

Research Article

Multivariate analysis on blackgram genotypes for bruchine (*Callosobruchus maculatus* F.) resistance towards selection of parental lines

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Abstract

Black gram (*Vigna mungo* (L.) Hepper) is one of the most important pulse crops in daily diets. However, black gram production and post-harvest preservation are still tedious due to the losses caused by the storage pest bruchine, *Callosobruchus maculatus* (F.), both quantitatively and qualitatively. Hence, the present study involves the utilization of the multivariate analysis by effectively understanding variation among the genotypes based on their level of bruchine infestation. The multivariate studies indicated that the traits viz., the total number of adult emergence (AE), seed damage % (SD) and seed weight loss % (SWL) had more variation and with more significant correlation among them. Also, these traits are the most influential principal component traits governing 88% of the variation among genotypes. The divergence analysis showed that the genotype TU 68 found in cluster II would have the potential to create the variation for bruchine infestation among the black gram genotypes involved in the study. As it has scored lesser adult emergence (AE) (7 adults), seed damage % (SD) (14 %) and seed weight loss % (SWL) (17.79 %) than the other genotypes. It shows the resistant nature of the genotype against bruchine beetles. Hence, TU 68 could be utilized in the future hybridization programme as a donor for bruchine resistance.

Keywords: Association, Blackgram, Bruchine, Cluster, PCA, Resistance

INTRODUCTION

Blackgram [*Vigna mungo* (L.) Hepper] is one of the important pulse crops of India. India is the largest producer and also consumer of blackgram. It is referred to as the “king of the pulses” due to its delicious taste and numerous other nutritional qualities. It is also popular for its fermented foods in Southern India. In India, the area that comes under blackgram is about 4.50 million hectares with a production of 2.83 million tonnes (Anonymous, 2019). Among various biotic and abiotic

yield limiting factors, storage pests that feed on seeds have always been one of the most important biotic constraints for the crop and cause severe economic loss during post-harvest storage. The net quantum of productivity and its economic value is constantly being affected by the storage pests, especially the storage pest bruchine (*Callosobruchus* sp.) (Swella and Mushobozy, 2009). Bruchine infestation primarily starts in the field condition and carry over into the storage condition where the seeds are completely damaged within 2-3 months. Damaged seeds lose their seed weight as well

as their nutritional quality. So, the post-harvest losses are the major obstacles to fulfil food security in developing nations (Somta *et al.*, 2007; Ponnusamy *et al.*, 2014). The post-harvest damage caused by the bruchine beetles varies from crop to crop, depending on the prevalent species and their respective biotype. In India, *C. maculatus* is responsible for 90 % yield loss in black gram (Soundararajan *et al.*, 2013). Hence, a study was undertaken to identify the resistant nature of 20 blackgram cultivars towards the infestation by *C. maculatus*. Principle component analysis (PCA) mainly emphasizes phenotypic traits components and their association by understanding the distribution among correlated and uncorrelated traits for the given phenotypes. Genetic diversity is an evolutionary platform where the created groups transfer the desirable genes through the hybridization process from the parent to progeny (Sharifi *et al.*, 2018; Perera *et al.*, 2017). The success of any breeding program depends on the magnitude of genetic variability and the extent of association between traits. Hence, the present study aimed at identifying the diverse set of parents through multivariate analysis among the blackgram genotypes against bruchine infestation.

MATERIALS AND METHODS

Source of seeds and insect culture

The seeds of twenty blackgram genotypes were obtained from the National Pulses Research Center (NPRC), Vamban, India and Bhabha Atomic Research Center, Trombay, India. Adults of *C. maculatus* were initially collected from infested seed lots from NPRC, Vamban. Among the various species, *C. maculatus* covers the major proportion of nearly 90% in the seed lots of Vamban. A representative sample of *C. maculatus* was collected and maintained on greengram [*Vigna radiata* (L.) Wilczek] seeds with temperature ranging of 29° -32°C and 60-70% RH for multiplication.

