Review Form 1.7

Journal Name:	Journal of Advances in Biology & Biotechnology
Manuscript Number:	**Ms_JABB_114560**
Title of the Manuscript:	**Biochemical Changes Play a Significant Role in Development of Fungal Disease Resistance in Mustard**
Type of the Article	

Review Form 1.7

PART 1: **Review Comments**

	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)*
Compulsory REVISION comments **1. Is the manuscript important for scientific community?** (Please write few sentences on this manuscript) **2. Is the title of the article suitable?** (If not please suggest an alternative title) **3. Is the abstract of the article comprehensive?** **4. Are subsections and structure of the manuscript appropriate?** **5. Do you think the manuscript is scientifically correct?** **6. Are the references sufficient and recent? If you have suggestion of additional references, please mention in the review form.** **(Apart from above mentioned 6 points, reviewers are free to provide additional suggestions/comments)**	**Title suggestions** Great! Let's review the title of the article "Biochemical Changes Play a Significant Role in Development of Fungal Disease Resistance in Mustard." Overall, the title provides a clear indication of the research focus. However, here are a few suggestions for improvement: **Clarity and Specificity:** • Consider specifying the type of mustard being studied (e.g., Indian mustard, Brassica juncea) for clarity. • If applicable, include the specific fungal pathogen or pathogens under investigation. Revised Title: "Biochemical Changes Driving Fungal Disease Resistance in Brassica juncea: Unraveling the Role of Specific Mustard Varieties" **Conciseness:** • While the title is informative, it can be made more concise without losing essential information. Revised Title: "Biochemical Drivers of Fungal Resistance in Brassica juncea" **Active Voice:** • Consider using active voice for a more direct and engaging tone. Revised Title: "Unveiling Biochemical Drivers of Fungal Resistance in Brassica juncea" **Inclusion of Keywords:** • Ensure that important keywords related to the research are included for better discoverability. Revised Title: "Exploring Biochemical Determinants of Fungal Resistance in Brassica juncea for Enhanced Disease Management" **Abstract suggestions** The abstract provides a comprehensive overview of the study on the biochemical changes associated with fungal disease resistance in mustard. While the content is informative, here are some suggestions to enhance clarity and precision: **Title Reference:** • Consider incorporating specific mention of the disease-resistant mustard genotypes in the title for better alignment with the abstract content. Revised Title: "Biochemical Basis of Fungal Disease Resistance in Disease-Resistant Mustard Genotypes" **Introduction of the Problem:** • Provide a brief introduction to the challenges faced by rape-seed mustard due to biotic stresses and the subsequent need for disease-resistant genotypes. Revised Abstract: "Rape-seed mustard, a crucial edible oilseed crop, faces significant challenges from biotic stresses, leading to the dependence on imported edible oils. This study focuses on screening mustard genotypes for disease resistance against major fungal diseases." **Clarity in Disease Resistance Findings:** • Reorganize the sentence about disease indexing to clearly state the genotypes that were found immune against specific fungal diseases. Revised Abstract: "Mustard genotypes L-4, GSC-7, and PC-6 exhibited immunity against Alternaria brassicae. Genotypes L-4, China, GSL-1, GSC-7, PC-5, PC-6, and RP-9 demonstrated immunity against Albugo candida, while L-4 and PC-5 were found immune against Erysiphe cruciferarum."	

Biochemical Parameters:

- Organize the information about biochemical constituents to improve readability and emphasize the key findings.

Revised Abstract: "The study of biochemical constituents revealed that immune genotypes accumulated higher osmolytes, including free proline and total phenol, under diseased conditions. These genotypes displayed tolerance by exhibiting lower lipid peroxidation rates, as indicated by reduced malondialdehyde content. Furthermore, they had a lesser impact on total chlorophyll, amino acids, proteins, and soluble sugars, including reducing and non-reducing sugars."

Closing Statement:

- Conclude the abstract by emphasizing the significance of the identified genotypes for future research and breeding efforts.

Revised Abstract: "The identified genotypes, characterized by their disease resistance and associated biochemical changes, present promising candidates for further phytopathological studies. Their utilization in breeding programs holds potential for the development of mustard varieties with enhanced fungal resistance."

The introduction provides a comprehensive overview of the significance of mustard as an oilseed crop, its various uses, and the challenges it faces due to fungal diseases. However, there are a few areas where the language can be refined for better clarity and flow. Here are some suggested improvements:

Mustard stands as a prominent oilseed crop, covering approximately 24% of the nation's total oilseed area and production [1-2]. Its global cultivation extends beyond its role as an agricultural staple, as it serves as a potential source for biodiesel production [3-7]. The natural compounds within the mustard crop make it a crucial industrial resource, with the residual cake serving as valuable animal feed [8-11, 3]. Boasting a protein content of 25-30%, mustard not only finds use as an oil in commercial and industrial applications but also contributes significantly to the food supply [12-15]. Moreover, the mustard plant serves as a natural immunity booster and aids in removing heavy metals from the biological pollution central system [16-22].

