

Reviewers' report and authors' answers for the manuscript submitted to Metabolites, MDPI, Special issue.

2nd reviewer

Comments and Suggestions for Authors

We sincerely appreciate your time and expertise in reviewing our work. We have carefully considered your suggestions and revised the manuscript accordingly.

In the revised manuscript, changes are highlighted in yellow.

Below, we also provide a point-by-point response explaining how we have addressed each reviewer's comment.

Major comment

Although the review comprehensively summarizes phytohormone-mediated signaling pathways under abiotic stress, it provides limited discussion on how these hormonal regulatory networks can be practically exploited for crop improvement through biochemical, molecular, and biotechnological interventions. The manuscript would be substantially strengthened by including a dedicated section discussing translational approaches such as exogenous hormone priming; metabolic engineering; synthetic biology; marker-assisted breeding; and especially CRISPR/Cas-mediated genome editing of hormone biosynthesis, transport, receptor, and signaling genes. Integration of recent advances in editing ABA, auxin, ethylene, JA, and strigolactone-related pathways for improving drought, salinity, and heat tolerance would significantly enhance the novelty, applicability, and future perspective of the review.

We sincerely thank the reviewer for this valuable suggestion. We agree that translational approaches such as hormone priming, metabolic engineering, synthetic biology, marker-assisted breeding, and CRISPR/Cas genome editing are highly important for improving abiotic stress tolerance in crops. However, we would like to clarify that the main scope of this review is the molecular and signaling mechanisms underlying phytohormone-mediated abiotic stress responses, with an emphasis on hormone biosynthesis, perception, signaling pathways, and crosstalk among major phytohormones. Therefore, the manuscript focuses on providing a mechanistic framework rather than detailed coverage of biotechnological and breeding applications. We believe that the topics suggested by the reviewer are better suited for a separate review specifically dedicated to translational and crop improvement strategies. We appreciate the reviewer's comment, which highlights an important future direction in this field.

Minor comments

Lines 30–31: The sentence “which are among the most significant limitations to crop growth and productivity worldwide” is somewhat generic and could be revised to a more precise scientific expression such as “major constraints on crop growth and productivity worldwide.”

The requested change has been applied, and the revised sentence has been highlighted in yellow in the manuscript.

Lines 31–33: The phrase “drought, salinity, extreme temperatures, and imbalances” is vague, particularly the term “imbalances.” The authors should clarify whether this refers to nutrient imbalance, ionic imbalance, or other environmental perturbations.

“Imbalances” was intended to refer to ion imbalances; however, to avoid ambiguity and in line with the reviewer’s suggestion, this term has been removed (highlighted in yellow on line 32). The sentence now focuses on drought, salinity, and temperature extremes, the main abiotic stresses addressed in this review.

Lines 34–36: The statement “Recent global assessments indicate that such stresses account for nearly half of the observed yield losses in key agronomic crops” is overly broad and requires stronger supporting evidence. The authors should provide recent high-impact references, specify the crop systems or geographic scope being discussed, and avoid generalized quantitative claims unless supported by robust meta-analyses or global datasets.

The statement has been revised to address the concern by replacing the original broad claim with a more specific one supported by a recent meta-analysis (Wan et al., 2022). The corresponding changes have been highlighted in the manuscript (lines 35 and 36).

Lines 37–39: The definition of drought stress as “sustained periods of below-normal soil moisture” is somewhat simplistic and does not fully capture the complexity of plant drought responses. The authors should consider including additional factors such as atmospheric water demand, evapotranspiration, developmental stage, and soil properties that collectively influence drought stress severity.

We agree that the initial definition in the Introduction was overly simplified and did not fully capture the complexity of drought stress in plants. However, due to the structural and length constraints of the Introduction section, it is not appropriate to provide an excessively detailed conceptual framework at that point. To address this, we have added a more comprehensive paragraph at the beginning of the drought section (lines 99–104), in which drought is defined more broadly, including soil moisture deficit, atmospheric demand, evapotranspiration, soil properties, and plant developmental stage.

