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Preharvest 24-epibrassinolide treatment prolongs harvest duration and shelf life in sweet corn

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ABSTRACT

Sweet corn is perishable and have limited harvest duration and shelf life due to their quality deterioration. Reactive oxygen species (ROS) are one of the most predominant factors for maintaining quality of sweet corn during and after harvest. Brassinosteroids (BRs) can enhance the activity of antioxidant enzymes and decrease the ROS level in plants. In this study, we found that a bioactive BR (24-epibrassinolide, EBR) treatment before harvest markedly inhibited change of quality indicators (MDA content, weight loss rate, and soluble sugar content) during and after harvest. Further analysis revealed that EBR promoted the activity and transcriptions of antioxidant enzymes, maintaining lower ROS level in kernels. Meanwhile, exogenous EBR increased the expression level of genes controlling sucrose transport in sweet corn kernels. Bioinformatics and binding analysis identified that BR transcription factor ZmBES1/ZmBZR1-10 might potentially bind to and upregulate transcriptions of antioxidant enzyme genes including *SOD* and *POD* genes, and sucrose transport-related genes including *SUT* and *SWEET* genes. These results indicated that exogenous application of EBR ameliorates quality during and after harvest by improving the antioxidant capacity and photosynthetic assimilates accumulation rate of sweet corn, thus prolonging harvest duration and shelf life in sweet corn.

1. Introduction

Sweet corn is one of the most important vegetables grown all over the world (Hu et al., 2021). Mutations in key enzymes involved in the starch synthesis pathway affected starch accumulation and increased soluble sugar content in endosperm (Greene & Hannah, 1998). Sweet corn is a good source of certain minerals, vitamins, dietary fiber, and phytonutrients. The optimal harvesting period of sweet corn is generally short, and inappropriate harvesting time often affects the quality of sweet corn. In addition, sweet corn is extremely perishable (Becerra-Sanchez & Taylor, 2021; Mehta et al., 2017; Subaedah, Edy, Mariana, & Clay, 2021), making it difficult to store and commercialize for long periods of time, and the loss rate is as high as 20%-35%. Therefore, prolonging the harvest period and shelf life of sweet corn is directly related to the edible quality and commodity value of sweet corn.

Reactive oxygen species (ROS) are continuously produced as by-products of various metabolic pathways (Ishchenko, Sanz, Privezentzev, Maksimenko, & Saparbaev, 2003), and also serve as secondary messengers in plant hormone responses (Waszczak, Carmody, & Kangasjärvi, 2018). Under normal physiology, plants themselves will produce an appropriate amount of ROS, acting as a signal to regulate the physiological and biochemical reactions inside and outside cells (Waszczak et al., 2018). In the meantime, plants employ both enzymatic and non-enzymatic antioxidant scavenging system to maintain the steady-state level of ROS (Fimognari et al., 2020). However, aging, drought, salinity, low temperature, and many other factors can disrupt cellular homeostasis, resulting in redox imbalances and the accumulation of ROS (Bhuyan et al., 2020; Sachdev, Ansari, Ansari, Fujita, & Hasanuzzaman, 2021). Excessive ROS accumulation affects many cellular functions, causing some unavoidable damage to carbohydrates, proteins, lipids, and DNA, ultimately leading to oxidative stress (Apel & Hirt, 2004; Gill & Tuteja, 2010). Sweetness and tenderness are important characters of sweet corn quality, which affect harvest duration and shelf life (Szymanek, Tanaś, & Kassar, 2015). Previous studies from our lab have shown that ROS level in kernels was closely related to sweetness and tenderness for sweet corn (Fang, Chen, Zhang, & Wang, 2023).

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Therefore, preventing excessive ROS synthesis and improving scavenging ability may be a strategy to delay deterioration in sweet corn quality during and after harvest.

Brassinosteroids (BRs), as a class of steroid hormone, are ubiquitously distributed throughout the plant kingdom (Clouse & Sasse, 1998). BRs can effectively increase chlorophyll content, improve photosynthetic efficiency, and promote the transport of photosynthetic products, thus helping in boosting crop production (Ahammed, Li, Liu, & Chen, 2020). Enhanced antioxidant enzyme activities have been shown to be closely associated with plant tolerance to abiotic stresses (Hu et al., 2020). BRs can induce plant tolerance by preventing the peroxidation and enhancing the activity of antioxidant enzymes (Cui et al., 2011). Recent studies have shown that BRs can slow down the aging process and quality deterioration of postharvest horticultural crops by regulating their antioxidant systems (Fang et al., 2021; Gao et al., 2016; Sun, Asghari, & Zahedipour-Sheshgelani, 2020).

Considering the impact of ROS on sweet corn quality, and the regulation of BRs on antioxidant enzymes and photosynthates' transport, we wonder whether BRs can regulate antioxidant enzyme activity and delay quality deterioration during and after harvest. 24-epibrassinolide (EBR), a highly active synthetic analog of BRs, is widely used in agriculture, with favorable safety and commercial availability (Wu, Zhang, Ervin, Yang, & Zhang, 2017). In this study, the effect of EBR treatment on the quality of sweet corn during and after harvest was explored. We also analyzed the expression and regulation of the antioxidant enzyme and sugar transporter genes by BRs transcription factor ZmBZR1. This study provided a new perspective for the effect of exogenous EBR on the quality of sweet corn during and after the picking period, and revealed the corresponding mechanisms from the perspective of antioxidant enzymes and sugar transporters, hence providing a theoretical basis for solving the problem of quality deterioration during and after the picking period in sweet corn.

