



# Pod Trichome Characterisation Using Foldscope, Morphological Characterization and Genetic Diversity Among Indigenous Collections of Pigeonpea [*Cajanus cajan* (L.) Millsp.]

K. Satish<sup>1</sup>, S. Muniswamy<sup>2</sup>, G. Girish<sup>3</sup>, Vikas Kulkarni<sup>1</sup>,  
J.R. Diwan<sup>1</sup>, Geeta<sup>2</sup>, Neeta Singh<sup>4</sup>, Sushil Pandey<sup>4</sup>, I.P. Singh<sup>5</sup>

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## ABSTRACT

**Background:** Association of several morphological traits such as trichome length and trichome density on the pod surface have been found to influence host plant resistance to insect pests. Genetic diversity analysis is used to identify the divergent genotypes and to utilize these genotypes to exploit heterosis. Further, morphological characters are stable across environments owing to oligogenic nature, they serve as morphological markers in breeding which can be used in varietal or genotypic identification, varietal purification and even in seed production. Hence, the current study aimed to investigate on host plant resistance using portable paper microscope 'foldscope', genetic diversity and morphological characters.

**Methods:** A total of 154 germplasm lines with three checks were evaluated in augmented block design (ABD) at Zonal Agricultural Research Station (ZARS), Kalaburagi, during *kharif*, 2018 to study their genetic diversity. Trichome length and trichome density were recorded using 'foldscope' 75 randomly selected genotypes, then correlated with shrivelled seed yield per plot due to pod fly incidence. Morphological characterization of 14 qualitative traits were recorded.

**Result:** Grouping of 157 germplasm lines into twelve clusters indicated a wider genetic diversity for the traits studied, of which 7 clusters were solitary with one entry each. The genotypes with more trichome density and length had less damage by the pod fly. Large variations for morphological characters was observed among the genotypes for qualitative traits such as pod colour, stem colour, flower colour, seed morphology and pod trichomes.

**Key words:** Correlation, Diversity, Morphological characterization, Trichomes, Variability.

## INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is one of the most multifaceted grain legume crops of rainfed agriculture in the semi-arid tropics. It engrosses a prime niche in sustainable farming systems of smallhold rainfed farmers. It plays an important role in food security, balanced diet and alleviation of poverty because of its diverse usages as a food, fodder and fuel (Rao *et al.*, 2002). It ranks sixth in global grain legume production and worldwide it is cultivated in about more than 5.0 m ha area. India is the largest producer and consumer of pigeonpea with an area of 4.4 m ha, with annual production of 3.68 m t and productivity of 832 kg/ha (Anonymous, 2019). The average yield in India remained around 900 kgs/ha for the past six decades (FAOSTAT, 2017).

In plant breeding, variability studies are important to know the genetically divergent genotypes. Considering the choice of diverse parental material in the study, lot of diversity is expected to be generated for various characters. Genetic diversity analysis was therefore used to identify the divergent genotypes and to utilize these genotypes to exploit heterosis. The genetically diverse parents are likely to produce not only heterotic effect but also desirable segregates to be selected.

Host plant resistance being one of the most exploited methods and is an important component for minimizing the losses due to insect pests. Association of several morphological traits such as trichome length and trichome density on the pod surface have been found to influence

<sup>1</sup>Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Raichur-584 104, Karnataka, India.

<sup>2</sup>Zonal Agricultural Research Station, Kalaburagi-585 104, Karnataka, India.

<sup>3</sup>Agricultural Research Station, Hagari, Bellary-583 138, Karnataka, India.

<sup>4</sup>National Bureau of Plant Genetic Resources, New Delhi-110 012, India.

<sup>5</sup>AICRP on Pigeonpea, ICAR-Indian Institute of Pulses Research, Kalyanpur, Kanpur-208 024, Uttar Pradesh, India.

**Corresponding Author:** S. Muniswamy, Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Raichur-584 104, Karnataka, India.

Email: muniswamygpb@gmail.com

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host plant resistance to insect pests. In this regard, association of pod trichomes with pod fly was studied in the present investigation using portable paper microscope 'foldscope'.

Morphological characters are stable across environments owing to oligogenic nature. Hence, they serve as morphological

markers in breeding which can be used in varietal or genotypic identification, varietal purification and even in seed production. Keeping above points in view, the genotypes were categorized based on morphological characters.

## MATERIALS AND METHODS

Present experiment was carried out during *Kharif* 2018 at Zonal Agriculture Research Station (ZARS), Kalaburagi, which is situated in agro-climatic zone-2 (North Eastern Dry Zone) of Karnataka state with 17° 20' Latitude (N), 76° 49' Longitude (E) and at an altitude of 443.88 meters above mean sea level.