Despite India ranking as the second-largest producer of rapeseed-mustard, the nation struggles to meet the demand, leading to substantial spending on edible oil imports due to the prevalence of diseases and insect pests [23-26]. White rust (Albugo candida), Alternaria blight (Alternaria brassicae), and powdery mildew (Erysiphe cruciferarum) cause annual yield losses ranging from 17% to as high as 71%, underscoring the urgent need for disease-resistant varieties [27-28, 4].

Plants respond to various biotic stresses, including fungal infections, at both cellular and molecular levels. The resistance exhibited by plants against diseases often correlates with specific biochemical parameters, whether inherent or induced in response to the disease [29]. While disease control through fungicides is discouraged due to challenges in achieving comprehensive foliage coverage and environmental concerns, breeding for resistance emerges as a highly advantageous alternative. However, the genetic foundation of cultivated Brassica is limited, with scarce resistance genes. The repetitive cultivation of susceptible varieties exacerbates yield losses, particularly in the absence of resistance sources against prevalent diseases like Alternaria blight and white rust [30, 5].

In this context, there is a critical need to identify and introduce mustard genotypes resistant to Alternaria blight, white rust, and powdery mildew. This study aims to screen such resistant genotypes based on disease indexing and explore the potential impact of biochemical responses on disease incidences.

Feel free to adapt these suggestions according to your preferences and the specific details of your research

The Methods section provides a detailed description of the experimental setup, plant material, disease screening, and biochemical analyses. Here are some suggestions for refining and organizing the content:

Experimental Setup: The study was conducted at the Research Farm of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India (26.22°N, 77.45°E) during the Rabi season of 2020–2021. Biochemical analyses were carried out at the Biochemical Analysis Laboratory, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, RVSKVV, Gwalior.

Plant Material: A total of 75 mustard genotypes were obtained from the All India Coordinated Research Project on Rapeseed and Mustard, Zonal Agricultural Research Station, Morena, RVSKVV, Gwalior, M.P., India (see Table 1).

Crop Raising: Mustard genotypes were sown in the experimental field of the Department of Genetics & Plant Breeding, College of Agriculture, RVSKVV, Gwalior. The sowing was done in a randomized block design with three replications under controlled and diseased conditions. Each genotype was planted in a 2-meter-long row with a spacing of 30 cm between rows and 15 cm between plants. Randomly selected plants were labeled for observation purposes.

Field Screening for Disease Assessment: The 0-9 disease rating scale was employed to calculate the percent disease intensity for three fungal diseases: Alternaria blight, white rust, and powdery mildew. Disease incidence was monitored regularly, and the severity score was used to determine the percent disease intensity (PDI) as per the provided formulas. The disease rating scale is detailed in List 1.

Biochemical Analysis: Biochemical parameters were assessed in healthy and diseased leaves twice: first on day 35 and then on day 70 after sowing.

Lipid Peroxidation Rate:

- MDA content was determined according to the method of Naserwafaei et al. [31].

Fungal Effect on Biochemical Parameters:

- Chlorophyll content was estimated following the method of Arnon [32].
- Total amino acids were determined by the method of Moore and Stein [33].
- Protein estimation was conducted using the Lowery et al. method [34].
- Total sugar estimation followed the protocol described by DuBois et al. [35].
- Reducing and non-reducing sugar were estimated using the DNSA method proposed by Miller [36].

Antioxidant Accumulation:

- Proline concentration was estimated following Bates et al.'s method [37].

- Phenol concentration was determined according to the procedure suggested by Swain and Hills [38].

Statistical Analysis: Biochemical responses from both healthy and diseased plants were subjected to diversity assessment among different mustard genotypes. Principal component analysis, dendrogram, and heatmap were generated to identify the significance of different biochemical responses towards the development of fungal disease resistance.

Feel free to adapt this structure according to your preferences and the specific details of your research.

Results and Discussion:

Assessment of Mustard Genotypes for Disease Resistance:

The study aimed to evaluate the tolerance/resistance of 75 mustard genotypes against Alternaria blight, white rust, and powdery mildew. Field screening based on a 0-9 disease intensity scale revealed distinctive symptoms for each fungal disease. Mustard genotypes were categorized based on their reactions to these diseases.

White Rust Invasion:

- Immune genotypes: Maya, L-4, China, GSL-1, GSC-7, PC-5, PC-6, RP-9.
- Highly resistant: JMWR-908-1.
- Resistant: WRR-15, WRR-25.
- Moderately resistant: Vasundhara, Pusa Jagannath, Kiran, PM-27, JMM-991, WRR-5, WRR-7, WRR-11, WRR-12, WRR-14, WRR-16, WRR-17, WRR-19, WRR-26, WRR-27, WRR-29, WRR-31, WRR-32.
- Susceptible: Others.

Powdery Mildew Infestation:

- Immune genotypes: L-4, PC-5.
- Highly resistant: China, RP-9.
- Resistant: GSC-7, PC-6.
- Moderately resistant: RB-50, Pusa Bold, WRR-10, GSL-1.
- Susceptible: Others.

