Agro-Science Journal of Tropical Agriculture, Food, Environment and Extension
Volume 21 Number 4 (October 2022) pp. 19 - 22
ISSN 1119-7455

DIGITAL IMAGING OF MORPHOMETRIC CHARACTERISTICS OF PIGEON PEA (*Cajanus cajan*) FOR GENOTYPE DISCRIMINATION

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ABSTRACT

Digital seed imaging was utilized in this study to evaluate the morphometric characteristics of pigeon pea for genotype discrimination. One hundred seeds in each of the pigeon pea accession were subjected to digital imaging analysis using the WinSEEDLE™ software to differentiate the seed metric characters. Seeds were placed on a lighting hood and were automatically analyzed by the scanner and the image of the seed was recorded by the WinSEEDLE™. The procedure was repeated three times. The parameters observed were projected area, straight length, curve length, straight width, curve width, volume circle, and projected perimeter. Data collected were subjected to analysis of variance, principal component analysis and product moment correlation coefficients between the parameters were obtained. The result revealed that the pigeon pea accessions were highly variable in all the seed metric traits evaluated. Accessions NSWCC-34 and NSWCC-32 showed superiority values in most of the seed metric traits evaluated. Accessions NSWCC-32 had 28.35 mm^2, 6.99 mm, 7.37 mm, 5.23 mm and 5.28 mm in seed projected area, seed straight length, curve length, straight width and curve width, respectively while NSWCC-34 recorded 28.34 mm^2, 6.75 mm, 7.10 mm, 5.22 mm and 5.27 mm for the same evaluated traits. Most of the seed traits showed significant positive and strong association. The study, therefore, recommends that accessions NSWCC-34 and NSWCC-32 as well as those traits with high contribution to the major variation (seed projected area, seed straight length, curve length, straight width, curve width and volume circle) could be included in the seed improvement programme for improved seed quality in pigeon pea.

Key words: accession, seed imaging, seed metric, seed quality

INTRODUCTION

Pigeon pea (*Cajanus cajan*) is a perennial legume from the family Fabaceae. It was domesticated in South Asia at least about 3,500 years ago and since then its seeds have become a common food grain in Asia, Africa, and Latin America (Singh and Singh, 2016). Pigeon peas are now widely cultivated in all tropical and semi-tropical regions of the world. Annual varieties are more suitable for seed production. It can, however, be perennial in nature such that the crop can last three to five years on the field (Gyulai *et al*., 2015). The crop has numerous uses: the wood is used as fuel, the leaves and husks are major component of livestock feed while the leaves provide green forage for animal feed especially in dry season when other forage crops have vanished due to insufficient rainfall (Sloan *et al*., 2009). The ratoon after harvest was reported to have yielded about 63.6% of the main yield, which was used to feed animals and other livestock (Dasbak and Asiegbu, 2012).

Image analysis of seeds and seedlings in agriculture is one potential technique which is widely used in research. This technique aside not been destructive also helps to relates attributes of seed morphology to seedling performance, which ultimately expresses seed vigor with greater precision and less subjectivity (Vasconcelos *et al*., 2018; Medeiros *et al*., 2019). The different metric measurements on seeds are very essential quantitative variables for determining the size and shape of seeds (Arapa-Carcasi and Padrón-Pereira, 2014). In crop production, seeds size and shape are major determinants of seed dispersal, potential seed loss, moisture imbibition, germination of seeds and grain grading quality (Gyulai *et al*., 2015). In addition, preference for seed grains for human consumption could have a relationship with the size and shape of seeds. However, the use of morphometric characteristics of seed may provide a useful management strategy for increasing establishment count in pigeon pea, thereby leading to higher yield. Medeiros and Pereira (2018) used seed imaging technique to determine the physiological attributes of soyabean seed during water imbibition analysis. Image analysis has become important laboratory tool for cultivar description for a long time. Apart from the use of image analysis for genetic comparison, it has been used in many ways to determine seed quality. Shende and Raut (2013) used an image analysis system to study the process of imbibitions in white cabbage *(Brassica oleracea* L.) seeds. Also, Núñez-Colín and Escobedo-López (2015) described computer image analysis as a very