A total of 154 indigenous collections of pigeonpea obtained from National Bureau of Plant Genetic Resource (NBPGR), New Delhi and three checks BSMR-736, Asha and PT-012 were sown in augmented block design (ABD) consisting of 11 blocks with 14 genotypes in each block and checks were repeated randomly. Each genotype was sown in three rows of three meter length with spacing of 90 cm between rows and 20 cm between plants. Standard agronomic practices were followed and plant protection measures were taken as and when required by following the recommended package of practices (Anonymous, 2017). Observations were recorded on nine quantitative traits in five randomly selected plants from each genotype, viz., days to 50% flowering, days to maturity, plant height, number of primary branches, number of secondary branches, pod bearing length, number of pods per plant, seed yield per plant and 100 seed weight. Data recorded on five plants were averaged and average values were subjected to statistical analysis. The analysis of variance (ANOVA) was carried out for all characters individually. The data was analyzed using WINDOSTAT ver. 8.5 software developed by Indostat services, Hyderabad as per the principles of

Mahalanobis (1936) and clustering by Tocher's method (Rao, 1952) respectively.

### Pod trichome length and density (per mm<sup>2</sup>)

Foldscope is an optical microscope that can be assembled from simple components, including a sheet of paper and a lens. It was developed by Dr. Manu Prakash and designed to cost less than US\$1 to build. It provides magnification upto 140X. The kit includes magnets that can be stuck onto the foldscope to attach it to a smartphone, allowing the user to take pictures of the magnification. The prepared slide is inserted into the paper microscope and the pod trichomes were observed and counted for trichome length and trichome density respectively and pictures were taken with the help of smartphone.

The pod's surface layer of test genotypes were cut into discs measuring 5 mm diameter and heated in 20 ml of water in glass test tubes on water bath at 85°C. The water is then poured off and 20 ml of 96% ethyl alcohol was added and the contents were boiled for 20 min at 80°C. Then alcohol was poured off and fresh alcohol was added until the chlorophyll content was completely removed. The sample was boiled at 85°C by adding concentrated lactic acid until the pod segments were cleared. The test tubes were cooled, length of trichomes on pods was measured by gently pressing a sticky transparent tape on the pod surface, then fixed to a glass slide. The length of trichomes was observed and measured using the image J software. Number of trichomes per unit area on the epidermal layer of pod was counted under a foldscope. Mean data of five pods (3 observation from each pod) in each entry was used for analysis as trichome density.

Trichome length and trichome density, were correlated with shrivelled seed yield per plot due to pod fly incidence in 75 randomly selected genotypes.

**Table 1:** Analysis of variance for yield and yield attributing traits of pigeonpea germplasm lines.

|                                     | DF  | DFF       | DM         | PH        | PB       | SB      | PBL      | NPPP      | SY/PP     | 100<br>SD Wt | Tr.Lgt  | Tr.den   |
|-------------------------------------|-----|-----------|------------|-----------|----------|---------|----------|-----------|-----------|--------------|---------|----------|
| Block<br>(Ignoring treatments)      | 10  | 311.14**  | 548.11**   | 890.26**  | 15.98**  | 26.09** | 162.56** | 9724.94** | 359.16**  | 3.32**       | 0.006** | 460.95** |
| Treatment<br>(eliminating blocks)   | 156 | 167.91**  | 326.00**   | 317.24**  | 7.00**   | 10.55** | 50.31**  | 2101.85** | 147.53**  | 3.67**       | 0.002** | 57.21**  |
| Checks                              | 2   | 1386.09** | 1827.84**  | 24.40     | 112.75** | 15.48** | 6.40*    | 184.75    | 242.43**  | 1.13**       | 0.008** | 50.66**  |
| Checks + var vs var                 | 154 | 152.09**  | 306.49**   | 321.04**  | 5.62**   | 10.49** | 50.88**  | 2126.75** | 146.30**  | 3.70**       | 0.002** | 57.30**  |
| Block<br>(Eliminating check + var.) | 10  | 4.19      | 5.60*      | 16.14     | 2.29**   | 1.20**  | 0.827    | 203.01    | 11.67     | 0.13         | 0.0001  | 1.66     |
| Entries<br>(Ignoring blocks)        | 156 | 187.57**  | 360.78**   | 373.27**  | 7.87**   | 12.15** | 60.68**  | 2712.23** | 169.81**  | 3.88**       | 0.003** | 86.65**  |
| Varieties                           | 153 | 151.12**  | 268.87**   | 360.79**  | 5.37**   | 11.66** | 60.88**  | 2756.59** | 144.15**  | 1.36**       | 0.003** | 83.24**  |
| Check vs var.                       | 1   | 3366.45** | 11488.54** | 2981.03** | 181.21** | 80.67** | 138.45** | 979.76*   | 3950.99** | 394.48**     | 0.03**  | 680.65** |
| Error                               | 20  | 1.62      | 1.71       | 10.39     | 0.62     | 0.15    | 1.74     | 156.49    | 24.10     | 0.12         | 0.0001  | 1.66     |

\*- Significant at 5 per cent; \*\*- Significant at 1 per cent.

