Research article

Ajayi AT. Genotypic differences in aluminum tolerance of cowpea accessions utilizing germination parameters. International Journal of Life Sciences and Biotechnology, 2021. 4(2): p. 254- 273. DOI: 10.38001/ijlsb.862549

Genotypic differences in aluminum tolerance of cowpea accessions utilizing germination parameters

Abiola Toyin Ajayi [1*]

ABSTRACT

One of the major factors which limit the productivity of cowpea on acid soils is aluminum toxicity. Reliable methods for identifying genetic variation for its tolerance is indispensable. Genetic variability for aluminum tolerance in 10 accessions of cowpea were studied in the laboratory. Fifteen seeds of each accession were sown in sterilized petri dishes containing filter papers and 5 ml of $AlCl_3$ at four levels (0, 50, 100 and 200 µm) and replicated three times in a completely randomised design (CRD). Petri dishes were incubated under room temperature in the dark for 48 hours. After 48 hours, they were exposed to photoperiod of 12 hr. / 12 hr. (day/night) at room temperature for another 48 hours. At day four after sowing, data were collected on percentage germination, number of roots per shoot, fresh weight of shoot, root length and hypocotyl length and fresh weight of shoot. Data were subjected to statistical analysis and accessions were arranged on their tolerance to aluminum stress by means of tolerance indices. Analysis of variance revealed significant effect of accessions on all parameters. Treatment was significant for all excluding percentage germination. Treatment by accession was significant for number of roots and root length. The observed genetic variation in cowpea for aluminum stress could be exploited by hybridisation to establish tolerant lines. Selection based on high heritability and GAM in percentage germination, hypocotyl length and number of roots in cowpea under aluminum stress can be exploited for selection.

ARTICLE HISTORY
Received
16 January 2021
Accepted
08 March 2021

KEYWORDS
Aluminum,
acid soils,
germination parameters,
tolerance indices

Introduction

In the nutrition and cropping systems of tropical and subtropical countries, the role cowpea plays can never be over stressed. Protein content of cowpea revolves around 25 percent; it is a fast growing crop with tremendous capacity to control erosions through ground surface cover and at the same time fixes atmospheric nitrogen in soil for soil improvement [1]. It can tolerate soils of diverse pH range compared to other legumes [2], with its productivity nonetheless limited by many factors.

[1] Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria
* Corresponding Author: Abiola Toyin Ajayi, E-mail: toyin.ajayi@aaua.edu.ng

One of the major factors which limit the productivity of cowpea on acid soils is aluminum toxicity. Exposure to micromolar concentrations in soil solution can rapidly hamper root elongation and consequently hinder the uptake of water and nutrients, thus causing substantial decrease of crop production on acid soils [3, 4]. Acid soils are toxic to plants for their nutritional disorder, deficiency, or restriction of vital nutrients such as phosphorus, calcium and magnesium. Toxicity resulting from aluminum, manganese and activity of hydrogen also play an important role in soil toxicity [5].

Plants lay open to aluminum stress have been shown to display distinct root elongation inhibition very early within hours or even minutes of exposure [6]. Plants grown in the fields find it hard to explore the soil for nutrients and moisture if the subsoil is acidic, making the plants to experience drought stress after only a few days of lack of rainfall. In addition to the reduced root system, plants suffering from aluminum stress have reduced shoot growth and cause a decline in crop yield. Aluminum toxicity hampers cell division in root tips and lateral roots, escalates cell wall inflexibility by cross linking pectins, decreases DNA replication, decreases phosphorus fixation in soil and on root surfaces, drops root respiration, disturbs enzyme action governing sugar phosphorylation and deposition of cell wall polysaccharides. Aluminum is available in all soils, but its toxicity becomes apparent only in acidic situations in which plant-toxic form, Al^{3+} predominates [7].

Acid soils occupy nearly 17 million hectares in Nigeria, making up 18% of the total land area [8], and about 35% of the world's arable land [9], and about 50% of the possible cultivated land [4, 10, 11, 12]. Soils of the humid tropics, especially the whole lands of the South Eastern Nigeria are acidic [13, 14, 15], due to high precipitations (1500 mm and above) in these regions which lead to the leaching of substantial amounts of exchangeable bases from soil surfaces [16]. Since there is a direct correlation between soil acidity and aluminum toxicity, liming of the soil could be adopted to raise the pH level to enhance plant growth and development under aluminum stress [17]. However, the practice of agriculture in developing countries has been mainly relegated to subsistence levels, hence it is unwise both economically and logistically to adopt liming of soil by the resource poor farmers [10, 16, 18]. Excess liming also have negative consequences, such as leaching of soil minerals causing Manganese (Mn) deficiencies in soils as well as acquisition by plants and Phosphorus

(P) deficiencies in soils [18]. Availability of higher aluminum tolerant cultivars of cowpea to farmers will contribute positively at ensuring food security, enhanced nourishment and also protect the soils against the negative effects of over liming.

