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ORIGINAL ARTICLE

24-Epibrassinoslide enhances plant tolerance to stress from low temperatures and poor light intensities in tomato (*Lycopersicon esculentum* Mill.)

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Abstract Brassinosteroids (Brs) are a newly recognized group of active steroidal hormones that occur at low concentrations in all plant parts and one of the active and stable forms is 24-epibrassinolide (EBR). We investigated the effect of EBR on tomato (*Lycopersicon esculentum* Mill.) and its mechanism when seedlings were exposed to low temperature and poor light stress conditions. Leaves of stress-tolerant 'Zhongza9' and stress-sensitive 'Zhongshu4' cultivars were pre-treated with spray solutions containing either 0.1 μM EBR or no EBR (control). The plants were then transferred to chambers where they were exposed to low temperatures of 12 °C/6 °C (day/night) under a low light (LL) level of 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Exogenous application of EBR significantly increased the antioxidant activity of superoxide dismutase, catalase and peroxidase, and decreased the rate of $O_2 \cdot^-$ formation and H_2O_2 and malondialdehyde contents. Additionally, the ATP synthase β subunit content was increased by exogenous hormone application. Based on these results, we conclude that exogenous EBR can elicit synergism between the antioxidant enzyme systems and the ATP synthase β subunit so that scavenging of reactive oxygen species becomes more efficient. These activities enable plants to cope better under combined low temperature and poor light stresses.

Keywords Antioxidant enzymes · ATP synthase β subunit · 24-Epibrassinolide · Mass spectrometry · Reactive oxygen species · Tomato

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Abbreviations

BRs	Brassinosteroids
CAT	Catalase
EBR	24-Epibrassinolide
H_2O_2	Hydrogen peroxide
MALDI-TOF/TOF MS	Matrix-assisted laser desorption ionization time-of-flight/time-of-flight mass spectrometry
MDA	Malondialdehyde
MS/MS	Tandem mass spectrometry
$O_2 \cdot^-$	Superoxide radical
POD	Peroxidase
ROS	Reactive oxygen species
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SOD	Superoxide dismutase

Introduction

Brassinosteroids (BRs), first discovered in pollen from rapeseed (*Brassica napus*) (Grove et al. 1979), are a newly recognized group of highly active steroidal hormones that occur at low concentrations in all plant parts. Because their physiological activity is significantly higher than that of the five other types of hormones, BR was confirmed as the sixth plant hormone during the 16th International Conference on Plant Growth Substances (Abbas et al. 2013; Fujii and Saka 2001). As naturally occurring substances that promote growth (Vardhini et al. 2011), BRs regulate various aspects of plant development, including cell expansion and division, xylem differentiation, proton activity, and photosynthesis (Vardhini 2012). They are also possibly involved in protecting plants against various environmental stresses, e.g., drought (Bajguz and Hayat 2009), high temperature (Kumar et al. 2012), chilling (Singh et al. 2012), salinity (Hayat et al.

2010), and heavy metals (Arora et al. 2010). One of the most active and stable forms of this phytohormone is 24-epibrassinolide (EBR).

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetables. Most cultivars are thermophilic and perform well under high light levels. Tomato plants are highly sensitive to low temperature and poor light at all stages of development, but especially during early seedling growth (Wang et al. 2010). These two stresses are the most significant factors restricting plant growth and crop productivity in Winter and Spring, when they can cause numerous metabolic changes by suppressing photosynthesis and respiration and inducing oxidative stress.

Under low temperature and poor light stress condition, crop plants with stronger resistance can improve the ability to resist stress by increasing the expression of specific genes and proteins. For examples, defensin and cold dehydrin genes were overexpressed in *Oxytropis* species and that enhanced the tolerance to low temperature (Archambault and Strömvik 2011). The increasing expressions of anthocyanidin synthase genes gave *Brassica rapa* strong tolerance to low temperature (Ahmed et al. 2015). Up-regulation of genes encoding chitinases and glucanases proteins enhanced the winter barley tolerance to low temperature (Janská et al. 2011). While, our experiment showed up-regulation of ATP synthase β subunit protein gave tomato a high capacity to cope with low temperature and poor light stresses.