Please cite as: Olufelo J.O. (2022). Digital imaging of morphometric characteristics of pigeon pea (*Cajanus cajan*) genotype discrimination. *Agro-Science*, **21 (4),** 19-22. DOI: https://dx.doi.org/10.4314/as.v21i4.3

promising technique to determine mechanical damage in seeds because the method is precise and examines seeds individually using enlarged images in which damaged areas as well as their exact location and extension can be found and examined in detail. Since this test is a non-destructive method, analyzed seeds can be submitted to other physiological tests to establish the relationship between the result of the image analysis and other physiological tests. This study was initiated to evaluate the seed digital imaging of morphometric characteristics of pigeon pea (*Cajanus cajan*) for genotype discrimination.

MATERIALS AND METHODS

This experiment was conducted at the laboratory of the Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta, Nigeria. Seeds of thirty accessions of pigeon pea obtained from the International Institute of Tropical Agriculture, (IITA), Ibadan and the Institute of Agricultural Research and Training (IAR&T), Ibadan were used for the study. An EPSON scanner was connected to a computer device to acquire the image and the Regent Instrument (Regent Instrument Inc, Canada) was used for the image analysis by running the custom written software WinSEEDLE™ (pro version). For every replication, one hundred pigeon pea seeds of each genotype were placed on a lighting hood in such a way that the embryo axis of the seed faced the image analysis system and the longitudinal axis ran parallel to the surface of the scanner. Seeds were automatically analyzed by the scanner and the image of the seed was recorded by the 'WinSEEDLE™'. The procedure was repeated three times. The parameters that were recorded included total projected area, average projected area, projected area, straight length, curve length, straight width, curve width, volume circle, surface area circle and projected perimeter.

Data Analysis

The data collected were subjected to Analysis of Variance (ANOVA), Principal component analysis (PCA) and Pearson's correlation analysis using the Statistical Analysis System (SAS™, 2017).

RESULTS

The accessions differed with respect to the total project area, project area, straight length, curve length, straight width, volume circle, surface area circle, width length and projected perimeter (Table 1).

Table 2 shows the mean values for the morphometric performance of thirty pigeon pea accessions. In terms of the total project area, accessions Tcc-8127 had the highest mean value of 4243.65 mm^2 which differed significantly from all the other accessions while accession Tcc-811 recorded the least performance. For project trait, all the accessions had statistically similar values except Tcc-8127 which had the least mean value of 3677.75 mm. In terms of the project area, accessions NSWCC-34C and NSWCC32 recorded the highest men values of 28.74 and 28.35 mm, respectively.

The mean value of straight length was highest in Tcc-8127 but Tcc-6 recorded the least value (4.65 mm). Also, Tcc-8127 recorded the highest value of curve length (11.69 mm) while accession TCc-6 recorded the least value (4.65 mm). For straight width trait, NSWCC32, NSWCC-19 and NSWCC-34 showed values of between 5.10 and 5.23 mm while Tcc6 had lowest value of 3.07 mm.

The correlation coefficients among seed morphometric characters in pigeon pea form digital imaging are shown in Table 3. The total project area recorded a positive and highly significant correlation with straight length ($r = 0.93$) and curve length ($r = 0.91$). However, seed projected area had a highly significant and positive correlation with straight length ($r = 0.33$), curve length ($r = 0.28$), straight width ($r = 0.91$), volume circle ($r = 0.91$), and projected perimeter ($r = 0.27$). The straight length of the seed had a highly significant and positive correlation with curve length ($r = 0.98$) but also had a significant and positive correlation with the curve width ($r = 0.22$). Curve length on the other hand, had a highly significant and positive correlation with the seed projected perimeter ($r = 0.27$), seed straight width ($r = 0.87$) and volume circle ($r = 0.78$). Also, seed curve width had a positive and highly significant correlation with seed volume circle ($r = 0.85$), but had a positive and significant correlation with projected perimeter ($r = 0.23$).