DFF: Days to 50% flowering; PB: Number of primary branches; NPPP: Number of pods per plant; DM: Days to maturity; SB: Number of secondary branches; 100 seed wt: 100 seed weight (g); PH: Plant height (cm); PBL: Pod bearing length (cm); SY/PP: Seed yield per plant (g); Tr.Lgt: Trichome length; Tr.den: Trichome density.

Morphological characterization of 14 qualitative traits were recorded as per the key guidelines provided by PPV & FR (Protection of Plant Varieties and Farmer Rights) Authority, New Delhi and seed characteristics were recorded as per ICRIASAT/ IBPGR (1993) (International Bureau of Plant Genetic Resources) guidelines.

## RESULTS AND DISCUSSION

Analysis of variance (ANOVA) exhibited highly significant differences among genotypes for all the traits (Table 1). Based on  $D^2$  values, the genotypes were grouped into 12 clusters using Tocher's method given by Rao (1952). Of the 12 clusters, cluster I was the largest comprising 60 genotypes followed by cluster II (55 genotypes), cluster III

(21 genotypes), cluster VII (11 genotypes), cluster X (3 genotypes) and seven clusters (IV, V, VI, VIII, IX, XI, XII) were solitary with single genotypes each. These results are similar to observations of Muniswamy *et al.* (2014) and Patel *et al.* (2018). Katiyar *et al.* (2004), observed 14 clusters while grouping of 221 genotypes whereas Nethravathi and Patil (2014) obtained nine clusters using 196 genotypes.

Generation of more clusters in general and sole clusters in specific is a representative of existence of huge amounts of diversity between the set of genotypes. The genotypes that fall into solitary cluster more usually have some distinctive characters which make them divergent. Furthermore, the genotypes which have congregated into a cluster exhibit narrow range of genetic diversity among them