2. Material and methods

2.1. Plant material and treatment

The sweet corn cultivar Zhetaitian 928 (ZTT) were grown at the farmland of Zhejiang Academy of Agricultural Sciences (Dongyang, China) under standard agronomic practices in the summers of 2021 and 2022. At 18 days after pollination, EBR treatment was performed. The plants were divided into two groups. One group was treated with 2 μM EBR (containing 2% EtOH), while the other was treated with distilled water (containing 2% EtOH) and served as control. Seed samples were collected with three biological replicates at 18 days after pollination, 20 days after pollination, 24 days after pollination, 27 days after pollination, 30 days after pollination, and 33 days after pollination, representing the 18DAP, 20DAP, 24DAP, 27DAP, 30DAP, and 33DAP stages, respectively. In addition, ears from EBR and H_2O treatments were harvested at 24 days after pollination. After picking, the ears were placed into 0.02 mm thick unsealed polyethylene bag and stored at room temperature (24 ± 1 ℃) for post-harvest treatments. Seed samples were collected with three biological replicates at 0 days after harvest, 3 days after harvest, and 6 days after harvest, representing the 0DAH, 3DAH, and 6DAH stages, respectively.

2.2. Determination of soluble sugar content

Soluble sugar content was determined using the Plant Soluble Sugar Content Assay Kit (Solarbio, Beijing, China). Soluble sugar content was articulated as mg/g (FW).

2.3. Determination of malondialdehyde (MDA) content

Measurement of MDA content was performed by the Malondialdehyde Assay Kit (Beyotime, Shanghai, China). MDA content was expressed on a fresh weight basis as μmol/mg.

2.4. Determination of weight loss (WL) rate

The weight loss rate was expressed according to the formula: WL% = (M1 − M2)/M1 × 100%, where M1 is the original ear weight and M2 represents the final ear weight (Ding et al., 2021). All tests were carried out in triplicate.

2.5. Determination of kernel hardness

Kernel hardness was measured by a fruit hardness tester (Model No HLY-YD5, Hanliny, Wuhan, China). The value was expressed in newtons (N).

2.6. Determination of ROS level

Nitro blue tetrazolium (NBT) and diaminobenzidine (DAB) staining was measured according to (Ren et al., 2019). For NBT staining, the kernels were completely immersed in 10 mM potassium phosphate buffer (pH 7.6) containing 0.5 mg/ml NBT (Sigma-Aldrich, St. Louis, USA) and incubated in 50 mM Tris-HCl buffer (pH 5.0) containing 1 mg/ml DAB (Sigma-Aldrich, St. Louis, USA) at room temperature. The kernels were not placed in 70% ethanol for decolorization until the spots appeared. After that, the sample was observed, and photos of the sample were taken. The relative intensities of DAB and NBT staining were analyzed using ImageJ.

2.7. Determination of antioxidant enzymatic activity

Measurement of peroxidase (POD) activity was performed by POD Activity Assay Kit (Solarbio, Beijing, China). POD activity was articulated as U/g. Measurement of superoxide dismutase (SOD) activity was performed by SOD Activity Assay Kit (Solarbio, Beijing, China). SOD activity was articulated as U/g. Measurement of catalase (CAT) activity was performed by CAT Activity Assay Kit (Solarbio, Beijing, China). CAT activity was articulated as U/g.

2.8. Quantitative reverse transcription PCR (qRT-PCR)

Total RNAs were extracted from kernels of three ears treated with H_2O and EBR as replicates. Synthesis of cDNA was performed using HiScript II Q Select RT SuperMix (Vazyme, Nanjing, China). The gene-specific primers for qRT-PCR analysis were listed in Table S1. *ZmTubulin1* (*Zm00001d013367*) was used as an internal control. The qRT-PCR was performed on CFX96 (Bio-Rad, California, USA) using AceQ qRT-PCR SYBR Green Master Mix (Vazyme, Nanjing, China). Three technical replicates were adopted for each gene.

2.9. Transactivation analysis

The promoter sequences of *POD4*, *SOD2*, *SUT4*, and *SWEET15a* were amplified from genomic DNA and inserted into pGreenII 0800-LUC, respectively. The full-length CDS sequences of ZmBES1/ZmBZR1-7 and ZmBES1/ZmBZR1-10 were synthesized by Genscript (GenScript, New Jersey, USA) and recombined into the overexpression plasmid pXY104, respectively. The mixture of GV3101 containing the vectors described above was infiltrated into the leaves of *N. benthamiana*. Dual-luciferase reporter (DLR) assay was performed with the Dual-Luciferase Reporter Assay System (Beyotime, Shanghai, China).

For *in vivo* bioluminescence imaging assay, the injected leaves were detached and sprayed with 2 mg/mL potassium luciferin (Beyotime, Shanghai, China). The luciferase luminescence from the infiltrated area was imaged using ChemiDoc MP Imaging System (Bio-Rad, California, USA).

