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Genetic analysis of seed protein content and its association with seed weight and yield in pigeonpea

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ABSTRACT

To investigate inheritance of seed protein content (SPC) and its relationships with agronomic traits in pigeonpea, four *elite* germplasm lines with diverse genetics blackgram were used to develop three crosses. Each cross consisted of six generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2). Generation mean analysis revealed the importance of dominance and epistatic effects for SPC. Duplicate and negative additive-by-additive epistasis was predominant and associated with transgressive segregation for SPC. Additive genetic variance component was higher than the environmental and dominance components. Broad-sense heritability ranged from 0.52 to 0.60. Predicted genetic gain after one cycle of selection was highest at 5% selection intensity. Seed weight (positively) and yield (negatively) correlated with SPC. It is inferred from the study that appropriate selection of parents and reciprocal recurrent selection could be effective for improving SPC in pigeonpea with stabilized yields.

Key words: Additive-by-additive epistasis, *Cajanus cajan*, Generation means, Genetic effects

One of the major forms of under nutrition is the inadequate intake of dietary protein. In communities where intake of animal protein is difficult or not affordable, food legumes provide the bulk of the needed dietary protein (Santos *et al.* 2012). Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the major legume crops cultivated as a source of food protein for over a billion people especially in the semi-arid tropics of Africa and Southern Asia (Mula and Saxena, 2010). The area cultivated with pigeonpea continues to increase annually and this can be attributed to its drought tolerance and ability to give relatively better yields in marginal soils than any other cultivated food legumes (Akibodeand Meridia, 2011).

The available genetic variability for seed protein content (SPC) in cultivated pigeonpea (Uphadyaya *et al.* 2007; Obala *et al.* 2018) indicates that there is potential for genetic enhancement of the trait through hybridization and selection. The genetics of SPC in pigeonpea has been reported to be quantitative in nature with the non-additive genetic effects being more important than the additive component (Vaghela *et al.* 2009). However, it is not clear which component of the non-additive effects, either dominance or epistasis, contributes more to the SPC variability in pigeonpea. Knowing whether non-additive effects are due to dominance or epistasis is important because certain types of epistasis suchas additive-by-additive and additive-by-dominance effects can confound and bias the estimates of additive and dominance components.

A robust approach for detecting both additive and non-additive genetic effects is generation mean analysis. GMA combines the study of population means, variances, and the detection of additive-dominance and epistatic effects (Hallauer and Miranda, 1988). However, there is no report of the use of GMA for elucidating the genetics of SPC in pigeonpea. Similarly, information on correlations of SPC with important traits such as seed weight and seed yield, although reported (Dahiya *et al.* 1977; Saxena e*t al.* 1987), remains limited. The scarce information impedes the effective use of the available genetic variability for enhancing SPC while stabilizing yield in the crop. The present study was therefore conducted with the objective of investigating the inheritance pattern for SPC and its relationships with 100-seed weight and seed yield in pigeonpea.

MATERIALS AND METHODS

Four cultivars including ICP 8863, ICP 14209, ICP 11605 and ICPL 87119 were selected based on their SPC and diverse genetic background (Obala *et al.* 2018). Pure seeds of the parental genotypes were obtained from ICRISAT's Gene bank and used to develop three crosses: ICP 11605×ICP 14209, ICP 8863×ICP 11605 and ICP 8863×ICPL 87119, hereafter referred to as Cross 1, Cross 2 and Cross 3, respectively. F_1 seeds of the three crosses were generated in 2012 rainy season. In the subsequent season of 2013, the F_1 populations were grown and selfed to generate F_2 seed. The F_1 plants were also crossed to the parents to generate backcross one to parent one (BC_1P_1) and parent two (BC_1P_2) with the parental as the seed plants. Additional F_1 seeds were also generated. At most two F_1 and two parental plants were used to generate the crosses. The hybridization was done manually after emasculating the unopened floral buds.

All six generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) in each cross were sown in the field in growing season of 2015 at ICRISAT, Patancheru, India (545 meters above sea level, 17°32'N and 78°16'E). Trial design was a randomized complete block design with two replications. Seedbed preparation, control of pests, plant spacing, harvesting and seed drying were as described in Obala *et al.* (2018). SPC was estimated at the Central Analytical Services Laboratory at ICRISAT, India as described in Obala *et al.* (2018, 2019). Besides SPC, data were also recorded for SW and SY in grams per plant. The total number of plants per generation evaluated for SPC, SW and SY are presented in Table 1.

