PUNJABI UNIVERSITY PATIALA

Seed Priming with Brassinolides Enhances Growth and Strengthens Antioxidative Defenses under Normal and Heat Stress Conditions in *Brassica juncea*

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GLOBAL CROP - GOLDEN OPPORTUNITIES

Presented by :

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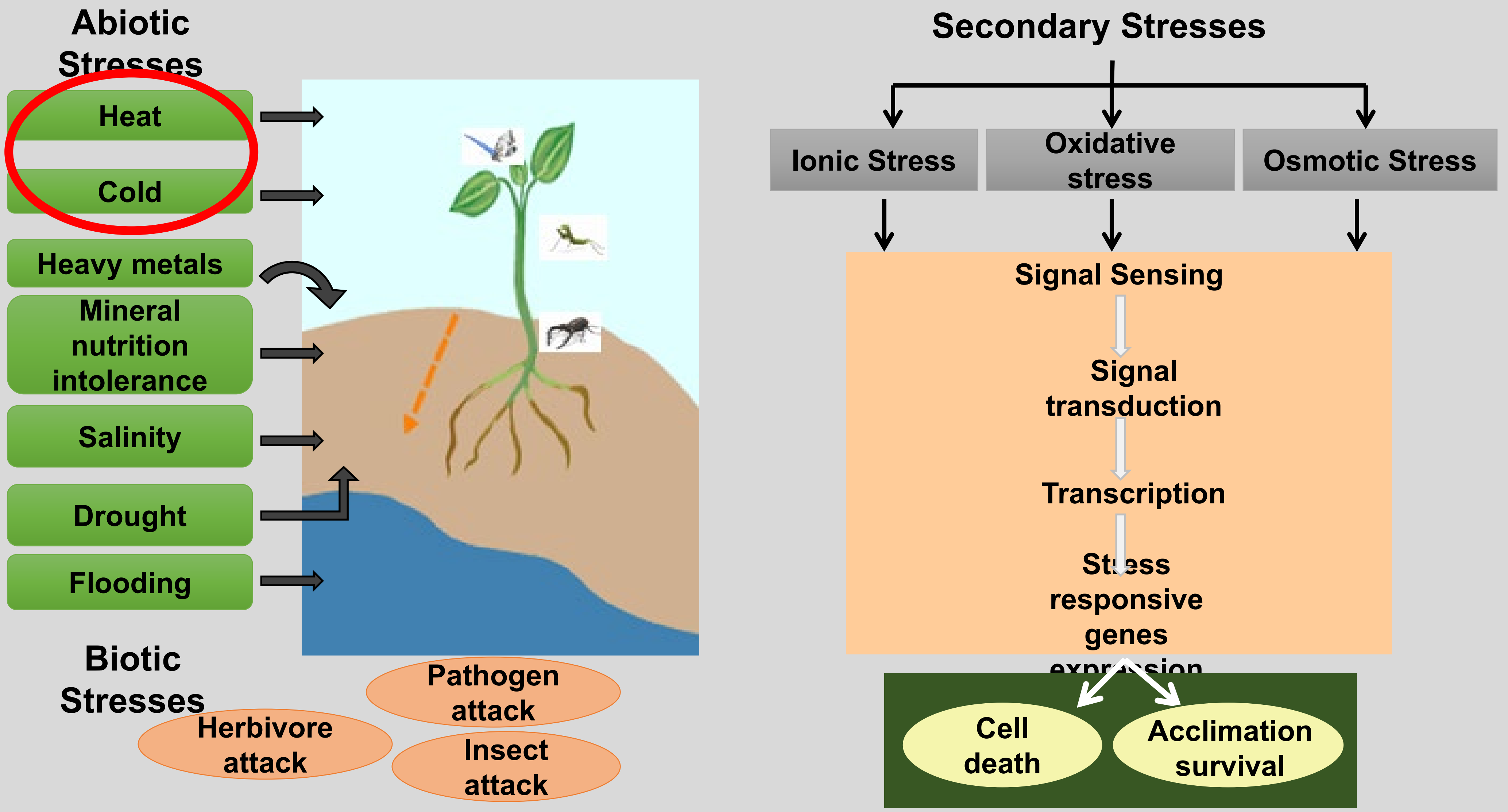


Abiotic Stresses
Heat
Cold
Heavy metals
Mineral nutrition intolerance
Salinity
Drought
Flooding
Biotic Stresses
Pathogen attack
Herbivore attack
Insect attack
Secondary Stresses
Ionic Stress
Oxidative stress
Osmotic Stress
Signal Sensing
Signal transduction
Transcription
Stress responsive genes
Cell death
Acclimation survival

