

INTEGRATED BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF CLIMATE-RESILIENT MUNGBEAN (*VIGNA RADIATA* (L.) R. WILCZEK) GENOTYPES FOR TOLERANCE TO TERMINAL HEAT AND WATER STRESS UNDER STAGGERED PLANTING ENVIRONMENTS

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ABSTRACT

Background: Terminal heat and water stress are major constraints affecting mungbean productivity under changing climatic conditions. The present study aimed to identify climate-resilient mungbean genotypes through integrated biochemical and molecular characterization under staggered planting environments.

Methods: Five mungbean genotypes (PDM 139, Pusa Ratna, Pusa Vishal, Pusa 1431, and TJM 3) were evaluated under three sowing dates (12 February, 27 February, and 14 March) during summer seasons of 2022–23 and 2023–24. Biochemical traits, yield attributes, and RAPD-based molecular diversity were assessed to determine stress tolerance.

Result: Delayed sowing enhanced stress-related biochemical responses, while early sowing recorded higher protein content. Among the genotypes, PDM 139 produced the highest seed yield (730.42 kg ha⁻¹), with maximum yield observed under 27 February sowing (826.02 kg ha⁻¹). RAPD analysis using 25 primers generated 75 loci, of which 54 were polymorphic, with an average polymorphism of 69.49%. Cluster analysis grouped the genotypes into three distinct clusters, revealing substantial genetic diversity. Integration of biochemical and molecular data identified PDM 139 as the most promising genotype for tolerance to terminal heat and water stress. The study highlights the potential of combining biochemical and molecular markers for developing climate-resilient mungbean cultivars.

KEYWORDS: Mungbean, climate resilience, terminal heat stress, water stress, RAPD, biochemical traits, genetic diversity, seed yield, abiotic stress tolerance.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) R. Wilczek) is an important short-duration grain legume cultivated extensively in tropical and subtropical regions of Asia, Africa, and Australia. It serves as a valuable source of dietary protein, carbohydrates, vitamins, minerals, and bioactive compounds, contributing significantly to nutritional security, particularly in developing countries (Mishra et al., 2025; Samanta et al., 2025). India is the largest producer and consumer of mungbean, accounting for a major share of the global pulse production. Owing to its short growth duration, nitrogen-fixing ability, and adaptability to diverse cropping systems, mungbean is widely cultivated during both kharif and summer seasons and plays a crucial role in sustaining soil fertility and agricultural productivity (Sakhare et al., 2026; Oktar et al., 2026). The increasing frequency and intensity of climatic extremes pose serious challenges to global agriculture. Among various abiotic stresses, terminal heat and drought stress are considered major constraints limiting mungbean productivity. Global climate projections indicate an increase in average temperature of approximately 1.8–4.0°C by the end of the twenty-first century, accompanied by greater variability in rainfall patterns and prolonged dry spells. Such climatic changes are expected to adversely affect crop growth, development, and yield, particularly in pulse crops that are highly sensitive to environmental fluctuations during reproductive stages (Basavaraj et al., 2026; Aina and Harris, 2026). Mungbean is particularly

vulnerable to terminal heat stress during flowering, pod formation, and seed filling stages. Exposure to high temperatures (more than 40°C) accelerates crop phenology, shortens the reproductive period, reduces pollen viability and fertilization, and ultimately lowers seed set and yield. Similarly, water deficit stress disrupts physiological and metabolic processes, leading to reduced leaf expansion, impaired photosynthesis, membrane damage, and decreased biomass accumulation. The combined occurrence of heat and drought stress during critical growth stages often results in severe yield penalties and threatens the sustainability of mungbean production under changing climatic conditions (Zhang et al., 2026; Sadeque et al., 2026).