Experimental design

The experimental design for this study was a completely randomized design under three replicates per entry. The bruchine screening experiment was carried out at Entomology laboratory at NPRC, Tamil Nadu Agricultural University, Vamban. The assay procedure of Dongre *et al.* (1996) was followed with some modifications. Modification such as instead of two pairs of adults, five pairs of adults were released on 50 seeds of each genotype with replication placed in a 15 cm diameter plastic Petri plates. The insects were allowed to remain in petriplates for five days for oviposition. After five days the adults were removed from petriplates.

Data observation for evaluation of bruchine infestation

Observations were recorded on oviposition on 50 seeds, the mean number of eggs per seed, developmental time (egg + larval + pupal stages) (days), adult emergence, mean developmental period (days), Howe's Index of susceptibility, seed damage (%) and seed weight loss (%). Seed damage and seed weight loss were estimated 50 days after adult infestation (DAI). The adults who emerged were counted on a daily basis and removed from the petriplates to avoid secondary infestation.

Statistical analysis

The data was analyzed by a completely randomized design. The descriptive statistics including range, mean, standard error and the principal component analysis (PCA) was done by the method described by Upadhyaya *et al.* (2002). The clustering was performed using Tocher's method. The multivariate analysis was performed using software PYTHON programming language. Variability parameters like phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2) and genetic advance as per cent of mean (GAM) were calculated as suggested by Johnson *et al.* 1955. The data were analyzed using the statistical software TNAUSTAT statistical package (Manivannan, 2014).

RESULTS AND DISCUSSION

The multivariate analysis is known to be good at evaluating the genotypes by identifying the potential traits that should be further evaluated at the genetic level (Mohammad *et al.*, 2009). Multivariate analysis is the best tool for choosing promising genotypes for the breeding programme (Ceolin *et al.* 2007). The basic pre-requisite thing in the multivariate analysis and the divergence analysis is the presence of significant differences among the genotypes taken for study. The Analysis of variance (ANOVA) of the present data revealed significant differences among the genotypes for all traits of bruchine infestation involved in multivariate analysis.

The descriptive data of bruchine resistance traits of 20 blackgram genotypes from three replications are furnished in Table. 1. The traits number of eggs per 50 seeds ranged from (48 - 147). The mean eggs per seed ranged from 1 - 2.9 ensures the presence of at least one egg per seed through the artificial screening method. The developmental time among the screened genotypes ranging from 21 - 39 days showed that the prevalence of the prolonged developmental time i.e., delayed emergence. Regarding the trait total number of adult emergence (6 - 50), the range indicated that the pres-

Table 1. Descriptive statistics of bruchine resistant traits on blackgram genotypes

| S. No. | Traits observed | Mean \pm Standard Error | | Min | Max |
|--------|--|---------------------------|------|------|-------|
| 1 | Number of eggs/50 seeds (NES) | 77.63 \pm | 2.59 | 48.0 | 147.0 |
| 2 | Mean No. of eggs/seed (MNES) | 1.55 \pm | 0.05 | 1.0 | 2.9 |
| 3 | Developmental time (days) (D.T) | 26.18 \pm | 0.43 | 21.0 | 39.0 |
| 4 | Total No. of Adult emergence (A.E) | 35.62 \pm | 1.40 | 6.0 | 50.0 |
| 5 | Mean developmental period (days) (MDP) | 32.78 \pm | 0.48 | 26.9 | 44.2 |
| 6 | Index of susceptibility (I.S) | 9.45 \pm | 0.22 | 3.5 | 12.4 |
| 7 | Seed damage (%) (SD) | 71.00 \pm | 2.76 | 12.0 | 98.0 |
| 8 | Seed weight loss (%) (SWL) | 34.37 \pm | 1.08 | 14.5 | 50.0 |