3. Results

3.1. Effects of exogenous EBR treatment before harvest on extending harvest duration and shelf life of sweet corn

We analyzed change in quality of sweet corn under two conditions: natural maturity and senescence on the plant (NMS), and postharvest maturity and senescence (PMS) (Fig. 1A). Under NMS, ears on the plants were used for analysis after EBR and H_2O treatments. Previous studies have found that the optimal harvest period for ZTT is 24DAP. Under PMS, ears from EBR and H_2O treatments were harvested at 24 days after pollination and stored at room temperature (24 ± 1 ℃) for post-harvest treatments. Sweetness and tenderness are two important characters of sweet corn quality (Szymanek et al., 2015). Under NMS, the soluble sugar content in control kernels reached peak at 24DAP and then markedly decreased (Fig. 1B). EBR treatment induced and maintained a higher level of soluble sugar content from 20DAP to 30DAP. Kernel hardness slightly increased for EBR and H_2O treatment under NMS (Fig. 1C). EBR treatment reduced the rate of increase in kernel hardness. MDA is an important index of lipid peroxidation, which is related to oxidative stress or senescence in organisms (Cao et al., 2018). Compared to H_2O treatment, EBR application significantly alleviated lipid peroxidation of sweet corn under NMS (Fig. 1D).

Under PMS, EBR treatment also retarded the soluble sugar content drops and blocked the speed of MDA and seed hardness rise (Fig. 1E–H). Moisture plays a vital part in maintaining sweet corn quality (Becerra-Sanchez & Taylor, 2021). Under NMS, WL was aggravated in control fruit as the storage time extended, while EBR treatment reduced water loss of harvested sweet corn ear. In addition, preharvest EBR treatment did not affect other agronomic traits (Fig. S1A–E). In summary, preharvest EBR treatment can significantly delay deterioration in sweet corn quality during and after harvest, thus prolonging harvest duration and shelf life.

3.2. EBR reduces reactive oxygen species (ROS) content in sweet corn kernels

The burst of ROS may be an important factor leading to deterioration in sweet corn quality during and after picking period (Fang et al., 2023). We used NBT and DAB staining to analyze the effect of EBR treatment on

