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MARKER-TRAIT ASSOCIATION IN SELECTED NIGERIAN MINI-CORE PIGEONPEA [Cajanus cajan (L.) Millsp.] ACCESSIONS USING SCoT MARKERS

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ABSTRACT

The pigeonpea [*Cajanus cajan* (L.) Millsp.] is a popular leguminous plant in the Fabaceae family. Its low yield is a major challenge in Nigeria with no improved varieties developed. The identification of markers linked to agronomical traits will accelerate agronomic yield improvements in pigeonpea. Hence, the study was conducted to identify SCoT markers associated with important agronomic traits within selected Nigerian pigeonpea lines. A total of 52 Nigerian pigeonpea were phenotyped for vegetative and yield traits. DNA was extracted from sampled accessions and evaluated with SCoT markers. Marker performance and association to agronomic traits were evaluated. The coefficient of variation (CoV) varied with agronomical traits, ranging from leaflet length (CoV = 15.77) to tertiary branches (CoV = 155.23). Broad sense heritability was high for all traits (H^2 > 75%) except for the number of seeds per pod (H^2 = 9.13%). Genetic advances ranged from 0.65 in leaflet width to 106.65 in pod number. Only seeds per pod showed more environmental variance than the genetic variance. SCoT markers showed 100% polymorphism with average Polymorphic Information Content values > 0.6. The effective marker ratio also ranged between 1.50 in SCoT-3 to 45.38 in SCoT-2. The cumulated phenotypic variance explained by associated markers ranged between 9.11% in 100 seed weight to 44.7% in leaflet width. Some markers were associated with more than one agronomic trait. These markers can be harnessed for their potential application in pigeonpea improvement programmes.

Keywords: Agronomical traits, Association study, *Cajanus cajan*, Marker-trait, Pigeonpea, SCoT.

INTRODUCTION

The pigeonpea [*Cajanus cajan* (L.) Millsp.] of the Fabaceae family is one of the world's most cultivated legumes. It is widely consumed in South Asia due to its high nutritional value (Srikanth *et al.,* 2013). Although it is regarded as the world's sixth most important leguminous crop by the Food and Agriculture Organization (FAOSTAT, 2013), pigeonpea is a subsidiary crop in West Africa, particularly for smallholder subsistence in Nigeria (Egbe and Vange, 2008), Ghana (Adjei-Nsiah, 2012), and Benin (Dansi *et al.,* 2012; Ayenan *et al.,* 2017). The potential yield of pigeonpea in Africa is low compared to other pigeonpea-producing continents. Hence, it is still considered an underutilized crop in Nigeria and many parts of Africa (Dutta *et al.,* 2011; Zavinon *et al.,* 2018).

This crop can be channelled towards achieving the 2030 Sustainable Development Goals, i.e., food security and poverty eradication if agronomic challenges are mitigated. Its economic and nutritional values are numerous. They include soil revitalization through nitrogen fixation, as a source of protein, and other nutritional benefits (e.g., carotene, and vitamin B). Most crop improvement strategies are based on the phenotypic selection of germplasm with desired and contrasting traits followed by conventional breeding, which takes time and wastes materials to achieve the desired goal (Amusa *et al*., 2019; Amusa *et al*., 2022). However, the introduction of molecular marker technology, which allows the identification of genomic regions associated with traits of interest has helped to overcome the time gap and reduced genetic resource wastage by taking advantage of the link between markers and traits during crop development programmes.

