Original Research Article

Multivariate Analysis andScreeningof moth bean accessions for biotic stressesin the arid region of western India

Abstract

The present investigation assesses the genetic diversity and resilience of moth bean (*Vigna aconitifolia*) in the arid zones of India, addressing the decline in cultivation area and productivity due to conventional cultivation practices and environmental stresses.(Rewrite)This research was carried out at the ICAR-Indian Institute of Pulses Research, Regional Research Centre, Bikaner, and employed an augmented design to analyze 300 accessions for morphological and agronomic traits. The study integrated Pearson's correlation, hierarchical clustering, and principal component analysis to understand trait interrelationships and genetic variance. The number of clusters per plant, number of pods per plant, plant height and test weight showed a highly significant and positive correlation, whereasdays to 50% flowering and number of branches per plant showeda negative correlation with seed yield per plant. Hierarchical clustering~~,~~ subdivided accessions into fourteen clusters, and cluster1 best suited to arid conditions with 21 accessions. Principal component analysis with eigenvalues classified the accessions into eight principal components. PC1 contributed the maximum ~~in~~ variation that is 32.21 ~~%~~percent, followed by other clusters. *Cercospora* leaf spot had the highest disease incidence among the three diseases (~~Yellow~~ yellow mosaic virus, ~~Cercospora~~ cercosporaleaf spot and ~~Leaf~~ leaf curl virus diseases). The findings underscore the potential of exploiting genetic variability in moth beans for breeding programs aimed at enhancing yield and stress tolerance, crucial for sustainable production in resource-poor arid ecosystems.

Keywords: Moth bean; arid ecosystem; principal component analysis; drought tolerance and yellow mosaic virus

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1. Introduction

Moth bean (*Vigna aconitifolia* (Jacq.) Maréchal) is an economically important pulse crop for arid regions, especially the Northern-Western desertic region of India (Kumar, 2002). It is one of the most drought-hardy and high-temperature tolerant crops. Due to its early maturity, relative drought tolerance, nitrogen fixation ability, and low input requirement, it is grown as a sole crop or can be integrated with other crops in different cropping systems. Therefore, it is the preferred choice of the farmers for sustained production under the extreme agro-climatic conditions of thisregion.

The western dry zone of India (part of Rajasthan), a prominent moth bean growingregion, contributes almost 98% (0.97 m ha) area of the country. Unfortunately, despite the highest acreage of moth bean in this region, it is ~~characterised~~ characterized by the lowest productivity (333 kg ha^{-1}) (Indiastat, 2021). In India, over the last decade, moth bean area has decreased drastically by 40% (1.65 to 0.99m ha), production by 58 % (0.80 to 0.34 m tons) and productivity by 29% (486 to 346 kg ha). Reduced productivity is mainly ~~because of~~due to the continued use of low-yielding conventional varieties, the limited genetic base of newly released varieties, cultivation in resource-poor soils, and repeated crop failure caused by abiotic (drought and heat) and biotic (YMV, *Cercospora* leaf spot and crinkle leaf virus) stresses. Abiotic stresses affect crop growth at different developmental stages and reduce the yield by up to 60 percent (Garg *et al*., 2004) or even cause permanent crop failure.

Among the biotic stresses,yellow mosaic virus is the most destructive disease affecting the yield potential of moth bean both qualitatively and quantitatively, and seed yield losses may vary from 20 to 100% (Mathur and Sharma, 2002; Varma and Malathi, 2003; Meghwal et al. 2015). Similarly, *Cercospora* leaf spot and crinkle leaf virus are other diseases ~~which~~ thatcause yield losses.So far, the progress achieved in moth bean for enhanced production is much less than in other pulses. Initially, moth bean varieties were released by selection in existing genetic diversity, but due to a gradual reduction in existing variability,

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interest was developed in mutation breeding, which led to several short-duration varieties (Sharma et al., 2021). (rewrite)However, the hybridiz~~s~~ation program~~me~~ in moth bean was neglected mainly because of a lack of genetic studies and difficulty in handling the small-sized flower and ~~their~~ itsmanipulation (Sharma and Majumdar, 1982). Earliness with high yield is of prime importance in drought-hit areas, as these genotypes reduce ~~the~~ disease incidences. Most of the moth bean-producing regions,especially ~~under~~ those inarid ecosystem,are covered by these varieties because of their short life cycle and higher yield.

