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Variability in Agro-Morphological and Morphometric of Pigeon Pea Genotypes Using Seed Image Analysis

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Abstract

This study investigated the variability in agro-morphological and morphometric of 30 pigeon pea genotypes in two environments using seed image analysis. The experimental seeds were sourced from IITA, and IART, Ibadan, Nigeria and conducted in the laboratory of plant breeding and seed technology of Federal University of Agriculture, Abeokuta, Nigeria.

An EPSON scanner was connected to a computer device to acquire image of the seeds and the Regent instrument was used for the image analysis, by running the custom written software WinSEEDLETM (Pro Version). For every replication, hundred seeds were placed on lighting hood. Seeds were automatically analyzed by the scanner and the image of the seed was recorded by the WinSEEDLETM. The procedure was repeated three times for all the genotypes and data collected were subjected to ANOVA, mean separation was done using tukey HSD at 5% level of probability, while Principal Component Analysis (PCA) were also determined. The result showed that, seed production environment effect and genotype effect were highly significant ($p<0.01$) on all the seed morphometric parameters examined. Seed morphometric attributes were variable among some genotypes implying that, it should be given due consideration in seed conditioning and improvement. Genotypes NSWCC-34C, NSWCC-34b, NSWCC-50 and NSWCC-7D were identified with consistent and high seed morphometric characteristic performance for most of the attributes evaluated within and across the two seed production environments.

Keywords: Seed production environment; Genotype NSWCC-34C; Image analysis; Principal component analysis

Introduction

Pigeon pea (*Cajanus cajan L. Mill* sp.) (*Leguminosae*) is a perennial legume of family; *Fabaceae*. It has been 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. Pigeon peas, which are popularly called as Toor dal in India, is widely used as one of the main ingredients in food globally, as it is rich in proteins [1].

One of the major requirements in developing machine vision systems for analyzing and sorting plant products (e.g., seeds, fruits, or vegetables) is the ability to analysis an image accurately and quickly. One of the challenges faced by the food industry is in assessing the quality and the type of grains and pulses. The quality detection by manual inspection in analyzing the grains and pulses may not give accurate results compared to automated system, for a common man, the colour, size and texture of pigeon pea are important parameters in deciding their quality. Seeds size and shape are major determinants of seed dispersal and probable loss, moisture imbibitions and germination of seed and grain grading quality. The colour, size, shape characteristics of plant products, and their capability to produce digital images suitable for further processing make modern image acquisition techniques highly adaptable tools. The importance of objective methods in analyzing seed is to be emphasized, because subjective monitoring can mask morphological variation when seed structures, with dimensions in the scale of millimeters or microns, pass from a steady state to a proliferating one. Apart from the use of image analysis for genetic comparison, it has been used in various ways to determine seed quality. Also, computer image analysis as a very promising technique to determine mechanical damage in seeds because the method is precise and examines seeds individually using enlarged images in great detail. Since this test is a non-destructive method, analyzed seeds can be submitted for other physiological tests to establish the relationship between the result of the image analysis and other physiological test. It also measures accurately the development on the seed, as regards its length, breath and width, which are essential parameters to measure the quality of a seed. This study was initiated to evaluate the effect of seed production environmental on the seed physical (metric) characteristics in some pigeon pea genotypes using seed image analysis [2].

Materials and Methods

The experiment was conducted in the laboratory of Plant Breeding and Seed Technology of Federal University of Agriculture, Abeokuta, Nigeria. Seed of 30 Pigeon pea genotypes obtained from International Institute of Tropical Agriculture (IITA) and Institute of Agricultural Research and Training (IART) Ibadan, Nigeria were used for the study. The seeds were multiply in two environments (derived savanna and rainforest) in 2016 and 2017 growing years [3].

An EPSON scanner was connected to a computer device to acquire image of the seeds and the Regent instrument (Regent Instrument Inc, Canada) was used for the image analysis, by running the custom written software WinSEEDLETM (Pro Version). For every replication, hundred seeds were placed on lighting hood in such a way that embryo axis of the seed faced image analysis system while 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 WinSEEDLETM. The procedure was repeated three times. The initial physical characteristics of the genotypes were evaluated using the seed imaging machine before and after the field experiments in both cropping years and agro-ecological conditions [4].