Plants respond to abiotic stresses through a range of biochemical and physiological adjustments. Accumulation of compatible solutes such as proline, free amino acids, soluble sugars, and stress-responsive proteins plays a vital role in osmotic adjustment, cellular protection, and maintenance of metabolic activities under adverse environments. These biochemical traits have been widely recognized as reliable indicators of stress tolerance and can be effectively utilized for screening climate-resilient genotypes. Evaluation of mungbean cultivars under staggered sowing environments provides an effective approach for exposing genotypes to varying levels of terminal heat and moisture stress and identifying superior performers under stress conditions (Abbas et al., 2026; Paramasivam et al., 2026).

Although morphological and biochemical traits are useful for assessing stress responses, their expression is often influenced by environmental conditions. Therefore, molecular markers provide a more reliable approach for assessing genetic diversity and identifying stress-tolerant genotypes. Among DNA-based marker systems, Random Amplified Polymorphic DNA (RAPD) markers have been extensively used for genetic characterization because they are rapid, cost-effective, independent of environmental influences, and capable of detecting a high level of polymorphism across the genome. RAPD analysis has proven valuable in assessing genetic relationships, varietal identification, diversity analysis, and the selection of genetically diverse parents for crop improvement programs. The integration of biochemical and molecular approaches offers a comprehensive strategy for identifying climate-resilient mungbean genotypes capable of withstanding terminal heat and water stress. Understanding the relationship between stress-responsive biochemical traits and molecular diversity can facilitate the development of improved cultivars with enhanced adaptability to future climatic scenarios. Therefore, the present investigation was undertaken to evaluate selected mungbean genotypes under staggered planting environments and to characterize their biochemical responses, yield performance, and genetic diversity using RAPD markers (Majhi et al., 2026; Altaf, 2026; Pal, 2026; Kankarwal et al., 2025). The study aimed to identify promising climate-resilient genotypes that could serve as potential breeding materials for developing heat- and drought-tolerant mungbean cultivars suitable for sustainable production under changing climate conditions.

MATERIALS AND METHODS

The present investigation was conducted during the summer seasons of 2019–20 at the Research Farm of the Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh, India, to evaluate climate resilience in summer mungbean (*Vigna radiata* (L.) R. Wilczek) through integrated biochemical, physiological, yield, and molecular characterization under terminal heat and water stress conditions. Jabalpur is located at 23°90' N latitude and 79°58' E longitude, at an altitude of 411.78 m above mean sea level, and falls under a semi-arid subtropical climatic zone characterized by hot summers and cool winters. The region receives an average annual rainfall of approximately 1284 mm. During the experimental period, maximum and minimum temperatures ranged from 37.6°C and 17.9°C, respectively, while relative humidity varied between 80 and 90%, creating suitable conditions for evaluating heat- and drought-stress responses in mungbean.

The field experiment was laid out in an asymmetrical factorial randomized complete block design (FRCBD) with three replications. The experimental treatments consisted of five summer mungbean genotypes, namely PDM 139, Pusa Ratna, Pusa Vishal, Pusa 1431, and TJM 3, grown under three sowing environments created through staggered planting dates, viz., 12 February, 27 February, and 14 March. The staggered sowing approach was adopted to expose the crop to varying intensities of terminal heat and moisture stress during reproductive growth stages. Standard agronomic practices recommended for summer mungbean cultivation were followed uniformly throughout the crop-growing period.

Observations on growth, physiological, biochemical, and yield-related traits were recorded at appropriate growth stages. Leaf area was measured through destructive sampling at 20, 30, 40, and 50 days after sowing and at harvest using a LI-COR portable leaf area meter (Model LI-3000/3100). Leaf area index (LAI) was calculated as the ratio of total leaf area to the corresponding ground area occupied by the plant. Net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was measured using an Infrared Gas Analyzer (IRGA; LI-6400 XT, LICOR Inc., Lincoln, Nebraska, USA) on fully expanded fourth leaves from the apex under ambient field conditions. Growth and yield-attributing parameters including plant height, number of branches per plant, number of nodes and effective nodes per plant, pod length, pod width, pod girth, number of pods per plant, number of seeds per pod, seed index (1000-seed weight), seed yield, and biological yield were recorded following standard procedures. Seed and biological yields were measured after harvesting, threshing, cleaning, and drying the produce and expressed on both plant and hectare basis.