Crop plants have adopted different mechanisms of tolerating toxic level of aluminum in acidic soils, these mechanisms include exclusion and internal control which are governed by the expression of multiple genes [4, 12]. Toxicity sensitivity is genotype-specific in cowpea germplasm. Understanding the physiological mechanisms of tolerance is very cogent in the improvement programs of the adapted genotypes [12]. However, improving the genetic and physiological tolerance of crop species to aluminum stress has been quiet challenging [4]. Genotypes of cowpea from Brazil were shown to possess different tolerance levels to acidic Alumihaplic Acrisol (pH 3.8) under phosphorus limiting conditions [19]. Genotypic differences in the performance of some cowpea genotypes on acid soils might be related with variation in the expression of aluminum tolerance mechanisms, especially under limited phosphorus conditions [20, 21]. Kushwala [22] reported significant genotypic differences for aluminum tolerance among twenty accessions of cowpea for important quantitative and physiological traits. Aluminum stress was found to decrease the yield and protein content of the seeds. Aluminum tolerance of eight cultivars of cowpea was studied at early growth and maturity by [23], and it was reported that both genotype and genotype x aluminum was significant for the growth and yield traits. Traits such as plant height at late growth stage, biomass yield and pod weight were all enhanced by aluminum stress, while plant height at earlier growth stage, nodulation and number of pods per plant were subdued by aluminum stress.

Impairment of root growth occurred only in the condition of exposing about 2 – 3 mm of the apical root to aluminum stress in maize, while other parts of the root if in contact with aluminum produced no effect on root growth [24]. Stubby and brittle roots proceeding aluminum exposure indicate that cytoskeleton may the point where toxicity of aluminum activities lies [25]. Aluminum tolerant maize genotypes have been found to excrete much citric acid from roots apices in response to aluminum stress compared to genotypes susceptible to aluminum [26]. Phosphorus deficiency with increased aluminum stress led to increased citrate exudation in a genotype of maize [27]. A study by [28] on genotypic differences for aluminum toxicity among fourteen inbred lines of maize for seedling root variation in hydroponics, employing tolerance membership index on actual root length, relative root length and root length response classified genotypes into three classes of highly tolerant, tolerant and intermediate. High heritability of the root traits indicated possibility of successful breeding programme for aluminum stress if selection is done among the maize genotypes. Root traits also displayed high expected genetic gain which proved that root traits are best for screening for tolerance in maize. A population of $F_{2:3}$ hybrids derived from the crosses between a tolerant maize parent and other susceptible lines of Kenyan maize showed high significant genotypic variability for relative net root growth in nutrient solution with heritability for aluminum tolerance reaching 97% [10]. However, information regarding estimates of genetic variations of germination and root traits under aluminum stress is limited in cowpea.

Germination parameters in hydroponics and petri dishes under laboratory conditions have been used successfully to screen for aluminum tolerance in many crop species including cowpea [21], maize [28], wheat [29], *Vigna* species [30], alfalfa [31], rice [32] and soy bean [33]. These were in a bid to avoid the hassles associated with field experiments since correlated responses have been achieved in many instances between field and laboratory performances among crops evaluated under aluminum stress [28].

The present study objectives were to screen for aluminum tolerance in cowpea using a simple and fast laboratory procedure and to pinpoint the best germination traits for which selection could be effective.

Materials and Methods

Procedure

The study was set up at the laboratory in the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria in July, 2016. Seeds of the 10 accessions were screened for tolerance to aluminum by employing the procedure of [34] and [35] with some modifications. A factorial experiment (10 x 4) was employed; seeds of uniform size were surface sterilized in sodium hypochlorite (NaClO) solution 5% (v/v) for 1 hr. and rinsed in distilled water five times. The seeds were sown in sterilized petri dishes containing two pieces of sterilized filter paper and 5 ml of aluminum chloride ($AlCl_3$) at four levels (0, 50, 100 and 200 μm). Fifteen seeds of each accession were placed on the filter paper in each petri dish and replicated three times for each treatment in a Completely Randomized Design (CRD). Petri dishes were incubated under room temperature in the dark for 48 hours. After 48 hours, they were exposed to photoperiod of 12 hr. / 12 hr. (day/night) at room temperature for another 48 hours. The 10 accessions are: TVu-199, TVu-207, TVu-218, TVu-235, TVu-236, TVu-241, IT98K-205-8, IT98-555-1, TVu-4886 and TVu-9256 coded as AC01, AC02, AC03, AC04, AC05, AC06, AC07, AC08, AC09 and AC10 respectively. Details of the accessions are described in [1].

Data gathering and analyses

At four days after sowing, data were collected on percentage germination, number of roots per shoot, root length, hypocotyl length and fresh weight of shoot. Data for were run through analysis of variance (ANOVA) using the Generalized Linear Model (GLM) procedure of the Statistical Package for Social Science (SPSS) version 20 (SPSS Inc., Chicago IL) [36]. Combination of duncan multiple range test (DMRT) and least significant difference (LSD) ($P \leq 0.05$ levels of significance) were adopted for mean separation where appropriate. Accessions were arranged on their tolerance to aluminum stress by means of tolerance indices (TI) according to [37], where the averages of other treatments were compared against that of control. The data on tolerance indices were run through cluster analysis with Palaeontological Statistic Software (PAST version 3.01) [38]. Estimates of heritability for each treatment was done with Plant Breeding Tools (PBTools version 1.4) [39], while the combined estimates of genetic parameters were done according to [28]. Pearson correlation coefficients were calculated using SPSS version 20, to determine the level of associations among all measured parameters. The data were also subjected to Principal Component (PC) analyses adopting the PAST.

