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Assessment of Genetic Variability and Traits Association in Pigeonpea [*Cajanus cajan* (L.) Millsp.] Germplasm

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ABSTRACT

Background: Pigeon pea is an important dietary protein source for humans but the production was constrained by various biotic and abiotic factors. Breeding strategies were followed to improve yield and developing high yielding varieties but at the same time utilization of genetic resources have declined. Pigeon pea is native to India with huge natural genetic variability in the local germplasm and its wild relatives. So it is necessary to identify and select breeding material from germplasm with considerable genetic and morphological variability to utilize in breeding programmes. As an initial study, 200 pre-breeding lines developed were evaluated for morphological variability patterns.

Methods: A total of two hundred lines selected from F4 generation of pigeon pea developed at ICAR-NBPGR were evaluated in randomized block design (RBD) during 2014-2015 *kharif* season under Indo-Swiss collaboration in Biotechnology at Agricultural College and Research Institute, Madurai (TNAU). The accessions found to be superior in seed yield than the local check APK1 were forwarded to the next generation (2015-2016) for assessment of genetic variability, heritability, genetic advance and association studies.

Result: Qualitative traits were evaluated and variation in leaflet shape, stem colour, pattern of streaks and base seed colour were observed. All tested lines expressed greater variability for most of the traits. Maximum coefficient of variation was observed for number of pods per plant followed by number of primary branches per plant. Selection of traits with moderate heritability coupled with high genetic advance like number of pods per plant, number of primary branches per plant could help in crop improvement program. Seed yield was positively correlated with number of seeds per pod, number of pods per plant and hundred seed weight. Potential genetic stocks and donors for high yield were selected based on hundred seed weight and seeds per pod. The accessions superior in number of pods and seed yield than check were forwarded to next generation for assessment. The identified trait-specific accessions will help in future breeding program.

Key words: Germplasm, Legumes, Prebreeding, Productivity, Yield.

INTRODUCTION

Pigeon pea, an often cross pollinated fast growing food legume and drought hardy crop known as arhar, tur or red gram, is grown widely in tropical and subtropical countries. The major pigeon pea producing countries are India and Myanmar; together producing around 83 per cent of the global production. These Asian countries are well ahead (14%) of the other major producers of Africa *viz.,* Malawi, Tanzania, Kenya and Uganda, (FAOSTAT, 2015). Globally, India is the largest consumer and second largest producer of pigeon pea with a production of 4.25 million tones (DES, 2018). Pigeon pea is known for its rich dietary protein needed predominantly for vegetarian population (Hari *et al*., 2006). The seeds contain crude protein (19.0-21.7%), crude fibre (9.8-13.0%), ash (3.9-4.3%) and dry matter (86.6-88.0%) (Amarteifio *et al.,* 2002) and is also rich in minerals like calcium, magnesium, iron and zinc (Faris and Singh 1990). Other than food, it can also be used as a fuel and animal feed. In addition, it has the capacity to fix atmospheric nitrogen and therefore helps improve the soil fertility.

Despite its hardiness and ability to grow on marginal lands, pigeonpea production is strained by various biotic and abiotic factors. For the last three decades, great emphasis was laid in developing high yielding varieties and hybrids. However, concurrently the insufficient exploitation of germplasm, limited its utilization. For an effective breeding

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programme, it is necessary to understand the extent of genotypic and phenotypic variation among the germplasm. Recent trend in crop production is exploring variations from total gene bank collections, selection of donors and prebreeding of selected donors in facilitating breeding program (Sharma *et al*., 2013; Sharma *et al*., 2019; Roy and Shil, 2020). Presence of intra-specific variation are a prerequisite for most of the crop improvement programme for utilization in selection for pest and disease resistance, better quality, early maturing and high yielding traits. Characterization and evaluation of available germplasm for different agro-morphological and biochemical traits is necessary to identify the effect of different genes on the phenotypes. We have already developed and evaluated high yielding lines in pigeon pea (Arumugam *et al.,* 2018), identified diverse markers and validated the developed high yielding lines (Singh *et al*., 2020), genes and genes network were also being cracked (Chaudhary *et al.,* 2017; Yasin *et al*., 2018; Yasin *et al*., 2019). In continuation to that, the present study was carried out with an objective to characterize two hundred selected pre-breeding accessions for the qualitative traits to estimate the genetic variability, heritability, coefficient of variation and character association analysis.