**Table 2:** Clustering pattern of pigeonpea germplasm lines based on  $D^2$  analysis.

| Cluster Number | No. of germplasm lines | Name of germplasm lines |          |    |          |    |          |    |          |    |                |
|----------------|------------------------|-------------------------|----------|----|----------|----|----------|----|----------|----|----------------|
| I              | 60                     | 1                       | IC468573 | 14 | IC523118 | 27 | IC523138 | 40 | IC523170 | 53 | IC523204       |
|                |                        | 2                       | IC468585 | 15 | IC523121 | 28 | IC523140 | 41 | IC523173 | 54 | IC523205       |
|                |                        | 3                       | IC468587 | 16 | IC523122 | 29 | IC523142 | 42 | IC523175 | 55 | IC523210       |
|                |                        | 4                       | IC523098 | 17 | IC523123 | 30 | IC523143 | 43 | IC523176 | 56 | IC523222       |
|                |                        | 5                       | IC523103 | 18 | IC523125 | 31 | IC523145 | 44 | IC523179 | 57 | IC523224       |
|                |                        | 6                       | IC523105 | 19 | IC523127 | 32 | IC523153 | 45 | IC523180 | 58 | IC523225       |
|                |                        | 7                       | IC523106 | 20 | IC523128 | 33 | IC523155 | 46 | IC523181 | 59 | IC523238       |
|                |                        | 8                       | IC523109 | 21 | IC253130 | 34 | IC523156 | 47 | IC523182 | 60 | <b>PT-0012</b> |
|                |                        | 9                       | IC523110 | 22 | IC523131 | 35 | IC523157 | 48 | IC523186 |    |                |
|                |                        | 10                      | IC523111 | 23 | IC523132 | 36 | IC523164 | 49 | IC523190 |    |                |
|                |                        | 11                      | IC523112 | 24 | IC523133 | 37 | IC523165 | 50 | IC523193 |    |                |
|                |                        | 12                      | IC523114 | 25 | IC523134 | 38 | IC523166 | 51 | IC523194 |    |                |
|                |                        | II                      | 55       | 13 | IC523116 | 26 | IC523135 | 39 | IC523168 | 52 | IC523195       |
| 1              | IC468569               |                         |          | 12 | IC523102 | 23 | IC523154 | 34 | IC523189 | 45 | IC523220       |
| 2              | IC468570               |                         |          | 13 | IC523104 | 24 | IC523160 | 35 | IC523191 | 46 | IC523221       |
| 3              | IC468571               |                         |          | 14 | IC523107 | 25 | IC523162 | 36 | IC523192 | 47 | IC523228       |
| 4              | IC468572               |                         |          | 15 | IC523113 | 26 | IC523163 | 37 | IC523196 | 48 | IC523230       |
| 5              | IC468578               |                         |          | 16 | IC523115 | 27 | IC523169 | 38 | IC523197 | 49 | IC523231       |
| 6              | IC468579               |                         |          | 17 | IC523117 | 28 | IC523177 | 39 | IC523198 | 50 | IC523232       |
| 7              | IC468580               |                         |          | 18 | IC523129 | 29 | IC523178 | 40 | IC523200 | 51 | IC523233       |
| 8              | IC468584               |                         |          | 19 | IC253148 | 30 | IC523183 | 41 | IC523207 | 52 | IC523235       |
| 9              | IC468586               |                         |          | 20 | IC523149 | 31 | IC523184 | 42 | IC523208 | 53 | IC523236       |
| 10             | IC468592               |                         |          | 21 | IC523150 | 32 | IC523187 | 43 | IC523209 | 54 | IC523237       |
| III            | 21                     | 11                      | IC523100 | 22 | IC523152 | 33 | IC523188 | 44 | IC523213 | 55 | IC523239       |
|                |                        | 1                       | IC468590 | 6  | IC523120 | 11 | IC523146 | 16 | IC523171 | 21 | IC523223       |
|                |                        | 2                       | IC468593 | 7  | IC523137 | 12 | IC523158 | 17 | IC523172 |    |                |
|                |                        | 3                       | IC523101 | 8  | IC523139 | 13 | IC523159 | 18 | IC523174 |    |                |
|                |                        | 4                       | IC523108 | 9  | IC523141 | 14 | IC523161 | 19 | IC523185 |    |                |
| IV             | 1                      | 5                       | IC523119 | 10 | IC523144 | 15 | IC523167 | 20 | IC523206 |    |                |
| V              | 1                      | 1                       | IC468574 |    |          |    |          |    |          |    |                |
| VI             | 1                      | 1                       | IC523234 |    |          |    |          |    |          |    |                |
| VII            | 1                      | 1                       | IC523226 |    |          |    |          |    |          |    |                |
| VIII           | 11                     | 1                       | IC468576 | 4  | IC468589 | 7  | IC523147 | 10 | ASHA     |    |                |
|                |                        | 2                       | IC468583 | 5  | IC523099 | 8  | IC523211 | 11 | BSMR-736 |    |                |
|                |                        | 3                       | IC468588 | 6  | IC523136 | 9  | IC523212 |    |          |    |                |
| IX             | 1                      | 1                       | IC523151 |    |          |    |          |    |          |    |                |
| X              | 1                      | 1                       | IC523126 |    |          |    |          |    |          |    |                |
| XI             | 3                      | 1                       | IC468575 | 2  | IC468577 | 3  | IC8581   |    |          |    |                |
| XII            | 1                      | 1                       | IC468591 |    |          |    |          |    |          |    |                |
| XII            | 1                      | 1                       | IC523124 |    |          |    |          |    |          |    |                |

while, between clusters had broad range of variability. The generation of such clusters may be due to total isolation arresting the gene flow or rigorous natural or human selection for diverse adaptive complexes. The grouping of 154 germplasm lines into twelve clusters is presented in Table 2.

The highest intra cluster distance was noticed in cluster VII (55.57), followed by III (47.15), I (43.47), II (41.52) and X (37.95). The inter cluster D<sup>2</sup> values were maximum (241.23) between clusters V and IX indicating these two clusters are distantly placed, followed by clusters VI and IX (238.89), IV and IX (238.63). It is sensible to select genotypes from clusters showing high inter cluster distance for further crossing programme (Table 3). These results are in agreement with the earlier findings of Sreelakshmi *et al.*, (2010), Muniswamy *et al.* (2014) and Patel *et al.* (2018).

Cluster mean analysis (Table 4) indicated that cluster IV (87.00) and II (92.45) are comprised of early flowering

genotypes. Cluster XI (133.00) comprised early maturing genotypes followed by cluster VIII (136.00). The highest cluster mean for number of pods per plant was recorded in cluster IX (286.00) followed by cluster III (231.52). The highest cluster mean for seed yield per plant was recorded cluster IX (51.70) followed by cluster III (40.77). The maximum cluster mean for 100 seed weight was recorded in cluster XI (12.00) followed by cluster IV (11.50). Similar results were also obtained by Muniswamy *et al.* (2014) and Patel *et al.* (2018).