ence of contrasting genotypes for the bruchine resistance among the screened genotypes. The mean developmental period ranged from 26.9 – 44.2 days after infestation, the index of susceptibility ranged from 3.5 – 12.4, the seed damage percentage and seed weight loss percentage ranged from 12.0 - 98.0 and 14.5 - 50.0, respectively. The descriptive statistics based on the bruchine resistance traits indicated that the presence of diverse range genotypes in the study. The principal component analysis (PCA) showed that PC1 and PC2 alone accounted for 88% of the cumulative proportion of variation (Table 2). The Eigen values of the principal components PC1 and PC2 were found to be greater than unity (one) were shown in the Scree plot (Fig. 1). In PC1, all traits were positively contributed with 63% of variation except traits *viz.*, development time and mean developmental period. Among them, more positive contribution was rendered by the index of susceptibility (0.41), the total number of adult emergence (0.41), seed damage (0.41) and seed weight loss (0.39). While rest of the components, *viz.*, number of eggs per 50 seeds (0.57), the mean number of eggs per seed (0.58), mean developmental period (0.42) and

developmental time (0.30) were positively contributed more variation in PC2 (Table. 2) (Fig. 2). Hence, the foremost importance has to be given to those traits with more contribution towards variation for the selection of parental lines for bruchine resistance. It will help to select bruchine resistant donor for future hybridization program.

The genetic divergence study grouped the 20 blackgram genotypes into four clusters based on Tocher's method (Table 3). The dendrogram heat map is furnished in Fig. 3. The results are in agreement with Ghosh *et al.* 2019. Among the clusters, cluster I was the major one with nine genotypes, followed by cluster III and cluster IV with eight and two genotypes, respectively. Cluster II was found solitary with only one genotype. The intra and inter cluster distance among the clusters is furnished in Table 4. The maximum intra cluster distance was shown by cluster I (12.7), followed by cluster III (12.2) and cluster IV (12.2). This maximum intra-cluster distance indicated that the genotypes present within these clusters were more diverse than the other clusters. Comparing intra and inter-cluster distance, inter-cluster distance will be helpful in the

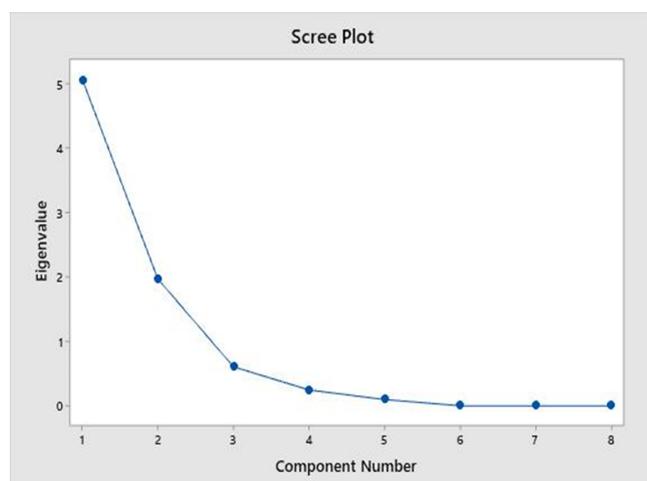
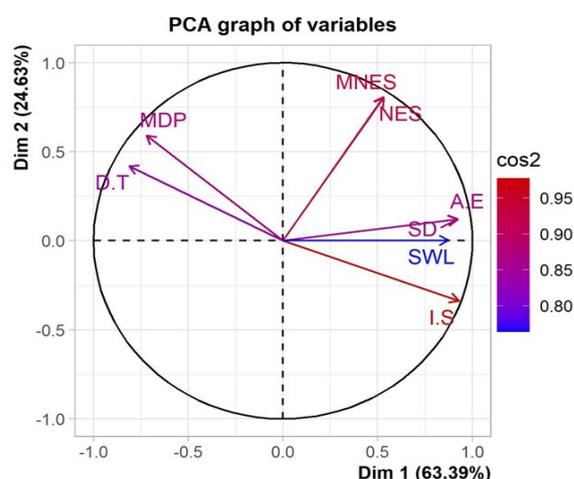
**Fig. 1.** Scree plot for the Eigen values of the principal components on bruchid infestation.**Fig. 2.** Biplot on various bruchine resistance trait on blackgram genotypes

