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# Evaluation of genetic divergence in cowpea (*Vigna unguiculata* ) for major insect pests*

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Cowpea [*Vigna unguiculata* (L) Walp.], an annual leguminous fodder crop has a great potential for sustainable agriculture in marginal lands and semi-arid regions of the country. The green cowpea fodder is rich in protein and is considered as balance diet to animals for higher milk and meat production. It is popular as intercrop with maize, sorghum, pearlmillet and teosinte.

This species is attacked by many insect pests that limit its productivity throughout the country. The losses in green fodder yield are as high as 30%. In Central India the crop is severely damaged by the defoliator insects, mainly flea beetle (*Pagaria signata* Motsch), semilooper (*Plusia nigrisigna* Wlk.), tobacco caterpillar (*Spodoptera litura* F.) and various species of grasshoppers (*Hieroglyphus nigrorepletus* Bol., *Cantontopes pinguis* Stol., *Oedalius abruptus* Thun., *Acridida exaltata*, *Chrotogonus tachypterus* Blanch., *Atractomorpha crenulata crenulata* Fabr.). Among all these insects, maximum losses were caused by the semiloopers (Saxena *et al*. 2002).

The knowledge of genetic diversity and association of component characters with insect tolerant is important for crop improvement programme (Hall 2003) and the host plant resistance is one of the major components of insect pest management. Lack of resistant cultivars is one of the major constrains for the integrated management programmes. Therefore, it is important to identify insect pest-resistant genotypes that could be used in resistance breeding programmes. Hence, a study was undertaken to identify the genotypes for insect pest tolerance, specifically towards semi-looper caterpillar (*Plusia nigrisigna* Wlk.) and to study the relationship of pest damage and the plant characters.

One hundred and eighty eight accessions (172 exotic and 16 indigenous) were screened under natural infestation conditions for two years 2006 and 2007 in monsoon season against major insect pests at Central Research Farm of Indian Grasslands and Fodder Research Institute, Jhansi.

The accessions were kept unsprayed during the experiment. The materials were planted in augmented design with four control varieties, viz ‘Bundel Lobia 1’ (BL 1), ‘Bundel Lobia 2’ (BL 2), ‘UPC 5286’ and ‘IGFRI 95 1’ (local control). Two rows (3 m length) were assigned for each entry. Row-to-row distance was 60 cm with plant-to-plant distance of 20 cm. Each entry was planted 100cm apart from the next entry.

Assessment of per cent leaf area damage was calculated using graph paper method. Ten central leaflets of fully opened leaf (third from top) from five randomly selected plants were selected for injury estimation. The observations were taken at three stages (15, 30, and 45 days after sowing) during every crop season. The pest resistance percentage for entries was calculated using Abbott’s formula.

$$\text{Pest resistance (\%)} = \frac{\text{Pest incidence in check-pest incidence in test entry}}{\text{Pest incidence in check}} \times 100$$

The genotypes were given pest resistance/susceptible rating (PRSR) based on pest resistance percentage score (100, 99 to 75, 74 to 50, 49 to 25, 24 to10, 9 to –10, –11 to –25, –26 to –50 and >–50 into 1 to 9 grading, respectively). Based on the following scale, low rating was the criteria of selection for tolerant entries. Data on 13 morphological descriptors, viz plant height, stem girth, stem weight, leaves/plant, leaf length, leaf width, leaf weight/plant, branch length, number of primary branches, number of secondary branches, leaf: stem ratio, fresh plant weight and dry plant weight were also recorded in the five randomly selected plants in each plot.

The step-wise partial regression analysis was performed

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(for all the accessions) with the ten plant parameters, viz plant height, stem girth, stem weight, leaves/plant, leaf length, leaf width, leaf weight/plant, branch length, no of primary and secondary branches. This analysis was used to correlate morphological descriptors with the insect damage. Cluster analysis and principal component analysis (PCA) were performed (for 15 insect tolerant genotypes) for understanding genetic diversity among the selected accessions. SPSS 13.0 window version was used for all the statistical analysis. The genotypes placed in different groups, and showing resistance to insect damage can be used in resistance breeding programmes to diversify the basis of resistance to this pest.

On the basis of data recorded during 2006 and 2007 the insect pest incidence on test entries varied from 5.6 to 46.7% as against 20.6–29.3% in check lines (Table 1). Ninetyeight lines were found better than the check ‘BL 1’. Out of which 15 accessions were the best in terms of the pest resistance/ susceptible rating (PRSR) value of 2 and 3. Least leaf area damage (5.6%) was recorded in the ‘EC 548976’. While 53 lines were at par with the check for the leaf area damage in the range of 21.2–29.3% (PRSR value of 6 and 7) and 38 lines were susceptible with leaf area damage in the range of 29.5–46.7% (PRSR value of 8 and 9). The most susceptible lines were ‘EC 548896’, ‘EC 548917’ and ‘EC 548959’. Using similar type of rating method for identification of insect pest tolerance in 162 cowpea genotypes during 2004–06, 4 lines (‘EC 24102 1’, ‘IL 1063’, ‘IL 05 08’ and ‘EC 240884’) emerged as most promising (Roy *et al*. 2008).