The parameters that were recorded included; straight length, curve length, seed area, curve width, width length and seed projected perimeter [5]. Data obtained from this study were subjected to analysis of variance. Significant means were separated using Tukey's HSD test. Principal component and correlation analyses were carried out on the seed physical characteristics [6].

Results

The ANOVA result showed that cropping year effects was highly significant ($p<0.01$) on all the seed parameters examined except seed area. Also, seed production environmental effect was highly significant ($p<0.01$) on straight width, curve width and width length. However, the genotype effect was highly significant ($p<0.01$) on all the seed morphometric parameters examined, while the same was recorded for seed production environment and genotype interaction except in seed perimeter. The 3-way interaction effect of cropping year, seed production environment and genotype also had a highly significant ($p<0.01$) effect on all examined parameters except in curve width, width length and seed perimeter (Table 1) [7,8].

Source	Df	Seed area	Straight length	Curve length	Straight width	Curve width	Width length	Seed perimeter
Rep	2	5.75	3.00*	1.24	2.29**	1.91**	0	34.77*
Year (Y)	1	77.98	14.47**	30.27**	51.46**	40.68**	0.98**	398.62**.
SPE (E)	1	9.93	0.52	1.92	18.58**	20.38**	0.29**	19.78
Genotype (G)	29	139.66**	2.44**	2.41**	1.07**	0.97**	0.16**	41.87**
E X G	29	172.41**	3.29**	3.41**	0.83**	0.75**	0.00**	4.41
Y X G	29	167.72**	2.78**	2.64**	1.13**	1.00**	0.01**	46.47**
Y X E	1	1300.22**	20.02**	19.44**	0.27**	0.6	0	27.55
YX E X G	29	196.90**	3.29**	3.51**	0.63**	0.49	0	1.82
Error	238	36.4	0.98	0.71	0.34	0.28	0.05	11.68

Note: **Significant at 1% probability level, *Significant at 5% probability level according to Tukey test, SPE-Seed production environment.

Table 1: Mean squares from analysis of variance for the effect of seed production environment on seed morphometric parameters of 30 pigeon pea genotypes grown in two cropping years.

The highest value was recorded in genotype area Nswcc-34 (38.76 mm^2) while the rest genotypes were not statistically different among one another ranging from 24.41 mm^2 in NSWCC-34c to 18.34 mm^2 in NSWCC-32. Similarly, seed straight length had the highest values in NSWCC-34b (7.45 mm) and NSWCC-34 (7.46 mm) while the least values of between 5.86 mm and 5.74 mm were recorded in genotypes AO/TB-78-9, CITA-1, NSWCC-18, NSWCC-18b, NSWCC-29b, NSWCC-34a, TCC-8127 and TCC-8129. Seed width length had the highest values of between 0.82 and 0.79 mm in CITA-2, NSWCC-19, NSWCC-28 and NSWCC-29b while the least value was in A078-99 (0.68 mm). Conversely, seed perimeter values were highest in NSWCC-18b (25.87 mm) and NSWCC-19 (22.92 mm) while A078-99 (17.04 mm) had the least value (Table 2) [9].

Genotype	Seed Area	Straight length	Curve length	Straight width	Curve width	Width length	Seed perimeter
A8125	19.69	5.68	6.12	4.49	4.35	0.72	18.14
AO78-99	21.23	5.95	6.34	3.91	4.00	0.68	17.04
AO/TB-78-9	21.51	5.86	6.31	4.57	4.66	0.75	20.63
CITA-1	21.50	5.82	6.24	4.43	4.50	0.73	19.34
CITA-2	24.11	6.19	6.53	4.94	4.98	0.79	18.85
CITA-3	19.18	5.64	6.17	3.96	4.06	0.71	21.96
1CPL 87	21.42	6.06	6.68	3.91	4.05	0.67	20.03
NSWCC-18	21.78	5.83	6.36	4.48	4.59	0.74	22.87

