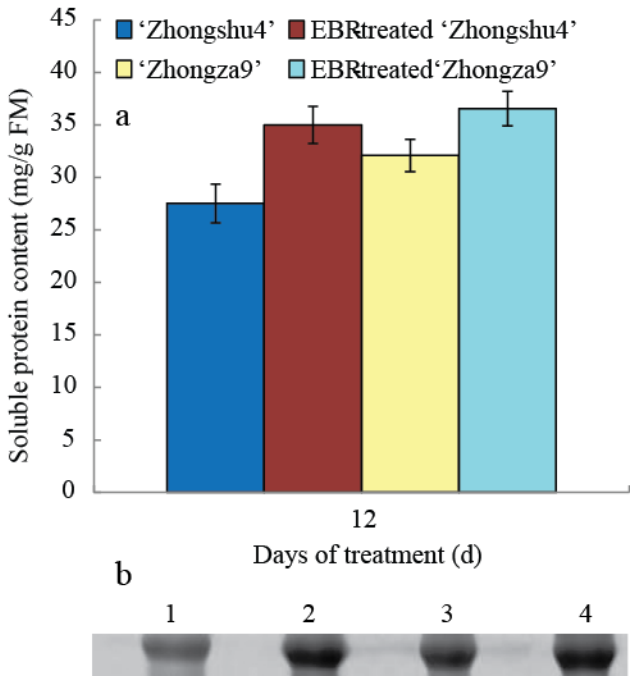

Fig. 3. Soluble protein content (a) and SDS-PAGE gel electrophoresis (b) on Day 12 of low temperature and poor light. Lane 1: 'Zhongshu4'; Lane 2: EBR-treated 'Zhongshu 4'; Line 3: 'Zhongza 9'; Line 4: EBR-treated 'Zhongza 9'.

Fig. 4. Three-dimensional model of ATP synthase β subunit with predicted binding sites. Those interacting with ligands are shown as red spheres.

Measurement of soluble protein content and SDS-PAGE: As shown in Figure 3a, the content of the soluble protein was higher in 'Zhongza 9' than 'Zhongshu 4'. While the soluble protein content of tomato seedling leaf with EBR was higher than that without EBR under low temperature and poor light stresses.

As shown in Figure 3b, a band was different in tomato with EBR and without EBR in two tomato varieties under low temperature and poor light stresses. Similar results can be seen from SDS-PAGE electrophoretogram, the content of the target protein was higher in 'Zhongza 9' than 'Zhongshu 4', Meanwhile, the value of the target protein content was higher in tomato with EBR than that without EBR.

Mass spectrometry analysis and prediction of binding sites and active sites of the target protein: Through MALDI-TOF/TOF MS method, we finally determined that this protein was ATP synthase β subunit (Cui *et al*., 2016). Predicted binding sites using the amino acid sequence showed that the ATP synthase β subunit and pdb 3fks (Kabaleeswaran *et al*., 2009) had the highest degree of similarity. The seven binding site residues were A_{174}, G_{175}, V_{176}, G_{177}, K_{178}, T_{179}, and R_{205} (Fig. 4). In addition, the results for predicted active sites indicated that our subunit was most similar to pdb 1kmh (Groth 2002), with seven active site residues at T_{304}, G_{307}, L_{309}, E_{311}, A_{340}, A_{344}, and L_{346} (Fig. 5).