Several molecular markers have been developed with different applications in crop improvement (Sahu *et al*., 2015; Singh *et al*., 2018). They include rapid amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), inter simple sequence repeats (ISSR), and single nucleotide polymorphism (SNP). The start codon targeted (SCoT) polymorphism markers is a fast,

cost-effective method that requires a small amount of DNA, and without prior DNA sequence knowledge of the organism (Collard and Mackill, 2009). Hence, it is widely applied in population and genetic studies, genetic mapping, and selection for crop production in different plants (Collard and Mackill, 2009; Patil *et al*., 2017; Saeidnia *et al*., 2021). Moreover, Rahimi *et al*. (2018) favoured SCoT markers over other markers, e.g., ISSRs and RAPDs, to be more efficiently applied in marker-assisted selection programmes. For SCoT markers, there are fewer recombination events between gene and trait, and the marker is anchored on the ATG start codon regions (Collard and Mackill, 2009). Hence, the study was conducted to identify SCoT markers associated with important agronomic traits within selected Nigerian pigeonpea lines.

MATERIALS AND METHODS

Collection of samples, planting, and phenotype evaluation

Pigeonpea accessions (Table 1) were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nigeria, and planted at the Botany Department screen house (Latitude 6° 30´ 52´´ N, Longitude 3° 25´ 56´´ E), University of Lagos, Nigeria. Accessions were planted with three seeds per hole (in a 2 kg pot filled with soil) and three replicates per accession, laid in a complete randomized design setup. Normal agronomic practices were performed throughout the experiment. The quantitative descriptor data for pigeonpea according to International Board for Plant Genetic Resources (IBPGR) and International Crops Research Institute for the Semi-Arid tropics (ICRISAT) was evaluated for each accession (Table 2; IBPGR and ICRISAT, 1993).

Table 1: List of pigeonpea accessions collected from ICRISAT.

SN	Accessions	SN	Accessions	SN	Accessions	SN	Accessions
1	ISC-107	14	ISC-168	27	ISC-201	40	ISC 46
2	ISC-11	15	ISC-169	28	ISC-202	41	ISC 51
3	ISC-111	16	ISC-171	29	ISC-23	42	ISC-63
4	ISC-115	17	ISC-172	30	ISC-24	43	ISC-66
5	ISC-118	18	ISC-174	31	ISC-25	44	ISC-76
6	ISC-120	19	ISC-176	32	ISC-3	45	ISC-77
7	ISC-123	20	ISC-178	33	ISC-30	46	ISC-78
8	ISC-124	21	ISC-179	34	ISC-31	47	ISC-82
9	ISC-131	22	ISC-183	35	ISC-34	48	ISC-84
10	ISC-133	23	ISC-184	36	ISC-35	49	ISC-86
11	ISC-136	24	ISC-185	37	ISC-4	50	ISC-91
12	ISC-140	25	ISC-186	38	ISC-40	51	ISC-95
13	ISC-158	26	ISC-2	39	ISC-42	52	ISC-129

Table 2: Description of traits evaluated in the study.

Characters evaluated	Acronym	Description
Leaflet length (cm)	LLNT	Length of leaves taken with (at maturity)
Leaflet width (cm)	LLWT	Width of leaves taken with a metre rule in cm (at maturity)
Vigour at 50% flowering	VIG	
Days to 50% flowering	D50F	Days on which 50% of the total number of flowers was observed
Pod length (cm)	PDL	Length of a pod in cm using vernier calliper (at maturity)
Pod number	PDN	Number of pods per plant (at maturity)
Seed per Pod	SDPD	Number of seeds per pod taken from 10 randomly selected pods
Plant height (cm)	PHT	From the plant's base to the highest canopy point
Days to 50% maturity	D50M	Number of days from sowing to 50% physiological maturity
100 seed weight (g)	SDWT	Weight of air-dried 100 seeds using a weighing balance (in grams)
Primary branches	PBRCH	Number of primary branches per plant
Secondary branches	SBRCH	Number of secondary branches per plant
Tertiary branches	TBRCH	Number of Tertiary branches

Source: IBPGR and ICRISAT (1993).