MATERIALS AND METHODS

A total of 200 pigeon pea pre-breeding lines selected from mapping population developed by crossing long duration high yielding accessions with extra early maturing poor yielding accessions at ICAR-NBPGR under Indo-Swiss collaboration in Biotechnology were evaluated in Randomized Block design (RBD) with two replications in each block along with an early flowering standard local check, APK 1 at Agricultural College and Research Institute, Madurai (TNAU). Each genotype was raised in two rows of three meters length with spacing of 60 × 30 cm. The experimental material was grown under irrigated conditions and recommended agronomic practices were followed. During first year 2014-2015 (*kharif*) all accessions were sown and evaluated for different qualitative traits and yield parameters. Based on yield, 26 better performing superior accessions than check were selected among 200 accessions and were evaluated in the consecutive season 2015-2016, for quantitative traits. The observations were recorded on five randomly selected plants in each replication. The data was recorded for qualitative characters from first season crop (F5) and quantitative traits from second season crop (F6) as listed in standard germplasm descriptors (IPGRI, 1993). The list of observed qualitative, quantitative data and their scores were mentioned in Table 1.

Analysis of variance was performed for all traits as prescribed earlier (Panse and Sukhathme 1967). The quantitative data was further used to estimate the mean, variance, phenotypic, genotypic coefficient of variation and correlation. Phenotypic (PCV) and genotypic coefficients of variance (GCV) were calculated based on the method advocated by Burton (1952). Heritability percentage in broad sense was estimated as per the method described by Lush (1940) and traits were classified as high (>60), moderate (31-60) and low heritability (0-30) as per the method of Robinson *et al.* (1949). Genetic advance was estimated according to the method suggested by Johnson *et al.* (1955). Traits were classified as having high, moderate or low genetic advance. Correlation coefficient (r) analysis (Robinson *et al.,* 1951) and significance among them were

Table 1: Morphological traits observed and their scores in pigeon pea.

Traits recorded	Code	Descriptions
Qualitative traits		
Growth habit	GH	1. Erect and compact, 2. Semi spreading, 3. Spreading, 4. Trailing
Stem colour	STC	1. Green, 2. Sunred, 3. Purple 4. Dark purple
Leaflet shape	LS	1. Lanceolate, 2. Narrow – elliptic, 3. Broad- elliptic, 4. Obcordate
Leaf hairiness	LH	1. Glabrous, 2. Pubescent
Pattern of streaks	PS	3. Sparse streaks, 5- Medium amount of streaks, 7. dense streaks, 9- uniform coverage of second colour
Flowering pattern	FP	1. Determinate, 2. Semi- determinate, 3. indeterminate
Pod form	PF	1. Flat, 2. Cylindrical
Pod Hairiness	PH	1. Glabrous, 2. Pubescent
Base seed colour	BSC	1. White, 2. Cream, 3. Orange, 4. Light brown, 5. Reddish brown, 6. Light grey, 7. Grey, 8. Purple, 9- dark purple, 10- dark grey.
Quantitative traits		
Days to 50% flowering	DFF	Total number of days from sowing to 50% of flower opening.
Plant height	PH	The height from the ground level to the tip of the plant
Number of primary branches per plant	NPB	The total number of branches on the main stem
Number of pods per plant	NPP	The total numbers of fully matured podsfrom individual plants
Number of seeds per pod	NSP	Total number of seeds from the pods of selected individual plant
Hundred seed weight	HSW	The dry weight of 100 seeds
Days to maturity	DM	Total number of days for maturing from sowing.
Single plant yield	SPY	The total seed yield of individual plant.

calculated by using (n-2) degrees of freedom where n is number of treatments or accessions. The obtained data was analyzed using RStudio using packages named 'variability' for variability studies, 'corrplot' for correlation and 'lavaan' and 'semPlot' for path analysis.