Results

Variability among accessions of cowpea for germination parameters under aluminum stress

Results from analysis of variance (ANOVA) revealed that the mean square values for effect of accession was significant ($P \leq 0.05$) for all measured germination parameters. Aluminum effect was significant for all germination parameters excluding percentage germination. Accession x treatment effect was also significant for number of roots per seedling and root length, but not significant for percentage germination, hypocotyl length and fresh weight of shoots. The most variable traits among the germination parameters under aluminum stress included root length (CV = 50.16%) and hypocotyl length (CV = 34.44%), while the least variable trait was percentage germination (CV = 20.23%), (Table 1).

Effects of accession and aluminum treatments on germination parameters are shown in Table 2. The highest value for germination (91.65%) was obtained in AC06, while the lowest value (51.65%) was obtained in AC01. Number of roots per seedling was highest (15.45) in AC02, while the least (6.30) was obtained in AC01. Hypocotyl length was highest (7.41 cm) in AC10, while AC02 had the least (2.92 cm). Root length was highest (1.48 cm) in AC06, while the least (0.61 cm) was obtained in AC09. The highest value (0.31 g) for fresh weight of shoot was obtained in AC06, while the least (0.19 g) was obtained in AC02. For effect of treatment, aluminum enhanced all germination parameters except in percentage germination, with the differences not significant in all except in fresh weight of shoots.

Effects of aluminum x accession on germination parameters of accessions of cowpea are presented in Table 3. Germination was generally inhibited in accessions AC01, AC03, AC04, AC05 and AC07, while it was enhanced in AC06, AC08 and AC09. Heritability estimate was high for all treatments with the highest value (79.00%) obtained in 50 μm of aluminum treatment, while the least (60.00%) was obtained in 100 μm of aluminum treatment. Number of roots per seedling was mostly enhanced above that of control by 50 μm and 100 μm, while 200 μm mostly inhibited number of roots in most accessions. Heritability for number of roots trait ranged from moderate to high, with the lowest (57.00%) in 100 μm, however the highest (84.00%) was obtained in the control. Hypocotyl length was majorly inhibited by 50 μm in

AC01, AC02, AC03, AC04 and AC05, while other treatments majorly enhance the hypocotyl length across accessions. Heritability estimate was high in all treatments, and it ranged between 75.00% in 100 µm and 87.00% in 200 µm aluminum treatment. Root length was enhanced above that of the control by 50 µm in most accessions, whereas 100 µm and 200 µm played the role inhibiting the root length compared to the control in most accessions. Heritability estimate was low (30.00%) in 50 µm of aluminum treatment, moderate (57.00%) in 100 µm and 200 µm of aluminum treatments and high (61.00%) in control treatment. Fresh shoot weight was majorly enhanced above that of control by aluminum treatment in almost all accessions, with the estimate of heritability ranging from low (0.00%) in 50 µm to moderate, 50.00% in 100 µm.

Table 1 Mean square values of accession, treatment and accession by treatment interaction of accessions of cowpea under aluminum stress in the laboratory

Source of Variation	DF	PG (%)	NR	HYPL (cm)	RL (cm)	FW (g)
Accession	9	2506.38**	113.67**	30.17**	1.25**	0.02**
Treatment	3	83.92^{ns}	16.28**	5.09**	0.2**	0.03**
Accession x treatment	27	63.23^{ns}	28.54**	2.47^{ns}	0.42**	0.01^{ns}
Error	80	251.78	14.03	3.22	0.31	0.01
CV (%)		20.23	30.58	34.44	50.16	40.00

**: Significant at $P \leq 0.05$; DF: Degree of freedom; PG: Percentage germination; NR: Number of roots; HYPL: Hypocotyl length; RL: Root length; FW: Fresh weight of shoot; CV: Coefficient of variation.

Table 2 Effects of accession and aluminum treatment on germination parameters of accessions of cowpea under aluminum stress

Accession	PG (%)	NR	HYPL (cm)	RL (cm)	FW (g)
AC01	51.65^{a}	6.30^{a}	3.41^{a}	0.67^{a}	0.22^{ab}
AC02	55.00^{a}	15.45^{e}	2.92^{a}	1.45^{c}	0.19^{a}
AC03	78.31^{bc}	$13.74d^{e}$	5.56^{b}	1.37^{c}	0.20^{ab}
AC04	79.97^{bc}	$12.68cd^{e}$	6.42^{bc}	1.05^{abc}	0.28^{ab}
AC05	83.87^{bc}	11.43^{bcd}	5.50^{b}	1.04^{abc}	0.26^{ab}
AC06	91.65^{c}	14.19^{de}	7.16^{c}	1.48^{c}	0.31^{b}
AC07	74.43^{b}	$9.65b^{c}$	3.83^{a}	0.79^{ab}	0.25^{ab}
AC08	89.98^{c}	14.91^{e}	5.87^{bc}	1.24^{bc}	0.25^{ab}
AC09	88.31^{bc}	$9.00a^{b}$	3.99^{a}	0.61^{a}	0.27^{ab}
AC10	91.08^{c}	15.15^{e}	7.41^{c}	1.34^{c}	0.28^{ab}
±SE	4.58	1.08	0.52	0.16	0.03
Treatment					
Control	79.76^{a}	11.37^{a}	4.66^{a}	0.99^{a}	0.21^{a}
50µm	79.09^{a}	12.89^{a}	5.13^{a}	1.13^{a}	0.27^{ab}
100µm	75.98^{a}	12.82^{a}	5.51^{a}	1.19^{a}	0.25^{ab}
200µm	78.87^{a}	11.91^{a}	5.54^{a}	1.09^{a}	0.29^{b}
±SE	2.89	0.68	0.33	0.10	0.02