Significance of temperature on biological life

- Different physical and chemical processes within the plants are governed by temperature
- The diffusion rate of gases and liquids
- Solubility of different substances
- Rate of reactions
- Stability of the enzyme system

Brassinosteroids

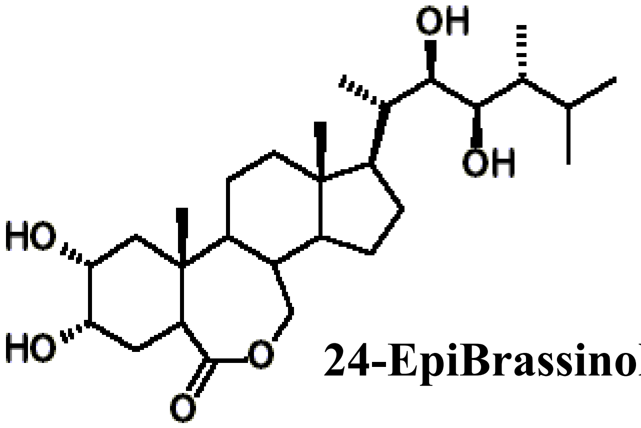
Brassinosteroids (BRs) are a family of about 70 structurally related compounds that contribute to:

•Growth

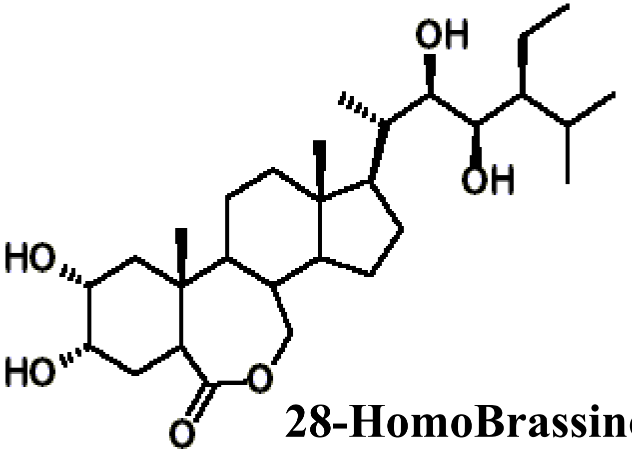
•Cell division, elongation, and differentiation

•Stress tolerance

•Reproductive development



24-EpiBrassinolide (EBL)



28-HomoBrassinolide (HBL)