The step-wise partial regression analysis indicated that out of ten plant morphological characters only leaf width (negatively) was significantly correlated ($r^2 = 0.02$, $P = 0.007$)

Table 1 Cowpea genotypes showing pest resistance grading

| Pest resistance/ susceptible rating | Leaf area damage (%) | Accessions* |
|---|---|---|
| 2 | 5.6 | EC 548976 (1) |
| 3 | 6.7–11.0 | EC 548886, EC 548932, EC 548936, EC 548944, EC 548987, EC 549003, EC 549011, EC 549012, EC 549016, EC 549017, IC 438361, IC 438372, IC 438373, IC 438375 (14) |
| 4 | 11.8–17.6 | EC 548850, EC 548853, EC 548854, EC 548856, EC 548857, EC 548862, EC 548865, EC 548876, EC 548879, EC 548880, EC 548897, EC 548898, EC 548901, EC 548906, EC 548907, EC 548912, EC 548914, EC 548919, EC 548931, EC 548933, EC 548934, EC 548935, EC 548938, EC 548939, EC 548941, EC 548942, EC 548947, EC 548949, EC 548957, EC 548964, EC 548969, EC 548973, EC 548977, EC 548982, EC 548996, EC 548999, EC 549004, EC 549014, EC 549015, EC 549018, IC 438362, IC 438364, IC 438368, IC 438369, IC 438374 (45) |
| 5 | 17.8–21.0 | EC 548858, EC 548863, EC 548864, EC 548900, EC 548904, EC 548908, EC 548909, EC 548911, EC 548920, EC 548921, EC 548925, EC 548929, EC 548930, EC 548943, EC 548948, EC 548951, EC 548953, EC 548965, EC 548966, EC 548968, EC 548970, EC 548974, EC 548978, EC 548981, EC 548985, EC 548998, EC 549000, EC 549002, EC 549006, EC 549008, EC 549010, EC 549013, EC 549020, EC 549021, IC 438365, IC 438367, IC 438370, IC 438371 (38) |
| 6 | 21.2–25.9 | EC 548855, EC 548872, EC 548874, EC 548877, EC 548878, EC 548895, EC 548905, EC 548915, EC 548916, EC 548926, EC 548952, EC 548955, EC 548958, EC 548960, EC 548967, EC 548971, EC 548979, EC 548980, EC 548983, EC 548984, EC 548988, EC 548989, EC 548990, EC 548991, EC 548995, EC 548997, EC 549007, EC 549019, IC 438363, IGFRI 95–1, EC 4216, BL–1 (29+3) |
| 7 | 26.0–29.3 | EC 548851, EC 548861, EC 548873, EC 548881, EC 548882, EC 548883, EC 548885, EC 548889, EC 548891, EC 548892, EC 548899, EC 548903, EC 548918, EC 548922, EC 548945, EC 548946, EC 548950, EC 548961, EC 548963, EC 548986, EC 548992, EC 548994, EC 549001, IC 438366, BL–2 (24+1) |
| 8 | 29.5–35.1 | EC 548852, EC 548860, EC 548867, EC 548869, EC 548875, EC 548884, EC 548887, EC 548888, EC 548893, EC 548894, EC 548910, EC 548913, EC 548923, EC 548924, EC 548927, EC 548928, EC 548937, EC 548956, EC 548962, EC 548972, EC 548975, EC 548993, EC 549009, IC 438360 (24) |
| 9 | 35.4–46.7 | EC 548859, EC 548866, EC 548868, EC 548870, EC 548871, EC 548890, EC 548896, EC 548902, EC 548917, EC 548940, EC 548954, EC 548959, EC 549005 (13) |

*Figures in parentheses are number of entries + check with the insect pest damage. The number of leaves/plant, leaf length, no of secondary branches showed collinearity with the leaf width. The relation ship thus can be depicted as follows:

Y=28.47–1.00X

where Y, pest damage (%) X, leaf width (cm)

Pandey *et al*. (1995) have earlier reported a positive correlation flea beetle damage, bacterial blight incidence and root knot index with the stem length. In the same study they have also observed a positive significant relation between leaf weight and flea beetle and semilooper damage in fodder cowpea.