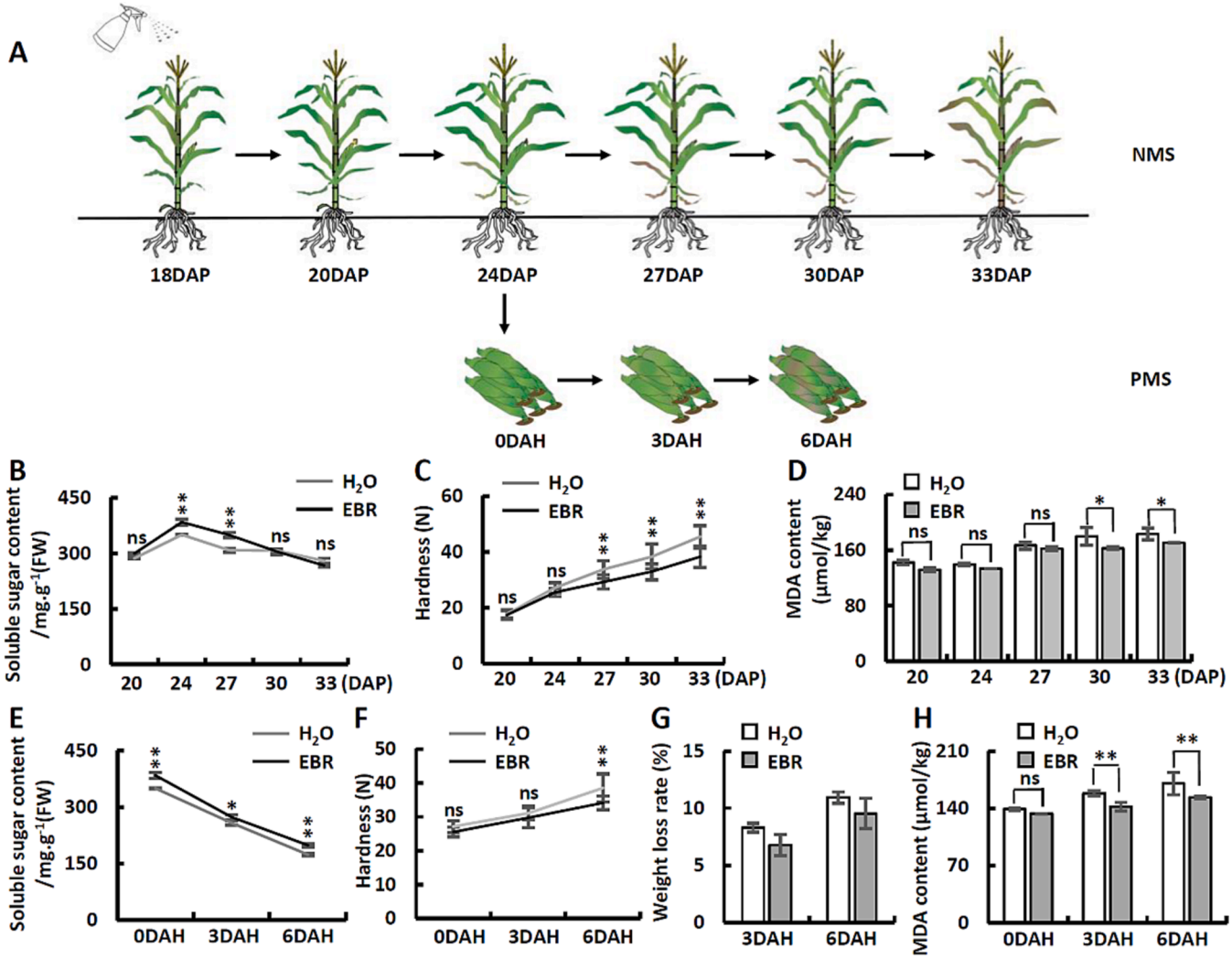

Fig. 1. Effect of 24-epibrassinolide (EBR) treatment on quality-related indicators of sweet corn during and after harvest. (A) A schematic diagram about time of EBR treatment and sampling. Change in quality was analyzed under two conditions: natural maturity and senescence on the plant (NMS); and postharvest maturity and senescence (PMS). Sweet corn ears were picked at 24DAP and stored at room temperature (24±1℃) for PMS analysis. (B-D) Dynamic changes of soluble sugar content (B), kernel hardness (C), and MDA content (D) in H_2O and EBR-treated kernels at 20DAP, 24DAP, 27DAP, 30DAP, and 33DAP. (E-H) Dynamic changes of soluble sugar content (E), kernel hardness (F), MDA content (G), and weight loss rate (H) in H_2O and EBR-treated kernels at 0DAH, 3DAH, and 6DAH. The data are presented as the mean ± SE of three biological replicates. Asterisks indicate the significant differences between the two groups (Student's *t* test, * $p < 0.05$; ** $p < 0.01$).

ROS level in kernels under NMS (Fig. 2A–C) and PMS (Fig. 2D–F). The results showed that exogenous EBR could significantly inhibit the accumulation of ROS in kernels (Fig. 2A–F), thus maintaining a plump appearance of grains (Fig. S2). Hence, we hypothesized that EBR can affect the quality of kernels during and after picking period by regulating the homeostasis of ROS.

3.3. EBR increases the activities of antioxidant enzymes in sweet corn kernels

CAT, SOD and POD are important antioxidant enzymes responsible for scavenging ROS (Jing, Guo, Li, & Li, 2020). We first analyzed these enzyme activity changes under NMS. As shown in Fig. 3A–C, SOD and POD activities slightly decreased in control kernels. EBR treatment induced and maintained higher levels of SOD and POD activities. CAT activity did not seem to be induced by EBR. During postharvest storage, the pattern of changes in antioxidant enzymes activities was similar to that under NMS (Fig. 3D–F). SOD activity was constant in control kernels. EBR treatment induced and maintained higher levels of SOD and POD activities. CAT activity was not induced by EBR. Taken together, EBR treatment can induce antioxidant enzyme activity before and after the picking period.

It is widely accepted that enzyme activity is often closely related to the corresponding gene expression. Therefore, we want to explore whether EBR influences the antioxidant enzyme activities by regulating the expression of corresponding genes. qRT-PCR analysis and expression profiling in MaizeGDB (https://maizegdb.org) showed that two *POD* genes and two *SOD* genes expressed in seeds was significantly increased after EBR treatment at 18DAP, 20DAP, and 24DAP (Fig. 3G–J, Fig. S3A–F), whereas these genes were not strongly affected by EBR in leaves (Fig. S4A–J). These results suggest that EBR may affect the antioxidant enzyme activities by regulating the expression of corresponding genes.

3.4. EBR induces expression of genes controlling sugar transport in sweet corn kernels

The photosynthetic assimilates sucrose produced in the leaves is transported to the seeds through various sugar transporters in the seeds during the seed filling stage. Therefore, we speculated that the content of soluble sugar in sweet corn kernels is closely related to the expression of sugar transporter genes. Sosso et al. (2015) have identified 22, 6, and 23 genes encoding ZmSTPs, ZmSUTs, and ZmSWEETs in maize, respectively (Sosso et al., 2015). Among these transporter families, one, three, and six genes encoding ZmSTPs, ZmSUTs, and ZmSWEETs, respectively, were found to be relatively highly expressed in kernels using MaizeGDB database (Fig. 4A–J). We further analyzed the effects of exogenous EBR on the expression of these genes in kernels before picking. The results showed that EBR can significantly induce the expression of these nine genes except *ZmSTP17* in kernels (Fig. 4A–J). These sugar transporter genes, like the aforementioned antioxidant enzyme genes, were not strongly affected by EBR in leaves (Fig. S5A–J).

3.5. Transcriptional regulation of ZmBZR1s on sucrose transport and ROS metabolisms

In order to further confirm the regulation of EBR treatment on the expression of sugar transporter genes and antioxidant enzyme genes, we