In an effort to devise new strategies for protection against such stresses, we conducted experiments in which the activities of antioxidant enzymes, the rate of $O_2\cdot^-$ formation and H_2O_2 and malondialdehyde contents were measured. Our objective was to determine whether exogenous EBR could improve the tolerance of tomato plants to low temperatures and poor light intensities. We also investigated the mechanism by means of which these changes might occur through analysis of the ATP synthase β subunit.

Materials and methods

Plant materials and treatments

Two tomato genotypes were used: 'Zhongza9,' which is tolerant of low temperature and poor light, and 'Zhongshu4,' which is sensitive. Seeds were placed on moistened filter paper for 7 days under darkness at 19 °C. The germinants were then sown in plastic pots containing 1:1 (*v*:*v*) peat moss and vermiculite. The seedlings were exposed to a 12-h photoperiod (light intensity of 350 $\mu mol\cdot m^{-2}\cdot s^{-1}$) with a day/night temperature regime of 25 °C/18 °C in a greenhouse at the College of Horticulture, Northwest A&F University, Yangling (34° 20′ N, 108° 24 E′), Shaanxi Province, China.

A 10-mM EBR stock solution was prepared by dissolving 0.048 g EBR in 10 mL absolute ethanol. A 0.1 μM EBR solution (containing 0.1 % Teepol) was prepared just before use, and 10 mL was sprayed on each plant (Singh et al. 2012). Uniformly sized seedlings were selected and when they had produced five true leaves, their foliage was pre-treated once a day for 7 days with spray solutions containing either no EBR (control) or 0.1 μM EBR (n=60 plants per treatment). The seedlings in each treatment group (−EBR or +EBR) were then transferred to environmental chambers and exposed to combined-stress conditions of 12 °C/6 °C (day/night) and a low light intensity of 80 $\mu mol\cdot m^{-2}\cdot s^{-1}$. This resulted in two treatment groups for each genotype: −EBR/stress and +EBR/stress. For each parameter, three tomato seedlings were randomly selected and sampled at a time. The plants were sampled after 0, 3, 6, 9, and 12 days of stress treatment, when they were immediately frozen in liquid N_2 and stored at −80 °C.

Measurements of antioxidant enzyme activities

Leaf samples (0.2 g) from each treatment group were homogenized on ice in 5 mL of 50 mM sodium phosphate buffer (pH 7.8) containing 1 mM EDTA and 2 % (*W*/*V*) PVP. The homogenate was centrifuged at 12,000×*g* for 30 min at 4 °C. The supernatant was used for monitoring enzyme activities (Wu et al. 2014a).

Superoxide dismutase (SOD) activity was assayed according to the method of Vardhini et al. (2011), which is based on the measurement of inhibition in the photochemical reduction of nitro blue tetrazolium spectrophotometrically at 560 nm. Peroxidase (POD) activity was determined at 436 nm based on its ability to convert guaiacol to tetraguaiacol, per the method of Abedi and Pakniyat (2010). Catalase (CAT) activity was studied by monitoring the disappearance of hydrogen peroxide (H_2O_2) at 240 nm, according to the method of Farhad et al. (2011).

Measurements of $O_2\cdot^-$ formation rate and H_2O_2 and malondialdehyde contents

For $O_2\cdot^-$ formation rate and H_2O_2 content measurements, leaf samples (0.2 g) from each treatment group were homogenized on ice in 5 mL of 50 mM potassium phosphate buffer (pH 7.8). The homogenate was centrifuged at 12,000×*g* for 10 min. Next, 0.5 mL 50 mM potassium phosphate buffer (pH 7.8) and 1 mL 1 mM hydroxylamine hydrochloride were added to 0.5 mL supernatant. After incubation at 25 °C for 20 min, an equal volume of ethyl ether was added and the sample was centrifuged at 12,000×*g* for 20 min (Hu et al. 2010).