NSWCC-18b	21.28	5.84	6.41	4.41	4.55	0.74	25.87
NSWCC-19	21.71	5.98	6.47	4.71	4.78	0.79	22.92
NSWCC-28	22.15	6.01	6.49	4.91	4.87	0.82	18.02
NSWCC-29b	22.76	5.80	6.45	4.93	5.05	0.82	19.42
NSWCC-32	18.34	5.48	5.86	4.22	4.13	0.72	19.11
NSWCC-34	38.76	7.46	7.96	4.19	4.33	0.74	19.17
NSWCC-34c	24.41	5.58	6.04	4.07	4.08	0.73	18.55
NSWCC-34b	23.07	7.45	7.87	4.74	4.67	0.77	18.59
NSWCC-35	20.54	6.18	6.79	4.03	4.23	0.70	21.83
NSWCC-35a	22.26	6.21	6.65	4.23	4.33	0.72	18.66
NSWCC-50	23.97	6.31	6.62	4.35	4.39	0.74	19.37
NSWCC-7D	23.13	6.22	6.58	4.62 a-d	4.65	0.78	19.00
NSWCC-34a	20.36	5.75	6.04	4.45	4.48	0.76	17.78
TCC-I	22.44	6.09	6.42	4.61	4.66	0.74	18.49
TCC-151	22.83	6.05	6.38	4.74	4.78	0.77	18.67
TCC-2	22.63	5.79	6.43	4.66	4.67	0.78	19.87
TCC-6 25	24.22	6.53	6.13	4.55	4.67	0.76	18.21
TCC-8	23.94	6.16	6.56	4.75	4.82	0.77	19.86
TCC-8111	20.18	5.67	6.02	4.27	4.29	0.73	17.76
TCC-8126	23.08	6.19	6.51	4.69	4.73	0.76	19.81
TCC-8127	23.16	6.15	6.53	4.51	4.53	0.75	18.15
TCC-8129	23.96	5.74	6.51	4.69	4.75	0.79	18.57
S.E	1.74	0.28	0.24	0.17	0.15	0.21	0.98

Table 2: Mean performance of genotypes for seed morphometric characteristics in 30 pigeon pea genotypes across seed production environments and cropping year.

Means followed by the same alphabets along the columns within a character are not significantly different from one another at 5% probability level according to Tukey test.

Seed straight width (4.69 mm), curve width (4.29 mm) and seed perimeter (19.33 mm) of seeds harvested at (rainforest) Ife environment was higher compared to(derived savanna) Ibadan. Conversely, seed curve length (6.56 mm) of seeds produced in derived savanna was higher in values compared to that of rainforest environment. Values of seed area, seed straight length and seed width were statistically similar between the two environments [10].

Seed area recorded positive and highly significant correlation with all the morphometric attributes examined (seed straight length, seed curve length, seed straight width, seed curve width, seed width length and seed perimeter) with values of between (r=0.67) to (r=0.11).

However seed straight length showed positive and highly significant correlation values with all the parameters examined except width length (r=0.04).

Seed curve length had positive and highly significant correlation values with seed perimeter (r=0.21) whereas seed straight width had positive and highly significant correlation values with seed curve width (r=0.94) and seed width length (r=0.85). Similarly, Seed curve width also showed positive and highly significant correlation values with width length (r=0.86) but had positive and significant correlation values with seed perimeter(r=0.11). No other seed morphometric attributes had significant correlation among themselves (Tables 3-5).

Parameter	Derived savannah	Rainforest	S.E	Lsd (0.05)
Seed area (mm^2)	22.73	22.38	0.45	0.74
Seed straight length (mm)	6.09	6.02	0.07	0.12
Seed curve length (mm	6.56	6.41	0.06	0.09
Seed straight width(mm)	4.24	4.69	0.04	0.06
Seed curve width (mm)	4.29	4.69	0.04	0.06
Seed width length (mm)	0.72	0.77	0.1	0.17
Seed perimeter (mm)	19.33	19.77	0.25	0.41

Table 3: Effect of seed production environments on seed morphometric attributes of pigeon pea across genotype and cropping years.

Variable	Curve length	Straight width	Curve width	Seed perimeter	Width length
Seed area	0.67**	0.23**	0.24**	0.11*	0.17**
Straight length	0.82**	0.15**	0.15**	0.13*	0.04
Curve length		0.04	0.08	0.21**	-0.08
Straight width			0.94**	0.06	0.85**
Curve width				0.11*	0.86**
Seed perimeter					-0.01
Note: **-Highly significant, *-Significant.					