RESULTS AND DISCUSSION

Frequency distribution for qualitative and quantitative traits

Morphological characterization of pigeon pea germplasm exhibited vast variation among the accessions for all the observed traits. The frequency distribution for different qualitative traits like leaflet shape, stem colour, pattern of streaks, seed coat colour are displayed in Fig 1a and the morphological variations are shown in Plate 1. lanceolate leaflet shape was observed in 99.5 % of accessions and only 0.5% accessions showed broad elliptic shape. The colour of the stem displayed maximum green in 96.0% followed by sun red in 2.0% and purple colour in 2.0% of accessions. For the flower pattern of streaks, medium amount of streaks was observed in 41.5%, followed by sparse streaks (29.5%), dense streaks (25%) and uniform coverage of second colour (4%). For base seed coat colour trait, reddish brown was predominant (51.5 %) followed by light brown (30.5%), grey seeds (8.5 %), white colour (7.5%) and cream colour (2%). No variation was observed for the traits *viz.,* growth habit (erect), leaf hairiness, flowering pattern, pod form and pod hairiness. The frequency of seven quantitative traits in 26 accessions of pigeon pea presented in Fig 1b indicated presence of wide variations for the traits like days to 50% flowering, hundred seed weight and single plant yield. It can be inferred that the available variation can be utilized for developing varieties with different duration and also for enhanced yield.

Mean performance, variability, heritability and genetic advance

Among the two-hundred accessions based on the seed yield from first season F_5 generation, only twenty-six accessions were selected on second season for variability and association studies. A total of twenty-six pigeon pea accessions showed greater amount of variability for the evaluated characters. The mean performances of twenty-six accessions were presented in Table 2. Among the evaluated genotypes, DGRg - 226 recorded early flowering (66 days) whereas, DGRg- 201 recorded late flowering (132 days) compared with grand mean. Minimum plant height was observed in DGRg - 247 (182.50 cm) and maximum was observed in DGRg- 263 (373.50 cm). Number of primary branches per plant was observed maximum for DGRg-263 (23.50) and minimum in DGRg- 287 (5.50). Maximum number of pods per plant was observed in DGRg-268 (136.3) and the minimum number of pods per plant was observed in the DGRg- 287 (81.4). Number of seeds per pod was observed maximum in DGRg- 226 (6) and the remaining all lines were observed minimum number of seeds per pod (4.00). The genotype DGRg- 233 was observed maximum hundred seed weight (18.5 g), whereas DGRg- 240 and DGRg 230 had minimum hundred seed

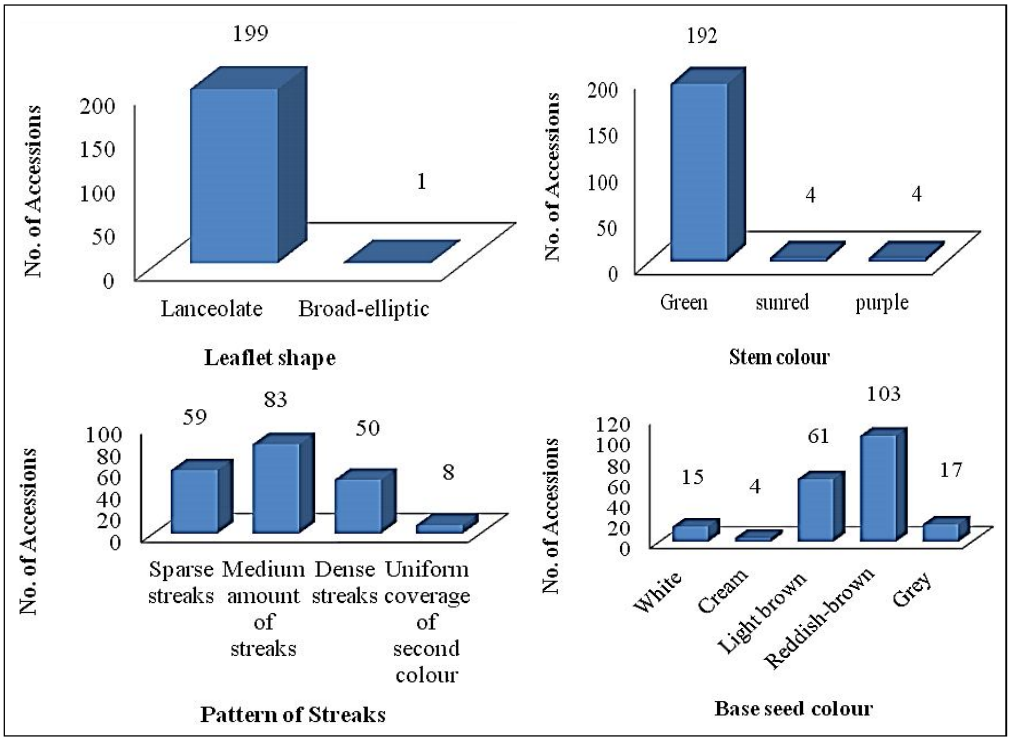


Fig 1a: Frequency distribution for qualitative traits.

weight (12 g). Days to maturity observed maximum 153.7 days for DGRg 202 and minimum days to maturity was observed in DGRg 226 (96 days). The single plant yield was observed maximum for DGRg-233 (78.83 g) and minimum was observed in DGRg- 287 (32.74 g).