The rate of $O_2\cdot^-$ formation and H_2O_2 content were assayed according to the method of Zhou et al. (2004). The rate of $O_2\cdot^-$ formation is based on the measurement of nitrite formation from hydroxylamine in the presence of $O_2\cdot^-$ and the

absorbance was read at 530 nm. The H_2O_2 content was measured at 410 nm of the titanium-peroxidase complex.

For malondialdehyde (MDA) measurements, leaf samples (0.2 g) from each treatment group were homogenized on ice in 5 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM EDTA and 2 % (*W*/*V*) PVP. The homogenate was centrifuged at 12,000×*g* for 10 min. The supernatant was used to monitor the MDA content. MDA contents were determined by performing thiobarbituric acid (TBA) reactions, as described by Hu et al. (2010). Measurements were corrected for nonspecific absorbance by subtracting the values obtained at 440, 532, and 600 nm.

SDS-PAGE and target protein content measurement

Protein extracts were prepared from the leaves of seedlings exposed to low temperature and poor light for 1, 6, and 12 days. The samples were ground to powder in liquid nitrogen and melted in ice-cold extraction buffer [50 mM Tris-HCl (pH 8.0), 10 mM NaCl, 1 % sodium dodecyl sulfate (SDS), 5 % 2-mercaptoethanol, 0.1 mM PMSF, and 0.1 mM DTT]. This was followed by centrifugation at 10,000×*g* for 15 min at 4 °C.

Equal amounts of concentrated protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using 15 % resolving gels and 4 % stacking gels. After 3 h of electrophoresis at 100 V/45 mA, the gels were stained with Coomassie Brilliant Blue R-250 (Khalifa 2012).

The grayscale percentages of the target protein in the crude protein were analyzed with the BrandScan software. Crude proteins were extracted from leaf samples and their contents (per gram) were calculated. Target protein contents were calculated using the formula: target protein content (mg/g)= crude protein content (mg/g)×grayscale percentage.

Mass spectrometry analysis

Target protein bands were excised from the gels, washed with a 100 mM NH_4HCO_3/30 % ACN solution, and dried in a vacuum concentrator. The proteins were digested overnight at 37 °C with 5 μL of 10 ng·μL^{-1} sequencing-grade modified trypsin (Promega, USA). Afterward, 100 μL of 60 % (*v*/*v*) ACN in 0.1 % aqueous trifluoroacetic acid (TFA) was added to the gel pieces, which were then sonicated for 15 min. The digested peptides were dissolved using 2 μL of 50 % acetonitrile/0.1 % TFA, and 0.5 μL aliquots were applied to the target disk. After air-drying, 0.5 μL of matrix solution [cyano-4-hydroxy cinnamic acid (CHCA) saturated in 50 % acetonitrile/0.1 % TFA] was added to the dried samples and again allowed to dry. The samples were then subjected to matrix-assisted laser desorption ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) tandem mass spectrometry (MS/MS) analysis using a 4800 Plus MALDI TOF/TOF™ Analyzer instrument (Applied Biosystems, USA) with an Nd:YAG smart laser beam (335 nm wavelength). The acceleration voltage in the ion source was operated at 20 kV and a minimum signal-to-noise ratio of 50 was selected for MS/MS. The laser power was set to 4600 for MS and to 5200 for MS/MS with the CID off. The MS spectra were acquired across a mass range of 800 to 4000 Da (Kang et al. 2012).