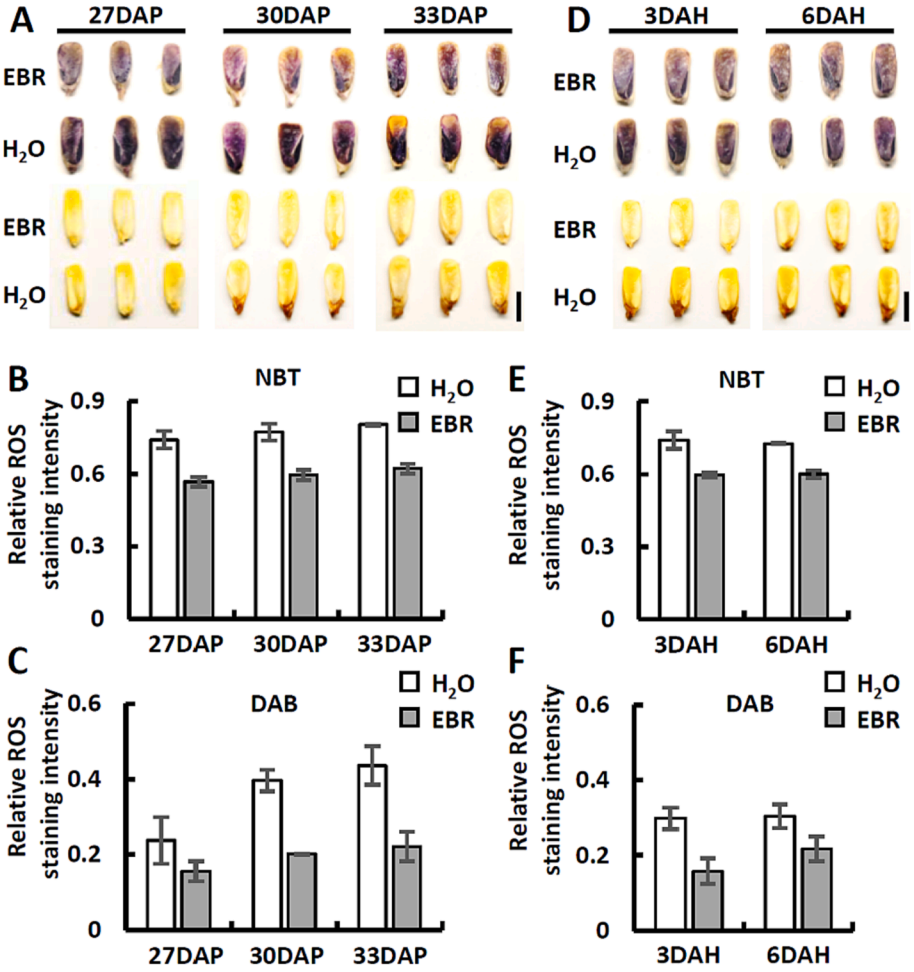

Fig. 2. EBR treatment reduced the accumulation of ROS in kernels during and after harvest. (A) NBT and DAB staining for the detection of ROS level in H_2O and EBR-treated kernels at 27DAP, 30DAP, and 33DAP. Scale bars = 1 cm. (B) Relative NBT staining intensity in H_2O and EBR-treated kernels at 27DAP, 30DAP, and 33DAP. (C) Relative DAB staining intensity in H_2O and EBR-treated kernels at 27DAP, 30DAP, and 33DAP. (D) NBT and DAB staining for the detection of ROS level in H_2O and EBR-treated kernels at 3DAH and 6DAH. Scale bars = 1 cm. (E) Relative NBT staining intensity in H_2O and EBR-treated kernels at 3DAH and 6DAH. (F) Relative DAB staining intensity in H_2O and EBR-treated kernels at 3DAH and 6DAH. The data are presented as the mean ± SE of three biological replicates.

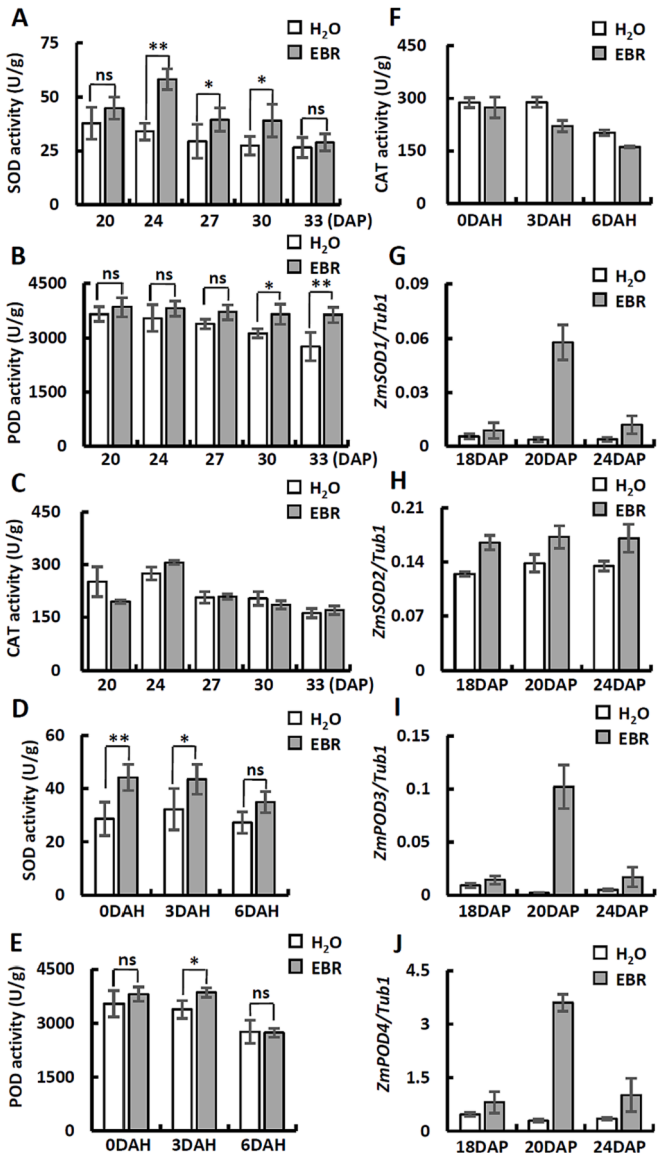

Fig. 3. Effect of EBR treatment on antioxidant enzymes system in kernels during and after harvest. (A-C) Dynamic changes of SOD (A), POD (B), and CAT (C) activities in H_2O and EBR-treated kernels at 20DAP, 24DAP, 27DAP, 30DAP, and 33DAP. (D-F) Dynamic changes of SOD (D), POD (E), and CAT (F) activities in H_2O and EBR-treated kernels at 0DAH, 3DAH, and 6DAH. (G-J) Dynamic changes of SOD genes and POD genes expression in H_2O and EBR-treated kernels at 18DAP, 20DAP, and 24DAP. The data are presented as the mean ± SE of three biological replicates. Asterisks indicate the significant differences between the two groups (Student's *t* test, * $p < 0.05$; ** $p < 0.01$).