Extraction of DNA from samples

The modified Dellaporta protocol (Dellaporta *et al*., 1983) was used for genomic DNA extraction. Young leaves (0.3 g) were harvested and ground in a mortar and pestle with an 800 µL extraction buffer. The mixture was homogenized with 100 µL of 20% sodium dodecyl sulphide and incubated at 65 °C. Ice-cold 5 M potassium acetate (300 µL) was added and mixed by gently inverting six times, then incubated on ice for 30 min. Then, the samples were centrifuged at 12000 rpm for 10 min, and the supernatant was transferred to another 1.5 mL Eppendorf tube. Ice-cold isopropanol (1 mL) was added to the tubes and gently inverted until DNA strands appeared. The sample was centrifuged again at 12000 rpm for 10 min, and the supernatant was gently decanted off till the isopropanol solution was removed. The pellets were re-suspended in 250 µL of high salt tris ethylene-diamine-tetra acetic acid (EDTA), 2 µL RNAse was added and incubated at 37 °C for 1 h. Ice cold isopropanol (1 mL) was then added and mixed by inverting gently. A volume of 500 µL 70% ethanol was included in the samples, centrifuged at 12000 rpm for 10 min, and the supernatant was gently decanted off. This was repeated twice. Samples were allowed to air dry for 2 h and 200 µL of Tris EDTA (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) was added to dissolve the pellets and stored at 4 °C until needed.

Optimization and amplification of SCoT markers

The optimization of SCoT markers was done using the gradient thermal cycler (Techne® Prime Thermal Cycler, UK). Thereafter, PCR reactions were carried out in a total volume of 10 µL reaction containing 2 µL of DNA (50 ng/mL), 1 µL of 1 µM primer, 2 µL of 5X FIREPOL® Master Mix (Solis BioDyne), and 5 µL of ddH_2O. PCR conditions are as follows: initial denaturation at 94 ºC for 3 min, followed by 40 cycles at 94 ºC for 1 min, 45 ºC for 1 min, and extension at 72 ºC for 2 min with a final extension at 72 ºC for 5 min. The amplified products were resolved on 2% agarose gel made in 0.5X TBE buffer, run for 1 h 30 min at 70 V, and stained with ethidium bromide (5 mg/mL). The image of banding patterns was captured under the UV light gel documentation system (UVTEC: UVIDOC H06, CAMBRIDGE). Amplicon band sizes were quantified using Gel Analyzer v1.9. Of the screened 20 SCoT markers, only five markers with a polymorphic information index (PIC) > 0.6 among samples were used in the study (Table 3).

Table 3: List of SCoT markers, sequences and amplicon range.

Markers	Sequences (5' – 3')	GC%	Amplicon range (bp)
SCoT-1	CAACAATGGCTACCACCA	50	268 – 505
SCoT-2	CAACAATGGCTACCACCC	56	264 – 1315
SCoT-3	CAACAATGGCTACCACCG	56	270 – 1704
SCoT-4	CAACAATGGCTACCACCT	50	422 – 565
SCoT-5	CAACAATGGCTACCACGA	50	435 – 665

Analysis of data

Descriptive statistics for morphological data were done using IBM SPSS v 26 (IBM Corp., Armonk, NY, USA). Other parameters which include phenotypic, environmental, and genetic variances were calculated according to Ahsan *et al.* (2014).

Genetic variance (Vg):

$$Vg = \frac{Genotype\ mean\ square - Error\ mean\ square}{Number\ of\ replicates\ (r)}$$

Environmental variance (Ve):

$$Ve = Error\ mean\ square$$

Phenotype variance (Vp):

$$Vp = Vg + \frac{Ve}{r}$$

Where r = number of replicates

Genotype coefficient of variation (GCV):

$$GCV = \sqrt{\frac{Vg}{mean}} \times 100$$

Environmental coefficient of variation (ECV):

$$ECV = \sqrt{\frac{Ve}{mean}} \times 100$$

Phenotype coefficient of variation (PCV):

$$PCV = \sqrt{\frac{Vp}{mean}} \times 100$$

Broad sense heritability (H^2):

$$H^2 = \frac{Vg}{Vp}$$

Genetic advance as percentage of means (GA%):

$$GA(\%) = \frac{K\sqrt{Vp}H^2}{mean} \times 100$$

Marker's Polymorphic Information Content (PIC) of each primer was calculated following the formula below;

$$PIC = 1 - \sum P_i^2$$

Where *Pi* is the frequency of the population carrying the i^{th} allele.