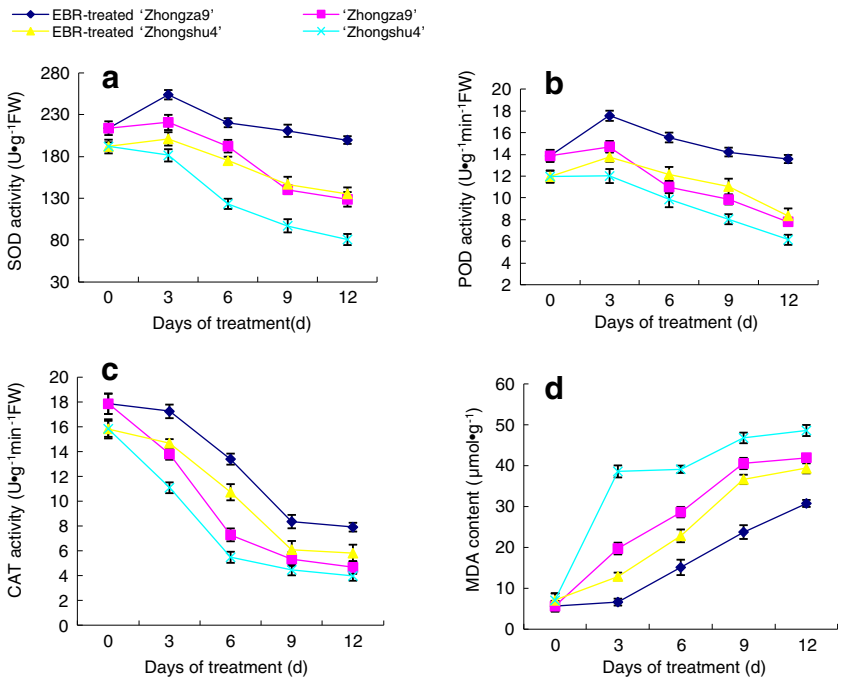

Fig. 1 Effects of EBR on activity by antioxidant systems and MDA content in tomato seedlings under combined low temperature/poor light stress. Mean values and standard errors (*bars*) were obtained from three independent experiments per time point

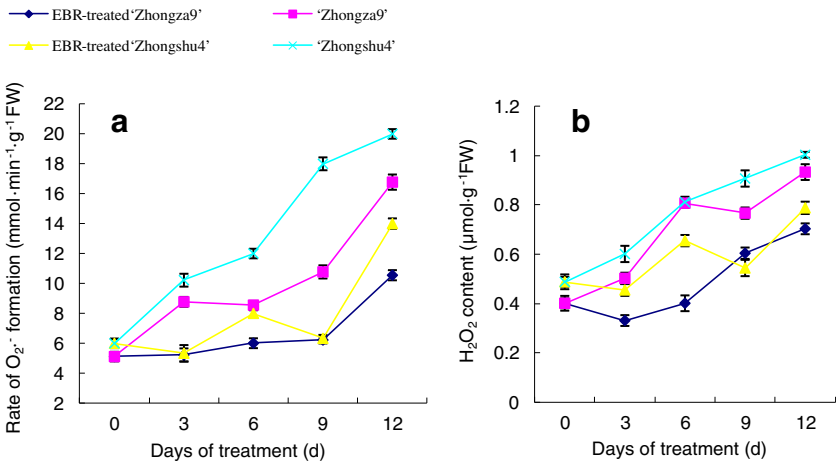

Fig. 2 Effects of EBR on rate of $O_2^{\cdot-}$ formation and H_2O_2 content in tomato seedling leaves under combined low temperature/poor light stress. Mean values and standard errors (*bars*) were obtained from three independent experiments per time point

The proteins were identified by searching the NCBI non-redundant database, using the MASCOT program (http://www.matrixscience.com, Matrix Science, UK). Search parameters included *L. esculentum*, set as taxonomy; trypsin cleavage, one missed cleavage allowed; carboxymethyl (C), set as a fixed modification; oxidation (Met) of methionines allowed as variable modification; peptide mass tolerance, within 100 ppm; and fragment mass tolerance of ±0.4 Da (Hu et al. 2012). According to the MASCOT probability analysis ($P<0.05$), only significant hits were accepted for protein identification (Wen et al. 2013).