Table 2. Principal components of bruchine resistant traits on blackgram genotypes

| S. No. | Traits observed | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 |
|--------|--|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 | Number of eggs/50 seeds (NES) | 0.24 | 0.57 | 0.33 | 0.00 | 0.06 | -0.03 | -0.03 | -0.71 |
| 2 | Mean No. of eggs/seed (MNES) | 0.24 | 0.58 | 0.33 | 0.01 | 0.06 | 0.03 | 0.03 | 0.71 |
| 3 | Developmental time (days) (DT) | -0.36 | 0.30 | -0.41 | 0.14 | 0.77 | -0.03 | -0.01 | 0.00 |
| 4 | Total No. of Adult emergence (AE) | 0.41 | 0.09 | -0.43 | 0.30 | -0.11 | 0.12 | 0.73 | -0.04 |
| 5 | Mean developmental period (days) (MDP) | -0.32 | 0.42 | -0.40 | -0.12 | -0.52 | -0.52 | -0.05 | 0.03 |
| 6 | Index of susceptibility (IS) | 0.41 | -0.24 | 0.07 | 0.21 | 0.26 | -0.81 | -0.08 | 0.03 |
| 7 | Seed damage (%) (SD) | 0.41 | 0.09 | -0.43 | 0.32 | -0.13 | 0.26 | -0.68 | 0.02 |
| 8 | Seed weight loss (%) (SWL) | 0.39 | 0.00 | -0.29 | -0.86 | 0.19 | 0.01 | -0.01 | 0.01 |
| | Eigen Values | 5.07 | 1.97 | 0.61 | 0.25 | 0.10 | 0.00 | 0.00 | 0.00 |
| | Proportion of Variance | 0.63 | 0.25 | 0.08 | 0.03 | 0.01 | 0.00 | 0.00 | 0.00 |
| | Cumulative Proportion | 0.63 | 0.88 | 0.96 | 0.99 | 1.00 | 1.00 | 1.00 | 1.00 |

Table 3. Clustering pattern among blackgram genotypes

| Cluster number | Number of genotypes | Constituent genotypes |
|----------------|---------------------|--|
| I | 9 | APK 1, KKM 1, MASH 1008, CO 6, MDU 1, LBG 752, VBN(Bg)7, ADT 6, VBN 11 |
| II | 1 | TU 68 |
| III | 8 | TMV 1, CO 5, VBN(Bg)4, ADT 3, VBN 6, VBN 9, LBG 787, VBN 8 |
| IV | 2 | ADT 5, MASH114 |

Table 4. Average intra (bold) and inter cluster distances in blackgram genotypes

| | Cluster I | Cluster II | Cluster III | Cluster IV |
|-------------|-------------|------------|-------------|-------------|
| Cluster I | 12.7 | | | |
| Cluster II | 92.7 | 0.0 | | |
| Cluster III | 40.0 | 57.6 | 12.2 | |
| Cluster IV | 36.6 | 64.9 | 33.0 | 12.2 |

identification of the genetic divergence among the genotypes of the experimental material (Reddy *et al.*, 2018). Maximum inter-cluster distance (92.7) was observed between cluster II and I followed by cluster IV and II (64.9) and cluster III and II (57.6). Inter cluster distances and mean values of clusters (Table. 5) indicated that cluster II (TU 68) found to be a potential donor as it showed the superior nature of bruchine resistance. It had superior performance on bruchine resistance traits *viz.*, number of eggs laid per 50 seeds (NES), the mean number of eggs per seed (MNES), developmental time (DT), adult emergence at 50 days after infestation (AE), mean developmental period (MDP) and less susceptibility index (IS). And also, it recorded less seed damage % (SD) and seed weight loss % (SWL). The principal component traits also clearly showed the genotype's superiority in cluster III (TU 68) for bruchine resistance among the genotypes. The genotype TU 68 confirmed its resistance in another study reported by Swamy *et al.*, 2016. Hence, this genotype TU 68 is confirmed for bruchine resistance and can be involved in further bruchine resistance breeding

programme.