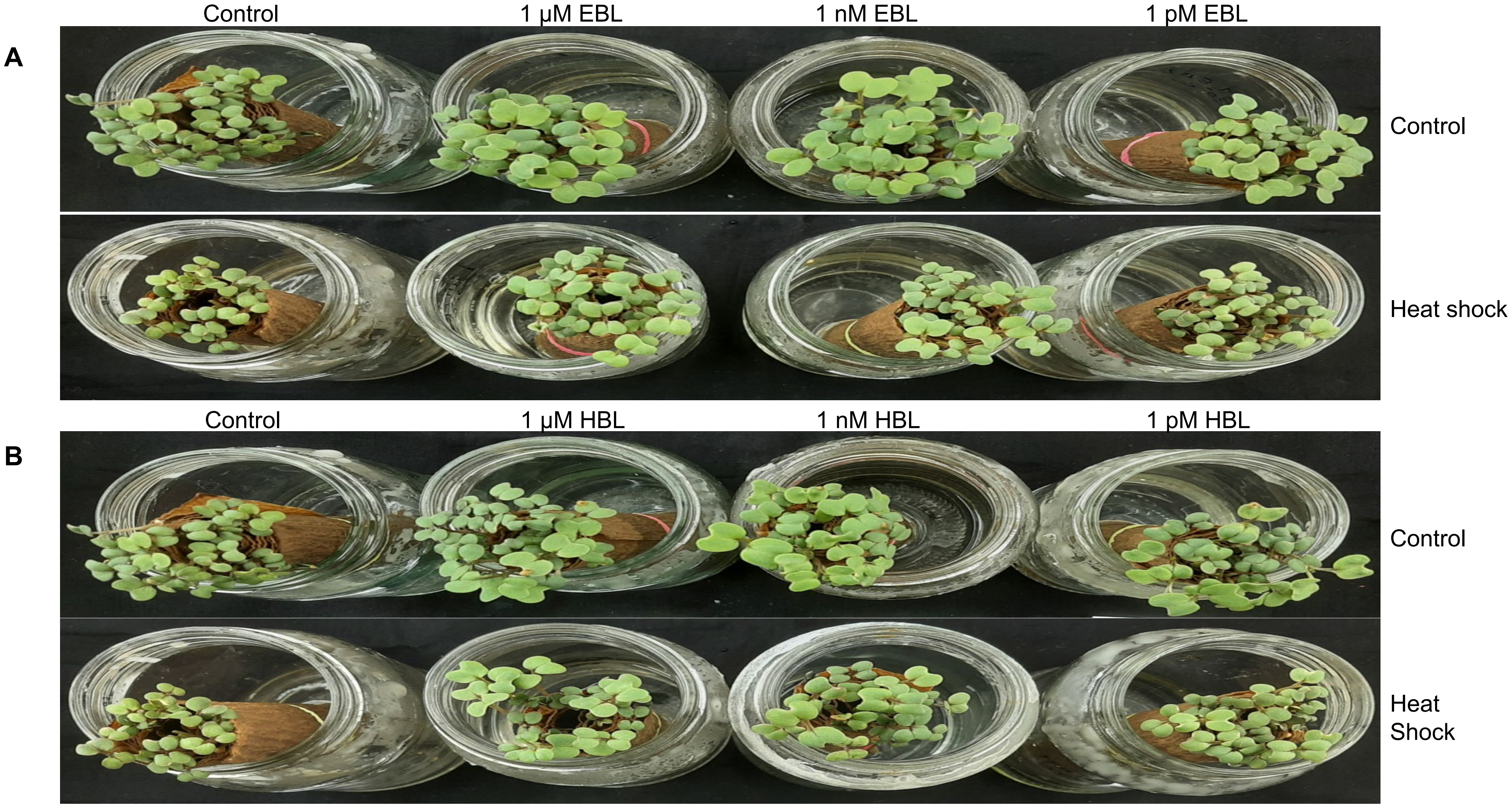


Figure 1. Brassinosteroids, EBL (A) and HBL (B) positively impacted the growth of normally grown and heat-shocked seedlings. Seeds pre-soaked in solutions containing different concentrations of EBL and HBL for 8 h, were grown under laboratory conditions. For control, seeds were pre-soaked in DDW. Eight-day-old seedlings primed with EBL, HBL, or DDW were maintained at 20 °C (as control) or subjected to 35 °C (heat shock) for 5 h daily for three consecutive days.

