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Genetic variation in physiological seed quality of some selected cowpea genotypes

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Abstract

The study was conducted using fifteen cowpea genotypes evaluated for their physiological seed quality attributes using viability and vigour tests. The laboratory experiments were set-up in a complete randomized design with three replicates at the seed quality control unit of the Institute of Agricultural Research and Training, Ibadan, Oyo State. Data collected were subjected to analysis of variance. Treatment means separated using Duncan Multiple Range Test at 5% level of probability. Results showed that variability was smaller in cowpea accessions as compared with local and improved genotypes, except root length and shoot length. Hundred seed weight ranged from 12.84-21.14, 9.90-31.44 and 12.14-15.33, SG ranged from 22.00-94.00, 72.00-100 and 46.00-96.00 and SGI (DAS) ranged from 2.43-15.45, 2.57-4.04 and 2.53-5.52 in landrace, accession and improved cowpea genotypes, respectively. Accelerated ageing germination percentage also ranged from 46.0-86.00, 64.00-82.00 and 24.00-78.00, conductivity in $\mu\text{S}/\text{cm}/\text{g}$ ranged from 7.34-28.89, 6.33-19.49 and 12.10- 29.72 and SVI ranged from 7.48-35.91, 20.99-41.65 and 14.12-39.74 in local, accession and improved cowpea genotypes, respectively. Marked differences were detected among genotypes for all the characters measured under local, accession and improved cowpea. Abewere was identified in this study to have high values for most germination and vigour parameters with good combination of most traits and is highly recommended for use in future breeding programs.

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Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is an annual, self-pollinated legume belonging to family Leguminaceae with a diploid chromosome number of $2n=2x=22$. It is native to India but tropical and Central Africa is considered as secondary centre of origin. Cowpea has been referred to as “Poor man’s meat” because of its high protein content (20- 25%) (Sabale *et al.*, 2018). It is considered as one of the oldest legume used as protein source for humans and livestock. It is being used as pulse in form of dry seed, immature pod and green leaf and growing twig can be utilized as vegetable. It is an important source of green as well as dry fodder. Cowpea is cultivated for both grain and fodder in all tropical and sub-tropical regions among fodder legumes (Nguyen *et al.*, 2017). The cowpea is widely distributed throughout the world, being one of the most important sources of protein (Guilhen, 2016) for low-income populations. Data available from the FAO (2016) on world cowpea production for 2013 show that 3.6 million tonnes were produced over 12.5 million hectares. Production takes place in 36 countries, with Nigeria, Niger (Castelletti and Costa, 2013) and Brazil being the greatest producers, accounting for 84.1% of the global area and 70.9% of global production (Brasil, 2016).

Cowpea production is wholly dependent on seed as propagation material. The quality of cowpea seed is therefore, an essential determinant of final quantity and quality of cowpea leaves and grain (Abukutsa-Onyango, 2011). Seeds are one of the greatest assets of agricultural activity in the world. Their physiological quality defines both the establishment of the crop and its productivity (Bagateli *et al.*, 2019). High-quality seeds are more capable of generating uniform seedlings that will give rise to individuals with ample productive potential and efficient use of the resources of the environment (Caverzan *et al.*, 2018). On the other hand, seeds with reduced quality will compromise the emergence of seedlings, and result in an uneven establishment in the field, which in turn will result in low yield (Ebone *et al.*, 2020). According to Shaibu and Ibrahim (2016), one of the factors that may affect crop production is the physiological quality of the seeds, which has an

indirect influence on the speed and percentage of seedling emergence and final stand, or a direct influence on plant vigour. Therefore, the use of seeds of high physiological quality is paramount to increasing productivity and improving the technological level of bean cultivation (Adebisi, 2013). In view of the above, the aim of this study was to evaluate the physiological quality of the seeds of fifteen cowpea genotypes produced in the Institute.

Materials and methods

Seeds of five (5) cowpea accessions, five (5) landraces and five (5) improved cowpea varieties were used for the experiment. The seeds were sourced from National Centre for Genetic Resource and Biotechnology (NACGRAB), local farmers in Oyo North and Institute of Agricultural Research and Training, Moor Plantation, Ibadan. The seed were planted between August and December, 2022 in Ibadan research field of the Institute. The plants were harvested processed and stored in paper envelop and placed under room temperature (28°C and RH 65%) in December of the same year. The laboratory analysis was carried out in the Institute seed quality control unit between February and March, 2023

Seed Quality Assessment

The quality of seeds of the 15 selected cowpea genotypes were assessed with the following tests in the seed testing laboratory of the Institute of Agricultural Research and Training, Moor Plantation, Ibadan.