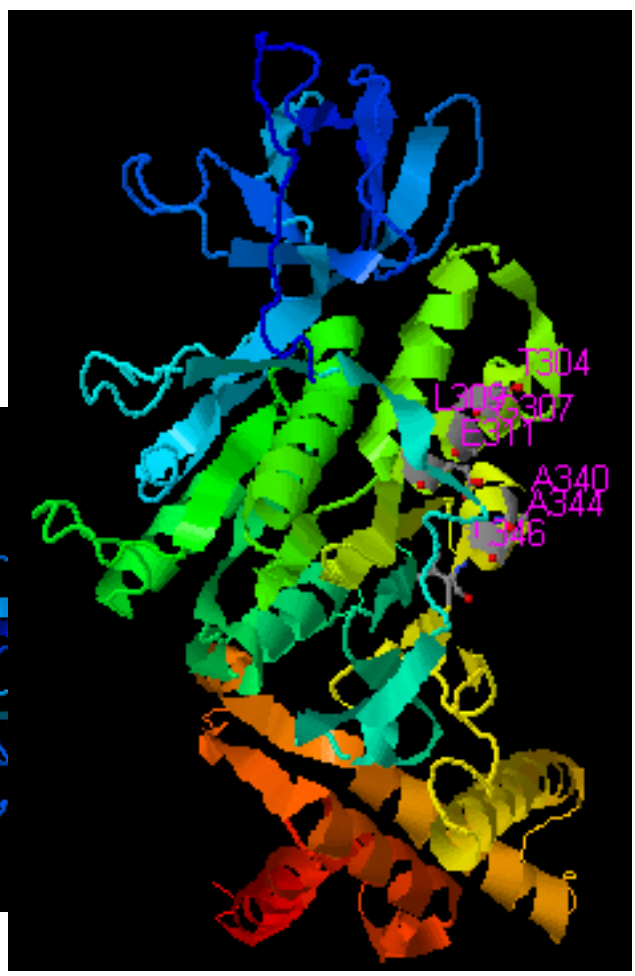

Fig. 5. Three-dimensional model of ATP synthase β subunit, with predicted active sites shown as red points.

Discussion

Photosynthesis, mainly affected by temperature and light, is one of the main determinants of plant biomass production. In our experiments, values for Pn, Gs, and Tr were significantly lower with extension of stress time in two tomato varieties. By contrast, Ci was elevated. The decrease of net photosynthesis was because of reduction in stomatal conductance limiting the transfer of CO_2 to the chloroplasts and causing CO_2 concentrations to decline in the leaves. So, these demonstrated that the limitation to Pn was non-stomatal.

Parameters of chlorophyll fluorescence are used to describe the physiological mechanism for photosynthesis, thereby reflecting the "internality features" of a plant and its relationship to the environment. In our experiments, values for Fv/Fm, Fv′/Fm′, Φ_{PSII}, and qP were reduced in two tomato varieties, indicating that low temperature and poor light could inhibit the photochemical activity of PSII. This would include the original light energy conversion efficiency of PSII and the potential photosynthetic activity of PSII in tomato seedling leaves.

In our study, exogenous EBR induced higher net photosynthetic rate and ATP synthase β subunit content in the tolerant variety 'Zhongza 9' than the sensitive variety 'Zhongshu4'. These results showed that exogenous EBR pretreatment potentially gave 'Zhongza 9' stronger protection against low temperature and poor light stresses.

EBR pretreatment enhanced the content of ATP synthase β subunit, while β subunit was the part of F_1-complex, which was a catalytic part of ATP synthase. EBR could activate the binding sites (A_{174}, G_{175}, V_{176}, G_{177}, K_{178}, T_{179}, R_{205}) and active sites (T_{304}, G_{307}, L_{309}, E_{311}, A_{340}, A_{344}, L_{346}), and made ATP synthase β subunit combinate with more ADP, finally, more ATP was catalyzed synthesis. While large amounts of energy were used for photosynthesis of tomato. So, photosynthetic capacity of tomato seedling enhanced, and growth and development accelerated, and finally the vegetable production enhanced under low temperature and poor light stresses. Our results are consistent with those reported by Hayat *et al*. (2011) and Jiang *et al*. (2013). But EBR how changed the binding sites and active sites residues and changed which one were still not known. So these were our important questions of next research.

Conclusion

Low temperature and poor light stresses made values for Pn, Gs, and Tr decrease, but Ci increase in two tomato varieties. Similarly, Fv/Fm, Fv′/Fm′, Φ_{PSII} and qP decreased. Meanwhile, Exogenous EBR induced higher net photosynthetic rate and ATP synthase β subunit content in the tolerant variety 'Zhongza9' than the sensitive variety 'Zhongshu4'. So, EBR pretreatment potentially gave 'Zhongza9' stronger protection ability against low temperature and poor light stresses.

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