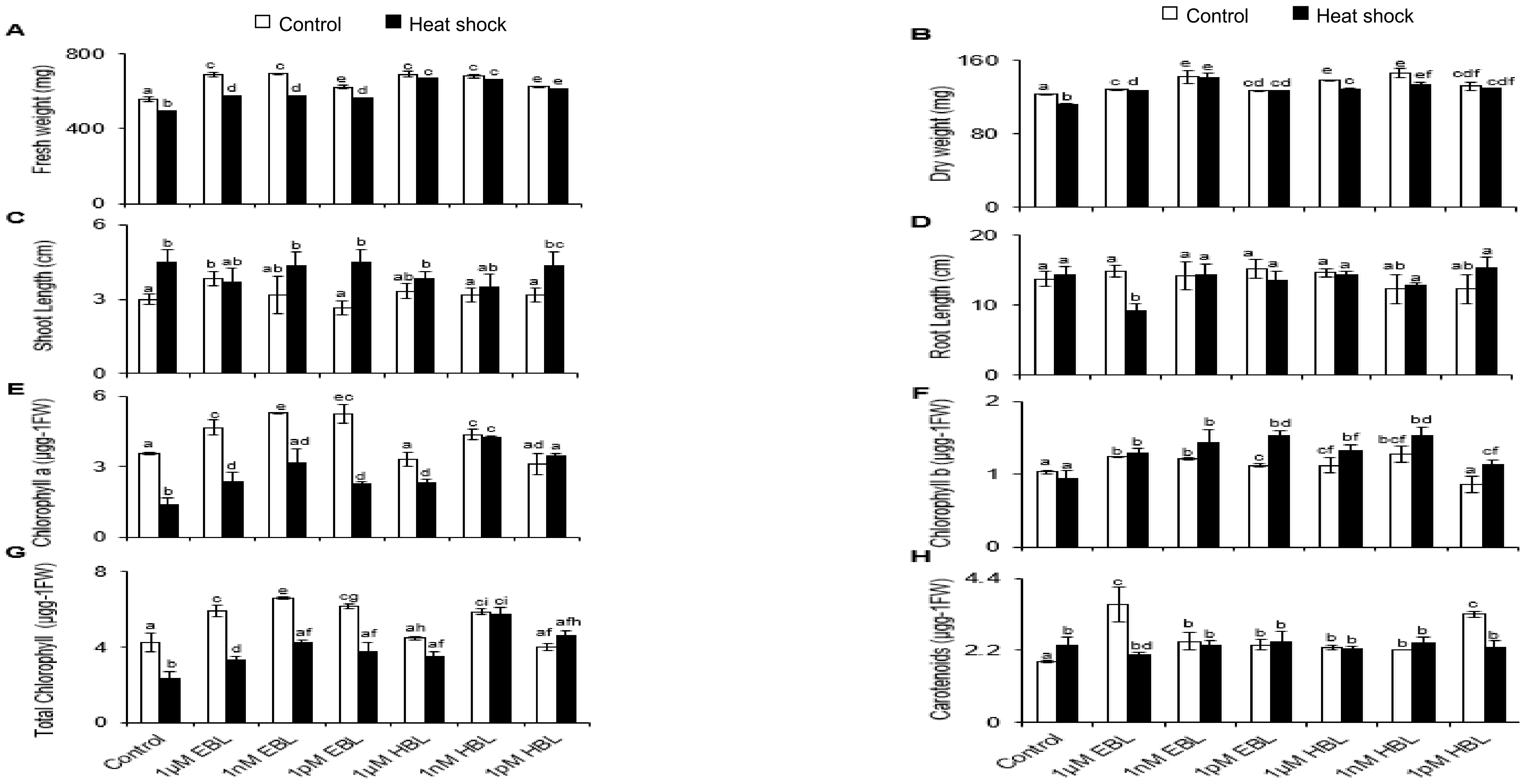


Figure 2. EBL and HBL showed positive impact on growth parameters and photosynthetic pigments in normally grown and heat-shocked seedlings. Measurement of growth parameters: (A) fresh weight, (B) dry weight, (C) shoot length, (D) root length, and photosynthetic pigments: (E) chlorophyll a, (F) chlorophyll b, (G) total chlorophyll, and (H) carotenoids. Seedlings primed with EBL, HBL, or DDW and subjected to heat stress or maintained under normal conditions (as shown in Fig. 2) were used. For fresh and dry weight, 25 seedlings were used. Root and shoot lengths were calculated using a ruler for 5 randomly selected seedlings. For pigments, seedlings weighing 200 mg were homogenized and used for quantification. Data represent the mean of three replicates. Error bars represent standard deviation (SD) and different letters above the bars represent a significant difference as determined by one-way ANOVA.