The variability parameters such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2) and genetic advance as per cent of mean (GAM) are presented in Table. 6. Among traits taken for the study, PCV was found to be slightly greater than the GCV, which showed less prevalence of environmental influence among the bruchine resistance traits. High PCV was recorded for the traits total number of adult emergence (AE) (30.91%) followed by seed damage % (SD) (30.63%), the mean number of eggs per seed (MNES) (26.03%), number of eggs per 50 seeds (NES) (26.02%) and seed weight loss % (SWL) (24.67%). The remaining traits recorded moderate PCV. High GCV was recorded by the traits total number of adult emergence (AE) (28.22%) followed by seed damage % (SD) (28.06%) and seed weight loss % (SWL) (20.08%). In comparison, all other traits recorded moderate GCV. High PCV and GCV indicated the presence of greater genetic variability for these bruchine resistance traits among the genotypes.

High heritability (h^2) was recorded for all traits, except

Table 5. Cluster mean values for four clusters obtained by Tocher's method

| | NES | MNES | DT | AE | MDP | IS | SD | SWL |
|-------------|-------|------|-------|-------|-------|-------|-------|-------|
| Cluster I | 87.78 | 1.76 | 25.48 | 44.70 | 32.29 | 10.29 | 88.89 | 39.32 |
| Cluster II | 60.00 | 1.20 | 38.00 | 7.00 | 43.47 | 3.88 | 14.00 | 17.79 |
| Cluster III | 63.71 | 1.27 | 25.46 | 30.63 | 31.90 | 9.36 | 61.25 | 31.55 |
| Cluster IV | 96.50 | 1.93 | 26.34 | 29.00 | 33.15 | 8.82 | 58.00 | 31.72 |

NES- Number of eggs/50 seeds, MNES - Mean No. of eggs/seed, DT- Developmental time (days), AE- Total No. of Adult emergence, MDP- Mean developmental period (days), IS- Index of susceptibility, SD- Seed damage (%), SWL- Seed weight loss (%)

Table 6: Variability analysis on various bruchine resistant traits on blackgram genotypes

| S. No. | Traits observed | PCV (%) | GCV (%) | h^2 (%) | GAM (%) |
|--------|--|---------|---------|-----------|---------|
| 1 | Number of eggs/50 seeds (NES) | 26.02 | 18.33 | 49.62 | 26.60 |
| 2 | Mean No. of eggs/seed (MNES) | 26.03 | 18.33 | 49.62 | 26.60 |
| 3 | Developmental time (days) (DT) | 13.00 | 12.66 | 94.82 | 25.39 |
| 4 | Total No. of Adult emergence (AE) | 30.91 | 28.22 | 83.37 | 53.08 |
| 5 | Mean developmental period (days) (MDP) | 11.50 | 10.29 | 80.03 | 18.96 |
| 6 | Index of susceptibility (IS) | 18.46 | 17.13 | 86.05 | 32.73 |
| 7 | Seed damage (%) (SD) | 30.63 | 28.06 | 83.89 | 52.94 |
| 8 | Seed weight loss (%) (SWL) | 24.67 | 20.08 | 66.23 | 33.66 |

Table 7. Correlation analysis on various bruchine resistant traits on blackgram genotypes