Biochemical analyses were carried out using fresh leaf samples collected during the reproductive stage. Protein content was estimated following the method of Lowry et al., (1951), total carbohydrate content according to Hodge

and Hofreiter (1962), free amino acids by the method of Lee and Takahashi (1966), and free proline content following Bates et al., (1973). These biochemical parameters were used as indicators of stress response and adaptation under heat- and moisture-stress environments.

For molecular characterization, seeds of the five mungbean genotypes were obtained from the Indian Agricultural Research Institute (IARI), New Delhi. Genomic DNA was isolated from young leaves of one-week-old seedlings using the cetyltrimethylammonium bromide (CTAB) method with minor modifications. Leaf tissues were ground in preheated CTAB extraction buffer and incubated at 60°C for one hour. The aqueous phase containing DNA was separated using chloroform:isoamyl alcohol (24:1), followed by precipitation with chilled isopropanol. The DNA pellet was washed with 70% ethanol, dissolved in T10E1 buffer, and treated with RNase to remove RNA contamination. DNA quality and integrity were verified by electrophoresis on 0.8% agarose gel and quantified using UV spectrophotometry. The DNA samples were subsequently diluted to a working concentration of 50 ng μL^{-1} .

Genetic diversity among the genotypes was assessed using Random Amplified Polymorphic DNA (RAPD) markers

Twenty-five arbitrary decamer primers belonging to the OPA, OPB, OPD, OPG, and OPX series (Operon Technologies, USA) were used for polymerase chain reaction (PCR) amplification. Each PCR reaction was carried out in a final volume of 20 μL containing 1 $\times$ PCR buffer, 2.5 mM MgCl_2 , 10 mM dNTPs, 10 pM primer, 50 ng genomic DNA, Taq DNA polymerase (5 U μL^{-1}), and nuclease-free water. Amplification was performed in a thermal cycler with an initial denaturation at 94°C for 4 min, followed by 45 cycles of denaturation at 94°C for 45 s, annealing at 38°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 5 min. Amplified products were resolved on 0.8% agarose gel in 1 $\times$ TBE buffer at 90 V for 3–4 h and visualized after staining with ethidium bromide using a gel documentation system. A 1 kb DNA ladder was used as a molecular size standard. RAPD bands were scored as present (1) or absent (0), and the binary data matrix was used to estimate genetic similarity among genotypes. Cluster analysis and dendrogram construction were performed using DARwin software (Version 6.0) to elucidate genetic relationships and diversity patterns among the mungbean genotypes.

The experimental data generated from field, physiological, biochemical, and molecular analyses were subjected to appropriate statistical analyses following the procedures for factorial randomized block design. Treatment means were compared using standard tests of significance at the 5% probability level to identify superior genotypes and sowing environments associated with enhanced tolerance to terminal heat and water stress (Gomez and Gomez, 1984).

Table 1. RAPD primers employed for molecular characterization of mungbean (*Vigna radiata* (L.) R. Wilczek) genotypes and their nucleotide sequences.

S. No.	Primers	Sequences (5'-3')	Annealing Temperature (°C)
1	OPA-12	TCGGCGATAG	36
2	OPA-18 A	AGGTGACCGT	36
3	OPA-18 B	GTGACGTAGG	36
4	OPB-01	CTTTCGCTCC	36
5	OPB-02	TGATCCCTGG	36
6	OPB-03	CATCCCCCTG	36
7	OPD05	TTGGCACGCG	36
8	OPG04	AGCGTGTCTG	36
9	OPG11	TGCCCCGTCGT	36
10	OPX3	TGGCGCAGTG	36

RESULTS AND DISCUSSION

Biochemical, Physiological and Yield Responses of Mungbean Genotypes under Terminal Heat and Water Stress