Extensive utiliz~~s~~ation of limited genetic resources to develop new varieties has resulted in a narrow genetic base. Consequently, newly developed varieties are more vulnerable to several abiotic and biotic stresses; these are the critical barriers to improving the production and productivity of this crop. As a result, its area and production have decreased considerably, and this valuable commodity is being eliminated from our cropping system. Genetic improvement of these crops will bring pulses to the forefront of ~~the~~ cereals. Characteriz~~s~~ing and assessing new genetic resources for various morphological and agronomic traits is necessary to reveal hidden genetic diversity and find potential genotypes adapted to arid conditions. Therefore, to measure genetic diversity, we investigated moth bean accessions for different qualitative and quantitative traits and screened for abiotic and biotic stresses.The chosen genotypes will be deployed in the breeding program~~me~~ to develop new varieties.

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2. Materials and Methods:

2.1 Description of the experimental site

The present investigation was carried out at the ICAR- Indian Institute of Pulses Research, Regional Research Centre, Bikaner, Rajasthan, representing the dry western region,situated at 28.06° N latitude and73.32°E longitude with an altitude of 226 m above mean sea level. This region is hyper-arid with a hot and dry summer (49^{0}C) and a cold winter (1^{0}C). The average annual rainfall in~~of~~ the area is 263 mm. The soil of the experimental site is loamy sand in texture ~~with~~ a pH of (8.5), low soil organic carbon (0.10%), low ~~in~~ available N (92 kg ha^{-1}), medium ~~in~~ P (15 kg ha^{-1}) and K (210 kg ha^{-1}).

2.2 Plant material and experimental design

A total of 300 moth bean accessions procured from ICAR-NBPGR, RS, Jodhpur were sown in an augmented design with eight blocks with four check varieties. RMO-225, RMO-

40, RMO-257, and RMB-101. The plots in each block were represented by two rows of 2.5 m length with 30 x 10 cm spacing. The crop was sown after one harrowing, followed by planking on July 25,2021, with the commencement of the rains. ~~Good~~ A good plant population was maintained in the experimental field by following all the recommended agronomic practices.

2.3 Weather during the crop growth period

The weather data was taken from the weather station installed in the ~~close~~ vicinity of the experimental block. From July to October, only 143 mm of rainfall was received at the ~~centre~~center, which was 54 percent less than the average monsoon season rainfall. The total number~~s~~ of rainy days ~~were~~was 11 during this period. The crop was sown in the 29th SMW on July 25, 2021, just after receiving considerable precipitation (20mm). After sowing ~~of~~ the crop, there was a long dry spell of almost 37 days with 9 mm of rains on August 7th ~~August~~ (32 SMW). However, from mid-September (37th SMW) to October 7 (39th SMW) experienced more rains (110 mm) which was 67.48% of total rainfall. Hence, from July to October ~~month~~ the distribution of rainfall was uneven. Throughout the crop-growing season (July to October) mean maximum temperature ranged from 40.5 °C to 29.6 °C and the minimum from 28.8 °C to 13.8 °C. ~~Maximum~~ The maximum relative humidity (67%) was recorded in 33rd SMD while the minimum was observed in the 45th SMD.

