Original Research Article

Screening of landraces of cluster bean for powdery mildew disease resistance and molecular characterization of selected landraces

ABSTRACT

Aim: To identify resistance landraces of cluster bean for management of powdery mildew disease. Cluster bean (*Cyamopsis tetragonoloba*) is a major legume crop. Powdery mildew is a devastating foliar disease affecting cluster beans. Fungicides are recommended for disease management; however, their use is costly and environmentally harmful.

Study design: Thirty-two landraces were screened against powdery mildew under natural epiphytotic conditions in the field to identify the resistance landrace. The disease severity was recorded at 25 days and 50 days after infection (DAI).

Place and duration: Department of Plant Biotechnology, College of Agricultural Biotechnology, Loni, between June 2024 and December 2024

Methodology: after 50 DAI, only 10 landraces were found to be resistant. However, twenty-two landraces were found to be highly susceptible, with a maximum grade of nine on the (0–9) scale. RAPD markers were used to characterize five landraces: two resistance landraces (P5 and P6), two susceptibility landraces (AS1 and AS4), and one control landrace (Phule Guar).

Result: The dendrogram analysis revealed that five cluster bean landraces may be classified into two major clusters: A and B. Only the control variety, Phule Guar, belonged to cluster B, indicating that it differed from the collected guar landrace. Cluster A1.1 contains AS4 and AS1, both of which were sensitive in nature but produced high yields. In contrast, landrace P6 from cluster A1.2 was resistant to powdery mildew disease but produced lower yields. Landrace P5 belonged to cluster A2, which was more diversified than AS4, AS1, and P6 landraces, as well as resistant and high yielding.

Keywords: Cluster bean, RAPD, landrace

Commented [ks1]: At least 5 keywords

Introduction

Cluster bean (*Cyamopsis tetragonoloba*) is an underutilized leguminous vegetable. It is known to as guar. It is one of the most important and promising vegetable cum industrial crops, known for its delicate pods and endospermic gum (Punia et al., 2009). For every 100g of edible portion, its tender pods have a high concentration of calories (16Kcal), moisture (81g), protein (3.2g), fat (1.4g), carbohydrate (10.8g), vitamin A (65.3IU), vitamin C (49mg), calcium (57mg), and iron (4.5mg) (Kumar et al. 2014). India produces around 80% of the world's guar gum, with the remaining 20% produced by Pakistan, the United States, Australia, South Africa, Sudan, and Argentina. Powdery mildew, caused by *Leveillula taurica*, is a significant foliar disease that can result in yield losses of up to 50-55% (Channamma *et al*. 2015). Although cluster beans are widely grown in India, production levels

are low due to diseases such as powdery mildew. Fungicides remain the primary method for controlling powdery mildew. Recent years have seen an increase in the use of new chemicals due to their rapid results and lack of resistance varirties (Vijaykuma *et al* 2025)

Commented [ks2]: Follow one pattern

The current study screened for novel and effective sources of resistance to powdery mildew, which can be a valuable source of genetic variation and resistance genes in both wild species and landraces. Landraces have significant potential for increasing genetic diversity in cultivated crops (Okoń S. and Kowalczyk K., 2020), taking into account the severity of the disease and the economic losses it causes. The present investigation was carried out to screen landraces of cluster bean for its resistance to powdery mildew.

Materials and Methods

The present investigation was carried out at Department of Plant Biotechnology, College of Agricultural Biotechnology, Loni. Total thirty two landraces were taken for screening (Table 1). Mrs. Rahibai Soma Popere, also known as SEED Mother for conserving indigenous seeds, has received the fourth highest civilian national honor, Padma Shree, for her work in the field. Mrs. Rahibai Soma Popere of Akole, District Ahmednagar, provided a collection of landraces for this study, as did Mrs. Kadlag of Pune and Tal. Ashti, District Ahmednagar.