Alternaria Infestation:

- Immune genotypes: L-4, GSC-7, PC-6.
- Highly resistant: China, GSL-1, RP-9.
- Resistant: Pusa Bold, Kranti, Maya, Kiran, JM-2.
- Moderately resistant: RB-50, Varuna, Rohini, Vardan, Vasundhara, Swarn Jyoti, Pusa Jagannath, Shraddha, DMH1, JMWR-908-1, NRC-HB-101, NRC-HB-506, RVM-3, RH-749, NRCDR-2, DRMRIJ-31, PC-5, JM-1, JM-3, RMM-10-01-01, RMM-12-01-18, WRR-5.
- Susceptible: Others.

The identified genotypes with immune or resistant characteristics have potential implications for enhancing resistance in mustard crops, providing valuable resources for further phytopathological studies and resistance breeding.

Biochemical Alterations in Mustard Leaves due to Fungal Infection:

The study investigated biochemical changes in healthy and diseased mustard leaves, focusing on parameters such as MDA, chlorophyll, amino acids, proteins, reducing/non-reducing sugars, total soluble sugar, phenols, and proline.

MDA Content:
- Increased in diseased leaves, indicating lipid peroxidation caused by biotic stress.
- Positive association with lipid peroxidation, potentially weakening cell wall integrity.

Chlorophyll Content:
- Decreased in diseased leaves, indicating reduced photosynthesis.
- Resistant genotypes exhibited higher chlorophyll content than susceptible ones.

Protein Content:
- Higher in genotypes vulnerable to white rust.
- Decreased with increasing white rust severity.

Amino Acid Levels:
- Increased under diseased conditions, indicating proteolysis and denaturation of membrane proteins.
- Proteins play a role in plant defense against invading pathogens.

Proline Levels:
- Amplified under fungal infection stress.
- Contributes to resistance by inducing proteolysis and denaturation of membrane proteins.

Total Phenol Content:
- Reduced as white rust severity increased.
- Resistant genotypes had higher total phenol content.

Sugar Levels:
- Higher in resistant genotypes.
- Decreased with increasing severity of fungal infection.

Heatmap Clustering:
- Classified 75 mustard genotypes into three major clusters based on biochemical parameters.
- Cluster I, Cluster II (subclusters IIA, IIB, IIC), and Cluster IV.
- Smallest Cluster IV had unique genotypes JMM-991 and NRC-HB-505.

The heatmap analysis highlights the diverse biochemical responses among mustard genotypes, providing insights into their resistance mechanisms.

Conclusion:

The research demonstrated the diverse responses of 75 mustard genotypes to Alternaria blight, white rust, and powdery mildew. The identified immune and resistant genotypes hold promise for enhancing disease resistance in mustard crops. Biochemical analyses revealed significant alterations in MDA, chlorophyll, amino acids, proteins, sugars, phenols, and proline, emphasizing the complex interplay between physiological and biochemical responses to fungal infections. The clustering of genotypes based on biochemical parameters further underscored the variability in their resistance mechanisms. These findings contribute valuable insights for further phytopathological studies and the development of mustard varieties with enhanced fungal disease resistance.

Conclusion:

In the quest for developing resistance against fungal infections in Brassicaceae cultivars, the role of genetic management has proven pivotal in shaping the dynamics of host-pathogen interactions. Beyond the realm of host genetics, the study emphasizes the significant impact of biochemical constituents on the severity of diseases. Regardless of their tolerance status, all biochemical

	constituents were found to influence the plant's response to fungal pathogens. The disease indexing revealed specific mustard genotypes that exhibited exceptional resistance against key fungal pathogens. Mustard genotypes L-4, GSC-7, and PC-6 were identified as immune against Alternaria brassicae, while Maya, L-4, China, GSL-1, GSC-7, PC-5, PC-6, and RP-9 demonstrated immunity against Albugo candida. Additionally, L-4 and PC-5 were classified as immune against Erysiphe cruciferarum. These immune genotypes, characterized through disease indexing, showcased their remarkable ability to resist fungal infections. The in-depth study of biochemical constituents provided insights into the mechanisms underlying the resistance of these genotypes. The immune genotypes exhibited lower susceptibility to pathogens by accumulating higher levels of osmolytes in diseased conditions. This accumulation of osmolytes in response to fungal infections highlighted the tolerance capacity of these genotypes. The identified immune genotypes, namely L-4, GSC-7, PC-6, Maya, China, GSL-1, PC-5, and RP-9, hold immense potential for further exploration in phytopathological studies and fungal resistance breeding. The broader implications of these findings extend to the development of mustard varieties with enhanced resistance, contributing to sustainable and resilient agricultural practices in the face of fungal challenges.	
Minor REVISION comments 1. **Is language/English quality of the article suitable for scholarly communications?**	Language is suitable but essential changes are added in comments	
Optional/General comments		

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:

Name:	**Anjum Faraz**
Department, University & Country	**University of Agriculture, Pakistan**