The effective multiplex ratio (EMR) was calculated using the formula;

$$EMR = n \times \beta$$

where n is the average number of fragments amplified by accession to a specific system marker (multiplex ratio) and β is estimated from the number of polymorphic loci (PB) and the number of non-polymorphic loci (MB);

$$\beta = \frac{PB}{PB + MB}$$

Marker Index (MI) for each SCoT marker was calculated as a product of polymorphic information content and effective multiplex.

$$MI = EMR \times PIC.$$

The resolving power (RP) of each primer was calculated as follows:

$$RP = RIb,$$

Where Ib represents the informative fragments. The Ib can be represented on a scale of 0/1 by the following formula; $Ib = 1 - (2 \times |0.5 - pi|)$, where *pi* is the proportion of accessions containing the i^{th} band.

The association between marker bands and traits was done using the stepwise multiple regression analysis following the method of Vazquez-Ovando *et al.* (2018) as shown below:

$$Y = a + b_1m_1 + b_2m_2 + ... + b_jm_j + ... + b_nm_n + d + e$$

Where Y is the dependent variable (trait) as a linear function of the set of independent variables (*mj*) represented by the SCoT markers. The *bj* terms are the partial regression coefficients that specify the empirical relationships between *Y* and *mj*, *d* represents the residual values between accessions after regression and *e* is the random error of *Y* that includes the environmental variation. To select the independent variables for the regression equation, *F* values with 0.045 and 0.099 probabilities were used as "enter" and "removal" criteria, respectively.

RESULTS

Phenotypic variability in quantitative traits of pigeonpea genotypes grown

A total of 13 quantitative traits were evaluated among the 52 accessions of Nigerian pigeonpea. The study revealed a wide variation within the traits evaluated (Table 4). The tertiary branches showed the highest variation (CoV = 155.23), while the least variation was observed in leaflet length (CoV = 15.77). Days to 50% flowering ranged between 64-173 days with a mean of 101 days, while days to 50% maturity ranged between 102-227 days with a mean of 149 days.

Table 4: Summary statistics for evaluated traits.

Descriptive Statistics	Mean	Min	Max	SD	CoV
Leaflet Length	7.97	5.50	12.30	1.26	15.77
Leaflet width	2.85	1.50	4.90	0.54	19.09
Vigour at 50% flowering	4.71	3.00	7.00	1.49	31.59
Days to 50% flowering	101.04	64.00	173.00	25.76	25.49
Pod length	5.03	3.60	9.10	0.82	16.32
Pod number	141.57	23.00	303.00	77.23	54.55
Seeds per pod	4.43	2.00	55.00	4.16	93.98
Plant height	147.98	48.00	287.00	48.1	32.50
Days to 50% maturity	149.21	102.00	227.00	23.55	15.78
100 seed weight	9.01	5.24	14.72	1.78	19.73
Primary branches	14.94	2.00	41.00	6.78	45.35
Secondary branches	16.76	2.00	52.00	10.34	61.69
Tertiary branches	8.81	0.00	57.00	13.67	155.23

SE: standard error; SD: standard deviation; CoV: coefficient of variation; H^2: broad-sense heritability.

Estimation of genetic parameters among the Nigerian pigeonpea genotypes

For each character, phenotypic and genotypic coefficients of variation (PCV and GCV), broad-sense heritability, and genetic advance were estimated for the evaluated traits (Table 5). The highest PCV and GCV were observed for tertiary branches (150.33 and 147.34%, respectively), while the lowest PCV and GCV were observed from the leaflet length (14.42 and 13.66%, respectively). Moderate PCV and GCV were observed in pod numbers (54.17% and 53.81%, respectively). However, some yield-related traits, including plant height, seed per pod, days of 50% flowering, days to 50% maturity, 100 seed weight, and pod length, gave a low GCV.