Table 1: Collection of landraces of cluster bean

Sr. no.	Name	No. of landraces
1	Cluster Ak (Aakole)	04
2	Cluster P (Pune)	11
3	Cluster AS(Ashti)	16
4	Phule Guar (Control)	01

The disease severity was recorded at 25 days and 50 days after infection. Five leaves from the lower, middle, and upper part of the plant were graded by using 0-9 scale (Wheeler BEJ 1969) (Table 2). Based on disease incidence, plants which were less affected or showing resistance was selected for diversity analysis.

Table 2: Disease scoring scale

Score	Description
0	No symptom of powdery mildew on leaves.
1	Small scattered powdery mildew specks covering 1%or less leaf area.
3	Small powdery lesions covering 1-10 % of leaf area.
5	Powdery lesion enlarged covering 11-25 % of leaf area.
7	Powdery lesions coalesce to form big patches covering 26-50% leaf area.
9	Big powdery mildew patches covering 51% or more leaf area and defoliation occurs.

RAPD profiling:

DNA extraction was performed using the CTAB technique (Saghai et al. 1984). The RAPD analysis was conducted. The reaction mixture was standardized and subjected to PCR amplification as described below. Mix 5µl of 2X PCR mixture (Taq DNA, dNTPs, MgCL2) with 3µl of Molecular Grade water, 1µl of DNA sample, and 1µl of primer. Total volume is around 10µl. The temperature profile for PCR amplification was as described in Table 3. The amplified PCR product was separated using a 1.2% agarose gel electrophoresis. After electrophoresis, the gel was carefully removed from the

casting tray and photographed using a UV transilluminator. Then Scoring of amplified fragments was done by using following formula.

Table 3: The temperature profile for PCR Amplification is as follows.

Steps	Temperature °C	Duration (min)	Number of Cycles
Initial Denaturation	94	10	1
Denaturation	94	1	35 cycles
Primer annealing	Variable (32-37)	1.30	
Extension	72	1	
Final extension	72	10	1
Hold	4	∞	……

Percent polymorphism =

$$\frac{\text{No. of polymorphic bands}}{\text{Total no. of bands}} \times 100$$

Jaccard's similarity coefficient was used to determine pairwise genetic similarities between Cluster bean cultivars. Clustering was performed using the symmetric matrix of similarity coefficients, and clusters were created using the unweighted pair group technique for arithmetic mean (UPGMA) with NTSYS-PC version 2.02.

Result and Discussion

Thirty-two landraces were screened against powdery mildew under natural epiphytotic conditions in the field to identify the resistance landrace (Plate 1), and the results are presented in Table 4 revealed that out of thirty-two landraces after 25 days, fifteen landraces were found to be resistant or moderately resistant, however two landraces were found to be moderately susceptible with five grades, and four landraces were found to be susceptible with seven grades. After 50 days, ten landraces were resistant, while twenty-two landraces were severely sensitive, with a maximum grade of nine (Table 4).

Plate 1: Leaves before disease incidence and after disease incidence

Table 4: Grouping of cluster bean landraces based on reaction against powdery mildew

GRADE	REACTION	GENOTYPES AFTER 25 DAS	NO. OF GENOTYPES	GENOTYPES AFTER 50 DAS	NO. OF GENOTYPES
0	I	AK2,AK3	2		
1	R	AK1,AS3,P4,P7,P11	5	P1,P2,P3,P4,P5,P6,P7,P8,P9,P11.	10
3	MR	P1,P2,P3,P5,P6,P8,P9,P10	8		
5	MS	AS1,PG	2		
7	S	AS4,AS17,AS14, AS16.	4		
9	HS	AK4	11	AK1,AK2,AK3,AK4,AS1,AS2,AS3, AS4,AS5,AS6,AS7,AS8,AS9,AS10, AS11,AS12,AS13,AS14,AS15,AS16	22
		AS2,AS5,AS6,AS7,,AS8, AS9,AS10,AS11,AS12,AS15.			
				P10,PG	

AK: Aakole, AS: Ashti, PG: Phule Guar, P: Pune

I: immune, R: resistant, MR: Moderate resistant, MS: Moderate susceptible, S: Susceptible, HS: Highly susceptible

Molecular characterization of selected landraces by using RAPD marker

Based on disease screening two resistance landraces (P5 and P6), two susceptibility landraces (AS1 and AS4), and one control landrace (Phule Guar) were selected for further molecular characterization.