Among the twenty-six accessions evaluated, fifteen genotypes showed significant decrease in flowering duration, plant height at maturity when compared to grand mean. Ten genotypes registered significantly higher mean performance to number of primary branches per plant, thirteen genotypes observed more number of pods per plant compared to grand mean, eleven genotypes for higher hundred seed weight, fourteen genotypes were early maturing then over all mean and fourteen genotypes for

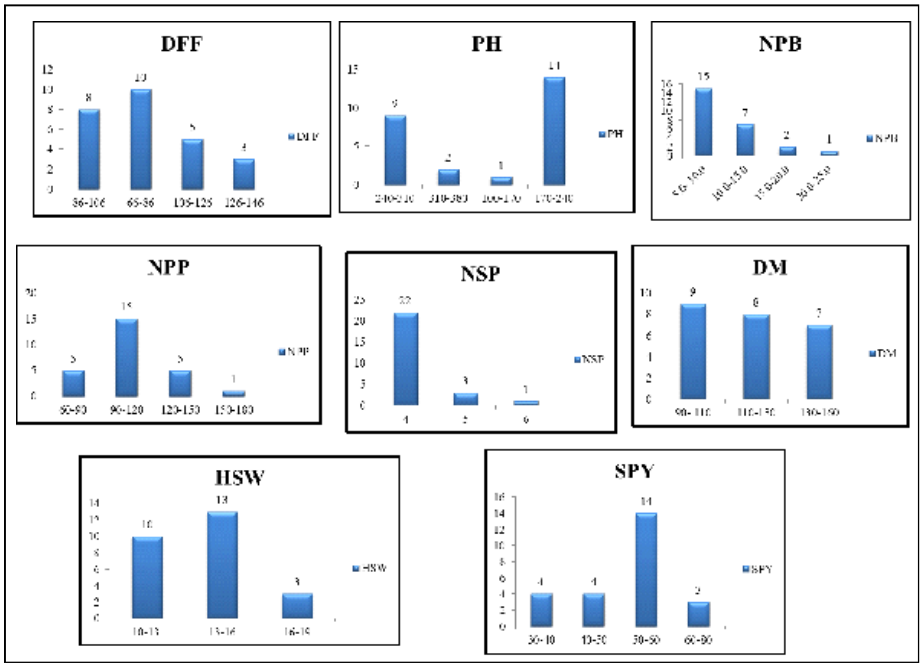


Fig 1b: Frequency distribution of accessions for qualitative traits.