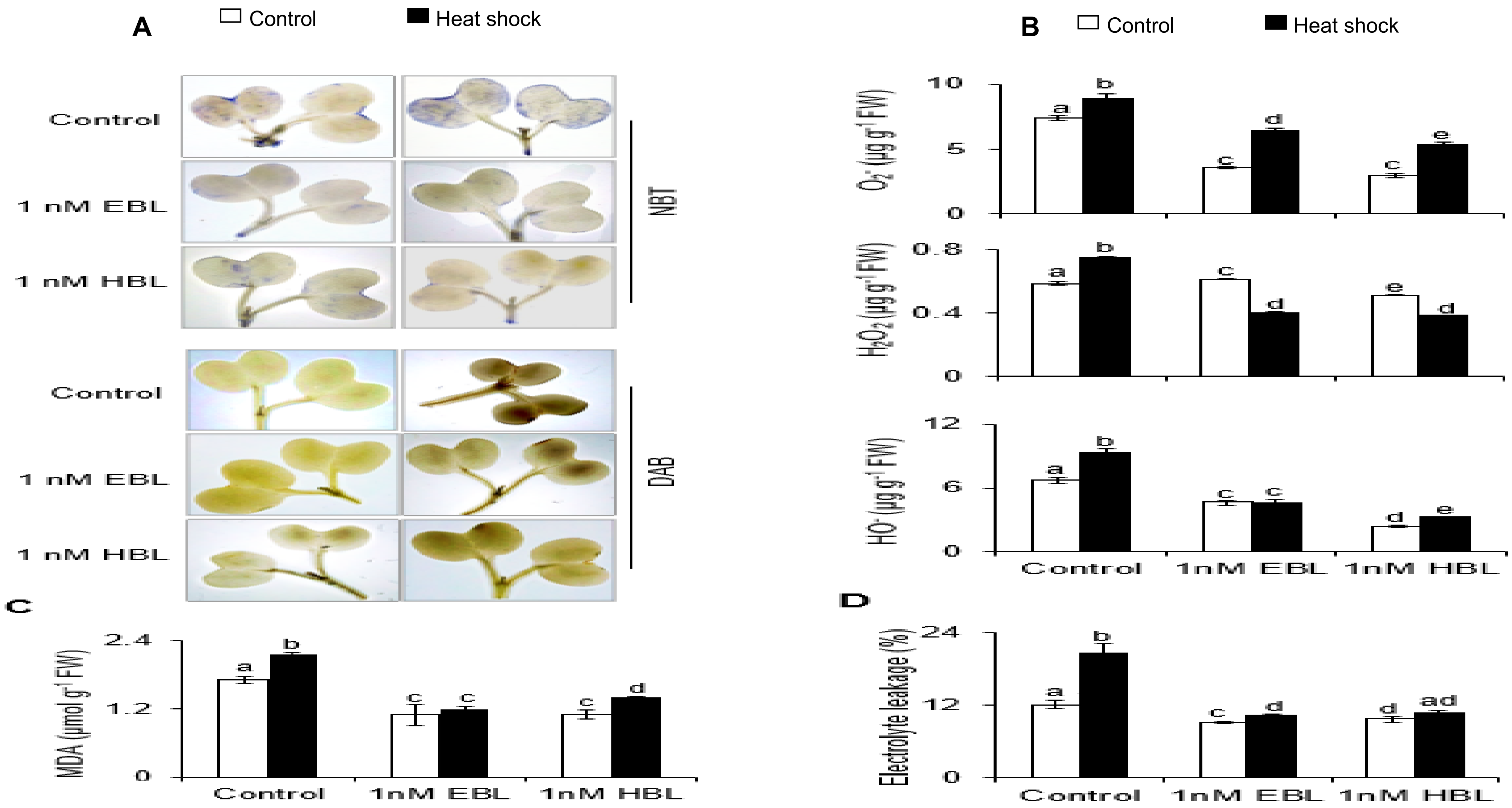


Fig. 3. EBL and HBL repress ROS accumulation and oxidative damage in both normally grown and heat-shocked seedlings. (A) Detection of O_2^- by NBT staining and H_2O_2 by DAB staining. (B) Quantification of O_2^-, H_2O_2, and HO^- levels. ROS accumulation analyses were carried out in 10-day-old seedlings established from seeds presoaked in different concentrations of EBL and HBL. (C) Lipid peroxidation quantified as malondialdehyde (MDA) levels. (D) Cell death represented by electrolyte leakage. For MDA levels, electrolyte leakage, and proline content, 10-day-old seedlings established from seeds presoaked in 1 nM each of EBL and HBL, were used. For MDA analysis, 500 mg of fresh tissue was homogenized and used for TBA-based assay. Electrolyte leakage was assessed in the leaves of 25 seedlings. Data represent the mean of three replicates. Error bars represent SD and different letters above the bars represent a significant difference as determined by one-way ANOVA.