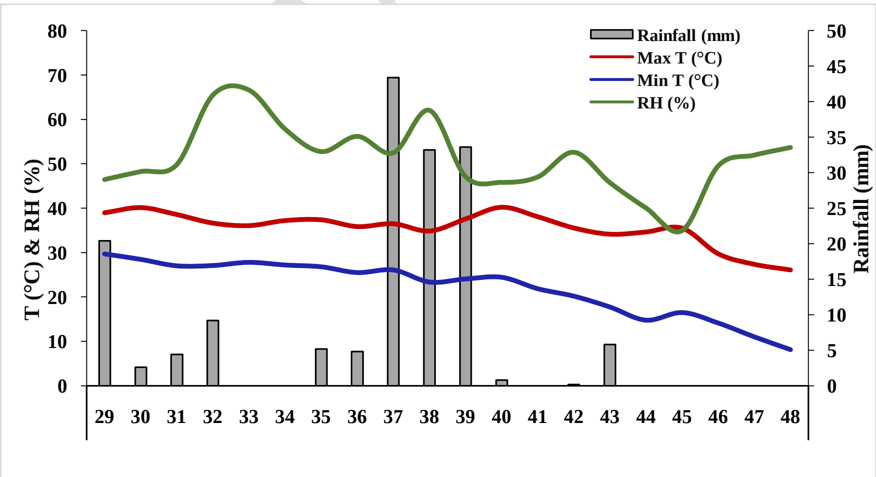

Fig.1 Weather during crop growing season

2.4 Data collection

At an early growth stage of the crop, five plants were selected from the ~~centre~~ centerof each plot, avoiding border plants and tagged. Observations were taken on fifteen (both qualitative and quantitative) traits using both IPGRI (International Plant Genetic Resource Institute) and NBPGR (National Bureau of Plant Genetic Resources) descriptors. These were early plant vigour, plant growth habit, leaf colour, ~~Lobing~~ lobing of the terminal leaflet, seed shape, seed colour, seed coat luster, days to 50% flowering, plant height (cm), number of branches per plant, number of clusters per plant, number of pods per plant, number of seeds per pod, seed yield per plant (gm), and test weight (gm). The ~~diseases~~ severity of the disease was recorded ~~by~~ using a standard disease rating scale. Observations were recorded during flowering to pod filling stages under natural field conditions. Observation on the incidence of Yellow mosaic virus was recorded on 1-9 rating scale (Park, 1978), disease incidence rating for Cercospora leaf spot was based on 1-9 rating scale (Alice and Nadarajan, 2007) and for Leaf curl virus disease (Ashfaq et al., 2007) 1-5 arbitrary scale was used. ~~Description~~ A description of all qualitative and quantitative traits are given in Table1.

Table 1 Description of traits

SN.	Morphological traits	Description
1	Early plant vigour	Recorded after 25 days of sowing. 1) poor, 2) Good, 3) Very good
2	Plant growth habit	Related to the position of branches on the stem. 1) Erect, 2) Semi erect, 3) Spreading
3	Leaf colour	Colour of the leaf at full foliage stage (after 30 days of sowing). 1) Green, 2) Dark green
4	Lobing of terminal leaflet	Recorded at first pod maturity. 1) Shallow, 2) Intermediate, 3) Deep
5	Seed shape	Dry seed shape. 1) Oval, 2) Drum
6	Seed colour	Dry seed colour. 1) Cream, 2) Light brown, 3) Dark brown
7	Seed coat luster	Dry seed coat luster 1) Shiny, 2) Dull
8	Days to 50% flowering	Number of days from sowing to when 50% of plant flowers
9	Plant height (cm)	From ground to tip of the plant recorded at maturity
10	Number of branches per plant	Number of branches on the main stem
11	Number of clusters per	Total number of clusters on the main stem

	plant	
12	Number of pods per plant	Total number of mature and effective pods per plant at maturity
13	Number of seeds per pod	Number of seeds per pod taken from 10 randomly selected pods
14	Seed yield per plant (gm)	The average yield of 5 random plants
15	Test weight (gm)	Weight of dried 100 random seeds

2.5 Statistical analysis

Multivariate approaches were also used to analyse the quantitative data. Pearson's correlation coefficients were calculated to determine the degree to which the eight traits are related to one another. The variation in genetic relationships between the collection's accessions was analysed using hierarchical clustering based on principal components (HCPC), as suggested by Cericolla et al. (2013). The level of dissimilarity among the accessions was assessed by doing cluster analysis on the principal components of the data. Based on Euclidean distances matrix, we applied ward's minimum variance clustering method to cluster accessions into different groups. To understand the dispersion and clustering of the accessions, the first two PCs were plotted, and the accessions were projected into the system of axes created by these two PCs.

3 Resultsand discussions

3.1.1Relationships among agro-morphological characters

The highest Pearson's correlation (0.857) was recorded between the number of clusters per plant and the number of pods per plant, followed by the number of pods per plant and seed yield per plant (0.787) (Table 2). As expected, the number of clusters per plant, number of pods per plant, plant height and the test weight showed a highly significant and positive correlation with seed yield per plant. These associations revealed the importance of these traits for improvement in seed yield as well as fodder yield.