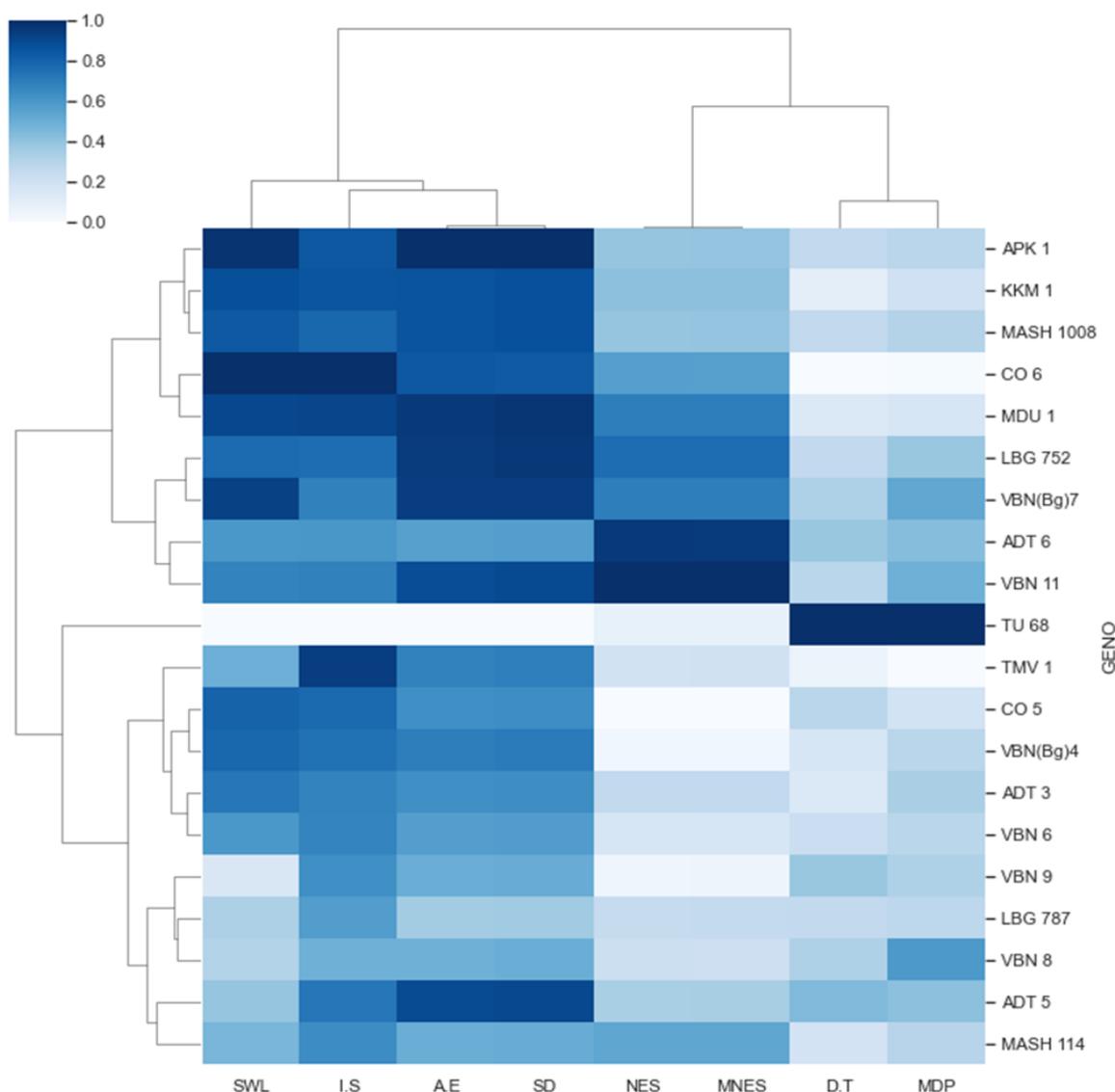
| | NES | MNES | DT | AE | MDP | IS | SD | SWL |
|------|-------|-------|--------|--------|--------|-------|-------|------|
| NES | 1.00 | | | | | | | |
| MNES | 1.00 | 1.00 | | | | | | |
| DT | -0.17 | -0.17 | 1.00 | | | | | |
| AE | 0.50* | 0.50* | -0.59* | 1.00 | | | | |
| MDP | 0.01 | 0.01 | 0.89* | -0.49* | 1.00 | | | |
| IS | 0.24 | 0.24 | -0.89* | 0.81* | -0.90* | 1.00 | | |
| SD | 0.50* | 0.50* | -0.59* | 1.00* | -0.49* | 0.81* | 1.00 | |
| SWL | 0.41 | 0.41 | -0.65* | 0.82* | -0.54* | 0.76* | 0.81* | 1.00 |