Table 1: Mean square values of morphometric traits evaluated in pigeon pea accessions

Character	Replicates	Genotype effect	Error
Total projected area	32888926.81	1158534.87**	358064.16
Project area	27.16	42.68**	7.31
Straight length	1.87	3.62**	1.04
Curved length	2.69	3.80**	1.06
Straight width	0.17	0.88**	0.22
Curved width	0.27	0.62**	0.11
Volume circle	202.31	1010.59^{ns}	784.94
Projected perimeter	30.93	50.13**	19.14
Degree of freedom	2	29	55

** - significant at 1% probability level, ^{ns} - not significant

Table 2: Mean performance of seed morphometric characters evaluated in 30 pigeon pea accessions

Accessions	Total projected area (mm^{2})	Seed projected	Seed projected area (mm^{2})	Seed straight length	Seed curved length	Seed straight width (mm)	Seed curved width (mm)	Volume circle (mm)
Tcc-8126	1030.28^{b}	10877.25^{a}	22.40^{c-f}	6.30^{b}	7.07^{b}	3.95^{c-e}	4.30^{c-e}	72.23^{c-i}
CITA-3	875.74^{c}	11366.28^{a}	20.43^{a-e}	6.00^{b}	6.59^{bc}	4.06^{a-d}	4.20^{a-d}	60.8l^{e-j}
ICPL-87	1083.62^{b}	10825.10^{a}	25.70^{acd}	6.70^{b}	7.85^{b}	4.49^{a-d}	4.88	86.48^{a-f}
Tcc-1	886.25^{c}	11031.44^{b}	18.61^{cd-f}	5.67^{b}	6.46^{bc}	3.98^{a-d}	4.22^{a-d}	58.07^{f-j}
Tcc-8129	891.67^{c}	11017.01^{b}	18.16^{cf}	5.77^{b}	6.72^{b}	3.80^{a-d}	4.22^{a-d}	54.04^{g-i}
CITA2	906.73^{c}	11001.96^{b}	18.87^{a-e}	5.70^{b}	6.13^{bc}	4.11^{a-d}	4.24^{a-d}	57.80^{f-i}
Tcc-2	801.77^{c}	11106.91^{b}	15.88d^{ef}	5.19^{b}	5.61^{c}	3.65^{b-d}	3.78^{c-e}	47.73^{ij}
AO/TB78-9	923.31^{c}	10985.52^{b}	20.08^{a-e}	5.72^{b}	6.63^{b}	3.99^{a-d}	4.29^{a-c}	64.60^{c-i}
A078-99	938.54^{c}	10970.07^{b}	17.80^{c-f}	5.43^{b}	6.10^{bc}	5.00^{ab}	4.18^{a-c}	66.62^{e-i}
Tcc-811.	672.96^{c}	11230.06^{b}	13.74^{f}	5.31^{b}	6.21^{bc}	3.19^{de}	3.66^{de}	3 3.82^{j}
Tcc-151	925.32^{c}	10930.20^{b}	25.39^{a-c}	6.31^{b}	6.77^{b}	4.70^{a-c}	4.70^{a-c}	75.57^{c-i}
