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Study of genetic variability, heritability and genetic advance in pigeonpea parental lines (*Cajanus cajan* L.)

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Abstract
Sixty pigeonpea genotypes were evaluated at International Crops Research Institute for the Semi-Arid Tropics during kharif 2021-22 for genetic parameters. Genetic variance (σ^2G) was recorded higher than environmental variance for all the traits *viz.*, days to 50 percent flowering (DTF), plant height (PltH) and seed protein content (SPC). Broad-sense heritability (H^2) estimates were highest for DTF followed by PltH. The range of genotypic coefficient of variation (GCV) observed was 6.43 to 10.42 percent for the traits under study indicating the extent of variability present among the pigeonpea genotypes. Moderate PCV and GCV was observed for the PltH and low phenotypic coefficient of variation (PCV) and GCV was observed for the traits DTF and SPC. High H^2 associated with high genetic advance as h of mean (GAM) was observed for PltH indicating that it was controlled by additive gene action and selection is advisable.

Keywords: Pigeonpea, genetic variability, heritability, genetic advance

Introduction
Cajanus cajan L., commonly known as pigeonpea, is a significant leguminous plant that is grown as an annual crop in semi-arid tropical and subtropical regions across the globe (Sarkar *et al.*, 2020) [26]. Typically, it is cultivated either as a monoculture or as an intercrop in conjunction with short-duration cereals or legumes, as well as with other crops like cotton and groundnut. The crop in question holds the sixth position in terms of global grain legume production. Its cultivation spans approximately 6.36 Mha worldwide, yielding an annual production of 5.96 Mt and a mean productivity of 852 kgha^{-1}. According to the Food and Agriculture Organisation's report (2023), India holds the top position in pigeonpea cultivation, with a vast area of 5.58 million hectares and a production of 4.29 Mt. This accounts for nearly 80 percent of the total production and area dedicated to pigeonpea cultivation worldwide. Myanmar, Malawi and Kenya are the next three countries on the list of pigeonpea producers. Pigeonpea is the second most extensively cultivated pulse crop in India, following chickpea. The crop in question provides not only nutritional benefits and contributes to ensuring food security, but its ability to thrive in various agro-ecological settings renders it an indispensable element of sustainable agricultural systems, as noted by Mula and Saxena (2010) [15] and Rao *et al.* (2010) [22]. According to Varshney *et al.* (2010) [34], the deposition of plant leaves on the ground plays a crucial role in providing essential nutrients to the subsequent crop. Additionally, this process contributes to the enrichment of soil through symbiotic nitrogen fixation.

Notwithstanding its capacity to thrive in various agro-ecological settings, the productivity of pigeonpea has remained comparatively inadequate, with an average range of 700–900 kgha^{-1}. The reasons for this phenomenon can be attributed to various factors, including but not limited to the restricted genetic diversity of cultivated pigeonpea, protracted crop duration, and inadequate uptake of improved cultivars, as highlighted by Saxena *et al.* (2010) [28]. In order to enhance the quality of cultivars, it is imperative to conduct a thorough investigation of the current genetic diversity present within the crop. The reliability of genetic parameters such as genotypic and phenotypic coefficients of variation, heritability, and genetic advance is significant in facilitating efficient selection in breeding materials. The aforementioned data holds significant value for breeders in their process of selecting suitable progenitors and breeding resources to facilitate the advancement of enhanced cultivars. The aim of the current study was to assess the diversity, determine the heritability, and evaluate the genetic progress of 60 parental genotypes of pigeonpea with regards to three traits: days to 50 percent

flowering, plant height, and seed protein content.

Materials and Methods

Plant material and field evaluation

The sixty genotypes (Table 2) utilised in the present study serve as parents in different breeding programs and mapping populations being developed and maintained at ICRISAT, Patancheru, India. The study was planned using an alpha lattice experimental design, which included three replications. A total of 60 genotypes were subjected to a planting scheme wherein each genotype was allotted a single row measuring 4 m in length. The spacing between the rows and within the rows was set at 75 cm and 30 cm, respectively. All customary cultural practises were performed in a regular manner at ICRISAT.