Means followed by similar alphabets in the same column are not significantly different from one another at $P \leq 0.05$ using Duncan Multiple Range Test (DMRT). SE: Standard error of means; PG: Percentage germination; NR: Number of roots; HYPL: Hypocotyl length; RL: Root length; FW: Fresh weight of shoot.

Table 3 Effects of accession and aluminum interaction on germination parameters of accessions of cowpea under aluminum stress

Treatment	AC01	AC02	AC03	AC04	AC05	AC06	AC07	AC08	AC09	AC10	H^2B
					Percentage germination (%)						
Control	55.53^a	53.33^a	86.63^a	86.63^a	91.07^a	88.87^a	77.77^a	86.63^a	80.00^a	91.10^a	69.00
50µm	53.30^a	53.33^a	80.00^a	75.53^a	84.43^a	93.30^a	75.53^a	88.87^a	93.30^a	93.30^a	79.00
100µm	48.87^a	60.00^a	66.63^a	75.53^a	77.77^a	91.10^a	68.87^a	91.10^a	91.07^a	88.87^a	60.00
200µm	48.90^a	53.33^a	79.97^a	82.17^a	82.20^a	93.33^a	75.57^a	93.30^a	88.87^a	91.07^a	66.00
±SE (9.16)											
LSD (NS)											
					Number of roots						
Control	7.55^a	11.42^a	16.73^b	13.27^a	11.62^a	13.60^a	11.80^a	13.40^a	2.66^a	14.33^a	84.00
50µm	5.17^a	11.53^a	15.53^b	12.23^a	12.04^a	17.07^a	9.47^a	17.27^a	12.00^b	16.67^a	71.00
100µm	4.25^a	19.07^b	12.47^{ab}	13.87^a	11.37^a	13.82^a	10.40^a	16.07^a	14.20^b	12.67^a	57.00
200µm	8.23^a	19.77^b	10.23^a	11.33^a	10.70^a	12.27^a	6.93^a	12.90^a	9.80^b	16.93^a	70.00
±SE (2.16)											
LSD (5.07)											
					Hypocotyl length (cm)						
Control	4.07^a	2.83^a	5.56^a	6.01^a	4.58^a	4.87^a	3.44^a	6.05^a	2.23^a	6.96^a	86.00
50µm	3.30^a	2.51^a	4.97^a	5.94^a	4.48^a	7.00^{ab}	3.93^a	6.81^a	4.47^a	7.84^a	82.00
100µm	3.37^a	3.31^a	6.48^a	7.33^a	7.25^b	7.48^b	4.20^a	4.85^a	4.34^a	6.49^a	75.00
200µm	2.91^a	3.03^a	5.22^a	6.39^a	5.71^{ab}	9.30^b	3.77^a	5.77^a	4.96^a	8.36^a	87.00
±SE (1.04)											
LSD (2.43)											

Table 4 cont'd

					Root length (cm)						
Control	0.48^{a}	1.45^{a}	1.47^{a}	0.81^{a}	1.53^{b}	0.91^{a}	1.13^{b}	0.95^{a}	1.67^{b}	1.27^{a}	61.00
50µm	0.33^{a}	1.61^{a}	1.65^{a}	1.32^{a}	0.67^{a}	1.70^{ab}	0.75^{ab}	1.17^{a}	0.77^{a}	1.34^{a}	30.00
100µm	0.24^{a}	1.49^{a}	1.22^{a}	1.30^{a}	0.99^{ab}	1.97^{b}	0.97^{ab}	1.59^{a}	0.96^{ab}	1.18^{a}	57.00
200µm	1.63^{b}	1.26^{a}	1.13^{a}	0.78^{a}	0.99^{ab}	1.35^{ab}	0.34^{a}	1.25^{a}	0.70^{a}	1.57^{a}	57.00
±SE (0.32)											
LSD (0.75)											
					Fresh weight of shoot (g)						
Control	0.17^{a}	0.18^{a}	0.26^{a}	0.21^{a}	0.24^{a}	0.31^{a}	0.19^{a}	0.14^{a}	0.11^{a}	0.27^{a}	27.00
50µm	0.31^{a}	0.19^{a}	0.24^{a}	0.27^{ab}	0.25^{a}	0.30^{a}	0.23^{a}	0.28^{ab}	0.30^{b}	0.30^{a}	0.00
100µm	0.13^{a}	0.18^{a}	0.14^{a}	0.37^{b}	0.27^{a}	0.30^{a}	0.23^{a}	0.26^{ab}	0.32^{b}	0.28^{a}	50.00
200µm	0.26^{a}	0.19^{a}	0.17^{a}	0.26^{ab}	0.29^{a}	0.31^{a}	0.33^{a}	0.31^{b}	0.35^{b}	0.29^{a}	39.00
±SE (0.07)											
LSD (0.15)											

Means followed by similar alphabets in the same column are not significantly different from one another at $P \leq 0.05$. NS: Non-significant; SE: Standard error of means; LSD: Least significant difference; H^2B: Heritability.