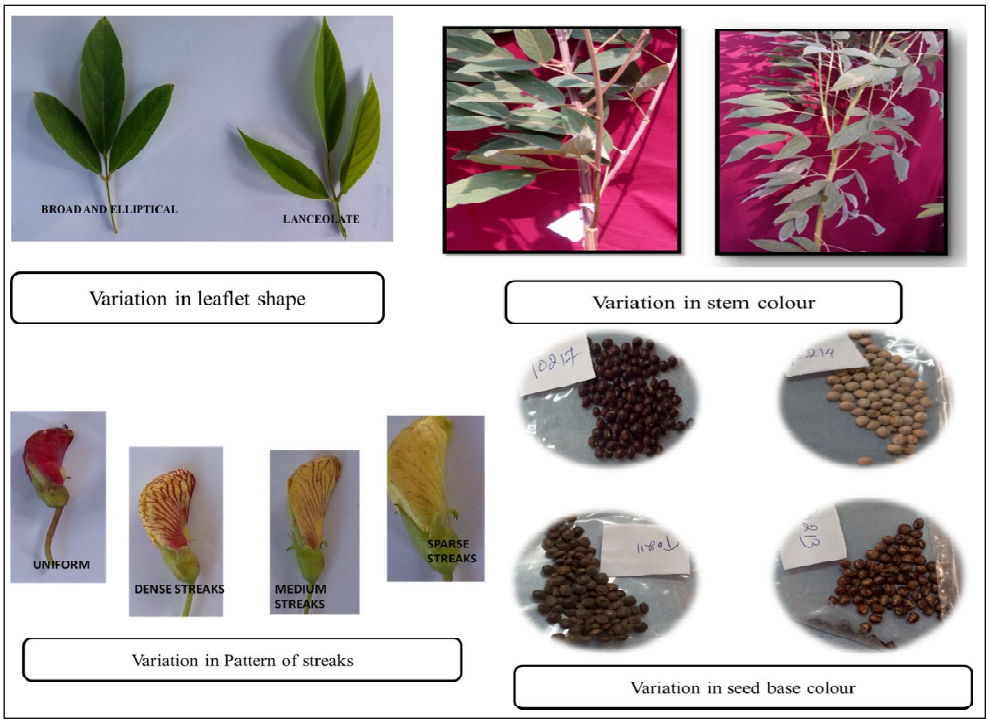


Plate 1: Morphological (Qualitative) variations observed in different traits.

seed yield per plant. In general, during crop improvement activities in any crop, the genotypes which are superior than the best control check should be given priority in selection programme.

The presence of variation among the genetic material is the basic requirement for crop improvement. The genetic variability parameters were analyzed and presented in Table 3. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) showed the presence of variability among the observed traits. The maximum coefficient of variation was observed for number of primary branches per plant, number of pods per plant, days to fifty percent flowering and followed by single plant yield. High GCV were observed for number of primary branches per plant (40.68%), days to fifty per cent flowering (22.4%) followed by plan height (20.67%) and number of pods per plan (19.02%). Similar results were reported in pigeon pea by Sreelakshmi *et al.* (2010), Singh *et al.* (2018) reported in cowpea and Linge *et al.* (2010) for plant height at maturity, days to fifty per cent flowering, seed yield per plant and number of pods per plant. It indicates that presence of very low environmental influences on these traits and mostly on genetic control so selection of these traits will be effective. Coefficient of variation studies indicated that the estimate of PCV was slightly higher than the corresponding GCV, indicating that the genetic components and expression of characters under study were slightly influenced by environmental factors and these traits were mainly under

Table 2: Mean performance of seven quantitative traits of twenty-six pigeon pea accessions.

Genotypes	DFF	PH	NPB	NPP	NSP	HSW	DM	SPY
APK 1	94.5	108	5.5	115	4	13.78	125.5	50.05
DGRg201	132	219.5	8.5	110.3	4	13	152.2	55.62
DGRg 202	122.5	200.5	7.3	86	5	13.8	153.7	42.73
DGRg 203	121	229	7.5	100.2	4	14.4	151	52.2
DGRg 206	72.5	220.5	9	123	4	14	103.1	53.79
DGRg 215	94	234	8.5	109.5	5	13.8	124	56.36
DGRg 226	66	198	7.5	101.5	6	16.6	96	65.34
DGRg 230	131.5	189.5	6.8	86.5	4	12	152.2	34.58
DGRg 233	98.5	253	17	170.8	4	18.5	129.1	78.83
DGRg 234	90.5	314	7.5	126	4	14	121	52.7
DGRg 235	91	270	8.5	94.6	4	12.8	121.4	38.84
DGRg 236	78	248	11	90	4	13	108	36.7
DGRg 237	97.5	230	7.5	119	4	13.9	128	50.12
DGRg 240	77.5	231	7.6	68.9	4	12	108.7	38.67
DGRg 243	131.5	231	15	100	5	14.2	152	50.22
DGRg 244	101.5	233.5	13	95.5	4	12.5	132.4	42.09
DGRg 247	122	182.5	9.5	109	4	12.4	152	55.37
DGRg 263	84.5	373.5	23.5	131.5	4	17.7	115.6	67.09
DGRg 265	78	305	13.5	120.5	4	13.8	108	56.06
DGRg 266	72.5	245.5	8.2	98.9	4	12.4	103.3	40.77
DGRg 268	90.5	296.5	18	136.3	4	13.2	121	57.64
DGRg 270	70.5	250	14.5	100	4	14	101	43.84
DGRg 272	68.5	213	12	114.7	4	14.2	99.6	58.32
DGRg 275	77	259.5	11.5	100.7	4	12.8	107.7	50.87
DGRg 276	121.5	266.5	8.5	117.3	4	14.6	142	55.28
DGRg 287	111	220	5.5	81.4	4	12.1	141.1	32.74
Mean	96	239.28	10.47	107.96	4.192	13.83	124.98	50.64

Table 3: Variability parameters for seven quantitative characters in pigeon pea.