Significant variations were observed among mungbean genotypes, sowing environments, and their interactions for biochemical, physiological, and yield-related traits under terminal heat and water stress conditions. Delayed sowing progressively increased stress-induced biochemical responses, while growth and yield parameters were adversely affected due to exposure to higher temperatures and moisture deficit during reproductive stages. Among the genotypes, PDM139 consistently exhibited superior performance for most biochemical and yield attributes. It recorded the highest protein content (15.79%), total carbohydrate content (57.03%), total free amino acids (1.92 mg g^{-1}), and proline accumulation (28.46 mg g^{-1}), whereas Pusa1431 showed the lowest values.

The higher accumulation of osmoprotectants such as proline and free amino acids in PDM139 suggests an efficient adaptive mechanism for maintaining cellular homeostasis under stress conditions. Similar findings have been reported in mungbean by Naresh et al., (2013) and Bangar et al., (2019), who observed greater proline accumulation in drought-tolerant genotypes, and by Sadiq et al., (2017) and Sadiq et al., (2018), who reported enhanced synthesis of compatible solutes under water-deficit conditions.

Sowing environments significantly influenced biochemical responses. Early sowing on 12 February recorded the highest protein content (15.71%), whereas delayed sowing on 14 March resulted in a decline in protein content (12.30%). The reduction in protein content under stress conditions may be attributed to degradation of soluble proteins and inhibition of protein synthesis under moisture deficit, as reported by Sadiq et al., (2017). In contrast, total carbohydrate content, free amino acids, and proline concentration increased significantly under late sowing conditions. Such accumulation of carbohydrates and amino acids under stress serves as an osmotic adjustment mechanism and has been widely documented in drought-stressed plants (Munns and Tester, 2008; Carillo et al., 2011; Pirasteh-Anosheh et al., 2016). Similarly, increased proline accumulation under stress conditions has been reported by Jaleel et al., (2007), Parida et al., (2008), Yamada et al., (2005), and Simon-Sarkadi et al., (2006). Growth and physiological traits were also markedly influenced by genotype and sowing environment. Pusa Ratna recorded the tallest plants (49.25 cm) and longest pods (8.43 cm), whereas PDM139 produced the highest number of branches, nodules, and effective nodules per plant.

Table 2. Effect of staggered sowing dates on biochemical and morpho-physiological traits of summer mungbean (*Vigna radiata* (L.) R. Wilczek) genotypes under terminal heat and water stress conditions.

Factor A	Protein (%)	Total carbohydrate content (%)	Total free amino acids (mg g ⁻¹)	Proline content (mg g ⁻¹)	Plant Height (cm)	Branches plant ⁻¹	Nodules plant ⁻¹	Effective Nodules plant ⁻¹
V1	15.79	57.03	1.92	28.46	47.33	5.89	24.95	23.20
V2	14.92	55.08	1.85	27.43	49.25	5.45	21.89	20.36
V3	13.32	51.09	1.67	24.38	42.43	4.50	18.28	17.00
V4	12.50	49.83	1.60	22.85	40.85	4.17	17.95	16.69
V5	14.06	52.88	1.75	25.57	44.77	5.06	19.44	18.08
SEm±	0.003	0.304	0.031	0.253	0.005	0.001	0.004	0.002
C.D. (P=0.05)	0.009	0.886	0.091	0.737	0.015	0.004	0.012	0.006
Factor B								
D1	15.71	48.73	1.54	19.34	46.55	5.33	19.00	17.67
D2	14.34	52.44	1.76	24.70	49.18	5.13	25.37	23.59
D3	12.30	58.38	1.96	33.18	39.05	4.57	17.13	15.93
SEm±	0.002	0.236	0.024	0.196	0.004	0.001	0.003	0.002
C.D. (P=0.05)	0.007	0.686	0.071	0.571	0.011	0.003	0.009	0.005

V1- PDM139, V2- Pusa Ratna, V3- Pusa Vishal, V4- Pusa 1431 and V5-TJM3

D1- First date of sowing (12th Feb), D2- Second date of sowing (27th Feb) and D3- Third date of sowing (14th Mar)

Table 3. Effect of staggered sowing dates on yield attributes, seed yield, and biological yield of summer mungbean (*Vigna radiata* (L.) R. Wilczek) genotypes under terminal heat and water stress conditions.