Statistical analysis

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

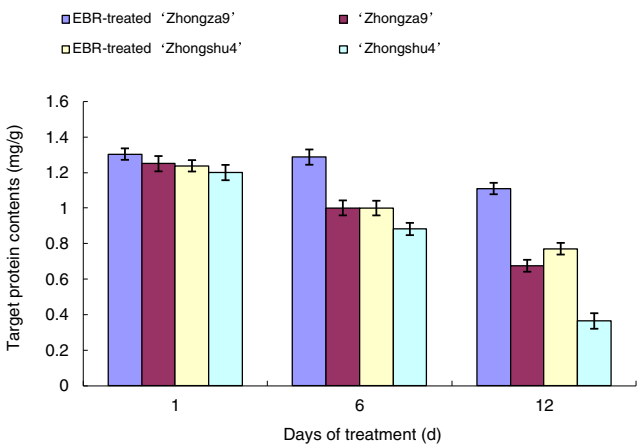

Fig. 3 Effects of EBR on target protein contents in tomato seedlings under combined low temperature/poor light stress

Results

Measurements of antioxidant enzyme activity

SOD activity followed similar trends in both cultivars (Fig. 1a), peaking on Day 3 before decreasing in the leaves of seedlings exposed to the combined low temperature and poor light stresses. However, SOD activity was stronger in the tolerant 'Zhongza9' than in the sensitive 'Zhongshu4.' Furthermore, SOD activity was higher in the +EBR seedlings. The subsequent decline in SOD activity was slower, and it remained at a relatively higher level, in the pre-treated seedlings. By Day 12, the SOD activities were 55.2 and 50.9 % greater in pre-treated 'Zhongza9' and 'Zhongshu4,' respectively, than in plants not exposed to EBR.

POD activity showed the same trend as SOD. By Day 12, POD activity had increased significantly by 73.9 and 35.9 % in +EBR 'Zhongza9' and +EBR 'Zhongshu4,' respectively, compared with the untreated samples (Fig. 1b).

CAT activity decreased over time for both +EBR genotypes, although the decline was more rapid in the sensitive cultivar. Compared with the non-pretreated seedlings, CAT

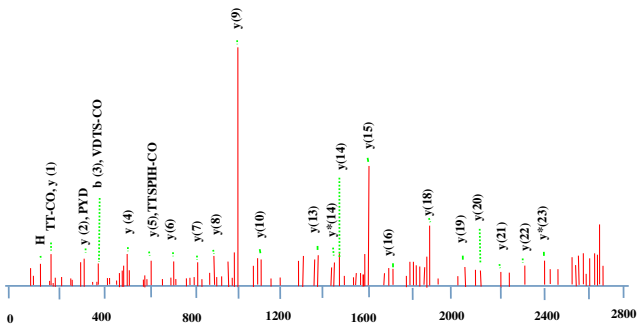

Fig. 4 Diagram of fragment ions MS/MS analysis. Each *mass spectra peak* was matched with corresponding ions