Contribution of individual characters towards divergence have been calculated (Table 5), which revealed that the relative contribution of number of pods per plant has maximum (71.62%) followed by plant height (13.06%), number of days to maturity (8.93%), number of days to 50 per cent flowering (2.94%), seed yield per plant (2.90%) and rest of the genotypes have shown very negligible or no

**Table 3:** Intra (diagonal) and intercluster distances (D<sup>2</sup> value) of 154 germplasm lines along with checks of pigeonpea.

| Cluster | I     | II    | III    | IV     | V      | VI     | VII    | VIII   | XI     | X      | XI     | XII    |
|---------|-------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| I       | 43.47 | 82.95 | 76.21  | 122.80 | 123.97 | 123.39 | 74.36  | 70.71  | 125.30 | 120.31 | 58.87  | 91.19  |
| II      |       | 41.52 | 140.14 | 56.58  | 57.49  | 57.80  | 88.82  | 122.64 | 194.49 | 104.75 | 93.84  | 134.34 |
| III     |       |       | 47.15  | 183.54 | 184.45 | 184.09 | 109.55 | 64.90  | 70.83  | 161.62 | 86.74  | 93.15  |
| IV      |       |       |        | 0.00   | 19.62  | 14.43  | 116.86 | 166.08 | 238.63 | 112.61 | 127.72 | 166.65 |
| V       |       |       |        |        | 0.00   | 19.37  | 118.89 | 163.06 | 241.23 | 111.65 | 133.24 | 172.35 |
| VI      |       |       |        |        |        | 0.00   | 115.80 | 167.43 | 238.89 | 107.48 | 125.73 | 166.71 |
| VII     |       |       |        |        |        |        | 55.57  | 118.30 | 153.89 | 76.86  | 74.20  | 79.04  |
| VIII    |       |       |        |        |        |        |        | 0.00   | 108.10 | 166.93 | 101.00 | 124.10 |
| IX      |       |       |        |        |        |        |        |        | 0.00   | 206.51 | 123.28 | 113.51 |
| X       |       |       |        |        |        |        |        |        |        | 37.95  | 114.48 | 116.53 |
| XI      |       |       |        |        |        |        |        |        |        |        | 0.00   | 72.18  |
| XII     |       |       |        |        |        |        |        |        |        |        |        | 0.00   |

\*Diagonal values indicate intra cluster distances.

\*Above diagonal values indicate inter cluster distances.

**Table 4:** Cluster means of 12 clusters for yield and its related traits in pigeonpea germplasm lines.

| Cluster no. | No. of germplasm lines | DFF        | DM         | PH        | PB       | SB       | PBL       | NPPP      | SY/PP     | 100 seed wt | Overall score | Rank |
|-------------|------------------------|------------|------------|-----------|----------|----------|-----------|-----------|-----------|-------------|---------------|------|
| I           | 60                     | 94.10(5)   | 143.53(7)  | 107.09(7) | 13.40(3) | 7.03(3)  | 22.33(7)  | 170.78(5) | 30.86(5)  | 7.64(10)    | 52            | 4    |
| II          | 55                     | 92.45(2)   | 139.91(5)  | 97.81(10) | 11.62(8) | 5.29(6)  | 20.26(9)  | 101.07(9) | 19.31(7)  | 7.66(9)     | 65            | 7    |
| III         | 21                     | 95.71(6)   | 146.90(9)  | 111.07(6) | 13.14(4) | 8.29(1)  | 23.31(6)  | 231.52(2) | 40.77(2)  | 8.12(7)     | 43            | 3    |
| IV          | 1                      | 87.00(1)   | 141.00(6)  | 101.70(9) | 9.00(11) | 2.00(10) | 22.00(8)  | 54.00(11) | 11.70(10) | 11.50(2)    | 68            | 8    |
| V           | 1                      | 97.00(8)   | 145.00(8)  | 89.00(11) | 10.00(9) | 4.00(8)  | 14.70(12) | 55.00(10) | 6.00(12)  | 7.50(11)    | 89            | 10   |
| VI          | 1                      | 96.00(7)   | 139.00(4)  | 107.00(8) | 12.00(7) | 6.00(5)  | 17.00(11) | 54.00(11) | 6.30(11)  | 7.50(11)    | 75            | 9    |
| VII         | 11                     | 119.27(10) | 176.09(10) | 133.19(5) | 12.09(6) | 4.39(7)  | 32.08(3)  | 144.73(7) | 36.92(3)  | 9.29(4)     | 55            | 6    |
| VIII        | 1                      | 93.00(3)   | 136.00(2)  | 63.30(12) | 13.00(5) | 8.00(2)  | 20.00(10) | 215.00(3) | 19.30(8)  | 8.00(8)     | 53            | 5    |
| IX          | 1                      | 96.00(7)   | 137.00(3)  | 136.70(4) | 15.00(1) | 7.00(4)  | 33.70(2)  | 286.00(1) | 51.70(1)  | 10.00(3)    | 26            | 1    |
| X           | 3                      | 154.33(11) | 198.00(12) | 146.37(3) | 9.67(10) | 3.33(9)  | 28.87(4)  | 102.00(8) | 19.67(6)  | 8.83(5)     | 68            | 8    |
| XI          | 1                      | 94.00(4)   | 133.00(1)  | 153.30(2) | 14.00(2) | 8.00(2)  | 25.30(5)  | 170.00(6) | 14.70(9)  | 12.00(1)    | 32            | 2    |
| XII         | 1                      | 105.00(9)  | 180.00(11) | 165.00(1) | 10.00(9) | 4.00(8)  | 67.70(1)  | 193.00(4) | 34.00(4)  | 8.50(6)     | 53            | 5    |