*significant at 5% probability, NES- Number of eggs/50 seeds, MNES - Mean No. of eggs/seed, DT- Developmental time (days), AE- Total No. of Adult emergence, MDP- Mean developmental period (days), IS- Index of susceptibility, SD- Seed damage (%), SWL- Seed weight loss (%)

the number of eggs per 50 seeds (NES) and mean the number of eggs per seed (MNES), which showed moderate heritability. Hence traits with high heritable nature can be subjected to selection for further trait improvement. High Genetic advance as per cent of mean (GAM) was recorded for all traits except mean developmental period (MDP) which showed moderate genetic advance as per cent of mean (GAM). Hence the selection for the bruchine resistance traits with high heritability and high genetic advance as per cent of mean is recommended for the genetic improvement program. Among the traits *viz.*, total number of adult emergence (AE), seed damage % (SD) and seed weight loss %

(SWL) indicated the presence of more variability among them. Similar type of results also reported by Indhu *et al.*, 2018 in screening other set of blackgram genotypes for bruchine resistance. Hence selection based on these traits can be utilized for crop improvement program.

The estimate of correlation coefficients for the bruchine resistant traits are presented in the Table 7. The Correlation heat map on bruchine resistance traits is furnished in Fig. 4. It indicates the trait seed weight loss had a significant positive association with total number of adult emergence (0.82), seed damage % (0.81) and index of susceptibility (0.76). However, the trait seed



Y-axis = Genotypes; X-axis = Bruchine resistance traits.