#	Immon.	a	a*	a⁰	b	b*	b⁰	d	d'	Seq.	v	w	w'	y	y*	y⁰	#
1	86.0964	86.0964			114.0913			44.0495		I							25
2	120.0808	233.1648			261.1598					F	2473.2318			2565.2944	2548.2678	2547.2838	24
3	87.0553	347.2078	330.1812		375.2027	358.1761		304.2020		N	2359.1888	2358.1936		2418.2259	2401.1994	2400.2154	23
4	72.0808	446.2762	429.2496		474.2711	457.2445		432.2605		V	2260.1204	2273.1408		***2304.1830***	2287.1565	2286.1725	22
5	86.0964	559.3602	542.3337		587.3552	570.3286		517.3133		L	2147.0364	2146.0411		***2205.1146***	2188.0881	2187.1040	21
6	30.0338	616.3817	599.3552		644.3766	627.3501				G				***2092.0305***	2075.0040	2074.0200	20
7	102.0550	745.4243	728.3978	727.4137	773.4192	756.3927	755.4087	687.4188		E	1960.9723	1959.9770		***2035.0091***	2017.9825	2016.9985	19
8	70.0651	842.4771	825.4505	824.4665	870.4720	853.4454	852.4614	816.4614		P	1863.9195	1862.9243		***1905.9665***	1888.9399	1887.9559	18
9	72.0808	941.5455	924.5189	923.5349	969.5404	952.5138	951.5298	927.5298		V	1764.8511	1777.8715		1808.9137	1791.8872	1790.9032	17
10	88.0393	1056.5724	1039.5459	1038.5619	1084.5673	1067.5408	1066.5568	1012.5826		D	1649.8242	1648.8289		***1709.8453***	1692.8188	1691.8347	16
11	87.0553	1170.6153	1153.5888	1152.6048	1198.6103	1181.5837	1180.5997	1127.6095		N	1535.7812	1534.7860		***1594.8184***	1577.7918	1576.8078	15
12	86.0964	1283.6994	1266.6729	1265.6888	1311.6943	1294.6678	1293.6838	1241.6525		L	1422.6972	1421.7019		***1480.7754***	1463.7489	1462.7649	14
13	30.0338	1340.7209	1323.6943	1322.7103	1368.7158	1351.6892	1350.7052			G				***1367.6914***	1350.6648	1349.6808	13
14	70.0651	1437.7736	1420.7471	1419.7631	1465.7686	1448.7420	1447.7580	1411.7580		P	1268.6230	1267.6277		1310.6699	1293.6434	1292.6593	12
15	72.0808	1536.8421	1519.8155	1518.8315	1564.8370	1547.8104	1546.8264	1522.8264		V	1169.5545	1182.5749		1213.6171	1196.5906	1195.6066	11
16	88.0393	1651.8690	1634.8424	1633.8584	1679.8639	1662.8374	1661.8533	1607.8792		D	1054.5276	1053.5324		***1114.5487***	1097.5222	1096.5382	10
17	74.0600	1752.9167	1735.8901	1734.9061	1780.9116	1763.8850	1762.9010	1736.9218	1738.9010	T	953.4799	966.5003	968.4796	***999.5218***	982.4952	981.5112	9
18	60.0444	1839.9487	1822.9222	1821.9381	1867.9436	1850.9171	1849.9331	1823.9538		S	866.4479	865.4526		***898.4741***	881.4476	880.4635	8
19	74.0600	1940.9964	1923.9698	1922.9858	1968.9913	1951.9648	1950.9807	1925.0015	1926.9807	T	765.4002	778.4206	780.3999	***811.4421***	794.4155	793.4315	7
20	74.0600	2042.0441	2025.0175	2024.0335	2070.0390	2053.0124	2052.0284	2026.0491	2028.0284	T	664.3525	677.3729	679.3522	***710.3944***	693.3679	692.3838	6
21	60.0444	2129.0761	2112.0495	2111.0655	2157.0710	2140.0445	2139.0604	2113.0812		S	577.3205	576.3253		***609.3467***	592.3202	591.3362	5
22	70.0651	2226.1289	2209.1023	2208.1183	2254.1238	2237.0972	2236.1132	2200.1132		P	480.2677	479.2725		***522.3147***	505.2881		4
23	86.0964	2339.2129	2322.1864	2321.2024	2367.2078	2350.1813	2349.1973	2311.1816	2325.1973	I	367.1837	380.2041	394.2197	425.2619	408.2354		3
24	110.0713	2476.2718	2459.2453	2458.2613	2504.2667	2487.2402	2486.2562			H	230.1248			***312.1779***	295.1513		2
25	129.1135									R	74.0237	73.0284		***175.1190***	158.0924		1

Fig. 5 Ion and amino acid alignments. Deduced peptide partial sequences were IFNVLGEPVDNLGPVDTSTTSPIHR when ions were aligned with amino acids

activity on Day 12 was 69.4 % higher in +EBR 'Zhongza9' and 46.1 % higher in +EBR 'Zhongshu4' (Fig. 1c).

