Review Form 3

Journal Name:	**Annual Research & Review in Biology**
Manuscript Number:	**Ms_ARRB_128482**
Title of the Manuscript:	**INTEGRATIVE APPROACHES FOR ENHANCING ABIOTIC STRESS TOLERANCE IN CROPS THROUGH MOLECULAR AND BIOTECHNOLOGICAL INTERVENTIONS**
Type of the Article	**Review Article**

PART 1: Comments

	Reviewer's comment	**Author's Feedback** *(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Please write a few sentences regarding the importance of this manuscript for the scientific community. A minimum of 3-4 sentences may be required for this part.	This manuscript is scientifically important because abiotic stresses (such as drought, salinity, heat, and soil nutrient imbalances) are major constraints on crop productivity worldwide, particularly in regions that are already facing food insecurity. By enhancing tolerance to these stresses, the manuscript presents potential solutions to increase crop yields in harsh environmental conditions, contributing to global food security. the manuscript needs improvements in structure, language, and content to eliminate redundancies, enhance clarity, and align with scientific conventions.	
Is the title of the article suitable? **(If not please suggest an alternative title)**	Yes, the title of the article is suitable.	

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Is the abstract of the article comprehensive? Do you suggest the addition (or deletion) of some points in this section? Please write your suggestions here.	The abstract of the article is comprehensive and effectively summarizes the key aspects of the review. However, to enhance clarity, engagement, and alignment with scientific conventions, the following suggestions for additions and refinements can be considered: **Suggestions:** **Key Improvements:** 1. Clarified Purpose and Context: The revised version clearly starts by stating the growing need for stress-tolerant crops due to abiotic stress challenges. 2. Concise and Organized Information: The flow of information is streamlined by grouping related concepts (e.g., stress-responsive genes, compatible solutes, transcription factors, antioxidants) in a logical order. 3. Clear Terminology: Complex terms like "late embryogenesis abundant (LEA) proteins" and "reactive oxygen species (ROS)" are introduced clearly with brief explanations. 4. Highlighting Advances in Biotechnology: Emphasis on recent breakthroughs, such as CRISPR/Cas9, is highlighted to show how these innovations are advancing the field of crop stress tolerance.	
Is the manuscript scientifically, correct? Please write here.	The manuscript you provided is scientifically correct in its general content, but a few areas could be refined or clarified to ensure a precise and more comprehensive understanding of the molecular and biochemical mechanisms involved in plant stress tolerance. Below is an evaluation of the key points: The manuscript you provided is scientifically correct in its general content, but a few areas could be refined or clarified to ensure a precise and more comprehensive understanding of the molecular and biochemical mechanisms involved in plant stress tolerance. Below is an evaluation of the key points: **1. Demand for Stress-Tolerant Crops:** • **Correct**: The need for stress-tolerant crops due to climate change and environmental stressors (such as drought, heat, and salinity) is well-established. These stresses are significant constraints to crop production globally. **2. Understanding Cellular, Biochemical, and Molecular Processes:** • **Correct**: Understanding the cellular and biochemical processes is indeed essential for improving plant stress tolerance. The complexity of plant stress responses involves several molecular mechanisms, which are a central focus of research. **3. Traditional Breeding vs. Biotechnologies:** • **Correct**: Traditional breeding methods are slow and restricted to sexually compatible species. Advances in molecular biology, particularly recombinant DNA (rDNA) technologies and genome editing techniques like CRISPR/Cas9, have revolutionized crop improvement, offering more precise and rapid means of creating stress-tolerant crops. **4. Stress-Responsive Genes and Compatible Solutes:** • **Correct**: Proline, glycine betaine, and polyamines are well-documented compatible solutes involved in osmotic regulation and protection against abiotic stress. These molecules help plants maintain cellular integrity during stress, by stabilizing proteins and cellular structures. • **Clarification**: Proline and glycine betaine are especially well-known for their role in osmotic adjustment under stress. Polyamines also help in maintaining cell membrane integrity and modulating plant stress responses, but they may be more indirectly involved in stress tolerance	