Factor A	Pod Length (cm)	Pod Width (mm)	Pod Girth (mm)	No. of pods plant ⁻¹	Seed Yield (Kg ha ⁻¹)	Biological yield (Kg ha ⁻¹)
V1	7.61	4.55	3.41	19.11	730.42	2281.82
V2	8.43	4.86	3.70	18.61	700.85	2194.33
V3	7.85	4.88	3.72	15.94	585.48	2032.59
V4	7.51	4.60	3.53	15.06	532.37	1973.01
V5	7.69	4.58	3.44	16.11	625.68	2076.36
SEm±	0.102	0.076	0.004	0.002	0.161	0.18
C.D. (P=0.05)	0.298	0.22	0.012	0.004	0.469	0.524
Factor B						
D1	7.86	4.71	3.60	15.90	659.27	2092.54
D2	8.15	4.81	3.78	19.78	736.07	2321.24
D3	7.44	4.49	3.37	15.23	509.54	1921.08
SEm±	0.079	0.058	0.003	0.001	0.125	0.139
C.D. (P=0.05)	0.231	0.17	0.009	0.003	0.364	0.406

V1- PDM139, V2- Pusa Ratna, V3- Pusa Vishal, V4- Pusa 1431 and V5-TJM3

D1- First date of sowing (12th Feb), D2- Second date of sowing (27th Feb) and D3- Third date of sowing (14th Mar)

Similar associations between nodulation and yield performance in mungbean have been reported by Kumar et al., (2011). The superiority of 27 February sowing for plant height, nodulation, and pod development agrees with the findings of Jahan and Adam (2014) and Navatre et al., (2024) who observed significant reductions in growth traits under delayed sowing and moisture-stress conditions.

Yield and yield-attributing traits followed a similar trend. PDM139 recorded the highest number of pods per plant, seed yield, and biological yield, while Pusa1431 recorded the lowest values. The superior yield performance of PDM139 may be attributed to enhanced assimilate production, efficient nodulation, and greater accumulation of stress-protective metabolites.

Table 4. Mean performance of physiological traits, yield components, and seed yield of mungbean (*Vigna radiata* (L.) R. Wilczek) genotypes under different sowing environments.

Factor A	Photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	LAI	No. of pods plant ⁻¹	No. of seeds pod ⁻¹	Seed Index (g)	Seed Yield (g plant ⁻¹)	Seed Yield (Kg ha ⁻¹)
V1	12.104	0.789	20.78	10.45	39.81	5.78	787.74
V2	11.348	0.682	20.67	10.89	45.47	5.61	754.22
V3	9.339	0.589	18.00	10.00	47.16	4.99	624.12
V4	9.122	0.557	16.89	9.67	46.14	4.65	571.62
V5	10.509	0.626	17.33	10.22	40.28	5.19	651.47
SE(m)±	0.228	0.014	0.169	0.069	0.153	0.002	0.035
C.D. (P=0.005)	0.664	0.042	0.492	0.2	0.444	0.005	0.102
Factor B							
D1	12.13	0.646	18.68	10.33	44.08	5.32	710.15
D2	10.439	0.691	20.93	9.80	44.99	6.12	792.33
D3	8.701	0.609	16.60	10.60	42.25	4.30	531.04
SE(m)±	0.177	0.011	0.131	0.053	0.118	0.001	0.027
C.D. (P=0.005)	0.514	0.032	0.381	0.155	0.344	0.004	0.079

Similar relationships between yield and branching, pod number, and nodulation have been reported by Jahan and Adam (2014) and Navatre et al., (2024). Sowing on 27 February produced significantly higher seed yield and biological yield compared with other sowing dates. These findings corroborate those of Meena et al., (2026), who reported higher mungbean productivity under optimum sowing conditions, and Miah et al., (2009), who observed significant yield reductions under delayed sowing. Likewise, Ahmad et al., (2021) reported a 20–40% reduction in biological yield under terminal heat stress. The interaction effect revealed that PDM139 sown on 27 February produced the highest seed yield (826.02 kg ha⁻¹) and biological yield (2473.09 kg ha⁻¹), confirming its superior adaptation to terminal heat and water stress environments.