Measurements of $O_2 \cdot^-$ formation rate and H_2O_2 and malondialdehyde contents

When the seedlings were exposed to low temperature and poor light, the MDA contents significantly increased (Fig. 1d). This rise was more dramatic in the sensitive genotype. However, when compared with plants that did not receive hormone pretreatment, the MDA levels on Day 12 were 36.4 and 23.5 % lower for +EBR 'Zhongza9' and +EBR 'Zhongshu4,' respectively.

Similarly, low temperature and poor light stresses increased the rate of $O_2 \cdot^-$ formation and H_2O_2 content in tomato seedling leaves, while EBR pretreatment significantly alleviated this increase (Fig. 2). The $O_2 \cdot^-$ formation rates on Day 12 were 59.0 and 42.8 % lower for +EBR 'Zhongza9' and +EBR 'Zhongshu4,' respectively. The H_2O_2 contents on Day 12 were 32.7 and 27.3 % lower for +EBR 'Zhongza9' and +EBR 'Zhongshu4,' respectively.

SDS-PAGE and target protein content measurement

After 1, 6, and 12 days of induced stress, total leaf proteins were extracted and separated by SDS-PAGE. We noted that one protein band expression was different in the two treatment groups for each genotype. Using the BandScan software, we calculated the content of this target protein. As shown in Fig. 3, with prolonged stress, the expression of the protein gradually decreased, albeit more slowly in leaves from the tolerant genotype 'Zhongza9.' Furthermore, under stress conditions, the protein levels were much higher in +EBR seedlings than in those that had not been pretreated.

Mass spectrometry analysis

We selected this band for analysis via MALDI-TOF/TOF MS. The protein was identified following a search of the NCBI non-redundant database. Most of the stronger mass spectra peaks were matched with y_1, y_2, $y_{4–10}$, $y_{13–16}$, $y_{18–22}$, y^*_{14}, y^*_{23}, b_3, and immonium ions (Fig. 4). Therefore, we deduced that the partial peptide sequence was IFNVLGEPVDNLGPVDTSTTSPIHR by aligning the ions with amino acids (Fig. 5). Through a BLAST alignment of this peptide, we confirmed that the protein was the ATP synthase β subunit of *L. esculentum* Mill (gi/6688527) with 17 % protein sequence coverage (Fig. 6).

Discussion

Low temperature and poor light stresses not only affect plant growth and development, but also lead to oxidative damage and even death of plant tissues (Ogweno et al. 2009). This is mainly caused by the excessive accumulation of reactive oxygen species (ROS) from plant membrane damage. These ROS can also react with nucleic acids, proteins and lipids, destroying cellular structure and function, and even causing cell death (Wu et al. 2014b). $O_2 \cdot^-$ and H_2O_2 are the main ROS and can be efficiently scavenged in vivo by three key antioxidant enzymes—SOD, CAT, and POD (Xi et al. 2013).

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  1  XXXXPTTSGS GVSTLEKKNP GRVVQIIGPV LDVAFPPGKM PNIYNALVVQ
 51  GRDSVGQPIN VACEVQQLLG NNRVRAVAMS ATEGLTRGMA VIDTGAPISV
101  PVGGATLGRI FNVLGEPVDN LGPVDTSTTS PIHRSAPAFI QLDTKLSIFE
151  TGIKVVDLLA PYRRGGKIGL FGGAGVGKTV LIMELINNIA KAHGGVSVFG
201  GVGERTREGN DLYMEMKESG VINKENIAES KVALVYGQMN EPPGARMRVG
251  LTALTMAEYF RDVNEQDVLL FIDNIFRFVQ AGSEVSALLG RMPSAVGYQP
301  TLSTEMGSLQ ERITSTKEGS ITSIQAVYVP ADDLTDPAPA TTFAHLDATT
351  VLSRGLAAKG IYPAVDPLDS TSTMLQPRIV GEEHYETAQR VKQTLQRYKE
401  LQDIIAILGL DELSEEDRLL VARARKIERF LSQPFFVAEV FTGSPGKYVG
451  LAETIRGFQL ILSGELDGLP EQAFYLVGTI DEATAKAMNL EMESNLKK
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Fig. 6 Protein sequence alignments. Matched peptides were shown in *bold red*