analyzed the transcriptional regulation of these genes by the key regulator of BR signaling. BES1/BZR1 is a key transcription factor in the brassinosteroid signal transduction pathway. To identify the maize ZmBES1/BZR1 genes, the BLASTP program was used to search NCBI (https://www.ncbi.nlm.nih.gov/) and MaizeGDB databases using the rice and Arabidopsis BZR1 protein sequences. A total of 10 maize ZmBES1/BZR1 family members were identified (Fig. 5A). Phylogenetic analysis found that ZmBES1/ZmBZR1-7 and ZmBES1/ZmBZR1-10 belonged to the same subclade as OsBZR1 and AtBZR1 (Fig. 5A). OsBZR1 and AtBZR1 have been confirmed to be involved in regulating brassinosteroid signal transduction and affect grain development (Gao et al., 2022; Jiang et al., 2013). Using MaizeGDB database to analyze the expression of 10 maize *ZmBES1/BZR1* genes, we confirmed that a total of 8 *ZmBES1/BZR1* genes were expressed in maize kernels, including *ZmBES1/ZmBZR1-7* and *ZmBES1/ZmBZR1-10*. These results suggested that ZmBES1/ZmBZR1-7 and ZmBES1/ZmBZR1-10 may be the key factors mediating brassinosteroid regulation of sweet corn quality.

Based on the previous analysis, we speculated that sugar transporter genes and antioxidant enzyme-related genes that were induced by preharvest EBR treatment in kernels might be the target genes of the BR signaling pathway (Fig. 3G–J, Fig. 4A–J). It was found that BZR1 binds to the BR response element (BRRE; CGTGT/CG) (He et al., 2005; Yin et al., 2005). We analyzed whether the promoter regions of sugar transporter genes and antioxidant enzyme-related genes contain this binding element (Fig. 5B). The results showed that multiple BRRE elements were found in the promoter region of sugar transporter genes and antioxidant enzyme-related genes (Fig. 5B), suggesting that sugar transporter genes and antioxidant enzyme-related genes were probably the target genes of the BR signaling pathway.

To detect transcriptional regulation relationship, we performed DLR assay to detect the regulatory relationship of ZmBES1/ZmBZR1-7 and ZmBES1/ZmBZR1-10 on *POD4*, *SOD2*, *SUT4*, and *SWEET15a*. pPOD4: LUC was co-transformed with either p35S: MCS (empty vector) only, p35S: ZmBES1/ZmBZR1-7, or p35S: ZmBES1/ZmBZR1-10 into tobacco leaves. For pSOD2: LUC, co-transformed with either p35S: MCS (empty vector) only, p35S: ZmBES1/ZmBZR1-7, or p35S: ZmBES1/ZmBZR1-10. For pSUT4: LUC, co-transformed with either p35S: MCS (empty vector) only, p35S: ZmBES1/ZmBZR1-7, or p35S: ZmBES1/ZmBZR1-10. For pSWEET15a: LUC, co-transformed with either p35S: MCS (empty vector) only, p35S: ZmBES1/ZmBZR1-7, or p35S: ZmBES1/ZmBZR1-10. The results showed that ZmBES1/ZmBZR1-10 significantly activates *POD4*, *SOD2*, and *SUT4* promoter-driven LUC expression. These results further confirmed the regulatory relationship of ZmBES1/ZmBZR1-10 on *POD4*, *SOD2*, and *SUT4*. The results of *in vivo* bioluminescence imaging assay further confirmed the regulatory relationship (Fig. 5C–G).

4. Discussion

Plants utilize CO_2 and synthesize carbohydrates during photosynthesis. Sucrose is the most common photoassimilate exported from leaves to nonphotosynthetic organs (Slewinski, Meeley, & Braun, 2009). Seed filling in plants relies on sucrose produced from photosynthesis in leaves, and capacity and efficiency of sucrose transport may affect ultimate seed weight (Ruan, 2012). Sweet corn is a genetic mutation of field corn (Greene & Hannah, 1998). The naturally-occurring genetic mutation reduce the synthesis of starch and increase the accumulation of sugars or other short chain polysaccharides. Sweetness is one of the important aspects of sweet corn quality (Szymanek et al., 2015). Soluble sugar content largely determines the quality in sweet corn. Thus, it is widely believed that the efficiency of photosynthesis and transportation of assimilation products determines soluble sugar level in kernels, thereby affecting the sweet corn quality. BRs can effectively increase chlorophyll content, improve photosynthetic efficiency, and promote the transport of photosynthetic products, thus helping in boosting crop production (Ahammed et al., 2020). Xu et al. (2015) reported that BRs altered the mRNA levels of sugar transporter genes (Xu, Xi, Zhang, Zhang, & Zhang, 2015). In the present study, we found that EBR treatment before harvest could increase soluble sugar content in kernels during and after harvest (Fig. 1B, 1E). qRT-PCR and binding analysis confirmed that BR transcription factor ZmBES1/ZmBZR1-10 might regulate expression of sugar transporter genes (Fig. 4A–J, Fig. 5A–G), thereby affecting the qualities of sweet corn.