Frequency distributions were constructed using F_2 SPC data in MS Excel 2013. Proportions of transgressive segregants in each cross were obtained as the percentage of F_2 plants with SPC falling outside the range of either parents. The least squares mean (μ) and variance (σ^2) of each generation were obtained using the general linear model procedure in SAS v9. 4. Means of the six generations per cross were separated using Fisher's LSD at 5% probability. The means of parental, F_1 and F_2 generations were used to test for significance of two a priori linear contrast parameters: (i) deviation of μF_1 from the mid-parent value as a measure of mid-parent heterosis (Holland 2001), and (ii) deviation of F_2 mean from the average of μF_1 and MPV as a measure of the overall effect of epistasis (Fenster and Galloway 2000).

Adequacy of additive-dominance model in explaining the observed phenotypic variation was tested using the ABCD scales (Mather 1949).The deviation of each of the scales from zero was tested using the't'-test. Where the additive-dominance model inadequately explained the observed variation, the additive ([a]), dominance ([d]) and their interactions [aa], [ad] and [dd] were estimated using a six-parameter model (Hayman 1958). Standard errors for the genetic effects were obtained as the square root of their respective variances. The phenotypic (σ^2P), environmental (σ^2E), genotypic (σ^2G) and additive (σ^2A) components of variance, and the broad-sense (H^2) and narrow-sense (h^2) heritability were estimated according to Mather and Jinks (1971). Three selection intensities of 5, 10 and 20% in order of increasing stringency were used to predict genetic gain for SPC from one cycle of selection using the model of Hallauer and Miranda (1988). The phenotypic (*rP*), environmental (*rE*) and genotypic (*rG*) correlations were calculated according Searle (1961).

RESULTS AND DISCUSSION

Parents of each of the three crosses significantly differed in SPC from each other (Table 1). Among the parental lines, ICP 14209 had the highest SPC (23.04 %) followed by ICP 8863 (22.09 %), ICP 11605 (21.14%) and ICP 87119 (19.3%). Significant difference between parents of a cross is a pre-requisite for accurate determination of genetic effects controlling a trait (Mather and Jinks 1982). Generally, non-segregating parental and F_1 generations had lower variances than the segregating F_2 and backcross generations, suggesting similar environmental influence on SPC in all generations whichis consistent with previous report (Saxena *et al.* 2002).The mean SPC of the F_1 was always lower than the mid-parent value, representing negative mid-parent heterosis, and closer to the low protein parent suggesting partial dominance of low SPC consistent with earlier observations in pigeonpea (Dahiya *et al.* 1977), and common beans (Noubissie *et al.* 2012). Frequency distributions of F_2 for SPC were continuous in all three crosses (Figure 1), indicating the involvement of several genes (Mackay 2009), which is also consistent with the presence of transgressive segregants. The proportion of transgressive segregants having SPC higher than that of

Table 1. Generation, sample size, least squares mean, variance, deviation of F_1 from mid-parent value (MPV), deviation of F_2 from average of F_1 and MPV and percentage of transgressive segregants for seed protein content in three crosses of pigeonpea[@]

	ICP 11605 (Low) × ICP 14209 (High)			ICP 8863 (High) × ICP 11605 (Low)			ICP 8863 (High) × ICPL 87119 (Low)		
Generation	**n**	**LsMean**	**σ^2**	**n**	**LsMean**	**σ^2**	**n**	**LsMean**	**σ^2**
P_1	18	21.42 bc	0.73	18	22.21 a	0.44	18	21.98 c	0.50
P_2	18	23.04 a	0.84	18	20.86 bc	0.65	18	19.34 a	0.07
F_1	12	21.60 bc	0.69	12	20.11 cd	1.90	6	20.86 b	1.08
F_2	237	21.97 b	1.86	236	21.36 b	2.73	253	19.58 a	1.42
BC_1P_1	40	20.99 c	1.76	40	20.23 cd	0.95	40	19.92 a	0.45
BC_1P_2	40	21.35 c	0.95	40	19.90 d	2.47	39	19.71 a	0.78
Mid-parent value (MPV)		22.23			21.54			20.66	
Deviation of F_1 from MPV		-0.63			-1.43^{***}			0.20	
Deviation of F_2 from [(F_1+MPV)/2]		0.05			0.53^{*}			-1.17^{**}	
Transgressive segregants< low parent (%)		32.1			35.6			41.9	
Transgressive segregants> high parent (%)		20.7			36.0			1.2	
Pooled over both extremes		52.7			71.6			43.1	