Table 2Correlation among different agro-morphological traits

Traits	DF	PH	NBPP	NCPP	NPPP	NSPP	TW	SYPP
DF	1	0.162*	0.088	-0.074	-0.064	0.026	-0.058	-0.085

PH	1	0.105	0.274**	0.247**	0.061	0.430**	0.242**
NBPP		1	0.063	0.038	0.280**	0.194**	-0.074
NCPP			1	0.857**	0.133	0.255**	0.667**
NPPP				1	0.090	0.237**	0.787**
NSPP					1	0.056	0.067
TW						1	0.288**
SYPP							1

Similar results were also reported by Patil et al., 2008, Rahim et al., 2010, and Srivastava and Singh 2012. Days to 50% flowering and the number of branches per plant were negatively correlated with seed yield per plant. This implied that early flowering genotypes could yield higher under rainfed conditions because they can escapeterminal drought, which is a common feature under dryland conditions(Bhandari,1990).

3.1.2 Cluster analysis

Hierarchical clustering was done to define the existing diversity in the collection and to investigate the resemblances and variations among the accessions based on the eight quantitative traits. As per Ward's method, hierarchical cluster analysis categorizsed the 213 moth bean genotypes into fourteen groups. Cluster 1 comprises the 21 accessions, characterizsed by early flowering type, the shortest plant height and much less branching, which are most adapted to arid climates; therefore, selecting accessions from this cluster is advantageous. Cluster II had the maximum number of accessions with 43 accessions=, while cluster IV (32 accessions) represented the 2nd largest group. However, clusters 11 and 14 contained the minimum number of accessions.

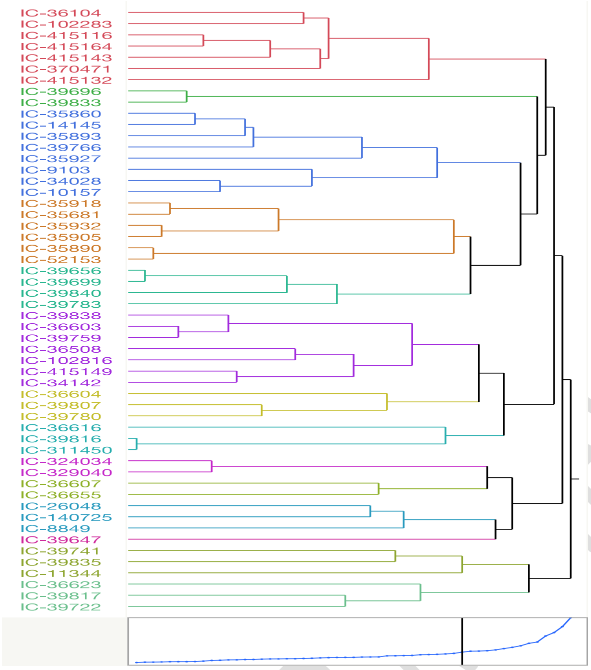

Fig 2. Hierarchical clustering by using PPS SRS method

To illustrate the dendrogram, the original dendrogram for 213 accessions was reconstructed using 54 accessions. These 54 accessions were picked randomly from each of the 14 clusters through the ~~P~~Probability Proportional to Size Simple Random Sampling (PPS SRS) technique. This way, we could get a true depiction of the original dendrogram.

Table 3 Inter and Intra-cluster distances

Cluster	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	2.14	2.74	3.59	3.54	3.45	2.81	4.13	3.92	3.98	4.52	7.81	5.33	4.61	3.64
2		1.84	3.01	2.60	3.54	3.22	3.65	3.53	3.48	4.13	7.12	4.58	3.76	2.56
3			2.34	2.80	2.99	2.93	3.12	3.32	3.44	4.26	6.34	3.79	3.61	3.31
4				2.11	2.95	3.33	3.46	3.91	3.57	4.19	6.96	4.27	3.93	2.84
5					2.07	2.53	4.04	4.47	4.12	4.73	6.99	4.32	4.39	3.69
6						1.97	3.77	3.98	4.23	4.71	6.50	4.06	3.83	3.35
7							2.14	3.32	4.29	4.83	6.66	4.45	4.03	3.91
8								2.41	3.10	3.45	6.96	4.96	4.34	14.19
9									2.08	3.06	7.87	5.63	5.07	4.54
10										2.78	7.90	5.76	5.41	4.71
11											3.86	4.85	4.83	5.96

12	2.90	3.51	3.62
13		2.66	2.98
14			1.82

The minimum inter-cluster distance was 2.53 units between clusters 5 and 6, and the maximum inter-cluster distance was recorded at 7.90 between cluster 10 and 11. The minimum intra-cluster distance was recorded in cluster 14 (1.82), having 14 accessions, whereas the maximum intra-cluster distance was found in cluster 11 (3.86) with 9 accessions (Table 5). To generate the maximum variability, accessions with more inter-cluster distance should be used in the crossing program. The genotypes of cluster 11 were diverse, indicating an opportunity for improvement through selection within the cluster.