Standard germination

The test was carried out in three replications of 100 seeds per replicate. Plastic germination bowls were filled with moistened sharp sand and seeds were evenly spaced on the sand. Thereafter the seeds were thinly covered with moistened sand and lightly pressed for a good seed-substratum contact. The bowls were covered with nylon sheets to prevent evaporation. Germination counts were taken daily from the 3rd to 8th day after planting. On the 9th day, seedling analysis was carried out and the numbers of normal and abnormal seedlings were recorded. Germination was interpreted as the percentage of seeds producing normal seedlings (International Seed Testing Association, 2018).

Standard Germination (SG) =

$$\frac{\text{Number of normal seedlings emerged}}{\text{Total number of seeds planted}} \times 100$$

From the germination data above, germination index (GI) was calculated for each replicate according to Ajayi and Fakorede (2000) as follows.

Standard Germination Index (SGI) =

$$\frac{\sum(N_x) \text{ DAP}}{\text{Total number of seedlings that emerged on the final day}}$$

Where N_x is the number of seedlings that emerged on day x after planting.

DAP is the days after planting.

100 Seed Weight

A 100- seed weight in 3 replicates from each genotype was determined and expressed in gramme

Evaluation of seedling traits

One out of every 10 normal seedlings, that is, 10% of the total number of normal seedlings, in each replicate, at the final germination count, was used to obtain data on the following seedling vigour parameters.

Shoot length (SHLT)

The length from the shoot level to the shoot tip of seedling in 10 randomly selected seedlings and expressed in cm.

Root Length (RLT)

The length from shoot level to the tip of the plant root of seedling in 10 randomly selected seedlings and expressed in cm.

Seedling Dry Weight (SDWT)

10 randomly selected seedlings oven dried at 108° C until constant weight and averaged.

Seedling Vigour Index (SVI)

The seedling vigour levels of each genotype was calculated by multiplying percent seed germination by average of seedling root and shoot length of each variety after 7 days of germination and divided by 100.

Seedling Vigour Index (SVI) =

$$\frac{\% \text{ germination} \times (\text{root length} + \text{shoot length})}{100}$$

Electrical conductivity

Fifty clean and apparently intact seeds in three replicates were counted, weighed, and placed in a glass flask containing 100 ml of distilled water. The flasks were covered with aluminum foil to prevent contamination and gently shaken intermittently. Conductivity measurements were taken after 24 h using Mettler Toledo MC126 conductivity meter. All measurements were expressed as $\mu\text{Scm}^{-1}\text{g}^{-1}$ and the results were interpreted as suggested by Hampton and TeKrony (1995).

Conductivity ($\mu\text{S/cm/g}$) =

$$\frac{\text{Conductivity } (\mu) \text{ of each flask} - \text{Conductivity of water}}{\text{Initial weight of seed sample}}$$

Accelerated ageing

Ageing of seeds were done by weighing fifty apparently intact seeds from each genotype in three replicates. Thereafter, the seeds were placed on a wire mesh suspended over water inside accelerated ageing boxes and then placed in an ageing chamber for 72 h at a constant temperature of 43°C. The seeds were re-weighed after ageing to determine the amount of moisture gained during the ageing process. After the ageing period, the seeds were subjected to standard germination test as described above and the germination count taken as similarly described. The results were expressed in percentage as done for standard germination. Ranking of genotype means across all vigour tests was done to identify outstanding genotypes. This was evaluated using a vigour index (VI), which was calculated as an average performance in each of the tests, as shown below

$$VI = \frac{(\text{SG} + \text{AAG} + \text{COND} (\%))}{3}$$

Where SG= Standard Germination (%), AAG= Accelerated Ageing Germination (%), COND=Electrical conductivity.

Conductivity values were converted to% as follows

$$\text{COND} (\%) = 100 \times (30 - \text{COND}) / 30$$

Data analysis

Data collected in the laboratory were subjected to analysis of variance (ANOVA) separately for each

genotype group and combined across genotype Treatment means were separated using Duncan Multiple Range Test (DMRT) at 5% level of probability.