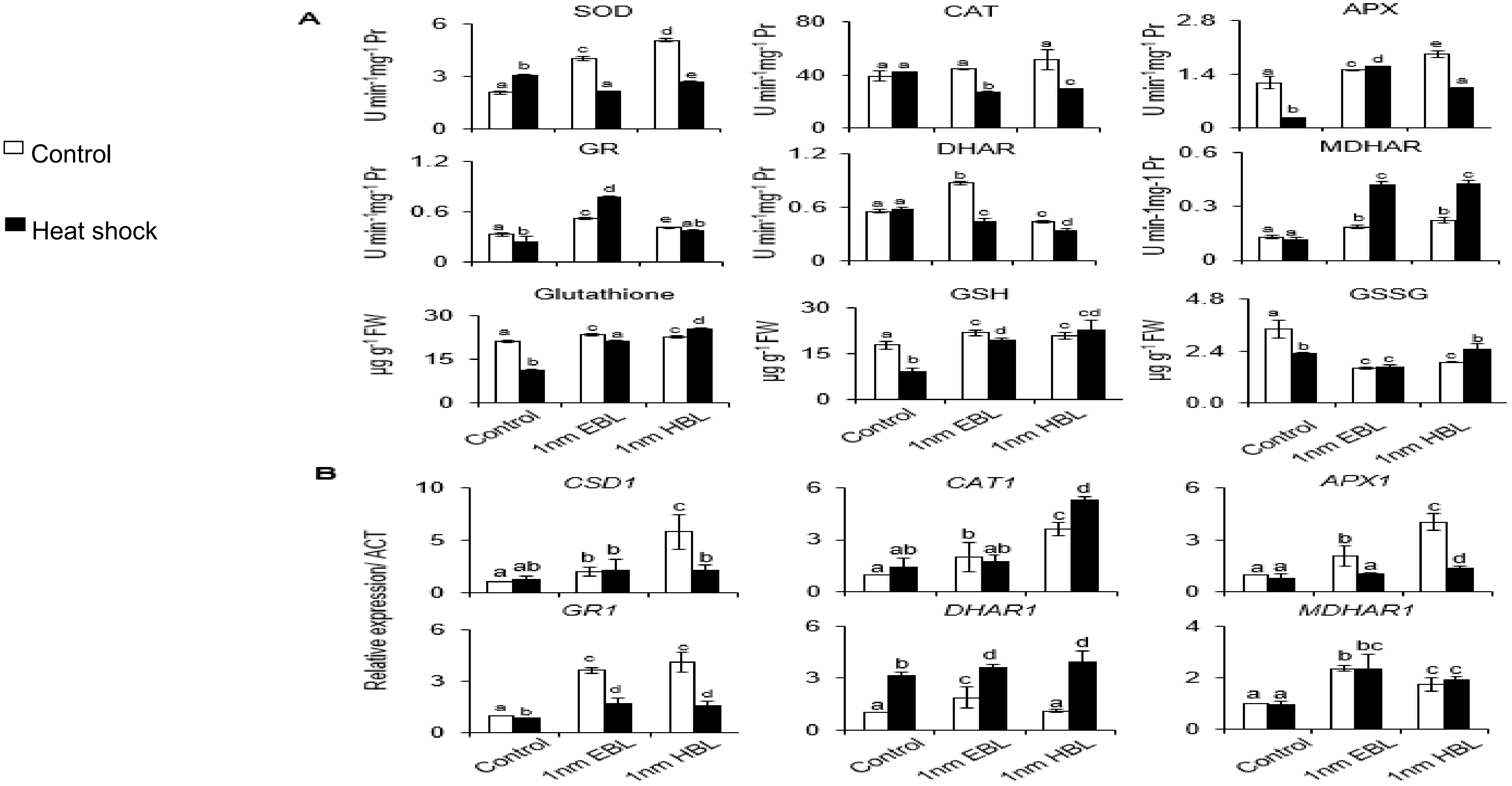


Figure 4. EBL and HBL positively affect the activities of antioxidants and their transcriptional expression. (A) Activities of enzymatic antioxidants and levels of non-enzymatic antioxidants. Ten-day-old seedlings were ground to a fine powder and homogenized in different buffers and used for enzyme assay and quantifications. (B) Transcriptional expression of enzymatic antioxidants. Transcript expression was determined by qRT-PCR using 10-day-old seedlings. Briefly, total RNA extracted from seedlings weighing 100 mg was used to prepare cDNA. ACTIN was used as an internal control. In both (A) and (B), data represent the mean of three replicates. Error bars represent SD and different letters above the bars represent a significant difference as determined by one-way ANOVA.