Correlation and estimates of genetic parameters of germination traits of cowpea under aluminum stress

Strong associations among germination parameters were experienced among all traits in this study, except between percentage germination and root length (0.089), and root length and fresh weight (0.119) (Table 4). Estimates of variances, Genotypic and Phenotypic coefficients (GCV and PCV) and Broad sense heritability (H^2B) of germination parameters of cowpea exposed to aluminum stress are presented in Table 5. GCV and PCV were high for all traits. In fresh shoot weight, the lowest (21.91%) GCV was obtained while in hypocotyl length, the highest (57.52%). Highest PCV (70.94%) was obtained in root length, while the lowest (40.39%) was obtained in percentage germination. Heritability in the broad sense ranged from low to high among parameters. Heritability was lowest (23.08%) in fresh weight of shoot, while the highest heritability (73.61%) was obtained in hypocotyl length.

Table 4 Pearson correlation of germination traits of accessions of cowpea under aluminum stress

	PG (%)	NR	HYPL (cm)	RL (cm)	FW (g)
PG	1	0.226*	0.571**	0.089	0.407**
NR		1	0.319**	0.600**	0.217*
HYPL			1	0.204*	0.361**
RL				1	0.119
FW					1

**: Significant at $P \leq 0.01$; *: Significant at $P \leq 0.05$; PG: Percentage germination; NR: Number of roots; HYPL: Hypocotyl length; RL: Root length; FW: Fresh weight of shoot.

Table 5 Estimates of genetic parameters of germination traits of accessions of cowpea under aluminum stress

Trait	GM	GV	PV	GCV (%)	PCV (%)	H^2B (%)
PG	78.42	751.53	1003.06	34.96	40.39	74.92
NR	12.25	33.21	47.24	47.04	56.11	70.59
HYPL	5.21	8.98	12.2	57.52	67.04	73.61
RL	1.11	0.31	0.62	50.16	70.94	50.00
FW	0.25	0.003	0.013	21.91	45.61	23.08

GM: Grand mean; GV: Genotypic variance; PV: Phenotypic variance; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; H^2B: Heritability in the broad sense; PG: Percentage germination; NR: Number of roots; HYPL: Hypocotyl length; RL: Root length; FW: Fresh weight of shoot.

Principal Component and bi-plot profiling of cowpea parameters under aluminum stress

The Principal Components centered on germination parameters under aluminum stress are presented in Table 6. Five Principal Components axis were extracted for all parameters out of which the first (eigen-values greater than 1.00) accounted for 89.29% of the total variation. The first PC axis accounted for 58.66% of the total variation with all traits having high loadings and making positive contributions to the total variation. The second PC accounted for 30.63% of the total variation with traits such as percentage germination (0.36) and fresh weight (0.55) with high positive contributions, root length (-0.55) and number of roots (-0.49) were negative contributors. Bi-plot of all the parameters under aluminum stress based on Principal Components 1 and 2 is presented in Figure 1. The bi-plot resulted in four major groups for the ten accessions under aluminum influence. Group I consisted of two accessions (AC03 and AC08), group II had four (AC10, AC06, AC04 and AC05), group III had three (AC09, AC01 and AC07) however, group IV had one (AC02). Accessions in group I and II were the most tolerant to aluminum toxicity and they were positively correlated with all the germination parameters. Accessions in group III were the sensitive accessions, while the one in group IV was the slightly sensitive one. These accessions were not strongly correlated with any of the germination parameters. Parameters like fresh weight, percentage germination and hypocotyl length were strongly correlated (angle < 90^0), while parameters like number of roots and root length were also strongly correlated (angle < 90^0). All traits were regarded as aluminum tolerant traits.

Table 6 Principal Component analysis of germination parameters of accessions of cowpea under aluminum stress

	Principal Components	
	PC1	**PC2**
Eigen-value	2.93	1.53
Cumulative eigen-value	2.93	4.46
Variability (%)	58.66	30.63
Cumulative variability	58.66	89.29
Variables	**PC1**	**PC2**
Percentage germination (%)	**0.46**	**0.36**
Number of roots	**0.45**	**-0.49**
Hypocotyl length (cm)	**0.54**	0.15
Root length (cm)	**0.41**	**-0.55**
Fresh weight (g)	**0.36**	**0.55**