[@]Means followed by the same letter within a column are not significantly different at $LSD_{0.05}$;*, ** and *** significant at 0.05, 0.01 and 0.001 probability levels, respectively; n, population size; LsMean, least squares mean; σ^2, variance

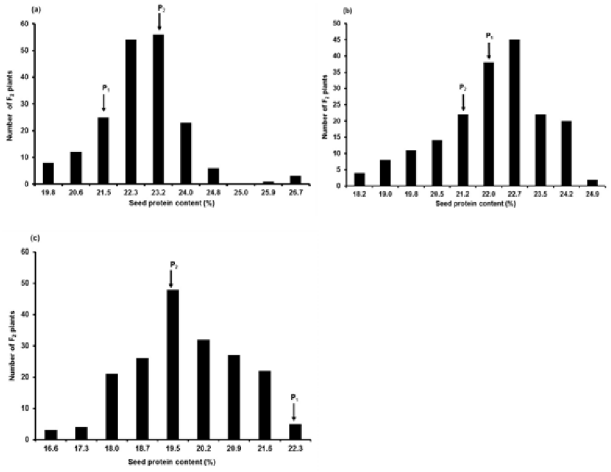

Fig. 1. F_2 frequency distribution for seed protein content in three crosses of pigeonpea: (a) Cross 1, ICP 11605 (P_1) × ICP 14209 (P_2) with P_1 and P_2 being low and high seed protein content parents, respectively; (b) Cross 2, ICP 8863 (P_1) × ICP 11605 (P_2), and (c) Cross 3, ICP 8863 (P_1) × ICPL 87119 (P_2) with P_1 and P_2 being high and low SPC parents, respectively.

the high parent were largest in Cross 2 (36.0%) and smallest in Cross 3 (1.2%), indicating that some specific parental combinations result in higher than expected progeny SPC compared to parental values. There was an overall transgression (41.9%) towards very low SPC in Cross-3.Selections from such a cross based on yield *per se*, without due consideration to SPC, may result in new cultivars with much lower SPC than either parents individually. Such low SPC cultivars would exacerbate the low protein intake in areas of cultivation and/or consumption.

Cross 1 and Cross 2 had negative heterosis though non-significant in Cross 1 but highly significant in Cross 2 (P d" 0.01) (Table 1). Cross 3 had positive but non-significant (P> 0.05) heterosis. Similarly, the deviation of F_2 mean from average of F_1 and MPV was positive but not significant (P < 0.05) in Cross 1 and positive but significant (P=0.05) in Cross 2. Cross 3 had a negative, highly significant (P d" 0.01) deviation of the F_2 from the average of MPV and F_1. Therefore, Cross 1 and Cross 2 had more favorable combination of genes from the parents for SPC than Cross 3, which reflects the pattern of transgressive segregation in the crosses.

The ABCD scaling test showed significance (P = 0.05) of at least one of the individual A, B, C, and D scales (Table 2) indicating the inadequacy of the additive-dominance model in explaining the observed SPC variation (Mather, 1949). Thus, Hayman's (1958) six parameter model was used to estimate the main and epistatic gene effects (Table 3). The mean effect [m] was significant and larger than all genetic effects measured in all three crosses (Table 3), indicating the quantitative nature of inheritance of SPC in the studied populations. The [a] was non-significant (P > 0.05) and smaller in magnitude than [d] in all three crosses indicating the limited influence off additive gene effect on SPC in pigeonpea. The [d], [aa] and [dd] were significant (P d" 0.05) in Cross 1 and Cross 2, and [ad] was significant only in Cross 3, indicating the importance of non-additive gene effects in controlling SPC which agrees with previous reports in pigeonpea (Vaghela *et al.* 2009), common bean (Iqbal *et al.* 2012) and mungbean (Tiwari *et al.* 1993). The negative [d], [ad] and [aa], indicates that these effects favored low SPC while the positive [dd] indicates favorable effect for increased SPC. The epistasis in Cross 1 and Cross 2 was duplicate while it was complementary in Cross 3 (Mather and Jinks, 1982; Table 2). Presence of duplicate epistasis and negative [aa] in the same cross was associated with increased SPC and desirable transgressive segregants while complementary epistasis resulted in low SPC and negligible number of desirable segregants in Cross 3.