Five RAPD primers were screened which successfully discriminated the landraces (Plate 2 and 3). The screened primers were able to generate 39 amplicons with an average of 7.8 amplicons per primer. Out of total amplicons, 35 amplicons were found polymorphic. They showed 88.33% polymorphism and the average number of polymorphic bands per primer was 7 (Table 5).

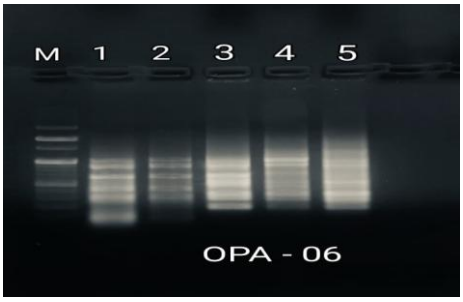

Plate 2: APD-PCR analysis with five selected landraces using OPA-06 primer M-ladder(1kb), 1- AS4, 2- AS1, 3- PG, 4- P5, 5- P6.

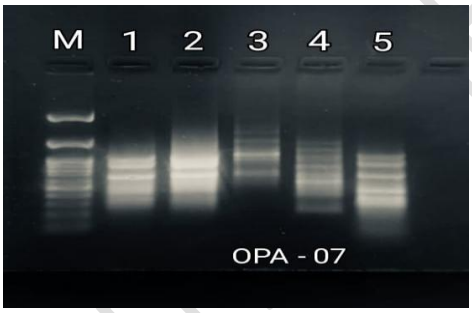

Plate 3: RAPD-PCR analysis with five selected landraces using OPA-07 primer. M-ladder(1kb), 1- AS4, 2- AS1, 3- PG, 4- P5, 5- P6.

Table 5: List of RAPD primers and polymorphic amplicons generated.

Sr. No.	Primer	Total no. of amplicons	Total no. of polymorphic amplicons	Percent Polymorphism (%)
1	OPH-05	6	5	83.33
2	OPA-01	8	8	100
3	OPA-02	4	3	75
4	OPA-06	9	9	100
5	OPA-07	12	10	83.33
	Total	39	35	88.33

Average number of amplicons per primer = 7.8
Average number of polymorphic amplicons per primer = 7
Total percent polymorphism by RAPD marker = 88.33%

A Jaccard's binary similarity matrix of combined data from five primers for the five landraces of cluster bean was prepared by scoring bands for presence or absence (Table 6). In the present study, the similarity coefficient value ranged from 0.34 to 0.63 across five landraces indicating a high degree of genetic variation. The highest genetic similarity to an extent of 0.63 was recorded between AS4 and AS1. Least genetic similarity 0.34 was observed in between AS1 and PG.

Table 6: Jaccard's binary similarity matrix for RAPD analysis

	AS4	AS1	PG	P5	P6
AS4	1.0				
AS1	0.63	1.0			
PG	0.48	0.34	1.0		
P5	0.53	0.44	0.40	1.0	
P6	0.56	0.45	0.35	0.50	1.0

The dendrogram RAPD analysis shows that five cluster bean landraces can be grouped into two major clusters viz. A and B. Only control variety i.e. Phule Guar belonged to cluster B indicated that it was diverse from collected guar landraces. Cluster A1.1 has AS4 and AS1, both the landraces were susceptible in nature but good yield. Whereas landrace P6 belonged to cluster A1.2 was resistance to powdery mildew disease but had fewer yields. Landrace P5 belonged to cluster A2 which was resistant and had good yield, and more diverse from AS4, AS1 and P6 landraces (Plate 4).