Molecular Characterization and Genetic Diversity Analysis

RAPD marker analysis revealed substantial genetic variation among the five mungbean genotypes. A total of 75 amplification products were generated using 25 arbitrary decamer primers, of which 54 were polymorphic, resulting in an average polymorphism of 69.49%.

Table 5. RAPD marker polymorphism and allele distribution among mungbean (*Vigna radiata* (L.) R. Wilczek) genotypes.

Name of primer	Total bands	Total Monomorphic band	Total Polymorphic band	% of Polymorphism
OPA18A	6	3	2	40%
OPG04	5	0	5	100%
OPA18B	6	4	2	33.33%
OPG11	9	0	9	100%
OPA12	13	2	11	84.61%
OPB03	7	3	4	57.14%
OPD05	8	2	6	75.00%
OPX03	7	2	5	71.42%
OPB01	7	2	5	71.42%
OPB02	8	3	5	62.50%

The average number of bands per primer was 7.5, demonstrating the effectiveness of RAPD markers in detecting genetic variation among mungbean cultivars. Similar levels of RAPD polymorphism in mungbean have been reported by Abna, (2013) and Chaudhary et al., (2022). The percentage polymorphism ranged from 33.33% to 100%, with primers OPG-04 and OPG-11 showing complete polymorphism. RAPD markers are considered efficient tools for genotype identification and diversity assessment because they are rapid, cost-effective, and independent of environmental influences (Williams et al., 1990; Welsh and McClelland, 1990; Zinn et al., 2010). The high level of polymorphism observed in the present investigation confirms their suitability for genetic diversity studies in mungbean. Cluster analysis based on RAPD data grouped the five genotypes into three distinct clusters. Cluster A comprised Pusa1431 and Pusa Vishal, Cluster B consisted solely of TJM3, while Cluster C included PDM139 and Pusa Ratna. The clustering pattern reflected the genetic relationships among the cultivars and corresponded closely with their biochemical and agronomic performance. Notably, PDM139, which exhibited superior biochemical responses and yield under terminal heat and water stress, was grouped separately from the comparatively susceptible genotype Pusa-1431.

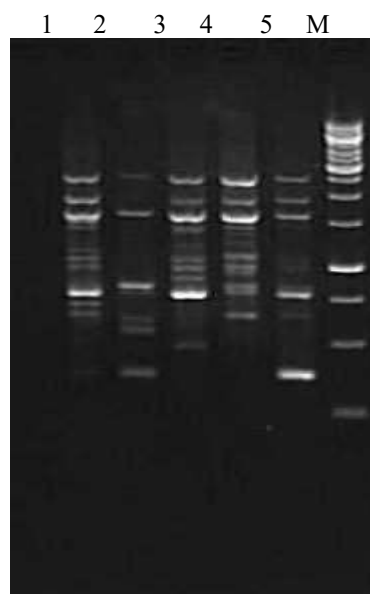


Fig.1. RAPD amplification profile of five mungbean (*Vigna radiata* (L.) R. Wilczek) genotypes generated using primer OPA-12. Lanes: 1 = PDM 139, 2 = Pusa Ratna, 3 = Pusa Vishal, 4 = Pusa 1431, 5 = TJM 3, and M = 1 kb DNA ladder (molecular size marker).