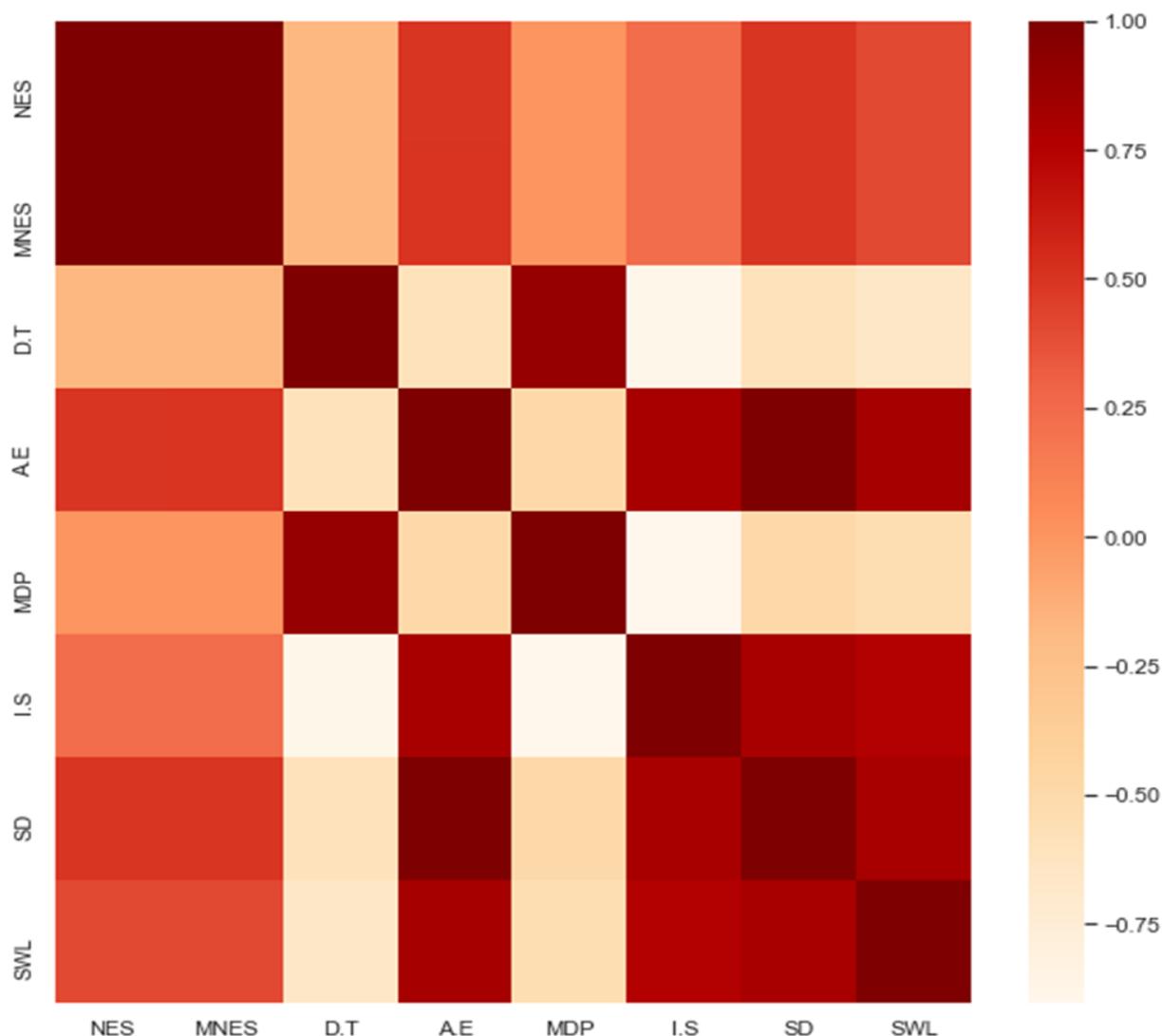
Fig. 3. Dendrogram heat map on various bruchine resistance traits on blackgram genotypes

weight loss percentage had recorded significant and negative association with developmental time (-0.65) and mean developmental period (-0.54). This indicates that the trait seed weight loss decreases with increasing developmental time and mean developmental period. The trait seed damage showed a significant and positive association with a total number of adult emergence (1.00), index of susceptibility (0.81), the number of eggs per 50 seeds (0.50) and mean number of eggs per seed (0.50). However, the trait seed damage had recorded a negative and significant association with developmental time (-0.59) and mean developmental period (-0.49). Index of susceptibility had a significant positive association with a total number of adult emergence (0.81). It also recorded a significant and negative association with developmental time (-0.89) and mean developmental period (-0.90). The trait mean develop-

mental period had a significant positive association with developmental time (0.86). Total number of adult emergence had significant positive association with the number of eggs per 50 seeds (0.41) and mean number of eggs per seed (0.41). It also recorded a negative and significant association with developmental time (-0.59). Hence these correlated traits can be directly involved in the genetic improvement program.

Conclusion

The present investigation on the identification of potential donors for bruchine infestation and in the framing of the hybridization programme based on the multivariate analysis indicated that the traits viz., total number of adult emergence (AE), seed damage % (SD) and seed weight loss % (SWL) had more variations in black gram



*Y-axis & X-axis = Bruchine resistance traits.

Fig. 4. Correlogram heat map on various bruchine resistance traits on blackgram genotypes

than other traits. Based on the D² study cluster II (TU 68) could be utilized as a donor parent in the development of high yielding black gram (*Vigna mungo* L. (Hepper)) variety with bruchine resistance. The trait seed weight loss had a significant positive association with a total number of adult emergence, seed damage percentage and index of susceptibility. All these traits had a positive inter-correlation among them. Hence, these traits can be given more importance in formulating the bruchine resistance breeding programme to identify the resistance among the cultivars.

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Conflict of interest

The authors declare that they have no conflict of interest.

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