Lines 44–47: The description of heat stress effects remains largely descriptive and would benefit from inclusion of molecular mechanisms such as protein denaturation, ROS accumulation, heat shock protein induction, and membrane fluidity alterations. This would improve the mechanistic depth of the introduction.

In the Introduction, we aimed to provide a concise overview of heat stress effects, while detailed molecular and cellular mechanisms—including ROS accumulation, protein misfolding, membrane destabilization, heat shock signaling, and HSP induction—are discussed extensively in the dedicated “Heat Stress” subsection of the abiotic stress section. Nevertheless, to enhance the mechanistic depth of the Introduction, we have revised the text to briefly incorporate key molecular aspects of heat stress (lines 43–49).

Lines 52–57: The salinity stress section is scientifically acceptable; however, the discussion remains descriptive and could be strengthened by briefly mentioning ionic toxicity, Na⁺/K⁺ homeostasis, SOS signaling pathways, or osmotic stress signaling mechanisms.

The mechanistic aspects of salinity stress, including ionic toxicity, Na⁺/K⁺ homeostasis, the SOS signaling pathway, and osmotic stress signaling, have been comprehensively covered in the dedicated “Salinity” subsection of the abiotic stress section. In the introduction, only a brief overview of salinity stress and its overall effects on plant growth and productivity was provided to maintain structural clarity and avoid redundancy.

Lines 63–66: The statement that “conventional breeding has shown limited success” is too generalized and potentially misleading. Significant progress has been achieved in breeding stress-tolerant cultivars in several crops. The statement should therefore be moderated and supported with more balanced discussion and recent references.

The sentence has been revised to provide a more balanced and accurate interpretation, acknowledging both the significant progress achieved through conventional breeding and the inherent limitations of conventional breeding in improving abiotic stress tolerance. The revised text is highlighted in yellow (lines 65–69). In addition, two recent references have been added to support this revised statement.

Lines 71–78: This section effectively summarizes major phytohormone-mediated responses under abiotic stress; however, it remains highly descriptive and lacks sufficient mechanistic integration. The Introduction would benefit from earlier mention of key signaling components such as ROS, Ca²⁺ signaling, MAPKs, SnRK2 kinases, transcription factors, and hormone receptor complexes.

We have revised this section to enhance its mechanistic depth and clarity, as suggested. The Introduction now includes earlier and more explicit mention of key signaling components involved in phytohormone-mediated abiotic stress responses, including ROS, Ca²⁺ signaling, MAPK cascades, SnRK2 kinases, transcription factors, and hormone receptor complexes. The revised text has been highlighted in yellow (lines 74–80).

Lines 79–83: The final objective statement is grammatically poor and requires revision for clarity. In particular, the phrase “While maintaining growth and productivity under unfavorable environmental conditions” appears incomplete and disconnected from the main sentence. The authors should revise this sentence to clearly state the scope, objectives, and novelty of the review article.

The sentence has been revised to improve clarity and grammar (lines 88–95). The incomplete phrase has been removed, and the statement has been rewritten to clearly define the objective of the review, including the roles of phytohormones in abiotic stress responses and their crosstalk and signaling interactions.

Lines 99–100: The sentence “Plants initiated rapid molecular signaling events” contains a grammatical inconsistency. It should be corrected (e.g., “plants initiate rapid molecular signaling events”).

The sentence has been corrected in line 119 (“initiated” → “initiate”).

Lines 130–132: The phrase “photosynthesis and respiration” is overly general. The authors may improve scientific depth by specifying impacts on photosystem stability, stomatal conductance, chloroplast ultrastructure, mitochondrial respiration, or Na⁺/K⁺ homeostasis.

We have added a new paragraph (lines 152–161) to provide a more detailed and mechanistic description of the effects of salinity stress on key physiological and cellular processes.