All traits showed high heritability ranging from 87.07% in pod length to 98.66% in pod number except for seeds per pod with the least heritability percentage (H^2 = 9.13%). The genetic advance percentage (GA %) ranged from 19.09% in pod length to 206.28% in tertiary branches. Other traits with high GA% are secondary branches, pod number, and primary branches. Days to 50% maturity showed the least environmental coefficient of variation (ECV = 5.58), while the highest environmental coefficient of variation was observed in seeds per pod (ECV = 92.47).

Table 5: Estimation of genetic parameters of quantitative traits among 52 pigeonpea genotypes.

Traits	Vg	Ve	Vp	Mean	GCV (%)	PCV (%)	ECV (%)	H^2_b	GA	GA (%)
LLNT	1.19	0.41	1.32	7.97	13.66	14.42	8.03	89.66	1.52	19.12
LLWT	0.21	0.08	0.24	2.85	16.22	17.26	10.22	88.32	0.65	22.71
VIG	1.23	1.00	1.56	4.71	23.52	26.53	21.25	78.61	1.55	32.93
D50F	635.25	36.47	647.41	101.04	24.94	25.18	5.98	98.12	35.29	34.92
PDL	0.47	0.21	0.54	5.03	13.63	14.61	9.10	87.07	0.96	19.09
PDN	5803.39	236.06	5882.08	141.57	53.81	54.17	10.85	98.66	106.65	75.33
SDPD	0.56	16.78	6.15	4.43	16.93	56.00	92.47	9.13	1.05	23.70
PHT	1971.25	367.77	2093.84	147.98	30.00	30.92	12.96	94.15	62.16	42.00
D50M	491.51	69.3	514.61	149.21	14.86	15.20	5.58	95.51	31.04	20.80
SDWT	2.33	0.86	2.62	9.01	16.93	17.95	10.32	88.99	2.14	23.71
PBRCH	39.23	7.21	41.63	14.94	41.92	43.18	17.96	94.23	8.77	58.68
SBRCH	83.23	24.78	91.49	16.76	54.42	57.06	29.69	90.97	12.77	76.19
TBRCH	168.42	20.69	175.31	8.81	147.34	150.33	51.64	96.07	18.17	206.28

Vg: Genetic; Ve: Environmental variance; Vp: Phenotype variance; GCV: Genotype coefficient of variance; PCV: Phenotype coefficient of variance; ECV: Environmental coefficient of variance; H^2_b: Broad sense heritability; GA: Genetic advance; GA(%): Genetic advance percentage; LLNT: Leaf length; LLWT: Leaflet width; VIG: Vigour at 50% flowering; D50F: Days to 50% Flowering (Days); PDL: Pod Length; PDN: Number of Pod; SDPD: Number of Seed Per Pod; PHT: Plant Height; D50M: Days of 50% Maturity; SDWT: 100 Seed Weight; PBRCH: Number of Primary Branches; SBRCH: Number of Secondary Branches; TBRCH: Number of Tertiary Branches.

Performance of SCoT markers

Amplicon amplification varied between markers (Supplementary Figures 1 to 5), with the highest amplification success observed with SCoT-2 and SCoT-3 having 60.44% and 57.69% successful amplification, respectively. A total of 54 polymorphic bands were observed from the five SCoT polymorphic markers (Table 6). The number of bands ranged from 6 bands in SCoT-4 and SCoT-5 to 20 bands in SCoT-2, and PIC was maximum in SCoT-5 (PIC = 0.99) and minimum in SCoT-2 (PIC = 0.68). SCoT-2 had the highest effective multiplex ratio (EMR = 45.38), while SCoT-5 had the lowest EMR of 1.50. A high marker index was observed in SCoT-2 and SCoT-1 with MI of 30.86 and 18.26, respectively, and SCoT-5 had the least marker index (MI = 1.49). Also, the maximum resolving power was observed in SCoT-2 (Rp = 4.54), while SCoT-5 had the minimum resolving power (Rp = 0.50).