Characters	Mean	GCV %	PCV %	Heritability (%)	GA (%) of mean
Days to fifty per cent flowering	96.00	22.4	22.56	99	36
Plant height (cm)	239.29	20.67	20.82	98	12
Number of primary branches per plant	10.47	40.68	43.55	87	32
Number of pods per plant	107.96	19.02	19.72	93	11
Number of seeds per pod	4.19	11.72	11.6	99	8.43
Hundred seed weight (g)	13.82	40.84	18.76	96	34
Days to maturity	124.98	15.52	16.32	90	8.77
Single plant yield (g)	50.64	20	21.79	84	11.23

the genetic control. Heritability is a good index of the transmission of characters from parents to offspring (Falconer, 1967). Heritability and genetic advance are the two important parameters, of which, former is used to estimate the expected genetic gain through selection. Relative comparison of heritability estimates and expected genetic advance as percentage of mean will give an idea about the nature of gene action governing a particular character. High heritability was found in days to fifty per cent flowering (99%) and number of seeds per pod (99%) followed by plant height (98%), hundred seed weight (96%), number of pods per plant (93%), number of primary branches per plant (87%) and single plant yield (84%). Similar results of maximum heritability for number of seeds per pod, days to 50% flowering and hundred seed weight was also observed by Chetukuri *et al.* (2013); Saroj *et al.* (2013). High genetic advance percent was observed for days to fifty percent flowering (36%), hundred seed weight (34%), number of primary branches per plant (32%), single plant yield (11.23%). Sharma *et al.* (2012) observed maximum genetic advance for number of pods per plant and number of primary branches per plant. In the present study, all the characters showed high heritability indicating low environmental effect and high capacity of the characters for the transmission to subsequent generation. All the seven quantitative characters studied had high heritability with high genetic advance suggesting these characters are governed by additive genetic effect to a great extent and improvement of these characters would be effective through phenotypic selection. The above report was already made by Vikas and Singh (1998) and Sarsamkar *et al.* (2008) for days to maturity, number of pods per plant, hundred seed weight and seed yield per plant in pigeon pea with the heritability more than 60 per cent.

Correlation and path analysis

The ultimate aim of plant breeding is to achieve a higher level of seed yield, which is a complex trait. It has been generally accepted that correlation between different characters represents a coordination of physiological processes, which is often achieved through gene linkages. The complex nature of seed yield is largely influenced by number of component traits. Hence, information on the strength and direction of association of these component characters with seed yield and also inter association among them would be very useful in formulating an effective and viable breeding programme for improvement of seed yield. Character association studies are of great significance in the process of selection by which simultaneous improvement of more than one character is possible. Correlation coefficients at genotypic level were generally of higher magnitude than the corresponding phenotypic level indicating the strong association between the characters. The results of correlation studies were presented in Fig 2. Estimation of correlation coefficients between different pair of traits under study revealed that not all traits were correlated to each other or with seed yield. Highest positive significant correlation was observed between days to fifty per cent flowering and days to maturity. Single plant yield was positive significantly correlated with number of pods per plant, number of seeds per plant, hundred seed weight, plant height and number of primary branches per plant. Hundred seed weight were found positive significantly correlated with number of pods per plants, number of primary branches per plant, plant height, number of seeds per pod. Considering the correlation between seed yield per plant