DFF: Days to 50% flowering; PB: Number of primary branches; NPPP: Number of pods per plant; DM: Days to maturity; SB: Number of secondary branches; 100 seed wt: 100 seed weight (g); PH: Plant height (cm); PBL: Pod bearing length (cm); SY/PP: Seed yield per plant (g).

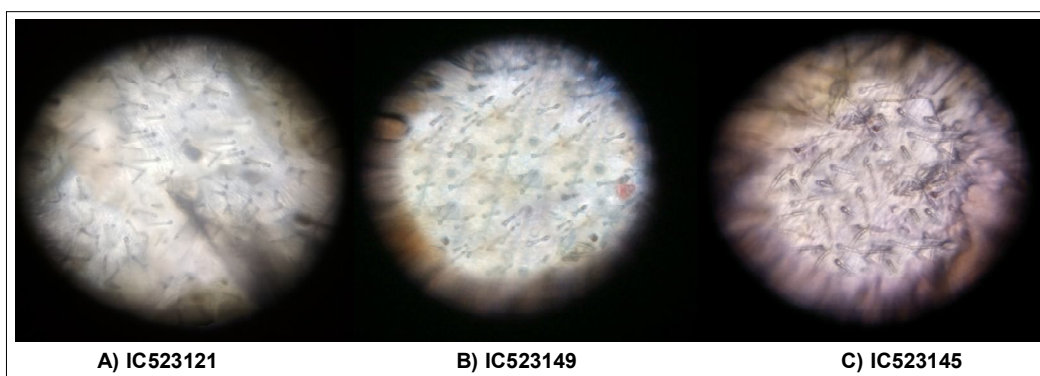
**Table 5:** Per cent contribution of each character towards genetic divergence in pigeonpea germplasm lines.

| Characters                   | Times ranked 1 <sup>st</sup> | Contributions (%) | Cumulative |
|------------------------------|------------------------------|-------------------|------------|
| Number of pods per plant     | 8771                         | 71.62             | 71.62      |
| Plant height                 | 1599                         | 13.06             | 84.68      |
| Days to maturity             | 1094                         | 8.93              | 93.61      |
| Days to 50% flowering        | 360                          | 2.94              | 96.55      |
| Seed yield per plant         | 355                          | 2.90              | 99.45      |
| Pod bearing length           | 53                           | 0.43              | 99.88      |
| Number of secondary branches | 13                           | 0.11              | 99.99      |
| Primary branches             | 1                            | 0.01              | 100        |
| 100 seed weight              | 0                            | 0.00              | 100        |

**Table 6:** Estimation of phenotypic correlation coefficients for shrivelled seed yield per plot due to pod fly incidence with pod trichomes for 75 randomly selected pigeonpea germplasm.

|   | Tr.length | Tr.density | Shrivelled seed yield per plot due to pod fly incidence |
|---|-----------|------------|---|
| Tr.length   | 1.000     | -0.138 NS  | -0.184 NS   |
| Tr.density  | -0.138 NS | 1.000      | 0.128 NS  |
| Shrivelled seed yield per plot due to pod fly incidence | -0.184 NS | 0.128 NS   | 1.000   |

\*NS- Non significant.

**Plate 1:** Morphological variation for pod trichome length and density in germplasm lines.**Plate 2:** Healthy vs shrivelled seeds of pigeonpea due to pod fly damage.

### Trichome length and density

The average pod trichome length obtained was 0.22 mm per mm<sup>2</sup> and it ranged from 0.10 mm (IC468576) to 0.39 mm (IC523145). The average pod trichome density recorded was 33.92 per 1 mm<sup>2</sup> and it ranged from 10.47 (IC468576) to 58.87 (IC523106). Estimation of phenotypic correlation coefficients for shrivelled seed yield per plot due to pod fly (*Melanagromyza obtusa*) incidence with pod trichomes in 75 randomly selected pigeonpea lines revealed negative non significant correlation indicating that the genotypes having high trichome length and trichome density suffered less damage by pod fly may due to antixenosis (Table 6). The genotypes IC523145 and IC553106 which had more trichome length and density respectively, had less shrivelled seed yield per plot (Plate 1 and 2). These genotypes can be used in the breeding programme against pod fly host plant resistance. The above results and findings are in line with the findings of Moudgal *et al.* (2008); Revathi (2014) and Dhakla *et al.* (2010). The foldscope was used to measure rice root hair length and rice lemma trichome (Diwan and Kashappa, 2019).