Table 6: SCoT primers and their amplification results generated in 52 accessions of pigeonpea.

Marker	Total bands	Polymorphic bands	PIC	EMR	MI	Rp
SCoT-1	14	14	0.70	26.12	18.26	3.73
SCoT-2	20	20	0.68	45.38	30.86	4.54
SCoT-3	8	8	0.96	4.00	3.84	1.00
SCoT-4	6	6	0.77	6.81	5.24	2.27
SCoT-5	6	6	0.99	1.50	1.49	0.50
Total	54					

PIC: Polymorphic information content; EMR: Effective marker ratio; MI: marker index; Rp: Resolving power.

Association of SCoT markers with agronomical traits in pigeonpea

Marker-trait association done using a stepwise regression analysis revealed SCoT marker bands associated with evaluated traits, explaining varied phenotypic variances (Table 6). The associated marker bands ranged from a single band observed for D50M, SDWT, and TBRCH to five bands observed for LLWT. The cumulative phenotypic variance explained also ranged from 9.11% in a

marker associated with SDWT to 44.7% in markers associated with LLWT. The study also showed that no marker band was associated with pod number, number of seeds per pod, and number of secondary branches.

Interestingly, some SCoT marker bands showed an association with more than one trait. These include SCoT-1_{565bp} which showed association with vigour at 50% flowering and number of primary branches and SCoT-2_{1315bp} with leaf width and plant length. Others include SCoT-2_{1100bp} which showed association with leaf length and days to 50% flowering, SCoT-2_{468bp} with the number of primary branches and plant height, SCoT-2_{318bp} with days to 50% maturity and days to 50% flowering, and SCoT-5_{453bp} with leaf width and plant height (Table 7).

Table 7: SCoT markers association with agronomical traits in pigeonpea.

Trait	Marker	Bp	β	R^2 (%)	Sig.
LLNT	SCoT-3	1349	-0.41	13.42	0.008
	SCoT-1	268	-0.40	9.83	0.016
	SCoT-2	504	0.42	8.59	0.018
	SCoT-2	1100	-0.27	5.65	0.045
LLWT	SCoT-2	998	-0.57	12.65	0.010
	SCoT-5	453	0.31	8.18	0.029
	SCoT-2	1315	0.35	7.02	0.036
	SCoT-1	531	0.50	8.07	0.019
	SCoT-1	433	-0.36	8.85	0.009
VIG	SCoT-2	754	0.44	15.12	0.004
	SCoT-1	270	0.30	8.05	0.028
	SCoT-1	565	0.25	6.21	0.045
D50F	SCoT-2	318	0.39	7.59	0.048
	SCoT-2	1100	-0.34	10.17	0.017
PDL	SCoT-2	1315	0.69	11.41	0.014
	SCoT-2	800	-0.41	17.17	0.001
	SCoT-4	518	-0.30	6.45	0.034
PHT	SCoT-5	453	-0.40	12.72	0.009
	SCoT-1	283	-0.35	9.70	0.017
	SCoT-3	1704	-0.29	7.72	0.026
	SCoT-2	468	-0.24	5.69	0.047
D50M	SCoT-2	318	0.35	12.47	0.010
SDWT	SCoT-3	506	0.30	9.11	0.030
PBRCH	SCoT-1	565	0.48	18.00	0.002
	SCoT-2	468	0.30	11.02	0.008
	SCoT-3	636	0.29	8.15	0.016
TBRCH	SCoT-2	275	0.37	13.86	0.007

Bp: base pair; β: Regression coefficient; R^2: Phenotypic variability explained; LLNT: leaf length; LLWT: Leaflet Width; VIG: Vigour at 50% flowering; D50F: Days to 50% Flowering; PDL: Pod Length; PHT: Plant Height; D50M: Days of 50% Maturity; SDWT: 100 Seed Weight; PBRCH: Number of Flowering Branches; TBRCH: Number of Tertiary Branches.