Among them, SOD is the first line of defense against ROS-associated damage, catalyzing the dismutation of $O_2 \cdot^-$ to H_2O_2 and molecular oxygen. Subsequently, H_2O_2 is further metabolized to simple water molecules through the action of POD and CAT. Therefore, the activities of antioxidant enzymes can serve as an important index of plant stress tolerance.

In our experiments, both SOD and POD activities increased in the early phase of induced stress in both tomato cultivars. This was a general adaptive strategy that plants used to overcome such challenges. After 3 days, the activities of SOD and POD decreased. However, the $O_2 \cdot^-$ formation rate and H_2O_2 and MDA contents increased, showing that the balance of ROS production and removal was disturbed. Cytoplasmic membranes under ROS attack produced peroxides. EBR pretreatment significantly increased the SOD, POD, and CAT activities and reduced the rate of $O_2 \cdot^-$ formation and H_2O_2 content in both tomato cultivars. Excess ROS were efficiently scavenged in vivo, and membrane lipid peroxidation was alleviated. Our observations were consistent with previous reports that EBR could improve plants' capacity to cope with the stresses of low temperature and poor light, especially during early seedling development (Sasse 2003; Divi and Krishna 2010). This was mainly because EBR induced stress tolerance by triggering the accumulation of H_2O_2 and $O_2 \cdot^-$ which subsequently up-regulated the antioxidant system (Jiang et al. 2012; Fariduddin et al. 2014).

As the main biological energy source, ATP is indispensable for many metabolic pathways in higher plants, while ATP synthase plays a key role in the synthesis of ATP in all living organisms (Cheng et al. 2010). ATP synthase comprises an integral membrane CF_0 portion and an extrinsic CF_1 portion. The enzyme complex CF_1 has five subunits. Of those, the β subunit, composed of a catalytic and ADP-binding unit, catalyzes ATP formation from ADP and Pi in the presence of a transmembrane proton gradient (Ye et al. 2013).

Our investigation was the first to show a link between EBR and the ATP synthase β subunit. In our experiment, the expression of the ATP synthase β subunit decreased in both tomato cultivars under low temperature and poor light stress conditions. This may be because H_2O_2 and $O_2 \cdot^-$ are signal molecules and can directly interact with ATP synthase β subunit (target or sensor). Accumulation of H_2O_2 and $O_2 \cdot^-$ under low temperature and poor light stresses inhibited ATP synthase β subunit synthesis. A decrease in ATP formation limited photosynthesis and respiration, ultimately inhibiting the growth and development (Rott et al. 2011). After pretreatment with exogenous EBR, the tomato seedlings showed increased antioxidant activity, and the excess H_2O_2 and $O_2 \cdot^-$ were effectively scavenged. Thus, inhibition of the ATP synthase β subunit was removed. The increase in ATP synthase β subunits activated biological energy metabolism and improved ATP levels, with most of that energy being used for growth and development. This additional energy enabled the plants to cope with the combined low temperature and poor light stresses. This demonstrated that exogenous EBR could make the antioxidant enzyme system and ATP synthase β subunit act synergistically, so that ROS were scavenged efficiently in vivo, and plant growth and development could gradually return to normal under low temperature and poor light stress conditions.

In this study, exogenous EBR induced higher antioxidant activities and ATP synthase β subunit content in the tolerant genotype 'Zhongza9' than the sensitive genotype 'Zhongshu4.' This gave 'Zhongza9' a higher capacity to removing H_2O_2 and $O_2 \cdot^-$. These results indicated that EBR pretreatment potentially gave 'Zhongza9' better protection against the oxidative damage caused by low temperature and poor light stresses.

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Conflict of interest The authors declare that they have no competing interests.

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