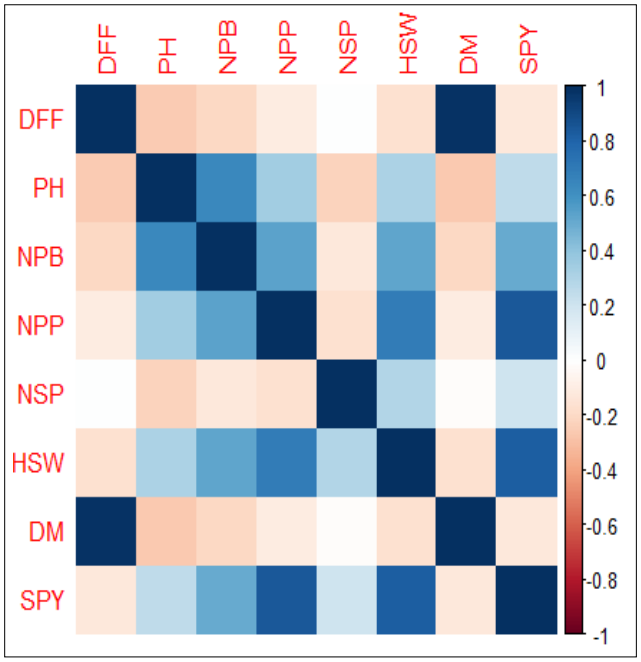


Fig 2: Correlation analysis for single plant yield and associated traits in pigeon pea.

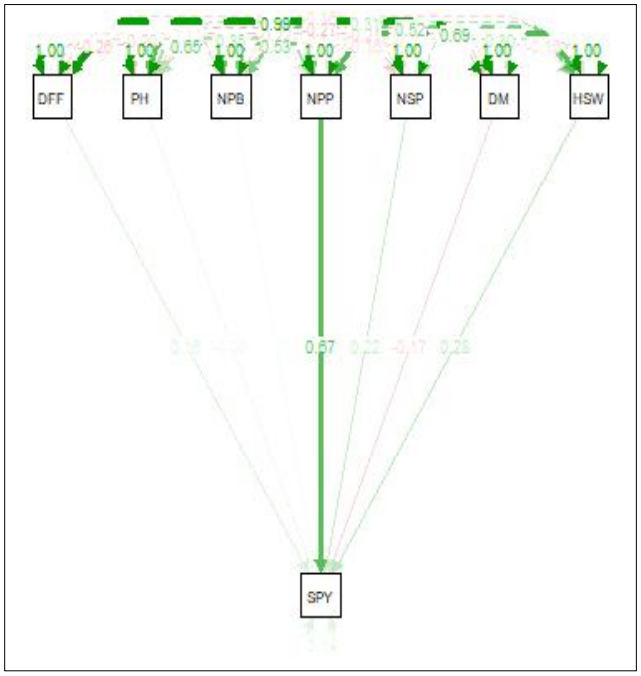


Fig 3: Path analysis for single plant yield and associated traits in pigeon pea.

and other characters, it was found that seed yield was positively correlated with number of pods per plant, number of seeds per pod and number of primary branches. Similar report was given by Bhadru (2010). The high yielding lines selected and studied for their molecular diversity using 62K SNP chip has confirmed the variations and correlations among the selected contrasting breeding lines (Singh *et al*., 2020). Hence, these characters namely seeds per pod and hundred seed weight have to be given importance during the selection programme to improve the yield potential of the crop.

The correlation coefficients between any two characters would not give a complete picture of a complex situation like yield of plant which is jointly determined by a number of traits either directly or indirectly. In such situation, path coefficient analysis would be useful, as it permits the separation of direct effect from indirect effects through other related traits by partitioning the genotypic correlation coefficient (Dewey and Lu, 1959). The correlation coefficient of seed yield with its component trait was further apportioned into direct and indirect effects. The results of path analysis showing the direct and indirect effects of all the seven characters on seed yield are furnished in Fig 3. Among the six characters analyzed, three characters showed positive direct effect and two characters showed negative direct effect on seed yield. The highest positive effect was recorded for number of pods per plant (0.67), hundred seed weight (0.28), number of seeds per plant (0.22), days to fifty percent flowering (0.16). Negative direct effect was recorded for plant height (-0.06), days to maturity (-0.17).

CONCLUSION

The present investigation was undertaken to identify genetically diverse and promising location specific breeding lines suitable for cultivation based on morphological traits, which could serve as donors in pigeon pea breeding and varietal development. Using this approach we have already developed some high yielding breeding lines and confirmed their molecular distinctness. The present study exhibited huge variation among the tested lines for seed yield as well as yield contributing traits which facilitated the selection and forwarding the selected lines for its further utilization. Superior genotypes identified in this study were forwarded for further breeding program.

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