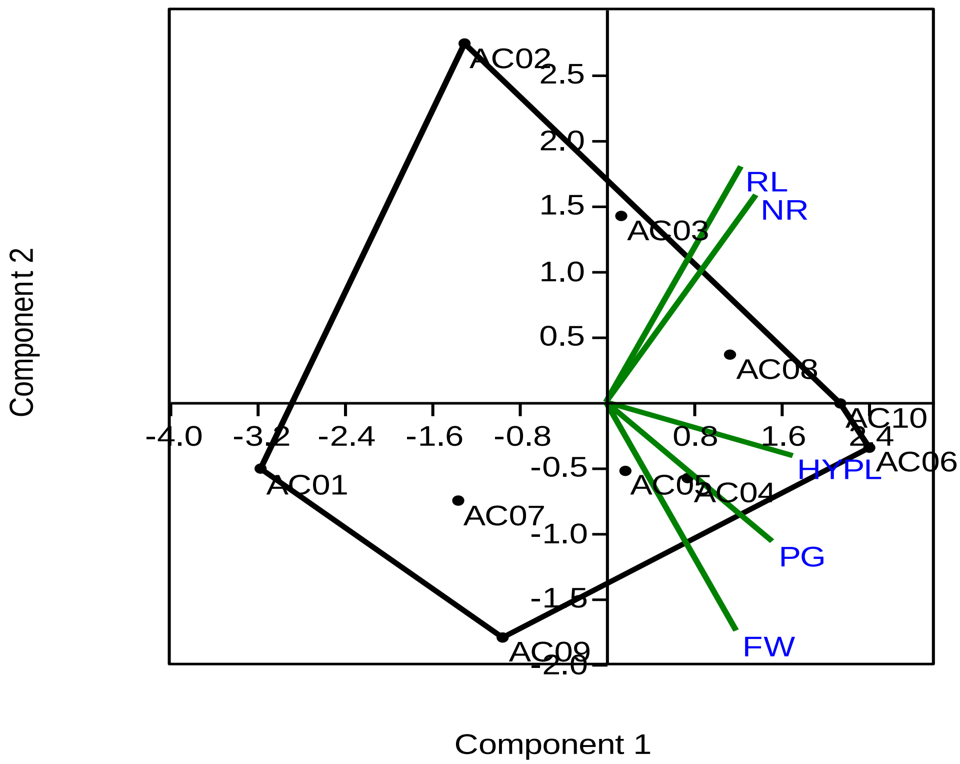

Fig 1 Bi plot of germination parameters of accessions of cowpea under aluminum stress

Aluminum tolerance indices based on germination parameters of accessions of cowpea

Table 7 presents the results of aluminum tolerance indices based on cowpea germination parameters. Nine groups were extracted from this based on similar mean index; groups 1 to 5 are those with mean indices ranging between 1.17 and 1.69 (AC05, AC04, AC08, AC03, AC06, and AC10). Groups 6 to 9 consisted of accessions with indices of between 0.51 and 0.92 (AC01, AC09, AC07 and AC02). Accessions in group 1 and 2 were the highly tolerant accessions, accessions in groups 3, 4 and 5 were moderately tolerant accessions, and accessions in groups 6, 7 and 8 were susceptible accessions, while the accession in group 9 was the highly susceptible accession.

Cluster analysis based on aluminum tolerance indices of germination parameters of accessions of cowpea under aluminum stress

A dendrogram of four major clusters was formed by the aluminum tolerance indices based on germination parameters of cowpea accessions (Figure 2). Cluster I consisted of two accessions (AC10 and AC06), highly tolerant accessions. Cluster II consisted of four accessions (AC05, AC04, AC08 and AC03), moderately tolerant accessions. Clusters III consisted of one susceptible accession (AC02), and IV consisted of two susceptible and one highly susceptible accessions (AC09, AC01 and AC07).

Table 7 Aluminum tolerance indices for germination parameters of accessions of cowpea under aluminum stress

Accession	PG (%)	NR	HYPL (cm)	RL (cm)	FW (g)	Mean	Rank
AC01	0.44	0.33	0.6	0.26	0.92	0.51	9
AC02	0.47	1.42	0.38	1.55	0.78	0.92	6
AC03	1.03	1.57	1.42	1.44	1.10	1.31	3
AC04	1.06	1.22	1.81	0.67	1.46	1.24	4
AC05	1.17	0.98	1.23	0.99	1.50	1.17	5
AC06	1.29	1.44	1.78	1.12	2.17	1.56	2
AC07	0.90	0.78	0.63	0.57	1.16	0.81	7
AC08	1.24	1.52	1.62	0.93	0.92	1.25	4
AC09	1.15	0.24	0.47	0.99	0.82	0.73	8
AC10	1.30	1.63	2.42	1.27	1.81	1.69	1

PG: Percentage germination; NRT: Number of roots; HYPL: Hypocotyl length; RL: Root length; FWS: Fresh weight of shoots.