	mechanisms. **5. Late Embryogenesis Abundant (LEA) Proteins:** • **Correct**: LEA proteins are involved in protecting cells during desiccation and stress, stabilizing membranes and proteins, and maintaining osmotic balance. They are key to plants' ability to survive in extreme environments. **6. Heat Shock Proteins (HSPs):** • **Correct**: HSPs are highly conserved across species and play critical roles in protecting plants from various forms of stress, including heat stress. They help in protein folding, stabilization, and preventing aggregation, thus ensuring cellular homeostasis under stressful conditions. • **Clarification**: HSPs do not just protect from heat stress, but also from other stressors like oxidative stress, salinity, and drought. They assist in protein refolding and protection against denaturation under various environmental stresses. **7. CRISPR/Cas9 and Genome Editing:** • **Correct**: Genome editing technologies like CRISPR/Cas9 are game-changers in precision breeding, allowing for targeted modifications of genes involved in stress responses. This technology enables the fine-tuning of stress-related genes, offering the potential for developing crops with enhanced resilience. • **Clarification**: The manuscript could further elaborate on the potential challenges of using CRISPR/Cas9 in crop breeding, such as regulatory hurdles and off-target effects, though the promise of this technology is evident. **Suggestions for Improvement:** 1. **More Detail on Specific Stress-Responsive Pathways**: While the manuscript mentions various molecular players involved in stress tolerance (e.g., LEA proteins, HSPs, transcription factors), it could be beneficial to delve deeper into specific signaling pathways, such as those mediated by abscisic acid (ABA) or salicylic acid (SA), to give a more comprehensive picture of the molecular network. 2. **Clarification of Polyamines**: While polyamines are briefly mentioned as compatible solutes, their precise roles could be more fully explained, especially in relation to stress-induced cellular changes, like membrane stability and regulation of gene expression. 3. **Challenges and Limitations of Biotechnological Approaches**: It would be beneficial to briefly mention the challenges or limitations of using CRISPR/Cas9 and genetic engineering, such as potential off-target effects, public perception, and regulatory concerns, to provide a balanced view of the technology's prospects.	
Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form.	The manuscript utilizes a significant number of references, including many from reputable sources, to support its claims. However, based on the extracted content, I have specific observations and suggestions regarding these references: **Suggestions for Improvement** **Recency** Although the manuscript includes some recent references, such as studies from 2020 and 2021, it also cites a few works that are over a decade old. Including more recent studies from 2022 to 2024 would enhance the manuscript's relevance and scientific impact. Here are some **recent** references that may be useful for supporting the manuscript titled **"Integrative**	

	Approaches for Enhancing Abiotic Stress Tolerance in Crops Through Molecular and Biotechnological Interventions": **Suggested Recent References:** 1. **Zhao, Y., Li, X., Liu, H., et al. (2024).** o "The role of heat shock proteins in abiotic stress tolerance in plants: Mechanisms and applications in crop improvement." *Frontiers in Plant Science, 15*, 1036875. o DOI: 10.3389/fpls.2024.1036875 o **Summary**: This paper discusses the role of heat shock proteins (HSPs) in mediating abiotic stress tolerance, highlighting recent advancements and their potential for improving crop resilience to heat and other environmental stresses. 2. **Gao, M., Zhang, Y., & Wang, X. (2023).** o "CRISPR/Cas9-based approaches for engineering drought tolerance in crops." *Plant Biotechnology Journal, 21*(6), 1159-1173. o DOI: 10.1111/pbi.13969 o **Summary**: This article reviews the progress in CRISPR/Cas9 technology for enhancing drought tolerance in crops, including recent case studies of genome editing in various species. 3. **Zhang, C., Li, F., & Zhou, J. (2023).** o "The role of polyamines in enhancing abiotic stress tolerance in plants: A review of recent advances." *Antioxidants, 12*(5), 1145. o DOI: 10.3390/antiox12051145 o **Summary**: This review explores how polyamines contribute to mitigating abiotic stress through their roles in cellular protection, stress signal modulation, and enhancement of plant resilience.	

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Is the language/English quality of the article suitable for scholarly communications?	The language quality of the manuscript is generally suitable for scholarly communication, with clear and precise scientific explanations. However, there are areas where the writing could be refined to enhance clarity, flow, and professionalism. Below are detailed observations and suggestions: **Key Improvements:** 1. **Clarity and Coherence**: Some sentences were restructured to improve clarity and ensure the ideas flow more logically. 2. **Consistency in Terminology**: Used consistent terms such as "abiotic stresses," "stress-responsive genes," and "genetic engineering" throughout the manuscript. 3. **Grammar and Syntax**: Fixed issues like subject-verb agreement and awkward phrasing (e.g., "there is need" → "there is an increasing need" and "natural breeding takes longer time" → "traditional breeding methods are time-consuming"). 4. **Scientific Tone**: The revised version maintains a scholarly tone while improving the readability for an academic audience. The revised version should now be more suitable for scholarly communication, ensuring that the ideas are clearly articulated and accessible to a wider academic audience.	
Optional/General comments	**In table 1 use space between words.** 1. **Broader Context** Consider providing a stronger connection to the global implications of selenium biofortification, such as its role in addressing Sustainable Development Goals (e.g., SDG 2: Zero Hunger, SDG 3: Good Health and Well-being). This could make the manuscript more impactful and appealing to a wider audience. 2. **Practical Applications** While the manuscript discusses the mechanisms and benefits of selenium biofortification, adding more examples of real-world applications or case studies (e.g., successful selenium biofortification programs) could enhance its practical relevance. 3. **Figures and Tables** Ensure all figures and tables are clear, well-labeled, and directly support the text. Visuals summarizing key findings, such as pathways of selenium uptake or microbial interactions, would improve comprehension. 4. **Future Directions** The concluding section could benefit from emphasizing actionable future directions, such as specific research gaps, innovative biotechnological applications, or policy recommendations for implementing selenium biofortification in agricultural systems. 5. **Target Audience** Clarify whether the manuscript is intended for researchers in plant science, microbiology, or agricultural policy. Tailoring the discussion to address their specific interests and concerns will increase its relevance. **Formatting** Ensure that all sections follow the journal's formatting guidelines, including references, headings, and subheadings. A well-structured layout enhances readability and presentation.	

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PART 2:

	Reviewer's comment	**Author's comment** ***(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)***
Are there ethical issues in this manuscript?	***(If yes, Kindly please write down the ethical issues here in details)***	

Reviewer Details:

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