ROS are generated as byproducts of metabolism or due to impaired respiration (Ishchenko et al., 2003). ROS can cause damage to various biological macromolecules such as proteins, lipids, and nucleic acids. It was found that ROS are elicitor of postharvest senescence and quality deterioration for fruits and vegetables (Ding et al., 2021). Our previous study found that ROS are important factor that affects sweet corn quality (Fang et al., 2023). BRs can induce plant tolerance by preventing the peroxidation and enhancing the activity of antioxidant enzymes. Recent

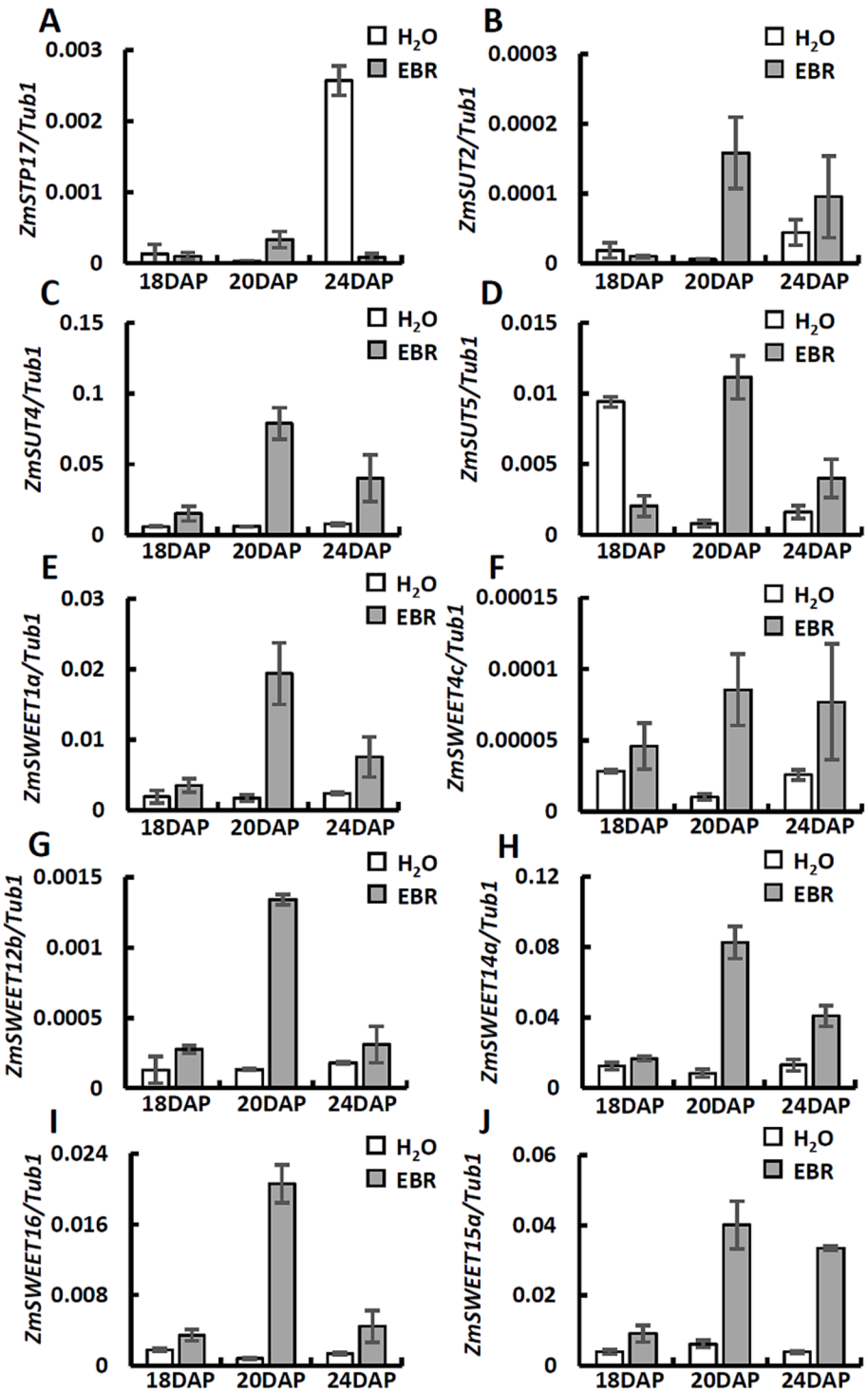

Fig. 4. Effect of EBR treatment on the expression of sugar transporters genes in kernels before harvest. (A) Dynamic changes of STP gene expression in H_2O and EBR-treated kernels at 18DAP, 20DAP, and 24DAP. (B–D) Dynamic changes of SUT genes expression in H_2O and EBR-treated kernels at 18DAP, 20DAP, and 24DAP. (E–J) Dynamic changes of SWEET genes expression in H_2O and EBR-treated kernels at 18DAP, 20DAP, and 24DAP. The data are presented as the mean ± SE of three biological replicates.

studies have shown that BRs can slow down the aging process and quality deterioration of postharvest horticultural crops by regulating their antioxidant systems (Fang et al., 2021; Gao et al., 2016; Sun et al., 2020). In this study, exogenous EBR before harvest increases antioxidative enzyme activities and corresponding gene expression in sweet corn (Fig. 3A–J). We further found that ZmBES1/ZmBZR1-10 might potentially bind to and activate *SOD* and *POD* genes expression (Fig. 5A–G). We therefore speculate that EBR treatment improved sweet corn quality by eliminating the inhibitory effect of ROS.