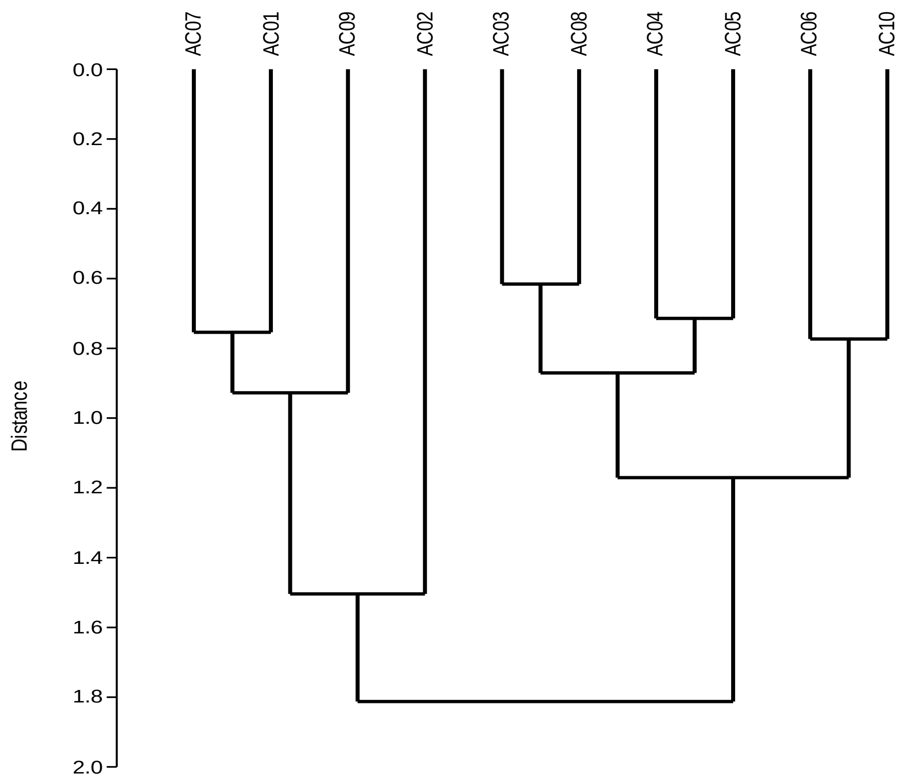

Fig 2 Dendrogram (Euclidean distance) based on aluminum tolerance indices of germination parameters of accessions of cowpea under aluminum stres

Discussion

Analysis of variance revealed significant effect of accession on all germination parameters. This indicated sufficient level of variations among the accessions involved. Aluminum treatment was significant for all parameters except for percentage germination. Aluminium and accession interaction was also significant for all parameters except for percentage germination, hypocotyl length and fresh weight of shoot. These were in agreement with the findings of [30] who reported no significant effect of aluminum treatment on germination of *Vigna radiata* and *Vigna sinensis*, but with significant effect on shoot and root growth. Five of the accessions (AC01, AC03, AC04, AC05, and AC07) had higher germination percentage under control than under aluminum stress, while germination under control was lower for the remaining accessions, indicating stimulatory effects. The number of roots in AC02 and AC09 increased significantly; the hypocotyl length increased significantly in AC06, the root length decreased significantly in AC01, while others had no significant changes in all of these parameters. These were in accordance with the findings of [30] who reported the observation of combination of inhibition symptoms and promotive effect of aluminum stress in *Vigna* species. Rout [7] reported that aluminum had no effect on seed germination, but promoted new root development and seedling establishment, while root growth was found to be more susceptible to aluminum stress than shoot in maize [3] and in wheat [40]. Low dosage of aluminum was also found to promote germination in *Vigna radiata* [41].

All germination parameters were observed to be negatively affected by aluminum in alfafa cultivars [35], in contrast, all germination parameters were generally enhanced in cowpea in this study, except for percentage germination which was generally reduced by treatment. Tolerance indices were able to group accessions into different classes of tolerance: AC10, AC06 and AC03 were highly tolerant; AC04, AC05, AC08 and AC02 were tolerant; AC07 and AC09 were moderately sensitive; whereas AC01 was highly sensitive. The dendrogram based on tolerance indices of germination parameters divided accessions into four key clusters. Cluster I clearly separated the most tolerant accessions (AC10 and AC06), while cluster IV clearly separated the most susceptible accessions (AC01, AC09 and AC07).

Target improvement for aluminum tolerance will depend on existence of sufficient genetic variability and identification of traits that are correlated under stress. Findings from many workers suggest that many approaches could be adopted in breeding for aluminum tolerance and identification of germane traits should be the focus of each breeding program [10, 28, 32, 42]. In this study, percentage germination was positively correlated with number of roots, hypocotyl length and fresh weight of shoot. Number of roots was positively correlated with hypocotyl length, root length and fresh weight of shoot. The root length and fresh weight of shoot were positively associated with hypocotyl length. This indicated that good germination will results in positive increment with all positively correlated traits; this was reflected in AC06 and AC10 with very good germination and subsequent superiority for number of roots, root length and hypocotyl length under aluminum stress. These accessions will be useful for aluminum tolerance breeding program. This agrees with the findings of [35]. GCV and PCV were very close in this study, suggesting strong genetic effect for most traits [43]. In control treatment of most traits, heritability was individually higher. Heritability was high for all traits apart from root length and fresh weight of shoot, meanwhile heritability was moderate for root length. Therefore, aluminum tolerant accessions can be selected based on percentage germination, hypocotyl length and number of roots in cowpea. This is similar to the findings of [28] in maize screened under aluminum stress.