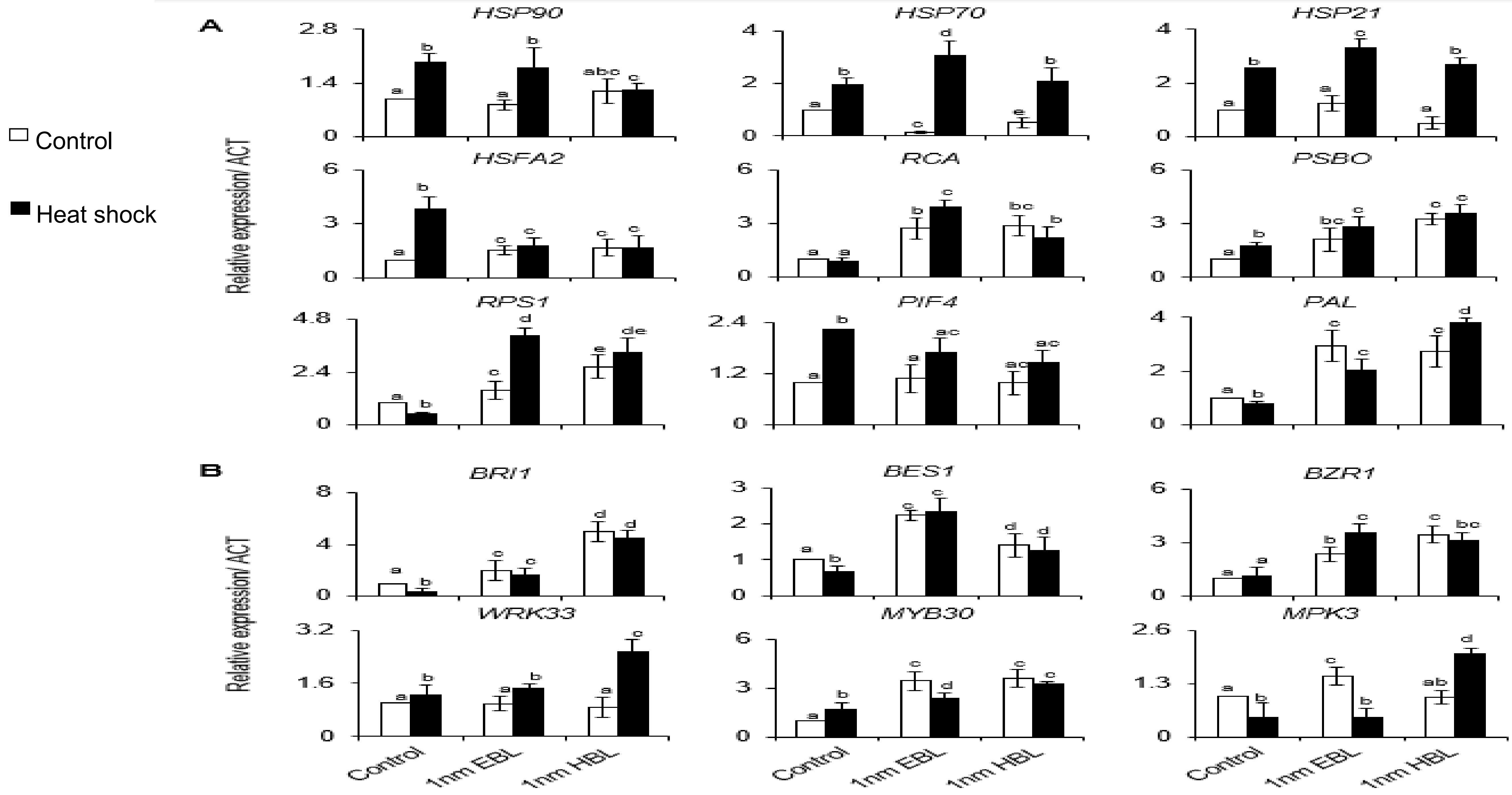


Figure 5. BRs treatment modulates the expression of genes involved in heat shock response and canonical BR signaling pathway. Genes involved in (A) general heat stress response and (B) BR signaling and downstream genes. Gene expression was determined by qRT-PCR using 10-day-old seedlings. Briefly, total RNA extracted from seedlings weighing 100 mg was used to prepare cDNA. ACTIN was used as an internal control. In both (A) and (B), data represent the mean of three replicates. Error bars represent SD and different letters above the bars represent a significant difference as determined by one-way ANOVA.