NSWCC-32	1056.07^{b}	10852.61^{b}	28.35^{a}	6.99^{b}	7.37^{b}	5.23^{a}	5.28^{a}	106.12^{a}
NSWCC-19	1124.91^{c}	10783.71^{b}	27.52^{a-c}	6.52^{b}	6.84^{b}	5.10^{a}	5.12^{a}	108.06^{a}
NSWCC-29B	802.68^{c}	11106.01^{b}	18.20^{c-f}	5.32^{b}	5.67^{bc}	4.10^{a-d}	4.20^{a-d}	63.1 1^{d-j}
NSWCC-18	864.43^{c}	11044.45^{b}	20.97^{a-e}	5.75^{b}	6.02^{bc}	4.51^{a-d}	4.55^{a-c}	71.34^{e-i}
NSWCC-18b	894.15^{c}	11068.87^{b}	21.17^{a-e}	5.78^{b}	6.10^{bc}	4.42^{a-d}	4.48^{a-c}	73.77^{c-i}
NSWCC-27a	838.24^{c}	11070.44^{b}	21.74^{a-e}	5.79^{b}	6.17^{bc}	4.48^{a-d}	4.54^{a-c}	78.94^{a-i}
NSWCC-46b	877.30^{c}	11032.39^{b}	22.33^{a-e}	6.01^{b}	6.36^{bc}	4.61^{a-d}	4.67^{a-c}	79.00^{a-h}
NSWCC-28	863.59^{c}	11037.76^{b}	21.52^{a-e}	5.74^{b}	6.00^{bc}	4.54^{a-d}	4.56^{a-c}	76.92^{c-i}
NSWCC-29b	1100.17^{b}	10810.32^{b}	21.25^{a-e}	5.71^{b}	5.93^{bc}	4.42^{a-d}	4.60^{a-c}	79.81^{a-h}
NSWCC18-B	875.35^{c}	11033.33^{b}	22.84^{a-f}	5.96^{b}	6.23^{be}	4.68^{a-c}	4.69^{a-c}	81.11^{a-g}
NSWCC-46	942:58^{c}	10966.80^{b}	24:53^{a-e}	6.13^{b}	6.42^{bc}	4.70^{a-c}	4.72^{a-c}	91.15^{a-d}
NSWCC-35	805.98^{c}	11102.74^{b}	19.57^{a-e}	5.62^{b}	5.91^{bc}	4.17^{a-d}	4.24^{a-d}	65.84^{c-i}
NSWCC-34A	970.31^{c}	10928.38^{b}	23.66^{a-e}	6.08^{b}	6.52^{b}	4.83^{ab}	4.85^{a-d}	89.03^{a-e}
NSWCC-34	1116.41^{b}	10792.28^{b}	28.74^{a}	6.75^{b}	7.10^{b}	5.22^{a}	5.27^{a}	107.54^{a}
NSWCC-51	948.84^{c}	10949.85^{b}	25.3l^{a-c}	6.27^{b}	6.53^{b}	4.95^{ab}	5.02^{a}	93.34^{a-c}
TCC-8127	4243.65^{a}	3677.75^{b}	18.47^{c-f}	11.17^{a}	11.69^{a}	3.78^{a-d}	3.91^{c-e}	56.08^{g-j}
CITA-3	737.26^{c}	11158.77^{a}	17.76^{c-f}	5.41^{b}	5.59^{c}	3.90^{a-d}	4.14^{a-d}	47.12^{ij}
Tcc-8	686.96^{c}	7879.06^{c}	20.43^{a-e}	5.22	5.77^{c}	4.31^{ab-d}	4.43^{a-d}	78.10^{a-h}
Tcc-6	725.47^{c}	11183.22^{a}	14.63^{ef}	4.65^{c}	5.19^{c}	3.07^{e}	3.26^{e}	50.84^{h-j}