Estimation of seed protein content

The estimation of SPC was conducted in accordance with the protocol outlined by Sahrawat *et al.* (2002) [25]. The method employed for determining the nitrogen content in seed samples was the selenium-sulfuric acid digestion technique. The proposed methodology relies on the chemical properties of selenium and sulfuric acid to facilitate the conversion of nitrogen present in seed tissue into ammonium ions, which were subsequently subjected to quantitative analysis. At the outset, a quantity of 10 g of fully developed, desiccated, and uncontaminated seeds from every genotype were subjected to phenotypic analysis of the SPC in pigeonpea. The seed samples were subjected to an overnight drying process at a temperature of 55 ºC within an oven. Approximately 0.200g of finely pulverised specimen was meticulously transferred into a 75 mL digestion tube, with a precision of 0.001g. Each series of test samples was accompanied by a standard sample and a blank sample. 3.5 mL of the digestion mixture, comprising sulphuric acid and selenium powder, was introduced and subjected to a temperature of 360 ºC for a duration of 2hrs. Selenium was commonly introduced in the form of selenium dioxide or selenium powder, whereas sulfuric acid was added in the concentrated solution state. The application of heat was employed to facilitate the chemical reaction between selenium, sulfuric acid, and plant tissue, resulting in the conversion of nitrogen into ammonium ions. Subsequently, the digestion tubes were extracted from the digestion block and allowed to cool. Subsequent to the cooling process, the mixture underwent filtration to isolate the particulate matter from the liquid phase, which had a total volume of 75 mL. The solution obtained was subsequently subjected to analysis via the Skalar Autoanalyzer, which employs a colorimetric approach. The Skalar Autoanalyzer operates based on the principle of spectrophotometry, whereby a beam of light is transmitted through the sample solution, and the extent of light absorption is quantified. The device employs a spectrophotometer to quantify the degree of light absorption exhibited by the specimen at a wavelength of 600nm. Following the analysis of the samples using the Skalar Autoanalyzer at a wavelength of 600 nm for total nitrogen estimation, the protein content was determined using the formula SPC (%) = N (%) × 6.25, where N represents the nitrogen content (%) and 6.25 is the conversion factor for pulses.

Phenotyping of agronomic traits

Besides SPC, data were also collected on DTF and PltH. The DTF was scored daily as described in Craufurd *et al.* (2001) [6]. It was recorded as the number of days taken from date of sowing to the day when 50 percent of the plants flower in each genotype in each replication. PltH was measured in centimeters of a stretched plant from ground level to the tip of the main stem at maturity.

Data analysis

The statistical analyses were conducted using the R software (Team, 2023) [19]. Best Linear Unbiased Predictions (BLUPs) provide an unbiased and optimal estimate of the true genotypic value of an individual, taking into account both the fixed and random effects of genetics and environment. The phenotype data was recorded and subsequently analysed using META-R software (Alvarado *et al*., 2020) [2] to generate BLUPs from the replicated data. An analysis of variance was carried out on replicated data, and means were separated using the least significant difference (LSD) at 5%.

The computation of genotypic (GCV) and phenotypic coefficients of variation (PCV) was carried out in accordance with the methodology outlined by Singh and Chaudhary (1979) [30], as follows: $P(\%)=(\sqrt{\sigma^2 P}/\mu)/\times 100$, and $GCV(\%)=(\sqrt{\sigma^2 G}/\mu)/\times 100$. The symbols σ^2P and σ^2G represent the phenotypic and genotypic variances, respectively. The categorization of phenotypic and genotypic coefficients of variations was based on their magnitudes as follows: low (<10%), moderate (10-20%), and high (>20%) as reported by Subramanian and Menon in 1973 [31]. The estimation of broad-sense heritability (H^2) was conducted through the utilisation of the formula: $H^2=(\sigma^2 G/\sigma^2 P)$. Johnson *et al.* (1955) [12] categorised the heritability into three groups based on their magnitude: low (0-0.3), moderate (0.3-0.6), and high (>0.6).