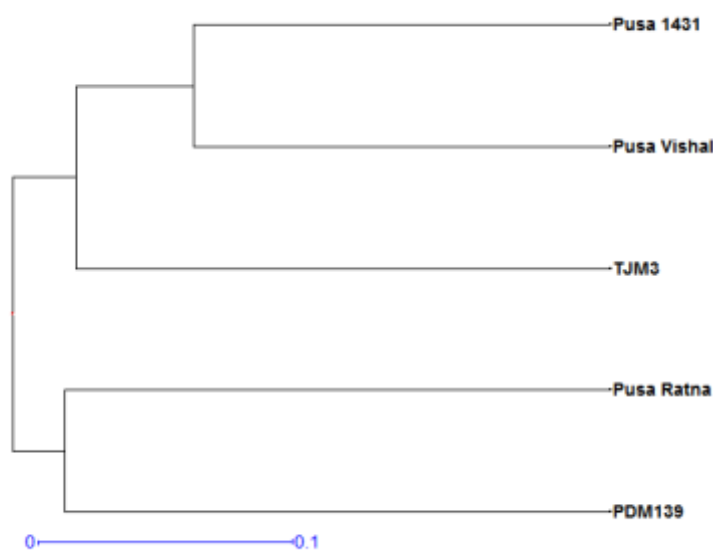


Fig. 2. Dendrogram depicting genetic relationships among mungbean (*Vigna radiata* (L.) R. Wilczek) genotypes based on RAPD marker analysis.

Integration of molecular and phenotypic information demonstrated that genotypes possessing superior stress-responsive biochemical traits also exhibited distinct genetic profiles. Such associations between molecular diversity and agronomic performance have been reported in mungbean by Sarfraz et al., (2025) and Wang et al.,

(2025). The RAPD analysis further revealed a relatively narrow genetic base among the cultivated mungbean varieties, which is consistent with earlier reports by Williams et al., (1990); Welsh and McClelland, (1990). The limited genetic diversity among released cultivars highlights the necessity of broadening the genetic base through the utilization of wild relatives, exotic germplasm, and pre-breeding materials. Therefore, the genetic information generated in the present study can be effectively utilized for parent selection and the development of climate-resilient mungbean cultivars. The combined biochemical and molecular analyses identified PDM139 as the most promising genotype for tolerance to terminal heat and water stress and a potential donor parent in future mungbean improvement programmes.

CONCLUSION

Terminal heat and water stress significantly affected the biochemical traits, growth, and productivity of mungbean under staggered sowing environments. Delayed sowing increased the accumulation of stress-responsive metabolites such as carbohydrates, free amino acids, and proline, indicating adaptive responses to stress conditions. Among the evaluated genotypes, PDM139 exhibited superior biochemical performance, higher nodulation, and the highest seed and biological yields, demonstrating greater tolerance to terminal heat and water stress. The optimum yield was obtained when PDM139 was sown on 27 February. RAPD analysis revealed considerable genetic diversity among the genotypes and grouped them into three distinct clusters. PDM139 and Pusa Ratna showed superior agronomic performance and belonged to the same genetically diverse cluster, suggesting their suitability as potential parents for developing climate-resilient and high-yielding mungbean cultivars. Overall, the integration of biochemical, yield, and molecular analyses identified PDM139 as the most promising climate-resilient genotype for future mungbean improvement programmes.

Abbreviations

RAPD – Random Amplified Polymorphic DNA

PCR – Polymerase Chain Reaction

CTAB – Cetyltrimethylammonium Bromide

LAI – Leaf Area Index

IRGA – Infrared Gas Analyzer

RCBD – Randomized Complete Block Design

UPGMA – Unweighted Pair Group Method with Arithmetic Mean

TBE – Tris–Borate–EDTA Buffer

DNA – Deoxyribonucleic Acid

EDTA – Ethylenediaminetetraacetic Acid

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Authors' Contribution

All authors contributed to the conception and design of the study, data collection, analysis, interpretation of results, and preparation of the manuscript. All authors have read and approved the final version of the manuscript.

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