3.1.3Principal component analysis (PCA)

Using standardised data, we performed principal component analysis on all eight quantitative traits to investigate the relationship among the traits and the factors determining trait variation. Principal component analysis with eigenvalue classified the accessions into eight principal components. When representing variation patterns among accessions, the first three principal components are the most relevant. PC1 contributed the maximum variation, 37.22 %, followed by PC2 with 17.62 % and PC3 contributed 15.22% to the total variation. Most of the variations were spread up to the 5th principal component, which accounted for 89.04% of the total variation.

Table 4Principal component analysis of moth bean accessions

Principal components (PCs)	Eigenvalue	% of variance	Cumulative %
1	2.977	37.216	37.216
2	1.409	17.624	54.840
3	1.217	15.215	70.055
4	0.880	11.004	81.059
5	0.638	7.981	89.040
6	0.447	5.588	94.629
7	0.310	3.879	98.508
8	0.119	1.492	100.000

PC1 contributed positively to the number of pods per plant, number of cluster per plant and yield per plant (g), PC2 (17.62 % variation) contributed more to number of branches per plant and number of seeds per pod and PC3 had greater weightage on days to 50% flowering and plant height. Similarly, the other five PCs (PC4, PC5, PC6, PC7 and PC8) contributed to the remaining variability with more weightage on the remaining traits. The traits associated with the first three PCs are more helpful (Cliffordand Stephenson,1975; Guei *et al.*,2005, Vir and Singh, 2015) in differentiating the accessions, as determined by eigenvalues.PCs having an eigenvalue greater than one need to be taken into consideration(Kaiser, 1961). Thus, it is effective for genetic improvement of characters with larger contributions to the variability rather than focusing on all the characters studied.

3.1.4 Biplot analysis

The biplot graph depicts the association between different yield and yield contributing traits and accession behaviour (Fig 5). The first two principal components were plotted to observe the relationship among the moth bean genotypes. An angle between vectors or lines < 90°C represents a positive correlation; if the angle is > 90°C, this indicates a negative correlation between the vectors. Except for the "days to 50% flowering," there was a positive association between most of the traits (Figure 5). Days to 50% flowering had negative correlation with seed yield per plant. This indicates that the yield is decreasing as the duration of flowering increases. The length of the vector line depicts the magnitude of the principal component's contribution to the trait. The PC1 contributed more to the traitsnumber of pods per plant, cluster per plant, number of pods per plant, seed yield per plant, plant height and test weight; whereas number of branches per plant and number of seeds per pod had more contribution of PC2. Days to 50% flowering had the minimum contribution of PC1 in variability.