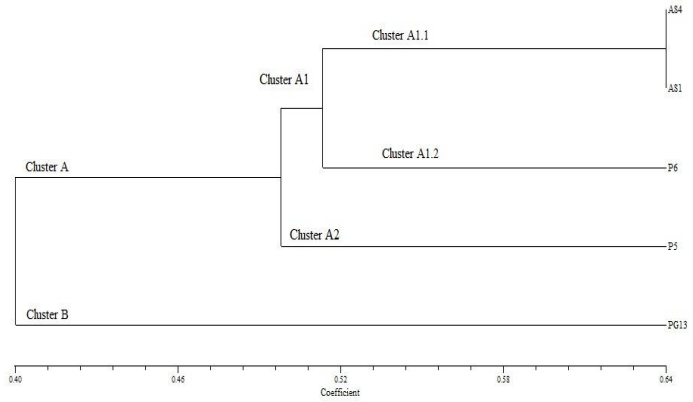

Plate 4: Dendrogram showing results of RAPD analysis

Powdery mildew is a serious foliar disease of cluster beans that causes significant economic loss. Fungicides have been advised for disease management; nevertheless, their use is expensive and environmentally unfriendly. The best way to control the disease is to use resistant landraces. Hence, in this study Thirty-two landraces were screened. The results revealed that after 50 DAS only ten landraces found to be resistant. However, twenty-two landraces were found highly susceptible with maximum grade nine in (0-9) scale.

Vijaykumar *et. al.,* 2021 screened forty-one landraces against powdery mildew disease, they revealed that none was found to be resistant however two landraces viz. Samrat and Deepti were found moderately susceptible, and Thirty-seven landraces were found susceptible. The remaining two landraces were found to highly susceptible with maximum grade nine in (0-9) scale.

Disease management through host plant resistance has proven to be the most effective approach in all disease management initiatives. The use of resistant cultivars in farming systems is the most straightforward, efficient, and cost-effective approach of disease management. Furthermore, as compared to traditional disease management strategies, these resistant cultivars conserve natural resources and save time, money, and energy.

References:

1. APEDA 2022. Available: https://www.apeda.gov.in/apedawebsite/SubHead_Product/Guargum.htm
2. Channamma, S. G., Mahesh, M., Arunkumar, K. (2015). *In vitro* evaluation of fungicides against spore germination of *Leveillula taurica* causing powdery mildew in guar. Indian J Tropic Agric 33(4), 3529-3531.
3. Kumar, V., Ram, R. B., Yadav, R. K. (2014). Genetic diversity in cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) for morphological characters. Indian J Sci Technol 7, 1144-1148
4. Vijaykumar, K. N., Kulkarni, S., Vijaykumar, A. G. and Channakeshava. (2021). Field evaluation of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub) landraces against powdery mildew under natural epiphytotic conditions. Journal of Pharmacognosy and Phytochemistry 10(1), 1541-1543
5. Punia, A., Yadav, R., Arora, P., Chaudhury, A. (2009). Molecular and morpho-physiological characterization of superior cluster bean (*Cymopsis tetragonoloba*) varieties. J Crop Sci Biotechnol 12(3),143-148
6. Saghai-Maroof, M., Soliman, K., Jorgensen, R., Allard, R. (1984). Ribosomal DNA spacer length polymorphism in barley: Mandelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. 81, 8014-8018.
7. Okoń, S. and Kowalczyk, K. (2020). Screening oat landraces for resistance to *Blumeria graminis* f. sp. Avenae. Journal of Plant Pathology. 102, 893-898
8. Vijaykumar, K. N., , Kulkarni, S., Hiremath S. M., (2025). Management of Powdery Mildew in Cluster Bean using Fungicides, Botanicals and Bioagents. Legume Research, 48(6), 1063-1067
9. Wheeler, B. E. J. (1969). An introduction to plant disease. John Wiley and Sons Ltd, London. 301.
10. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., Tingey S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, Nucleic Acids Research, 18,6531-6353.