Results and discussion

The result shows that genotype effect was significant for all the characters evaluated with the exception of root length and shoot length, respectively. However, the replication effect was not significant on any of the quality attributes evaluated in the laboratory (Table 1). Highest mean values for hundred seed weight (HSW), standard germination (SG), accelerated ageing germination percentage (AAG), seedling vigour index (SVI) and lowest values of standard germination index (SGI) and conductivity ($\mu\text{S}/\text{cm}/\text{g}$) were recorded by cowpea accessions (Table 2) The variability in physiological quality traits did not follow a definite pattern as presented in Table 2. The variability as measured by standard deviation (SD) was smaller in cowpea accessions as compared with local and improved genotypes, except HSW and shoot length. Hundred seed weight ranged from 12.84-21.14, 9.90-31.44 and 12.14-15.33, SG ranged from 22.00-94.00, 72.00-100 and 46.00-96.00 and SGI (DAS) ranged from 2.43-15.45, 2.57-4.04 and 2.53-5.52 in landrace, accession and improved cowpea genotypes, respectively. Accelerated ageing germination percentage also ranged from 46.0-86.00, 64.00-82.00 and 24.00-78.00, conductivity in $\mu\text{S}/\text{cm}/\text{g}$ ranged from 7.34-28.89, 6.33-19.49 and 12.10-29.72 and SVI ranged from 7.48-35.91, 20.99-41.65 and 14.12-39.74 in landrace, accession and improved cowpea genotypes, respectively. The little variability recorded by the cowpea accessions could be as a result of observed improvement in viability and vigour potentials for all the genotypes under cowpea accession. This variability could also be attributed to several factors among such as the initial physical and physiological qualities of the seed lots (Wang and Hampton, 1991) Hundred seed weight ranged from 10.03g (150-EX) to 30.56g (NGB07632). Kawoleri, NGB07592, NGB07614 with seed weight of between 21.53-28.77g while 150-EX recorded the lowest seed weight of 10.03 g (Table 3). This result corroborates the finding of Henshaw (2008) who reported wide variation in the seed quantitative traits

for 28 cowpea genotypes in Nigeria. Doumbia *et al.* (2013) attributed the differences in seed weight to the time factor for accumulation of assimilates in the seeds or genetic makeup of the different genotypes. On the other hand, Makanur *et al.* (2012) reported that the variation observed in seed size might be due to crop growth, seed development and maturation. Germination percentage ranged from 35.33% (Kawoleri) to 94%. (NGB07614) Twelve genotypes showed 100% germination. Genotype Kawoleri recorded the least germination during the duration of the experiment. It might be due to the fact that the seeds had rapidly lost their viability. High germination percentages indicate good seed quality and may be due to the activity of α -amylase as reported by Banik *et al.* (2015). All the genotypes with lower, medium and larger seed size showed higher germination percentage compared with Kawoleri genotype with medium seed size. Marked variation was also observed in the speed of germination as measured by standard germination index. This variation might be due to the seed quality, physiological factors such as chemicals and hormones as well as genotypic differences among the genotypes. Generally, the speed of germination was higher in genotypes with smaller seeds compared with genotypes with larger seeds. AAG% ranged from 26.67% in Ife Brown to 78.67% (NGB07632).

Accelerated ageing test is one of the most lucrative tests for seed vigour. Artificial exposure of the seeds to higher temperature and humidity for a prescribed time period provides the simulation results with natural ageing (TeKrony *et al.*, 1980). According to Woltz and Tekrony (2001), seed lots of high quality showing 70% to 80% germination after the aging test can be considered to have met acceptable minimum level. In this study, three landrace genotypes (Olomoyoyo, Gombe and Abewere), two improved genotypes (BBT Brown and Modupe) and all the accessions had germination after ageing between 70% and 80%. The result confirm the findings of Rastegar *et al.* (2011) who reported that highly vigorous seed lots are more tolerant to stressful condition and produce higher percentages of normal seedlings. The conductivity also ranged from 8.18 $\mu\text{S}/\text{cm}/\text{g}$