**Morphological characterisation**

Out of 154 genotypes, morphological variation was not observed for plant growth habit, leaf pubescence and pod pubescence. Unique genotypes were observed for characters like Dark purple stem colour (IC523231), cylindrical pod shape (IC 468590), mottled, speckled seeds (IC468584) and Elongate seed shape (IC523168). The traits like early plant vigour, branching pattern, base flower colour,

stem colour, streak pattern of base petal, pod colour, seed characteristics exhibited lot of variation and the results of characterization of genotypes for morphological traits are presented in Table 7. Similar findings for plant vigor and plant growth habit were observed by Muniswamy *et al.* (2014) and Kumar *et al.* (2016). Hariprasad (2018) found similar results for branching pattern and stem colour. Kallihal *et al.* (2016) observed similar morphology in case of streaks

**Table 7:** Morphological characterization of 154 pigeonpea germplasm lines.

| Qualitative characters | Type                 | No. of genotypes | Frequency (%) / genotype |
|------------------------|----------------------|------------------|--------------------------|
| Early plant vigour     | Poor                 | 2                | 1.29                     |
|                        | Good                 | 121              | 78.57                    |
|                        | Very good            | 31               | 20.12                    |
| Branching pattern      | Erect and compact    | 56               | 36.36                    |
|                        | Semi spreading       | 51               | 33.11                    |
|                        | Spreading            | 47               | 30.51                    |
| Plant growth habit     | Determinate          | 0                | 0                        |
|                        | Semi determinate     | 0                | 0                        |
|                        | Indeterminate        | 154              | 100                      |
| Base flower colour     | Light yellow         | 7                | 4.54                     |
|                        | Yellow               | 136              | 88.31                    |
|                        | Orange yellow        | 8                | 5.19                     |
|                        | Red                  | 3                | 1.94                     |
| Stem colour            | Green                | 121              | 78.57                    |
|                        | Sun red              | 4                | 2.59                     |
|                        | Purple               | 28               | 18.18                    |
|                        | Dark purple          | 1                | IC 523231                |
| Leaf pubescence        | Glabrous             | 154              | 100                      |
|                        | Pubescent            | 0                | 0                        |
|                        | Sparse streaks       | 84               | 54.54                    |
| Streaks pattern        | Medium streaks       | 57               | 37.01                    |
|                        | Dense streaks        | 13               | 8.44                     |
|                        | Glabrous             | 0                | 0                        |
| Pod pubescence         | Pubescent            | 154              | 100                      |
|                        | Flat                 | 153              | 99.35                    |
| Pod shape              | Cylindrical          | 1                | IC 468590                |
|                        | Green                | 11               | 7.14                     |
| Pod colour             | Purple               | 2                | 1.29                     |
|                        | Dark purple          | 2                | 1.29                     |
|                        | Green and purple     | 138              | 89.61                    |
|                        | Plain                | 150              | 97.40                    |
| Seed colour pattern    | Mottled              | 0                | 0                        |
|                        | Speckled             | 3                | 1.94                     |
|                        | Mottled and speckled | 1                | IC 468584                |
|                        | Narrow               | 62               | 40.25                    |
| Seed eye width         | Medium               | 71               | 46.10                    |
|                        | Wide                 | 21               | 13.63                    |
|                        | White                | 6                | 3.89                     |
|                        | Cream                | 20               | 12.98                    |
| Base seed colour       | Orange               | 8                | 5.19                     |
|                        | Light brown          | 71               | 46.10                    |
|                        | Reddish brown        | 43               | 27.92                    |
|                        | Dark grey            | 0                | 0                        |
|                        | Dark purple          | 6                | 3.89                     |
|                        | Oval                 | 110              | 71.42                    |
| Seed shape             | Globular             | 26               | 16.88                    |
|                        | Square               | 17               | 11.03                    |
|                        | Elongate             | 1                | IC 523168                |

pattern on base petal, pod shape and pod colour. Similar findings for seed colour pattern, base seed colour were found by Muniswamy *et al.* (2014).

## CONCLUSION

Germplasm lines IC468574, IC523234, IC523226, IC523151, IC523126, IC468591 and IC523124 which fall into solitary cluster more often have some unique characters which make them divergent. Based mean performances of yield and yield related traits, the genotypes IC523144, IC523171, IC468593, IC523126, IC468590 were found promising. Hence, afore said genotypes can be used for the further studies for improving the yield as a parent in the future breeding programme. IC523145 and IC553106 genotypes which are having more trichome density and length can be used against the pod fly host plant resistance in plant breeding.