Table 4: Correlation co-efficient among seed morphometric attributes across seed production environment and cropping years (N= 336).

Variable	PC2	PC3
Seed area	0.56	0.5
Seed straight length	0.67	0.15
Seed curve length	0.79	0.1
Seed straight width	0.78	0.16
Seed curve width	0.45	0.21
Seed width length	-0.59	0.1
Seed perimeter	-0.31	0.84
Eigen value	2.44	1.24
Proportion of variation%	22.16	11.57
Cumulative variation%	64.15	75.72

Table 5: Principal component based on correlation co-efficient matrix for seed morphometric attributes across two seed production environments and cropping years.

Three of the components axes had Eigen values that are greater than 1.0 and accounted for 75.72% of the total variation. Relative discriminating power of the PCA as revealed by Eigen values were 4.62, 2.44 and 1.24 for PC1, PC2, and PC3, respectively. The arithmetic sign of the co-efficient is irrelevant since a common rule of thumb for determining the significant of a trait co-efficient is to treat co-efficient greater than 0.03 as having enough effect to be considered important.

PC1 accounted for 41.90% of the variation and was loaded with, seed area, seed straight length, seed curve length, seed straight width, seed curve width and seed width length with values 0.05, 0.56, 0.51, 0.82, 0.83, and 0.69, respectively while PC2 was relatively loaded with seed area, seed straight length, seed curve length, seed straight width, seed curve width, seed width length and seed perimeter with values between 0.79 and 0.31. In PC3, only two characters (seed area (0.50) and seed perimeter (0.84)) loaded the axis whereas every other seed shape parameters examined showed no significant contribution to the variation among the entries.

Discussion

The result showed variability among the thirty genotypes for all the metric characters. The variability may be due to inherent genetic differences among genotypes, irrespective of cropping year and seed production environment. A cursory look at the result of the seed morphometric analysis from this study reveals that the pigeon pea genotypes were different from each other as there were significant differences among the parameters measured at 5% probability level. This also, indicates that descriptors of seed differences can be effectively exploited in physiological studies as stated by Dell' Aquila (2004), and in cultivar and genotype description. Genotypes NSWCC-34C, NSWCC-34b, NSWCC-50 and NSWCC-7D were identified with consistent and high seed morphometric characteristic performance for most of the attributes evaluated within and across the two seed production environments. The implication of these performances is that it provides opportunity for selecting pigeon pea genotypes with superior seed physical traits. Similar observations were earlier reported on genetic diversity in some kenaf genotypes, in white cabbage seeds [11].

Correlation coefficients of most of the morphometric traits across seed production environment and cropping year in this study revealed significant positive correlation between seed area and all other seed morphometric parameters. The positive and significant relationship that exists between these seed metric characters indicates that improving on any of the parameters will significantly increase other characters. Also, since they are significantly associated, each can be used as substitute in any evaluation and classification studies and as such these characters possessed greater practical value for seed discrimination in Pigeon pea. The seed size and seed metrics were important discriminators of *Zostera marina*. The result of the principal component analysis across the two seed production environments and cropping years showed that different characters contributed differently to the total variation as indicated by the Eigen values as well as loading on different principal axis. Between the three principal axes that had Eigen values higher than one, PC1 and PC2 accounted for 41.90% and 22.16%, respectively of the variance recorded among the seeds was loaded largely with seed area, seed straight length, curved length, seed straight width and seed width length. These results suggest that these traits are the main seed metric variables to select for effective discrimination among pigeon pea seeds. Seed area, seed length, perimeter and flatness index contributed largely to the variability in the first two principal component axes of tropical inbred maize genotypes [12].

Conclusion

Genotypes NSWCC-34C, NSWCC-34b, NSWCC-50 and NSWCC-7D had consistent and high seed morphometric characteristic performance within and across the two seed production environments. Hence, it can be used in selecting pigeon pea genotypes with superior seed physical traits. Also, relationship among morphometric traits revealed significant positive association between seed area and all other seed morphometric parameters examined, hence, an important tools in selecting superior seed physical traits.

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