The σ^2G had consistently larger effects than the σ^2E in all three crosses (Table 4), indicating the importance of genotype in determining the SPC variation. Despite the predominance of non-additive gene effects, the σ^2A for

Table 2. ABCD scaling test for seed protein content in three crosses of pigeonpea[@]

	Scale			
Cross	**A**	**B**	**C**	**D**
ICP 11605 × ICP 14209	$-1.05^{NS} \pm 0.87$	$-1.94^{*} \pm 0.82$	$0.21^{NS} \pm 1.44$	$1.60^{**} \pm 0.61$
ICP 8863 × ICP 11605	$-1.86^{NS} \pm 1.43$	$-1.18^{NS} \pm 1.53$	$2.06^{NS} \pm 3.12$	$2.55^{*} \pm 1.13$
ICP 8863 × ICPL 87119	$-3.0^{**} \pm 0.77$	$-0.81^{NS} \pm 1.27$	$-4.70^{**} \pm 1.48$	$-0.44^{NS} \pm 0.54$

[@]NS, not significant at 0.05 probability level; *, and ** significant at 0.05 and 0.01probability levels, respectively

Table 3. Hayman's main and epistatic gene effect estimates, priori linear contrasts and proportion of transgressive segregation for seed protein content in three crosses of pigeonpea[@]

Gene model	**ICP 11605 × ICP 14209**	**ICP 8863 × ICP 11605**	**ICP 8863 × ICPL 87119**
[m]	$21.97^{***} \pm 0.09$	$21.36^{***} \pm 0.11$	$19.58^{***} \pm 0.07$
[a]	$-0.37^{NS} \pm 0.36$	$0.33^{NS} \pm 0.40$	$0.22^{NS} \pm 0.25$
[d]	$-3.84^{*} \pm 1.53$	$-6.64^{***} \pm 1.81$	$1.15^{NS} \pm 1.34$
[aa]	$-3.21^{**} \pm 1.08$	$-5.21^{***} \pm 1.24$	$0.94^{NS} \pm 0.80$
[ad]	$0.44^{NS} \pm 0.57$	$-0.34^{NS} \pm 0.58$	$-1.10^{**} \pm 0.36$
[dd]	$6.20^{*} \pm 2.71$	$8.25^{*} \pm 3.18$	$2.84^{NS} \pm 2.37$
Epistasis	Duplicate	Duplicate	Complementary

[@]NS, not significant at 0.05 probability level; *, ** and *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 4. Variance components and heritability estimates for seed protein content in three crosses of pigeonpea[@]

Population	Variance components					Heritability		Genetic gain at 3 selection intensities		
	σ^2P	σ^2E	σ^2G	σ^2A	σ^2D	H^2	h^2	5%	10%	20%
ICP 11605 × ICP 14209	1.86	0.74	1.12	1.02	0.10	0.60	0.55	1.5	1.3	1.0
ICP 8863 × ICP 11605	2.74	1.22	1.51	2.05	-0.54	0.55	0.75	2.6	2.2	1.8
ICP 8863 × ICPL 87119	1.42	0.68	0.74	1.61	-0.87	0.52	1.13	2.8	2.4	1.9
							Average	2.3	2.0	1.6

[@]σ^2P, Phenotypic variance; σ^2E, Environmental; σ^2G, Genotypic variance: σ^2A, Additive variance: σ^2D, Dominance variance ; H^2, Broad-sense heritability ; h^2, Narrow-sense heritability; Values of h^2 underlined are greater than their corresponding H^2 values as result of high σ^2A; 5%, 10% and 20% are selection intensities used for predicting genetic gain after one cycle of selection

SPC in all three crosses were higher than σ^2D. The high σ^2A amidst pervasive epistatic effects seems to suggest that epistasis contributed to the σ^2A which agrees with earlier studies that epistasis contributes to, and increases σ^2A (Monnahan and Kelly, 2015). The negative σ^2D in Cross 2 and Cross 3 led to higher than expected σ^2A, which in turn led to uncertainty in the estimation of h^2, with h^2 being greater than H^2 in the two crosses (Table 4). H^2 is the maximum value for h^2, and h^2 must always be less or equal to the H^2 (Hartland Jones, 2011). Therefore, h^2 values greater than the H^2 in Cross 2 and Cross 3 (underlined in Table 4) were omitted from further discussions but only presented here for future references. Thus, the h^2 on plant basis of 0.55 is close to 0.65 previously reported in pigeonpea (Saxena *et al.* 2002), 0.47 in cowpea (Santos *et al.* 2012) and 0.63 to 0.73 in common beans (Kelly and Bliss, 1975). The H^2 of 0.52 to 0.60 is within the range of 0.34 to 0.62 reported earlier in pigeonpea (Dahiya *et al.* 1977). Estimates of genetic gain (Table 4) suggests that selection at 5% intensity would give the highest genetic gain for SPC, although the amount of gain is genetic background dependent.