Since incidence of insect pest damage is influenced by various plant characters and environmental factors, the pest resistance/susceptible rating alone cannot be relied on to use the accessions for insect resistance breeding programmes (Aipala *et al*. 2002). The application of cluster analysis and PCA were used in this study to realize the genetic diversity of insect tolerant-genotypes for their successful exploitation in advance hybridization programmes.

Based on cluster analysis using average linkage between groups using morphological descriptors the accessions were grouped into three clusters (Fig 1). Cluster I, II, and III comprised 9, 2 and 4 accessions, respectively. The means of plant characters for individual clusters are presented in Table 2. Derived from intra cluster distance Cluster I was more distinct from other cluster. The cluster I contained taller, thicker, and plants having longer branches, and more number of leaves. The leaves of cluster I genotypes were also broader and longer in comparison to the accessions of group II and group III. The cluster II was characterized by low pest damage, lesser number of secondary branches. The maximum inter-cluster distance was observed in cluster I and II, followed by II and III. Parental lines from these clusters may be used for developing high-yielding insect-tolerant lines.

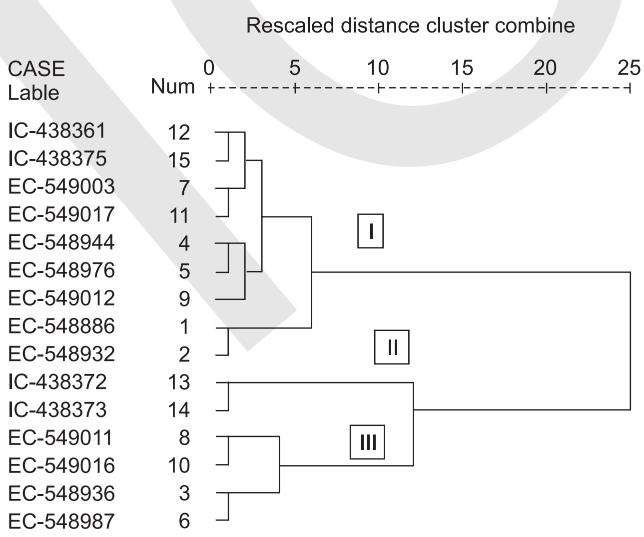


Fig 1 Clustering of cowpea genotypes by average linkage method

Table 2 Means of plant characters in different clusters

| Descriptor | Cluster I | Cluster II | Cluster III |
|---|---|---|---|
| Insect damage (%) | 8.73 | 6.70 | 8.60 |
| Plant height (cm) | 81.08 | 117.95 | 123.52 |
| Branch length (cm) | 86.63 | 131.02 | 146.00 |
| Number of primary branches | 5.94 | 7.92 | 8.48 |
| Number of secondary branches | 1.51 | 0.91 | 2.27 |
| Stem girth (cm) | 0.87 | 1.55 | 1.44 |
| Number of leaves/plant | 62.62 | 85.25 | 61.01 |
| Leaf length (cm) | 9.48 | 11.23 | 10.89 |
| Leaf width (cm) | 6.37 | 9.39 | 8.13 |
| Leaf weight/ plant (g) | 55.53 | 121.20 | 94.28 |
| Stem weight/plant (g) | 101.82 | 296.05 | 198.51 |
| Green biomass (g) | 157.35 | 417.24 | 292.79 |
| Leaf stem ratio | 61.72 | 320.38 | 188.94 |
| Dry weight/plant (g) | 0.58 | 0.42 | 0.49 |

The principal component analysis performed on these descriptors revealed that the first three components accounted for 76.95% variation (Table 3). The principal component 1 showed greater weight to plant height, stem girth, leaf width, number of primary branches, branch length, leaf weight/plant, stem weight/plant, leaf: stem ratio, fresh weight/plant and dry weight/plant. Leaf length and number of secondary branches were given higher weight in PC2. While insect damage and Leaves/plant received greater weight in PC3.

The scatter plot for principal component score of first two components has further refined the groups (Fig 2) and is in conformity with the cluster analysis. The accessions in clusters II and III were regrouped except the 'EC 549011' of