DISCUSSION

Genetic variation has a strong influence on crop improvement. Adequate variability provides options from which selections are made for improvement and possible hybridization (Amusa *et al*., 2022). The high variability observed in pod number, and low variability observed in 100 seed weight, days to 50% maturity, and pod length corroborate the report. of Didas *et al.* (2021). The study showed higher variability among evaluated traits compared to the report of Zavinon *et al.* (2019) from the Benin Republic. This observation suggests that the pigeonpea accessions from Nigeria are more diverse than those from the Benin Republic. There have been studies on the variability estimation in pigeonpea using various methods, which include morphological (Manyasa *et al*., 2008; Zavinon *et al*., 2019), biochemical (Joshi *et al*., 2013), and molecular (Njung'e *et al*., 2016; Bohra *et al*., 2017) techniques. The heterogeneity observed for traits evaluated in pigeonpea germplasm depends on its natural outcrossing rate, which ranges from 3-26% and varies across locations, genotype and the intensity of insect population, and the time of flowering (Zavinon *et al*., 2019).

The knowledge of genetic inheritance of agronomically important traits, especially yield related, is important in deciding breeding strategies for crop improvement (Amusa *et al*., 2019; Amusa *et al*., 2022). However, relatively few efforts have been made to understand the genetics of important traits in pigeonpea (Randive *et al*., 2018). The study revealed that most agronomically important traits evaluated showed ~80% and above heritability. High values of heritability for the yield-associated traits can be considered favourable for pigeonpea improvement, through an effective phenotypic selection of these traits. Hence, a high expected genetic gain can be achieved from the selection of these characters (Malek *et al*., 2014). The low heritability observed for the number of seeds per pod in our study implies that these traits validate the report of Zavinon *et al.* (2019). However, our investigation did not agree with the low heritability reported for plant height, days to 50% flowering, pod length, days to 50% maturity, pod number, seeds per pod, 100 seed weight, primary branches, and secondary branches, as described previously (Zavinon *et al*., 2019). The lower heritability status reported by these authors might be due to a higher environmental variance experienced due to the multi-locational environmental influences compared with the one-location evaluation of samples in this study.

High genetic advances with high heritability estimates offer the most suitable condition for selection. It also indicates the presence of additive genes in the trait and further suggests reliable crop improvement through the selection of such traits (Ogunniyan and Olakojo, 2014). These authors stated that the higher the additive gene action, the higher the genetic variance, while higher non-additive action favours a lower genetic advance of a trait. However, since high heritability does not always indicate a high genetic gain, these authors recommended heritability be considered in association with genetic advances to predict the effect of the selection of superior crop varieties. Most of the evaluated traits showed high heritability with low to moderate genetic advance, implying both additive-gene and non-additive gene actions.

The use of molecular breeding could accelerate the utilization of the substantial variability among the pigeonpea germplasm lines for various morphological, physiological, and agronomic traits (Randive *et al*., 2018). SCoT markers are simple to design because they rely on a conserved sequence, such as ATG, that surrounds the translation start codon (Xiong *et al*., 2012). Hence, the use of SCoT markers can improve the efficiency of artificial selection among phenotypes evaluated. SCoT markers have been reliably used in several plants, such as Plantago (Rahimi *et al*., 2018) and Orchardgrass (Yan *et al*., 2016). The selected SCoT markers used in this study showed 100% polymorphism, with high PIC values indicating a high level of marker informativeness. Studies have shown more marker efficiencies in SCoT compared to expressed sequence tag (EST-SSRs) in tetraploid potato (Gorji *et al*., 2011; Yan *et al*., 2016), inter-retrotransposon amplified polymorphism (IRAP) in mango (Alikhani *et al*., 2014) and ISSR markers in mango and *Quercus brantii* (Luo *et al*., 2011; Alikhani *et al*., 2014).

