Review Form 3

Journal Name:	**Journal of Advances in Biology & Biotechnology**
Manuscript Number:	**Ms_JABB_127341**
Title of the Manuscript:	**Identification of Superior Forage Pearl Millet Genotypes through Multi-Trait Analysis and Cluster Grouping in Diverse Temporal Semi-Arid Environments**
Type of the Article	**Research paper**

PART 1: Review Comments

Compulsory REVISION 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. Why do you like (or dislike) this manuscript? A minimum of 3-4 sentences may be required for this part.	This manuscript is important for the scientific community. It highlights genetic variability and key traits in forage pearl millet. The study identifies high-yielding genotypes for breeding programs. It supports sustainable livestock systems in arid regions. These findings address the global demand for quality fodder. I like this manuscript as it focuses on improving forage pearl millet, vital for arid regions. It provides detailed analysis of genetic variability and trait associations. Advanced tools like PCA and clustering enhance the reliability of results. The study supports breeding programs to meet rising fodder demand. It aligns with global goals for sustainable livestock farming.	
Is the title of the article suitable? **(If not please suggest an alternative title)**	The title is clear and informative. However, for better readability and focus, an alternative could be "Multi-Trait Analysis and Clustering of Forage Pearl Millet Genotypes in Semi-Arid Conditions"	
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.	**Too much long.** The abstract is generally comprehensive and effectively summarizes the key aspects of the study. "The present study evaluated genetic variability and trait associations in 103 forage pearl millet genotypes (97 genotypes and 6 checks) across three distinct environments (e.g., the Kharif season of 2022 (E1), Summer season of 2023 (E2), and Kharif season of 2023 (E3)) during 2022-23. Sixteen morphological traits were assessed using a randomized complete block design with two replications. Significant differences were observed among genotypes, environments, and genotype × environment interactions. Correlation analysis identified key traits positively associated with green fodder yield, such as leaf length, leaf width, and plant height at first cut. Principal component analysis revealed that four principal components explained 72% of the total variability, with the first component influenced by leaf count and width. Hierarchical cluster analysis grouped genotypes into five clusters, with Cluster IV showing superior forage traits. This study identifies promising genotypes for future breeding programs focused on improving forage productivity in arid and semi-arid regions.	
Are subsections and structure of the manuscript appropriate?	The manuscript structure is generally appropriate	
Please write a few sentences regarding the scientific correctness of this manuscript. Why do you think that this manuscript is scientifically robust and technically sound? A minimum of 3-4 sentences may be required for this part.	The manuscript is scientifically robust and technically sound, as it employs standard and widely accepted analytical methods, such as Pearson correlation, principal component analysis (PCA), and hierarchical clustering, to assess the relationship between traits and their contribution to forage yield in pearl millet. The study is well-grounded in established research, with results that align with findings from previous studies on forage millet, such as those by Chaudhary et al. (2015) and Gupta et al. (2022). The use of PCA and cluster analysis provides valuable insights into trait associations and genetic diversity, which are essential for targeted breeding strategies. The manuscript also incorporates a strong theoretical framework, making it a valuable contribution to the field of forage crop improvement.	

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Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form. **-**	**Sufficient**	

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Minor REVISION comments **Is the language/English quality of the article suitable for scholarly communications?**	Yes, the language and English quality of the article are suitable for scholarly communication, but there is room for improvement. Some sections could benefit from clearer sentence structure and more precise phrasing to enhance readability. Additionally, refining the flow of ideas and ensuring consistent use of terminology would strengthen the manuscript. Addressing these aspects would make the article more polished and better suited for academic audiences.	
Optional/General comments	**Introduction** Here are some suggestions for the authors to improve the introduction in a concise and structured manner: o Consider reducing the details of the crop's geographical distribution and production statistics. Focus on key points relevant to the study, such as the importance of forage pearl millet in arid and semi-arid regions. o Combine the first and second paragraphs to streamline the explanation of pearl millet's significance, both as a crop and its role in fodder production. o Emphasize the need for genetic diversity and high-yielding varieties, specifically in the context of the breeding challenges for forage pearl millet. This can be done in a single sentence summarizing the aim of the study. o Provide a brief mention of the methods (ANOVA, PCA, cluster analysis) without going into too much detail. Focus on how these methods will be used to address the research question. o Eliminate repeated information about the importance of statistical tools like correlation analysis and PCA. Combine similar points for clarity and flow. o The last part of the introduction should clearly outline the aim of the study, i.e., evaluating genetic variability, trait associations, and genetic diversity in pearl millet. o Use transitions to connect the discussion of the statistical methods and the context of the study, ensuring a smooth flow of ideas. By making these adjustments, the introduction can be made more concise and focused, while retaining the necessary background and context for the study. Materials and methods : some suggestions for improving the "Materials and Methods" section: **2.1 Experimental Material and Design:** o Provide more details on how the 103 genotypes were selected (e.g., selection criteria, diversity, etc.) to give readers a clearer understanding of the genetic material used. o Mention the number of replications in each block for clarity. For example, how many plants were included per plot for each replication. o Briefly mention the key seasonal differences (e.g., temperature, rainfall) between the three environments to highlight why these were chosen as distinct temporal environments. o In the section describing the design, "germplasm subdivided into four distinct trials" could be rephrased as “experimental material divided into four trial groups” for clearer expression. **2.2 Data Collection and Statistical Analysis:** o The list of traits is comprehensive, but it may be better to group similar traits together for easier reading. For example, group all yield-related traits (e.g., GFYF, DFYF, TGFY) under one subheading or point. o Clearly mention if all 16 traits were measured across all environments or if any environment-specific measurements were made. A brief explanation of how data were collected (e.g., sample size, timing) would be helpful. o When mentioning software packages like "Metan," "MASS," and "factoextra," briefly explain their purpose and why they were chosen (e.g., "Metan for Genotype-by-environment interaction analysis" for context). o The sentence “Data processing and statistical computations were executed using R statistical software version 4.4.0 (2024-04-24 ucrt) within the RStudio” can be	

	simplified to: “Data analysis was performed using R version 4.4.0 (2024-04-24 ucrt) in RStudio.” o It would be helpful to mention how many clusters were formed or the criterion used for the cluster analysis to provide more context on the genetic diversity exploration. o Include a brief explanation of how PCA helped in the analysis. For example: “PCA was used to identify key patterns and reduce dimensionality, revealing the main drivers of variation in the genotypes.” o Ensure that terms like "genotype-by-environment interaction effects" and "trait-genotype association biplots" are consistently defined and explained, especially for readers unfamiliar with advanced statistical methods. **PLEASE SEE ATTACHMENT**	

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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