Table 3 Eigen values, variance and factor loadings of the main components of the descriptors

| | PC1 | PC2 | PC3 |
|---|---|---|---|
| Eigen value | 7.32 | 2.06 | 1.39 |
| Variance (%) | 52.31 | 14.71 | 9.93 |
| Cumulative variance (%) | 52.31 | 67.03 | 76.95 |
| Descriptors | | *Factor loadings* | |
| Insect damage (%) | –0.285 | 0.226 | 0.369 |
| Plant height (cm) | 0.733 | 0.484 | –0.004 |
| Stem girth (cm) | 0.910 | 0.053 | 0.102 |
| Number of leaves/plant | 0.158 | 0.379 | –0.793 |
| Leaf length (cm) | 0.395 | –0.777 | 0.291 |
| Leaf width (cm) | 0.720 | –0.623 | 0.157 |
| Branch length (cm) | 0.847 | 0.385 | 0.194 |
| Number of primary branches | 0.586 | 0.256 | 0.230 |
| Number of secondary branches | 0.209 | 0.544 | 0.529 |
| Leaf weight/plant (g) | 0.954 | 0.116 | 0.047 |
| Stem weight/plant (g) | 0.960 | –0.070 | –0.120 |
| Leaf : stem ratio | –0.643 | 0.323 | 0.279 |
| Fresh weight/plant (g) | 0.970 | –0.021 | –0.076 |
| Dry weight/plant (g) | 0.941 | –0.053 | –0.186 |

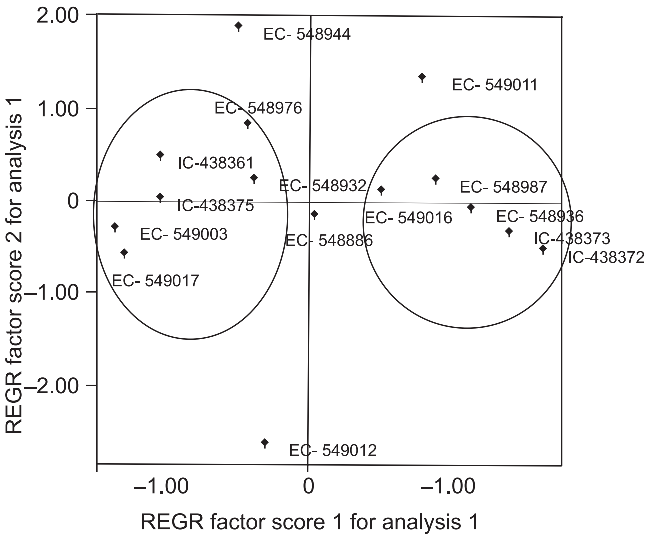


Fig 2 Principal component analysis for 15 cowpea genotypes

cluster III. Similarly, the accessions of cluster I are tightly grouped except the accessions 'EC 548944', 'EC 548886', 'EC 549012'. This far apart placement of the four accessions *ie* 'EC 548886', 'EC 548944', 'EC 549011' and 'EC 549012', were collected from US, South Africa, Kenya and Botswana indicative of that these germplasm are yet not under free flow or exchange. The accession 'EC 549012' was found promising for lower insect damage and big leaves contributing to green fodder yield. The PCA also exposed the opportunity to use accessions from centre/focus and the accessions placed at a significant distance for genetic diversity to use in breeding programme.

Similar kind of analysis to isolate resistance genotypes was applied by Gichimu *et al*. (2008) for identifying disease- and pest-resistant genotypes in watermelon. They have performed a principle component (PC) analysis using severity scores and accessions, plotted on two dimensions using the first two principle components (PC1 and PC2). Results demonstrated significant variation among accessions in susceptibility/resistance to various diseases, pests and non–pathogenic disorders. Kumar *et al*. (2006) used the PCA to indicate the diversity in sorghum genotypes for *Chilo partellus*. Genotypes placed in different groups can be used in resistance breeding programmes to diversify the basis of resistance to this pest.

Based on pest resistance grading, cluster technique and principal component analysis the accessions 'IC 438372', 'IC 438373' and 'EC 549012' were found to be highly insect tolerant and may be used as donor for resistant breeding. The present study also suggests that the narrow leaf genotypes are resistant to the insect damage and may be considered for on field screening of the germplasms and advance breeding experiments.


## SUMMARY

188 genotypes (172 exotic and 16 indigenous) of cowpea (*Vigna unguiculata* (L) Walp.), an important leguminous fodder crop for tropical and semi-arid regions, were screened for insect pest resistance. In promising genotypes the mean insect damage ranged from 5.6 to 11.0%. Fifteen genotypes ('EC 548976', 'EC 548886', 'EC 548932', 'EC 548936', 'EC 548944', 'EC 548987', 'EC 549003', 'EC 549011', 'EC 549012', 'EC 549016', 'EC 549017', 'IC 438361', 'IC 438372', 'IC 438373' and 'IC 438375') were identified as tolerant towards insect pests under field condition. Partial regression analysis indicated a significant negative relationship between per cent insect damage and leaf width of genotypes. Finally, application of cluster technique coupled with principal component analysis identified the accessions 'IC 438372', 'IC 438373' and 'EC 549012' as the superior insect tolerant lines for use in insect resistance breeding programme.


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