5. Conclusion

In this study, we found that EBR treatment before harvest delayed the decline in quality of sweet corn during and after harvest, maintaining soluble sugar content and antioxidant enzymes activity, thus improving sweetness and tenderness. In addition, we identified a key regulator of BR signaling, ZmBES1/ZmBZR1-10, that is highly expressed in kernels and strongly responsive to EBR signaling, positively regulating the expression of the sugar transporter genes including *SUT* and *SWEET* genes, and the antioxidant enzyme-related genes including *SOD* and one *POD* genes. These results indicated that exogenous EBR before

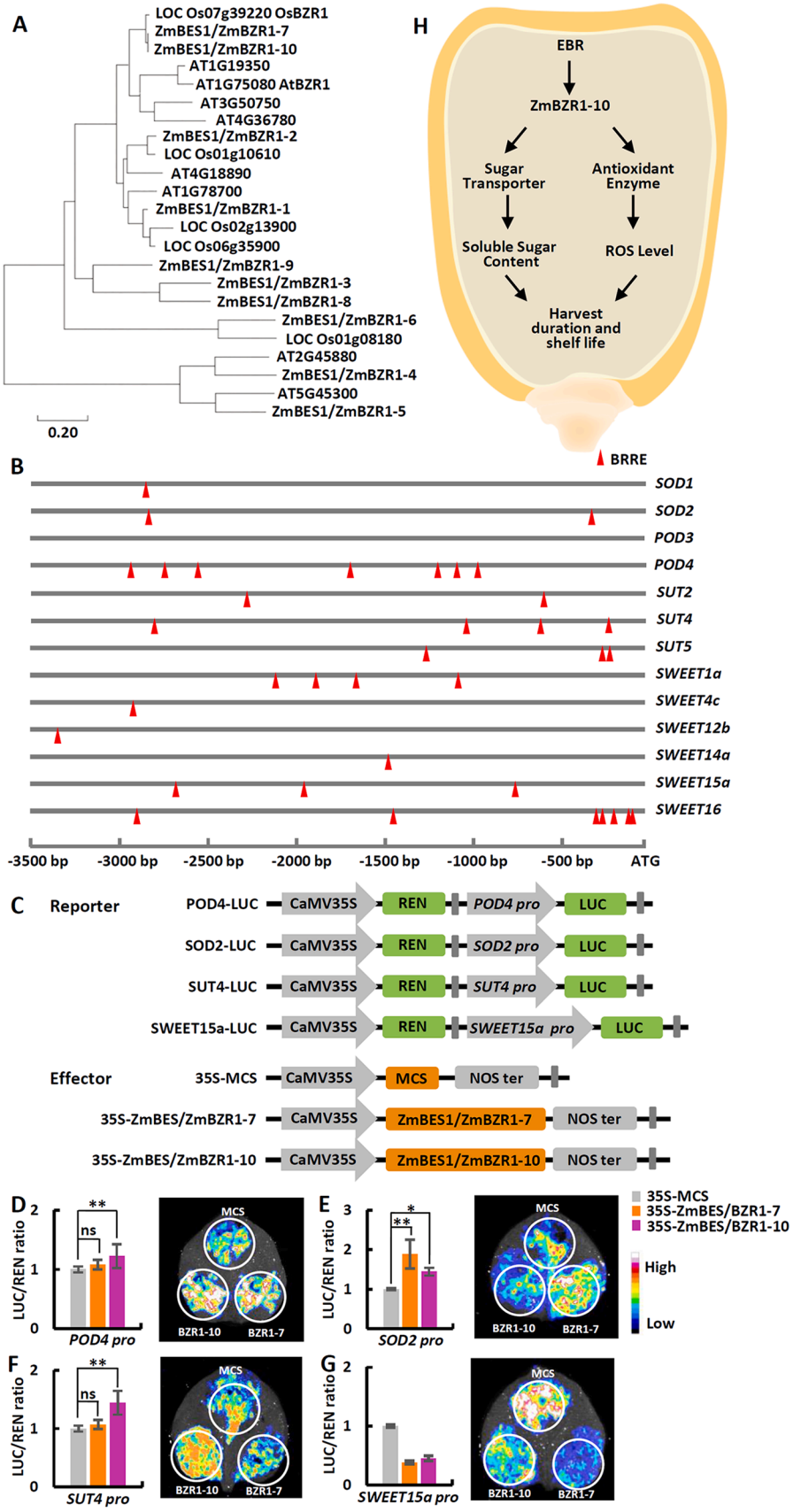

Fig. 5. ZmBES1/BZR1-10 act as positive modulator of antioxidant system and sugar transporters. (A) Phylogenetic tree of the BES1/BZR1s of maize, Arabidopsis, and rice. (B) Prediction of BR response element (BRRE; CGTGT/CG) in promoters of antioxidant enzyme-encoding genes and sugar transporter-encoding genes. (C) Diagrams of the reporter and effector constructs used in the dual-luciferase reporter assay. (D-G) Double luciferase assay and *in vivo* bioluminescence imaging of ZmBES1/BZR1-7 and ZmBES1/BZR1-10 to *POD4*, *SOD2*, *SUT4*, and *SWEET15a* transcriptional regulation. The data are presented as the mean ± SE of three biological replicates. Asterisks indicate the significant differences between the two groups (Student's *t* test, * $p < 0.05$; ** $p < 0.01$). (H) In sweet corn kernels, ZmBES1/BZR1-10 mediates activation of antioxidant enzymes and sugar transporters by EBR treatment, thus maintaining quality of sweet corn during and after harvest.

harvest appeared to enhance transcriptional regulation on the sugar transporter genes and antioxidant enzyme-related genes through ZmBZR1s (Fig. 5H), thus promoting accumulation of soluble sugars, reducing the level of ROS, improving the quality before and after the picking period, and prolonging the harvest period and shelf life of sweet corn.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2023.100179.

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