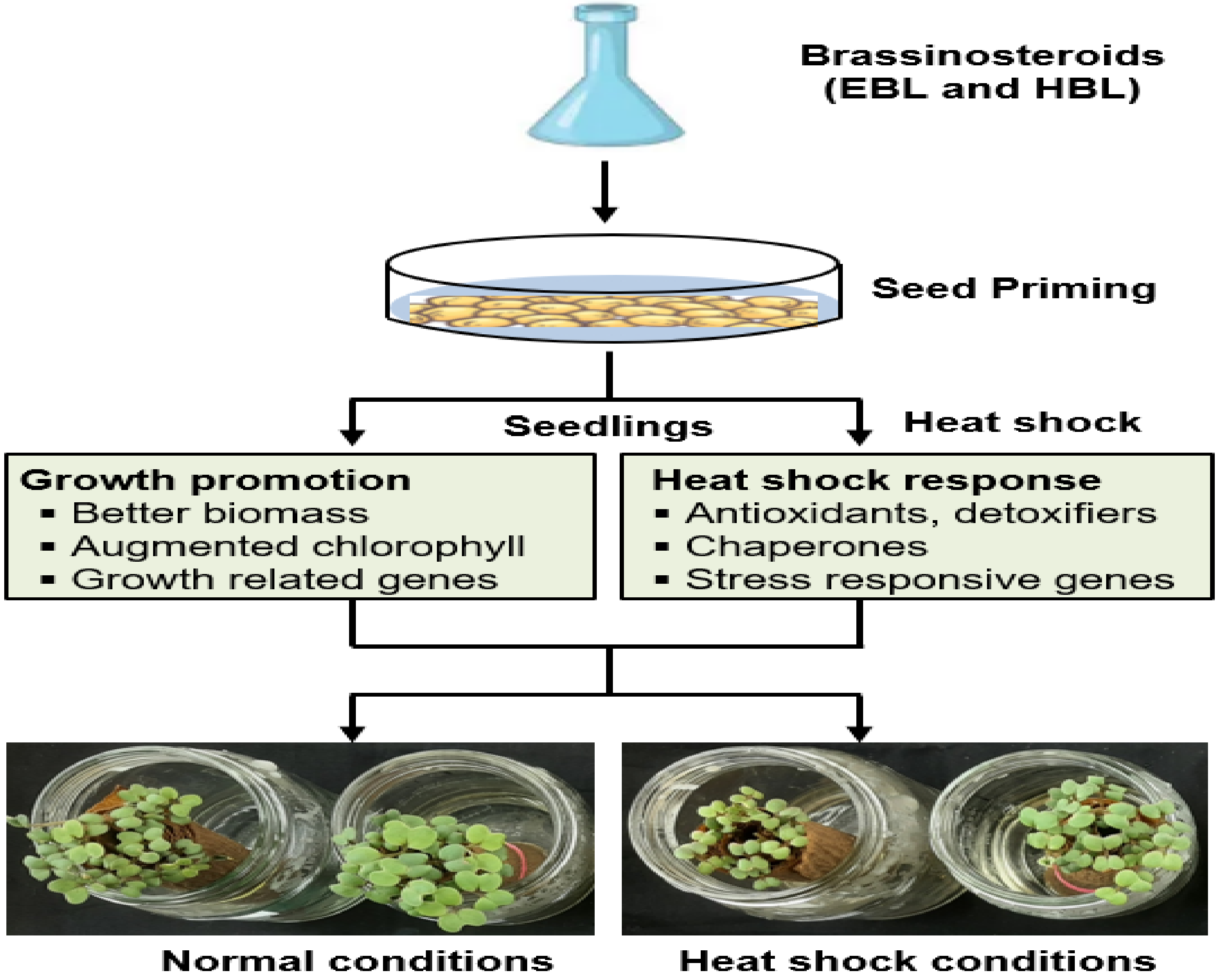


Figure 6. BRs-priming induced growth promotion and heat shock response protect *B. juncea* seedlings against heat shock. Seed priming with BRs leads to growth promotion and pro-activation of HSR. BRs-priming enhances biomass, photosynthetic pigments, and the expression of many growth-related genes. It also augments antioxidants, detoxifiers, chaperones and the expression of stress-responsive genes. Thus, seed priming with BRs enhances growth of seedlings under normal conditions and provides resilience to stress conditions.

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ORIGINAL RESEARCH

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Seed priming with brassinolides improves growth and reinforces antioxidative defenses under normal and heat stress conditions in seedlings of *Brassica juncea*

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Abstract

Environmental stresses pose a major challenge for plant researchers to fulfill increasing food demand. Researchers are trying to generate high-yielding and stress-tolerant or resistant varieties using classical genetics and modern gene-editing tools; however, both approaches have limitations. Chemical treatments emerged as an alternative to improve yield and impart stress resilience. Brassinosteroids (BRs) are a group of phytohormones that regulate various biological processes, including stress management. With foliar spray methods, BR treatments showed promising results but are not economically feasible. We hypothesize that priming of seeds, which requires lesser amounts of BRs, could be equally effective in promoting growth and stress tolerance. Owing to this notion, we analyzed the impact of priming seeds with selected BRs, namely, 24-epibrassinolide (EBL) and 28-homobrassinolide (HBL), in *Brassica juncea* under normal and heat shock stress conditions. Seeds primed with BRs and grown until seedlings stage at normal conditions (20°C) were subjected to a heat shock (35°C) for a few hours, relating to what plants experience in natural conditions. Heat shock reduced the growth and biomass with an increased accumulation of reactive oxygen species. As anticipated, BRs treatments significantly improved the growth and physiological parameters with an enhanced antioxidant defense under both conditions. Transcriptional analyses revealed that BRs concomitantly induce growth and oxidative stress-responsive gene expression via the canonical BR-signaling pathway. Transfer of unstressed and heat-shock-treated seedlings to field conditions demonstrated the long-term effectivity of BR-priming. Our results showed seed priming with BRs could improve growth and resilience against heat shock; hence, it appears to be a viable strategy to enhance crop yields and stress tolerance.

1 | INTRODUCTION

Abiotic stresses resulting from environmental perturbations pose a constant challenge to plants. These environmental constraints deteriorate overall productivity. Among various environmental cues, increasing temperature because of industrialization and modernization presents a significant threat to plants (Dash & Mallick, 2017; Yang et al., 2013). It is a severe hazard for nourishing crop production in the productive regions of tropical and subtropical areas. The global average temperature has enhanced by 1°C over the past 100 years and is also anticipated to rise at a quick pace in the future. Hence, increased temperature or resulting heat stress has become a major threat limiting crop production around the globe (Abdelrahman et al., 2017). In general, plants throve well at a specific temperature range; however, fluctuations in threshold temperature negatively impact growth, nutritional values, and overall productivity. It has been predicted that every

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