Lines 155–158 / Figure 2: The figure legend refers to the “bZIP” transcription factor family, whereas the corresponding label inside Figure 2 is written as “BZIP.” The terminology should be standardized throughout the figure and legend for consistency.

The abbreviation has been standardized throughout the manuscript and in the figure as “bZIP” for consistency.

Lines 165–166: The phrase “transport and nutrient movement” is somewhat vague. The authors should specify whether the phrase refers to membrane transport processes, phloem transport, ion transport, or aquaporin-mediated water movement.

The sentence has been revised to clarify which transport processes are affected by cold stress, including ion transport and aquaporin-mediated water movement (lines 193–196).

Lines 204–206: The heat-shock protein section is scientifically sound; however, the manuscript could be improved by distinguishing among major HSP classes (e.g., HSP70, HSP90, small HSPs) and their specific protective roles under thermal stress

We have revised the manuscript by adding a new paragraph that more clearly distinguishes the major heat-shock protein classes and their specific protective roles during thermal stress, including HSP70, HSP90, and sHSPs. The added section is highlighted in yellow in lines 236–245.

Lines 217–219 / Figure 4: The manuscript uses inconsistent notation for calcium ions throughout the text and figures (e.g., “Ca²⁺”, “Ca++”, and “Calcium”). The authors should standardize calcium ion representation consistently throughout the manuscript and figures. The conventional scientific notation “Ca²⁺” is recommended for clarity and consistency with plant signaling literature.

The notation for calcium ions has been standardized throughout the manuscript and figures as “Ca²⁺” to align with the plant signaling literature, and Figure 4 has been revised accordingly. Exceptions include the first mention of “calcium” in the text for clarity (followed by Ca²⁺), as well as established terms such as “calcium-dependent protein kinases” and phrases like “calcium-dependent signaling cascade/pathway,” where “calcium” is retained as part of accepted biological nomenclature.

Lines 229–494: Section 3 (“Phytohormones”) covers multiple hormone-mediated stress signaling pathways and hormonal interactions; however, the section would greatly benefit from the inclusion of a comprehensive integrative figure summarizing phytohormonal abiotic stress responses.

A comprehensive integrative figure summarizing phytohormone-mediated abiotic stress signaling pathways and their crosstalk has already been included in Section 4 (Figure 5). It collectively illustrates the interactions among the major hormonal networks under stress conditions.

Lines 231–233: The sentence describing ABA biosynthesis is grammatically incomplete and scientifically unclear. In particular, the phrase “establishing a direct metabolic link between stress” appears truncated and should be revised for clarity and completeness.

The sentence has been revised to improve grammatical structure and scientific clarity. The changes appear in lines 268–271.

Lines 231–233: The abbreviation “NCED” should be defined at its first occurrence as “9-cis-epoxycarotenoid dioxygenase (NCED).

The abbreviation NCED has been defined at its first occurrence in the revised manuscript (lines 265 and 266) as 9-cis-epoxycarotenoid dioxygenase (NCED).

Lines 463–494: The strigolactone section is informative, but the discussion would benefit from clearer explanation of SL biosynthesis, MAX signaling components, and interactions with ABA and auxin during abiotic stress adaptation

we have revised and reorganized the strigolactone section to improve clarity and logical flow. The text has been rewritten so that SL biosynthesis, perception/signaling via the MAX2-dependent pathway, and hormonal crosstalk with ABA and auxin during abiotic stress are presented in a clearer and sequential manner. The beginning of each section has been highlighted in yellow.

Lines 495–502: The introductory paragraph effectively introduces phytohormonal crosstalk during abiotic stress; however, the discussion remains highly condensed and would benefit from clearer conceptual framing distinguishing synergistic, antagonistic, and hierarchical hormone interactions.

A new paragraph has been added to the section (lines 539–559) to clarify and distinguish among synergistic, antagonistic, and hierarchical phytohormonal interactions, thereby improving the conceptual framework of the discussion.

Authors are thankful for the time you spent reading our manuscript.
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