The formula used to calculate genetic advance (GA) is expressed as follows: $GA=H^2\times\sqrt{\sigma^2 P}\times K$, where K is the selection differential (2.06 at 5%). The conversion of GA to percent genetic gain was achieved through the formula: Genetic gain = GA x 100. This measure was subsequently classified into three categories based on the percentage range, namely low (0-10%), moderate (10-20%), and high (>20%). This categorization was proposed by Johnson *et al.* in 1955 [12].

Results and Discussion

Performance of genotypes

The fundamental concept of systematic plant breeding involves the utilisation of existing natural variability and diversity to enhance crop improvement programmes, as stated by Bhandari *et al*. (2017) [4]. The current investigation entails the evaluation of the genetic variance inherent in the pigeonpea genotypes. The results of the analysis of variance indicate that there were statistically significant differences observed among the genotypes with respect to the studied traits. Table 1 presents the mean and range values for all traits. The observed range for each trait exhibited a broad spectrum of values, implying the potential existence of genetic variability among the genotypes. The present study reports a range of DTF values between 86.8 days (ICPB2039) and 125.1 days (ICPL20097), with a mean value of 111.5 days. The study identified 30 pigeonpea genotypes with a lower DTF compared to the overall mean. These genotypes hold potential for the development of early-maturity pigeonpea varieties. The PltH measurements ranged between 114.9 cm (ICPB2039) and 204.2 cm (ICP1156), with a mean

value of 169.8 cm. In this study, it was observed that 31 pigeonpea genotypes exhibited a plant height (PH) that was lower than the overall mean. These genotypes may be utilized in the development of dwarf and semi-dwarf pigeonpea genotypes. While, SPC ranged from 21.6 percent (ICPB2078) to 28.4 percent (ICP7076) with a mean of 25.7 percent. The study identified 35 pigeonpea genotypes that exhibited a higher SPC than the mean value. These genotypes hold potential for the advancement of pigeonpea genotypes with elevated SPC. Pigeonpea genotypes that exhibit early flowering, superior plant stature, and high seed protein content may be identified and chosen for future breeding programmes based on their mean values. The study's utilisation of highly inbred landraces or breeding lines is likely the cause for the expected low coefficient of variation (CV) values observed across traits, as presented in Table 2.

Table 1: Mean squares for seed protein content and agronomic traits in 60 pigeonpea genotypes.

	Mean square	
Trait	**Genotype (DF = 59)**	**Error (DF = 91)**
Seed protein content (%)	9.9***	1.6
Days to 50% flowering	268.0***	26.4
Plant height (cm)	1129.1***	177.8
DF, Degrees of freedom, *** significant at P = 0.001.		

Table 2: BLUP, range and coefficient of variation for seed protein content and agronomic traits studied in 60 pigeonpea genotypes.

Genotype	SPC (%)	DTF50	PltH (cm)
ICP10397	27.0	118.2	177.6
ICP1071	27.3	110.7	178.2
ICP11015	25.8	109.5	162.6
ICP11494	26.1	97.0	165.5
ICP1156	27.1	107.8	204.2
ICP11737	26.6	109.8	198.9
ICP12298	27.2	110.1	171.4
ICP12515	26.5	110.1	155.9
ICP14209	27.0	117.0	176.9
ICP14303	26.3	100.6	149.2
ICP14524	25.8	115.2	180.7
ICP14722	25.2	108.9	170.6
ICP14903	26.2	103.0	169.2
ICP15068	25.2	101.5	160.0
ICP16180	26.7	98.5	168.2
ICP16309	26.4	108.9	162.2
ICP202	26.7	114.9	187.0
ICP3451	24.9	122.7	177.7
ICP3576	27.0	119.7	188.4
ICP4029	26.0	113.4	168.9
ICP4317	28.1	115.8	187.1
ICP6123	27.0	114.6	188.6
ICP6370	26.7	111.9	156.2
ICP655	25.3	122.1	184.3
ICP6845	26.9	117.6	180.4
ICP6971	27.7	123.0	192.8
ICP7076	28.4	105.4	182.9
ICP7426	25.8	116.7	170.7
ICP7803	26.6	110.7	186.4
ICP8255	25.5	110.7	192.0
ICP8757	24.8	112.8	199.6
ICP8863	23.8	96.4	165.3
ICP9045	23.7	107.5	158.3
ICP9499	24.5	111.3	186.2
ICP964	26.5	114.6	173.1
ICP9671	27.1	117.0	181.5
ICPB2039	25.7	86.8	114.9
ICPB2043	23.6	94.0	153.7
ICPB2047	25.2	123.3	154.1
ICPB2048	23.7	105.7	164.6
ICPB2078	21.6	116.1	161.6
ICPB2092	23.4	120.6	173.0
ICPB2156	23.2	94.6	182.6
ICPL20094	25.1	110.7	154.9
ICPL20096	26.6	122.1	155.7
ICPL20097	25.8	125.1	189.2
ICPL20098	26.0	122.4	160.6
ICPL20101	22.6	117.0	165.6
ICPL20102	23.0	120.6	160.7
ICPL20103	26.6	114.3	169.7