(NGB04654) to 27.41 μ S/cm/g (Ife Brown). According to Matos *et al.* (2015) the test for electrical conductivity (EC) is based on the permeability of the cell membranes, i.e. during the loss of seed viability, there is an increase in the exudates of seed cells soaked in water, due to the loss of integrity of these membranes. The higher the conductivity value the lower the vigour (ISTA, 2018). The lower values EC, the greater the physiological quality of the seeds (Moura, 2016). Seed of Abewere (landrace) and NGB04654 (accession) leaked the least ions when soaked, depicting high vigour and greater tendency of producing a healthy plants stand when sown on the field. Seedling dry weight was highest in Ife BPC with a value of 0.28 g followed by NGB07632 (0.07g). The measurement of root and shoot length shows significant differences ($p < 0.01$) among the genotypes of cowpea used. Highest root length was recorded in Modupe with value of 15.04cm and least in Sadu (8.70cm). Shoot length ranged from 18.60cm in NGB07592 to 26.70cm in 150-EX. Seedling vigour

index was highest in 150-EX (accession) but not statistically different from Modupe (improved). The least SVI was recorded in Kawoleri (landrace) with a value of 11.99. Generally, all the accession genotypes recorded high SVI. Marked differences were detected among genotypes for all the characters measured under landrace, accession and improved cowpea genotypes (Table 4). Abewere, a landrace was ranked the most stable among the genotypes used for the experiment with a vigour index of 1. The least vigour index recorded by few of the genotypes could be as a result of decrease in germination percentage and some other vigour indexes (accelerated ageing and conductivity). This finding corroborates the findings of Ghassemi-Golezani *et al.* (2010). They reported that the decrease in germination percent and other indexes due to physiological and biochemical changes during seed ageing led to reduction in vigour index. Thus, the vigour index offers the possibility of categorizing seed lots into classes of seed quality.

Table 1. Mean Squares from the combined analysis of variance of cowpea vigour tests.

Tests	Replication (df=2)	Genotype (df=14)	Error (df=30)	CV (%)	R ² (%)
Hundred Seed Weight (g)	2.31	87.55**	0.83	5.45	97.9
Standard Germination (%)	101.21	846.06**	78.66	11.52	83.33
Standard Germination Index (Days)	3.63	8.16*	2.25	40.34	62.90
Acceleration Ageing Germination (%)	04.5	732.00**	41.33	9.84	89.20
Conductivity (μ S/cm/g)	6.67	110.73**	91.21	10.24	94.44
Seedling Dry Weight (g)	0.003	0.008**	0.0016	28.34	71.08
Root Length (cm)	2.27	8.95	6.52	21.97	39.05
Shoot Length (cm)	8.38	19.86	8.66	13.05	51.68
Seedling Vigour Index	18.11	106.16**	20.64	17.33	70.58

*, ** Significant at $p < 0.05, 0.01$, respectively

Table 2. Indices of variability in physiological seed quality and seedling vigour traits of different cowpea genotypes.

Traits	Landrace			Accession			Improved		
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
Seed Vigor									
HSW (g)	16.37	12.84-21.74	3.25	19.61	9.90-31.44	7.73	13.86	12.14-15.53	1.15
SG (%)	71.33	22.00-94.00	21.50	89.20	72.00-100	8.41	70.53	46.00-96.00	15.52
SIG(DAS)	4.29	2.43-15.45	3.34	3.13	2.57-4.04	0.41	3.72	2.53-5.52	0.98
AAG (%)	67.66	46.0-86.00	11.65	75.20	64.00-82.00	5.39	52.93	24.00-78.00	19.49
COND.	17.00	7.34-28.59	6.99	13.28	6.33-19.49	4.20	20.80	12.10-29.72	4.52
Seedling Vigor									
RLT(cm)	10.64	6.70-15.40	2.73	11.95	8.70-17.00	2.38	12.27	7.80-17.00	2.86
SHLT(cm)	22.13	15.80-27.90	2.82	21.13	12.20-28.20	4.31	24.41	19.30-28.90	2.41
SDW(g)	0.14	0.07-0.20	0.05	0.12	0.07-0.20	0.05	0.16	0.07-0.37	0.08
SVI	23.23	7.48-35.91	7.44	29.40	20.99-41.65	5.33	26.02	14.12-39.74	6.79

HSW=Hundred Seed Weight; SG%=Standard Germination Percentage; SGI= Standard Germination Index; AAG=Accelerated ageing germination percentage; COND=Conductivity (μ S/cm/g). RN= RTL=Root Length; SHLT=Shoot Length; RDW=SDW= Seedling Dry Weight; SVI=Seedling Vigour Index; SD= Standard Deviation (\pm)

Table 3. Mean values of physiological seed quality and seedling quality traits of 15 cowpea genotypes.