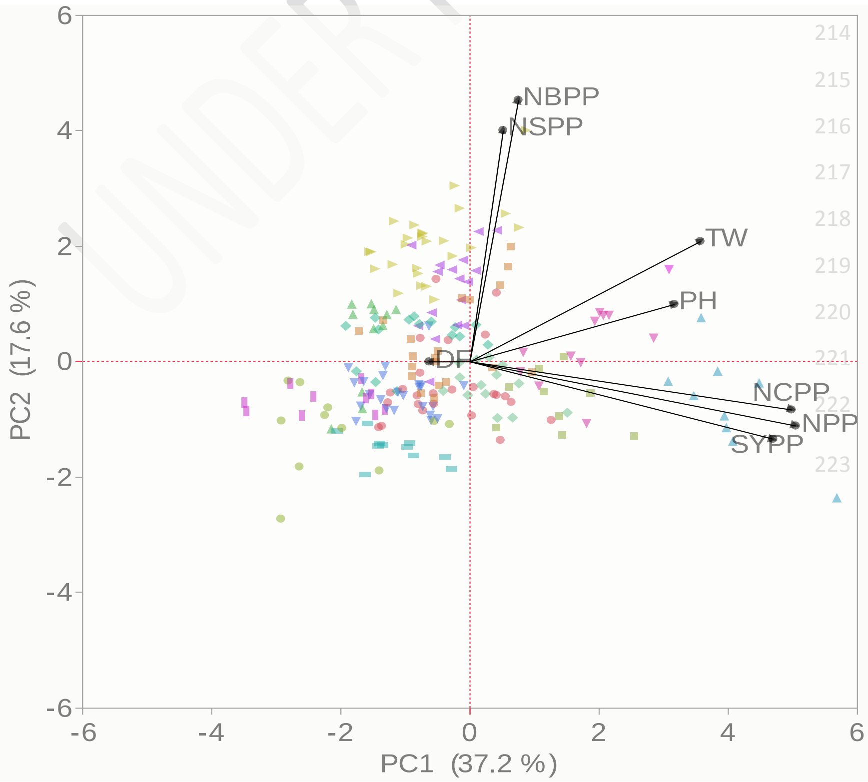

Fig 3 Biplot analysis of principal components

3.2Screening of moth bean accessions for different biotic (Yellow mosaic virus, Cercospora leaf spot and Leaf curl virus) stresses

Several pathogens affect the moth bean crop, which causes significant yield and quality losses in grain and fodder production. Under natural field conditions, yellow mosaic virus, leaf crinkle virus and Cercospora leaf spot diseases were detected, spreading through a large white fly population. Accessions were screened for all three diseases, and different degrees of disease reactions and their severity were recorded.

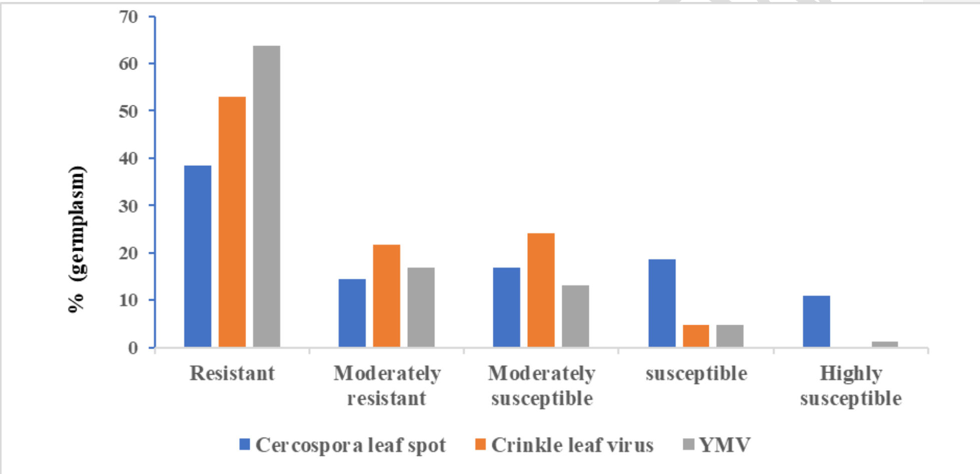

Fig 4. Behaviour of moth bean accessions against biotic stresses

The screening results indicate considerable genetic variability in the accessions for resistance. The disease incidence varies for each biotic stress because this depends on favourable environmental conditions, inoculum levels, and accession type. The response of moth bean accessions against the yellow mosaic virus, Cercospora leaf spot and leaf curl virus are presented in fig 4. *Cercospora* leaf spot had the highest disease incidence among the three diseases, ranging from 5-90%, followed by crinkle leaf virus (10-50%) and yellow mosaic virus (5-80%). It was observed that the accessions with early and medium maturity were more affected byCercospora leaf spot and accessions with late maturity had more incidence of yellow mosaic virus and crinkle leaf virus (Sharma et al., 2021). Based on screening results, the accessions IC-329051, IC-36623 IC-415104, IC-415116, IC-415127, IC-415139, IC-415155 were found highly susceptible to *Cercospora* leaf spot and one accession IC-103154 was found highly susceptible to yellow mosaic virus. Three accessions IC-39734, IC-39827 and IC-10158 were identified, which were showing high resistance to all the three diseases. Accessions with genetic resistance may be used directly or via hybridisation to develop high yielding resistant moth bean cultivars. Varietal resistance is the most effective approach to managing diseases in rainfed environments.

4. Conclusion

This study revealed that based on hierarchical clustering, 213 accessions were grouped into fourteen clusters. Accessions with more inter-cluster distance can be utilised in the crossing program, to generate the maximum variability.It was also observed that the accessions with early and medium maturity were more affected with Cercospora leaf spot and accessions with late maturity had more incidence of yellow mosaic virus and crinkle leaf virus.Further, this study will help the researchersto select potential genotypes most suited to extremeconditions and can be utilised in developing new varieties.

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