Phenotypic correlations between SPC and SW were moderate, positive and significant (P d" 0.01) in Cross 1 and Cross 2 but very weak, negative and not significant (P > 0.05) in Cross 3 (Table 5). It was negative between SPC and SY in all three crosses but only significant (P = 0.01) in Cross 2. These observations suggest that the *r*P among the studied characters are dependent on genetic background. The *r*E between SPC and SW were consistently negative, highly significant (P = 0.01) and larger than *r*P in all three crosses, indicating that genetic factors controlling the two traits responded similarly to the environment. In the case of SPC and SY, *r*E was non-significant in Cross-1 and Cross-2 but significant (P =0.05) in Cross 3, indicating that genes controlling the two traits differentially responded to the environment. However, *r*G between SPC and SW, and between SPC and SY could not be estimated in all crosses (Table 5). Failure to estimate rG could have resulted from the use of small sample sizes in the early generations (Hébert *et al.* 1994). It was difficult to obtain large number of seeds in early generations (F_1, F_2 and backcross F_1) in our study. We therefore base our discussion of genetic correlations on *r*G obtained in Cross-2 for SPC and SW, and in Cross-1 for SPC and SY (Table 5). The strong, positive and highly significant genetic correlation (*r*G = 0.87; P = 0.01) between SPC and SW in Cross 2 (Table 5) indicates that the two traits can be simultaneously improved, which agrees with the report of an earlier study in pigeonpea (Saxena *et al.* 1987). Similarly, the moderate, negative and significant genetic correlation (*r*G = -0.33; P = 0.01) between SPC and SY in our study agrees with the report of Dahiya *et al.* (1977) and indicates that simultaneous selection for both high SPC and SY could be possible. Given that estimates of *r*G between SPC and SW, and between SPC and SY in the present study could be obtained only from one cross each, generalization of these observations may not be very appropriate.

Continuous distribution of the F_2 data, the presence of transgressive segregants and epistasis point to polygenic control of SPC in pigeonpea. It may be possible to derive genotypes with SPC as high as 25 to 27 % from crosses between the well-adapted but low to moderate SPC parents. The selection would most be effective at later generations followed by reciprocal recurrent selection procedures. SPC and SW maybe simultaneously improved owing to the generally positive or non-significant correlations between them. The negative association of SPC and SY suggests that selection for yield *per se* without considering SPC may lead to reduction in SPC. Selection of

Table 5. Phenotypic, environmental and genotypic correlation coefficients between seed protein content and 100-seed weight, and between seed protein content and seed yield in three crosses of pigeonpea[@]

Correlated traits (X × Y)		ICP 11605 × ICP 14209	ICP 8863 × ICP 11605	ICP 8863 × ICPL 87119
SPC × SW	*r*P	0.23^{**}	0.20^{**}	-0.07^{NS}
	*r*E	-0.44^{**}	-0.39^{**}	-0.76^{**}
	*r*G	n/a	0.87^{**}	n/a
SPC × SY	*r*P	-0.07^{NS}	-0.27^{**}	-0.04^{NS}
	*r*E	0.20^{NS}	-0.21^{NS}	-0.31^{*}
	*r*G	-0.33^{**}	n/a	n/a

[@]NS, not significant at 0.05 probability level; * and ** significantly different from zero at 0.05 and 0.01 probability levels, respectively; SPC, seed protein content (%) per plant; SW, 100-seed weight in g per plant; SY, seed yield in g per plant; n/a, could not be estimated due to excessively high σ^2A as a result of negative σ^2D; *r*P, *r*E and *r*G are the phenotypic, environmental and genotypic correlation coefficients, respectively

parents based on their combining ability for SPC and SY may be a necessary first step that would facilitate their simultaneous genetic improvement.

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