Genotype	HSW	SG (%)	SIG	AAG	COND	SDWT	RLT	SHLT	SVI
Landrace									
Kawoleri	21.53b	35.33f	9.29a	52.67c	27.04a	0.02b	11.20bcd	22.57abcd	11.99e
Olomoyoyo	12.80f	82.00abcd	2.90b	72.00ab	21.29b	0.11cd	11.80abcd	21.43 abcde	27.05 bc
Gombe	16.66d	75.33bcd	3.33b	77.33a	12.18fg	0.11cd	10.70bcd	24.03abc	26.11bcd
Sadu	18.93	72.66cd	3.29b	63.33bc	16.31de	0.13cd	8.70d	22.80abcd	22.90bcd
Abewere	14.45ef	91.33a	2.68b	74.00ab	8.21h	0.13cd	10.83bcd	19.80cde	28.00ab
Accession									
NGBo7592	21.80b	86.00abc	3.60b	74.67a	17.60cd	0.11cd	12.40abcd	18.60de	26.73bcd
NGBo7614	28.77b	94.00a	2.93b	74.67a	13.65ef	0.13cd	10.67bcd	21.27bcde	30.67ab
NGBo7632	30.56a	87.33abc	3.31b	78.67a	9.63gh	0.20b	12.47abcd	17.63e	25.41bcd
NGBo4654	12.87f	88.69ab	2.81b	72.00ab	8.18h	0.07d	11.03bcd	21.93abcde	29.29ab
150-EX	10.03g	90.00ab	3.02b	76.00a	17.34d	0.11cd	13.17abc	26.70a	35.53a
Improved									
Ife-Brown	14.01ef	56.00e	4.26b	26.67d	20.35bc	0.13cd	11.43abcd	24.37abc	20.32cd
Ife BPC	15.02e	69.33de	4.13b	36.67d	27.41a	0.28a	13.97ab	23.33abcd	25.80bcd
BBT-White	14.99e	54.67e	4.59b	57.33c	20.93b	0.15bc	9.13cd	26.30a	19.45de
BBT-Brown	12.79f	88.08ab	2.75b	74.00ab	14.76def	0.13cd	11.33abcd	22.60abcd	29.86ab
Modupe	12.49f	84.67abc	2.86b	70.00ab	20.58b	0.09cd	15.46a	25.46ab	34.69a

Means with same value(s) on each column is (are) not statistically significant at $p < 0.05$

HSW=Hundred Seed Weigooht; SG%=Standard Germination Percentage; SGI= Standard Germination Index; AAG=Accelerated ageing germination percentage; COND=Conductivity ($\mu\text{S}/\text{cm}/\text{g}$). RN= RTL=Root Length; SHLT=Shoot Length; RDW=SDW= Seedling Dry Weight; SVI=Seedling Vigor Index

Table 4. Performance of individual cowpea genotypes among vigour tests.

Genotype	SG (%)	AAG (%)	COND (%)	VI	Rank
Landrace					
Kawoleri	35.35	52.67	9.36	32.62	15
Olomoyoyo	82.00	72.00	24.04	61.02	10
Gombe	75.33	77.33	34.40	70.69	6
Sadu	72.66	63.53	40.62	60.54	11
Abewere	91.33	74.00	72.63	79.33	1
Accession					
NGBo7592	86.00	74.67	41.23	67.31	8
NGBo7614	94.00	74.67	54.50	74.39	4
NGBo7632	87.33	78.67	67.91	77.88	2
NGBo4654	88.69	72.00	72.74	77.86	3
150-EX	90.00	76.00	42.21	69.40	7
Improved					
Ife-Brown	56.00	26.67	32.87	38.28	13
Ife BPC	69.33	36.67	8.64	38.22	14
BBT-White	54.67	57.33	30.32	42.44	12
BBT-Brown	88.08	74.00	50.80	70.93	5
Modupe	84.67	70.00	31.39	62.02	9

SG%=Standard Germination Percentage;

AAG=Accelerated ageing germination percentage;

COND=Conductivity (%); Vigour Index

Conclusion

The study has provided useful information on the physiological parameters of 15 cowpea genotypes. It also revealed the genetic relationships among the genotypes. This will assist in the selection of suitable

parental genotypes in the cowpea seed improvement program. The genotype Abewere was identified in this study to have high values for most germination and vigour parameters with good combination of most traits and is highly recommended for use in future breeding programs.

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Competing interests

The authors declare that they have no competing interests

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