An important advantage of the stepwise multiple regression association analysis is that this method does not require the preparation of segregating the population, which takes more time. Hannachi *et al.* (2013) opined that this method is useful in removing the effect of non-effective characteristics in the regression model when used for association studies. The efficiency of this association method has been shown in identifying and mapping the controlling genes of both Mendelian and non-Mendelian traits (Bayat *et al.*, 2018). Hence, informative markers that are identified in association analyses and showed a high phenotypic variation with high R^2 in the regression model can be isolated and cloned, and used in a breeding program. The present study revealed SCoT markers associated with some agronomical traits. They include leaflet length, leaf width, vigour at 50% flowering, days to 50% maturity, number of primary branches, number of tertiary branches, pod length, plant height, days to 50% maturity, and 100 seed weight with varied phenotypic variances (R^2). It was observed that the percentage of variation, which is explained by identified SCoT markers associated with yield traits was low. This low proportion of variation values for each trait may be explained by the contribution of numerous minor genes controlling the trait, markers with weak quantitative effects, rare alleles, and intricate allelic interactions (Saeidnia *et al.*, 2021). However, moderate cumulative phenotypic variability of several of these associated markers was observed for some of the traits evaluated, probably due to the limitation of a few informative markers explored.

The study revealed some SCoT markers (including SCoT-1_{565bp}, SCoT-2_{1315bp}, SCoT-2_{1100bp}, SCoT-2_{468bp}, SCoT-2_{318bp}, and SCoT-5_{453bp}) were associated with more than one agronomical trait. These linked SCoT markers could be considered loci, especially since this marker type anchors on the start codon of a gene. These observations suggest that the traits might be controlled by similar genes and/or genes close to each other. Bayat *et al.* (2018) mentioned that markers associated with more than one trait could be in the coding regions of these traits. They can be useful in breeding programs when entered into the regression model, and to explain trait variations observed. Hence, this information on the marker-trait association and the knowledge about the loci controlling interesting traits in pigeonpea would be suitable for pigeonpea improvement programmes.

CONCLUSION

This present study evaluated the level of variability, heritability, and SCoT markers associated with important agronomic traits within the collected Nigerian pigeonpea germplasm. The genetic markers used in this study were associated with various traits that accounted for the varied phenotypic variances except for pod number, number of seeds per pod, and number of secondary branches. Several markers were linked to loci controlling a particular trait. These linked loci could be incorporated into pigeonpea improvement programmes via marker-assisted selection or breeding. More markers should be evaluated to obtain those that account for a large amount of phenotypic variance.

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SUPPLEMENTARY DATA

Figure 1: SCoT-1 amplicon pattern among pigeonpea genotypes evaluated.
Figure 2a: SCoT-2 amplicon pattern among pigeonpea genotypes evaluated.
Figure 2b: SCoT-2 amplicon pattern among pigeonpea genotypes evaluated (rerun for unamplified genotypes).
Figure 3a: SCoT-3a amplicon pattern among pigeonpea genotypes evaluated (1-30).
Figure 3b: SCoT-3 amplicon pattern among pigeonpea genotypes evaluated (31-52, and some rerun for unamplified genotypes).
Figure 4a: SCoT-4a amplicon pattern among pigeonpea genotypes evaluated.
Figure 4b: SCoT-4 amplicon pattern among pigeonpea genotypes evaluated (rerun for some unamplified genotypes).
Figure 5a: SCoT-5 amplicon pattern among pigeonpea genotypes evaluated.

Figure 5b: SCoT-5 amplicon pattern among pigeonpea genotypes evaluated (rerun for unamplified genotypes).

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