ICPL20104	24.1	115.8	145.1
ICPL20105	25.6	123.9	170.0
ICPL20237	24.0	103.0	171.0
ICPL332	27.0	102.4	159.7
ICPL85063	26.0	100.6	157.2
ICPL99004	25.5	106.6	148.2
ICPL99046	25.5	114.9	134.6
ICPL99048	25.9	119.7	163.4
ICPL99050	25.2	110.7	159.8
ICPL99051	24.3	111.3	157.1
Mean	25.7	111.5	169.8
Range	21.6-28.4	86.8-125.1	114.9-204.2
CV (%)	5.3	4.7	7.9
LSD 5%	2.2	8.0	20.3

CV, Coefficient of variation; SPC, Seed protein content; DTF50, Days to 50% flowering; PltH, Plant height;

Genotypic and phenotypic coefficients of variation, heritability and genetic gain

The statistical measures of mean, range, and coefficient of variation provide insight into the potential for improvement of a particular trait. However, they do not provide any information regarding the impact of genotype on the variation of the trait. Therefore, the current investigation involved the estimation of various parameters, including genotypic (σ^2G), environmental (σ^2E), and phenotypic (σ^2P) variances, GCV and PCV, broad-sense heritability (H^2), and genetic gain, as presented in Table 3. Understanding the genetic variation associated with a particular trait is a crucial aspect of formulating a breeding strategy. The proportion of σ^2G was high suggesting that the observed variation is predominantly attributed to genetic factors rather than environmental influences. Comparable outcomes were noted by Gohil (2006) [9] and Vange and Moses (2009) [33]. Overall, the values of σ^2G and GCV were consistently proximate to those of σ^2P and PCV, respectively. It is noteworthy that σ^2G consistently exceeded σ^2E for all traits. The estimates of GCV reflect the total amount of σ^2G present in the material studied. Table 3 demonstrates the superiority of GCV and PCV in depicting the variability that exists among the genotypes. For all the traits, the PCV exhibited a greater magnitude compared to the GCV. The moderate values of GCV and PCV observed for PltH (10.42, 13.20%) suggest a considerable degree of variability for this trait, which can facilitate the identification of genotypes with desirable characteristics based on this trait. The GCV and PCV values exhibited low levels for DTF (8.04, 9.29%) and SPC (6.43, 8.26%). This suggests that the selection process for said traits lacks statistical significance. Obala *et al.* (2018) [16] presented comparable findings regarding plant height and SPC. In their study on pigeonpea, Reddy *et al.* (2019) [23] noted that a majority of the quantitative traits exhibited low GCV and PCV. The magnitude difference between GCV and PCV was minimal for all traits, suggesting a reduced impact of environmental factors on trait expression. Several studies, including those conducted by Ranjani *et al.* (2018) [21], Hemavathy *et al.* (2019) [11], and Ranjani *et al.* (2021) [20], have reported minimal variation between GCV and PCV for diverse characteristics in pigeonpea. According to Burton's (1952) [5] proposal, an assessment of genetic variation and heritability estimates would provide a more comprehensive understanding of the potential effectiveness of selection. The GCV, which quantifies the degree of genetic diversity of a particular trait, is typically incorporated alongside heritability and genetic gain when evaluating the impact of phenotypic selection. The heritability estimates for the three traits, namely DTF (0.75), PltH (0.62), and SPC (0.60), were found to be high, suggesting that there is potential for genetic enhancement of these traits. Obala *et al.* (2018) [16] and Sharma *et al.* (2021) [29] have previously reported high heritability values exceeding 95 percent for the traits of number of pods per plant and 100-seed weight. Comparable findings were documented by Patel and Patel (1998) [18], Pansuriya *et al.