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## REFERENCES

- Anonymous, (2017). Improved agronomic practices, UAS Dharwad and UAS, Raichur, pp 100-112.
- Anonymous, (2019). Project Coordinator's Report, AICRP on Pigeonpea.
- Braham, M.A., Mahmud, A.A., Zeba, F. and Afrin, K.S. (2010). Genetic variability, character association and genetic divergence in Mungbean. *Indian J. Crop Sci.* 3(1): 1-6.
- Bhadru, D. (2011). Variability and genetic divergence studies in white seed coated pigeonpea [*Cajanus cajan* (L.) Millsp.]. *Crop Res.* 42(1/2/3): 263-268.
- Diwan, J.R. and Kashappa, C. (2019). Foldscope as a Research Tool and its Applications. In: Book Foldscope and Its Applications [(Ed.) Arundev Sharma] published by National Press Associates, New Delhi.
- Dhakla, K., Yadav, G.S. and Rohilla, H.R. (2010). Assessment of pigeonpea genotypes for resistance to pod fly, *Melanagromyza obtusa* (Malloch). *J. Insect Sci.* 23(1): 70-75.
- FAOSTAT, (2017). Food and Agriculture Organization of the United Nations. Rome, Italy. <http://faostat.fao.org/database>.
- Hariprasad, C., (2018). Genetic diversity studies and morphological characterization in germplasm lines of pigeonpea [*Cajanus cajan* (L.) Millsp.]. M. Sc. (Agri.) Thesis, Univ. Agril. Sci., Raichur, Karnataka, India.
- Kallihal, P.K., Chandrashekhar, S.S., Shwetha, K.S., Salimath, P.M. and Dhone, V.K., (2016). Characterization of pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes based on morphological traits. *BIOINFOLET-A Quat. J. Life Sci.* 13(2a): 212-215.
- Katiyar, P.K., Dua, R.P., Singh, I.P., Singh, B.B. and Singh, F. (2004). Multivariate analysis for genetic diversity in early pigeonpea accessions. *Legume Res.* 27(3): 164-170.
- Kumar, A., Singh, A., Kumar, R. and Singh, B. (2016). Genetic variability, character association and path analysis in pigeonpea [*Cajanus cajan* (L.) Millspaugh]. *Bull. Env. Pharmacol. Life Sci.* 7(3): 63-68.
- Mahalanobis, P.C. (1936). On the generalized distance in statistics. *Proc. Natl. Inst. Sci. India.* 2: 49-55.
- Moudgal, R.K., Lakra, R.K., Dahiya, B. and Dhillon, M.K. (2008). Physico-chemical traits of [*Cajanus cajan* (L.) Millsp.] pod wall affecting *Melanagromyza obtusa* (Malloch) damage. *Euphytica.* 161: 429-436.
- Muniswamy, S., Loksha, R., Dharmaraj, P.S., Yamanura and Diwan, J.R. (2014). Morphological characterization and assessment of genetic diversity in minicore collection of pigeonpea [*Cajanus cajan* (L.) Millsp.]. *Electronic J. Pl. Breed.* 5(2): 179-186.
- Nethravathi, K.H. and Patil, B.R. (2014). Character association and path coefficient analysis for yield and its components in pigeonpea. *Karnataka J. Agric. Sci.* 27(2): 225-226.
- Patel, H.N., Patel, A.M., Patel, J.M. and Patel, N.I., (2018). Genetic divergence study through D<sup>2</sup> analysis in pigeonpea [*Cajanus cajan* (L.) Millsp.]. *International Journal of Chemical Studies.* 6(5): 1947-1950.
- Rao, C.R. (1952). *Advanced Statistical Methods in Biometrical Research.* John Wiley and Sons, New York, pp: 22.
- Rao, S.C., Coleman, S.W. and Mayeux, H.S., (2002). Forage production and nutritive value of selected pigeonpea ecotypes in the southern great plains. *Crop Sci.* 42: 1259-1263.
- Revathi, K. (2014). Evaluation of pigeonpea genotypes against pod fly, *Melanagromyza obtusa* (malloch). M. Sc. (Agri.) Thesis, Univ. Agril. Sci., Hyderabad, Karnataka, India.
- Singh, M.N., Awasthi, S.K. and Singh, R.S. (2010). Genetic divergence in pigeonpea. *J. Food Legume.* 23(1): 82-83.
- Sreelakshmi, C., Shivani, D. and Sameer Kumar, C.V. (2010). Genetic divergence and stability analysis in pigeonpea [*Cajanus cajan* (L.) Millsp.]. *Electron. J. Pl. Breed.* 1(4): 530-535.