Means followed by same alphabet along column are not different from one another at 5% probability level.

Table 3: Correlation coefficients among seed morphometric characters in pigeon pea form digital imaging

	B	C	D	E	F	G	H	I
Total projected area (A)	-0.51**	0.03	0.93**	0.91**	-0.05	-0.07	-0.01	-0.05
Seed project (B)		0.02	-0.48**	-0.46**	-0.01	-0.003	0.02	0.05
Seed projected area (C)			0.33**	0.28**	0.79	0.91**	0.91**	0.27**
Seed straight length (D)				0.98**	0.81**	0.22*	0.22*	0.11
Seed curve length (E)					0.11	0.17	0.17	0.27**
Seed straight width (F)						0.87**	0.78**	0.08
Seed curve width (G)							0.85**	0.23*
Volume circle (H)								0.08

** - correlation significant at 1% level, * - correlation significant at 5% level

The results of principal component analysis for seed morphometric characters of pigeon pea form digital imaging are shown in Table 4. The first three components explained 94.9% of the total variation among the accessions. The first principal component PC1 captured 40.82% of the variation, and this variation was increased to 64.97% in PC 2 and 94.90% in PC3. In PC1, characters such as seed projected area, seed straight, curve length, straight width, curve width and volume circle, accounted for major variation among the pigeon pea genotypes with values of between 0.35 and 0.96. In PC 2, three characters (total projected area, straight length and curve length) with values of 0.61, 0.90 and 0.91, respectively accounted for major variation among the entries. Similarly, in PC 3, characters such as total projected area (0.34) and seed projected perimeter (0.86) dominated the major variation I (Table 4).

Table 4: Result of principal component analysis for seed morphometric character of pigeon pea form digital imaging

Seed parameters	PCI	PC2	PC3
Eigen value	5.31	3.14	1.29
Variance (%)	40.82	24.15	9.92
Cumulative (%)	40.82	64.97	94.90
Total projected area	0.15	**0.95**	-0.13
Seed projected area	-0.10	**-0.61**	**0.34**
Seed straight length	**0.41**	**0.90**	-0.02
Seed curve length	**0.35**	**0.91**	0.14
Seed straight width	**0.87**	-0.23	-0.07
Seed curve width	**0.95**	-0.21	0.07
Seed volume circle	**0.93**	-0.17	0.01
Seed projected perimeter	0.18	-0.10	**0.86**

Bolded numerals represent significant contribution (values > 0.30).

DISCUSSION

The results showed variability among the 30 genotypes for all the metric characters except volume circle, surface area, thus offering the opportunity for selecting pigeon pea accessions with superior seed physical traits. Gotmare *et al.* (2000) in cotton, Wendel and Grover (2015) in cotton, Arapa-Carcasi and Padrón-Pereira (2014) in quinoa seed and Olaitan *et al*. (2017) in kenaf, all reported genetic diversity in some seed morphometric traits in using seed digital imaging analysis. The NSWCC-32 and NSWCC-34 showed superiority values in most of the evaluated seed metric traits. The NSWCC-32 had 28.35 mm^2, 6.99 mm, 7.37 mm, 5.23 mm and 5.28 mm in seed projected area, seed straight length, curve length, straight width and curve width, respectively, while NSWCC-34 recorded 28.34 mm^2, 6.75 mm, 7.10 mm, 5.22 mm and 5.27 mm in seed projected area, seed straight length, curve length, straight width and curve width, respectively.

The principal components data indicated that seed projected area, seed straight length, curve length, straight width, curve width, volume circle and surface area circle were traits that contributed significantly to the variation within the pigeon pea accessions examined. These traits with high contribution to the major variation could be included in the seed improvement programme for improved seed quality in pigeon pea. The study showed strong and positive associations among mapping of seed morphometric characters, implying that selection for one morphometric character will lead to improvement in the others. Lee and Lee (2018) and Zavinon *et al*. (2018) noted positive and strong associations among some morphometric characters in kenaf after seed digital images analysis.

Conversely, there was a negative association between seed projected and characters such as seed curve length and seed straight length, suggesting that an increase in one character will lead to a decrease in other characters and the relationship is therefore worthless in seed improvement if the relationship could not bring a desirable improvement.

CONCLUSION

The results showed that the pigeon pea genotypes evaluated were highly variable in all the seed metric traits. NSWCC-34 and NSWCC-32 showed superiority values in most of the evaluated seed metric traits. Most of the seed traits showed significant positive and strong associations among themselves. The data for the principal components indicated that seed projected area, seed straight length, curve length, straight width, curve width, volume circle and surface area circle were traits that contributed highly to the variation within the pigeon pea accessions examined. The study, therefore, recommends that NSWCC-34 and NSWCC-32 as well as those traits with high contribution to the major variation (seed projected area, seed straight length, curve length, straight width, curve width and volume circle) could be included in the seed improvement programme for improved seed quality in pigeon pea.

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