* (1998) [17], Bhadru (2008) [3], Linge *et al.* (2010) [13], Hemavathy *et al.* (2019) [11] and Ranjani *et al.* (2021) [20]. Although high heritability suggests that selection based on phenotypic performance is effective, it does not provide insight into the magnitude of genetic improvement that can be achieved through selecting the most favourable individuals. The concept of genetic advance refers to the enhancement in the average of chosen lineages in comparison to the initial population, as stated by Lush (1940) [14] and Johnson *et al.* (1955) [12]. The phenomenon can be described as a directional change in the frequency of genes towards the advantageous end under the influence of selection pressure exerted by environmental factors. The term "genetic gain" refers to the percentage increase in genetics over the mean. According to Johnson and colleagues' (1955) [12] findings, heritability estimates and genetic gain tend to be more advantageous. The characters DTF (14.33%) and PltH (16.94%) displayed a moderate degree of genetic advance relative to the mean, suggesting that the observed variations in these traits can be attributed, to some extent, to additive gene effects. Without genetic advance, the estimates of heritability alone will not be of practical value and emphasized the concurrent use of genetic advance along with heritability. These results are in accordance with the findings of Satish Kumar *et al.* (2005) [27] and Vange and Moses (2009) [33]. The high heritability combined with high genetic advance as percent mean was reported for PltH by Rekha *et al.* (2013) [14]. Ajay *et al.* (2014) [1] observed a high heritability and genetic advance as percent mean in pigeonpea for PltH. Obala *et al.* (2018) [16] reported high heritability and moderate genetic advance as percent of mean for SPC and high heritability and moderate genetic advance as percent of mean for DTF and PltH.

Table 3: Estimates of broad-sense heritability, genotypic and phenotypic coefficients of variation, and genetic gain for three traits in 60 pigeonpea genotypes.

Trait	σ^2G	σ^2E	σ^2P	*H2*	GCV (%)	PCV (%)	GA	GAM (%)
DTF50	80.36	26.93	107.29	0.75	8.04	9.29	15.98	14.33
PltH	313.10	189.78	502.88	0.62	10.42	13.20	28.76	16.94
SPC	2.72	1.76	4.48	0.60	6.43	8.26	2.64	10.33

Conclusion

The significant differences among pigeonpea genotypes in the present study indicated presence of variability for all traits measured. This is supported by the H^2 indicating influence of genetic factors on phenotype. Whereas H^2 estimates can be used to predict the reliability of the phenotypic value as a guide to breeding value (Falconer and Mackay, 1996) [7], H^2 alone does not reveal the extent of response to selection. H^2 along with GCV and GA provide reliable estimates of the amount of genetic gain to be expected through phenotypic selection (Burton, 1952) [5]. The combination of high H^2, GCV, GA and genetic gain for DTF, and PltH indicates that the variation in these traits is largely due to genetic factors, and selection would be effective for these traits. However, SPC had high H^2 but low GCV and low genetic gain estimates, depicting a low response to selection. The variations and relationships among traits are dependent upon the set of materials evaluated and the environment in which they are tested (Hamdi *et al*., 1991; Wray and Visscher, 2008) [10, 35], re-evaluating the 60 and other potentially useful genotypes for SPC and agronomic traits in multiple sets of environments may be necessary.

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