The PCA can be used to identify the most powerful traits, therefore, first two PCs with Eigen values greater than 1 were used to ascertain important traits. All important traits with positive loadings greater than 0.30 in PC1 and PC2 were considered as best. Bi-plot was used for inter-relationships between the traits, and all traits were identified to have selection potential. These traits had high heritability except for fresh weight which had very low heritability. Bi-plot clearly separated highly tolerant (AC06 and AC10) accessions, tolerant (AC03, AC04, AC05 and AC08) accessions, and moderately susceptible accessions (AC09, AC07 and AC02) and the highly susceptible accession (AC01) from one another. Fresh weight of shoot, percentage germination and hypocotyl length were highly positively correlated, while number of roots and root length were also highly correlated according to the bi-plot. The most tolerant accessions were the only vertex accessions corresponding to the important traits sector. PCA and cluster analysis have been used to group genotypes of maize under aluminum stress [28].

Conclusion

The observed genetic variation in aluminum stressed cowpea accessions could be exploited by hybridisation to establish tolerant lines. High heritability and GAM observed in percentage germination, hypocotyl length and number of roots in cowpea can be exploited for selection. Consequently, selection for these traits would result in genetic gain and breeding progress. Crosses involving the tolerant AC10 and AC06 with the sensitive AC01 and AC09 would contribute positively to improvement programs for aluminum tolerance. Also, this level of genetic variability in the present accessions can be exploited in molecular breeding programs especially in generating population of hybrids for QTL mapping for aluminum tolerance.

Acknowledgments

The author extends his appreciation to Adekunle Ajasin University, Akungba-Akoko, Nigeria for the financial support under the PhD Grant. Sincere appreciation is also extended to the Genetic Resources Centre (GRC), International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria for providing the present accessions of cowpea.

References

1. Ajayi, A.T., A.E. Gbadamosi, and V.O. Olumekun, Screening for drought tolerance in cowpea (*Vigna unguiculata* L. Walp) at seedling stage under the screen house condition. International Journal of Biosciences and Technology, 2018. 11 (1): p. 1-19.
2. Abdou-Razakou, I.B.Y., et al., Using morpho-physiological parameters to evaluate cowpea varieties for drought tolerance. International Journal of Agricultural Science Research, 2013. 2 (5): p. 153–162.
3. Giannakoula, A., et al., Aluminum stress induces up-regulation of an efficient antioxidant system in the Al-tolerant maize line but not in the Al-sensitive line. Environmental and Experimental Botany, 2010. 67: p. 487–494.
4. Du, H., Y., et al., A maize ZmAT6 gene confers aluminum tolerance via reactive oxygen species scavenging. Frontiers in Plant Science, 2020. 11: p. 1016.
5. Jayasundara, H.P.S., B.D. Thomson, and C. Tang, Responses of cool season grain legumes to soil abiotic stresses. Advances in Agronomy, 1998. 63: p. 77–151.
6. Kochian, L.V., M.A. Pineros, and O.A. Hoekenga, The physiology, genetics and molecular biology of plant aluminium resistance and toxicity. Plant and Soil, 2005. 274: p. 175–195.
7. Rout, G.R., S. Samantara, and P. Dasp, Aluminum toxicity in plants. Agronomie, 2001. 21: p. 3–21.
8. Oluwatoyinbo, F.I., M.O. Akande, and J.A. Adeniran, Response of Okra (*Abelmoschus esculentus*) to lime and phosphorus fertilization in acid soil. World Journal Agricultural Sciences, 2005. 1: p. 178–183.
9. Ryan, P.R., et al., The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. Journal of Experimental Botany, 2011. 62 (1): p. 9–20.
10. Matonyei, T.K., et al., Aluminum tolerance mechanisms in Kenyan maize germplasm are independent from the citrate transporter ZmMATE1. Scientific Reports, 2020. 10: p. 7320.
11. Rasheed, A., et al., A review on aluminum toxicity and quantitative trait loci mapping in rice (*Oryza sativa* L.). Applied Ecology and Environmental Research, 2020. 18 (3): p. 3951–3964.
12. Wei, Y., et al., Recent advances in understanding mechanisms of plant tolerance and response to aluminum toxicity. Sustainability, 2021. 13: p. 1782.
13. Opara-Nadi, O. A., Liming and organic matter interaction in two Nigerian Ultisols: effect on soil pH, organic carbon and early growth of maize (*Zea mays*. L). In 16^{th} annual conference of Soil Science Society of Nigeria. 1988.
14. Hakim, N., Organic matter for increasing P fertilizer use efficiency of maize in Ultisol by using 32 P technique. In 17^{th} World Congress of Soil Science. 2002.
15. Uzoho, B.U., and N.N. Oti, Phosphorus adsorption characteristics of selected Southeastern Nigerian soils. Journal of Agriculture, Food, Environment and Extension, 2005. 4 (1): p. 50–55.
16. Akinrinde, E.A., Strategies for improving crop use efficiencies of fertilizer nutrients in sustainable agricultural systems. Pakistan Journal Nutrition, 2006. 5: p. 185–193.
17. Agrahari, R.K., et al., Aluminum-specific upregulation of GmALS3 in the shoots of soybeans: A potential biomarker for managing soybean production in acid soil regions. Agronomy, 2020. 10: p. 1228.

18. Bessho, T., and L.C. Bell, Soil solid and solution phase changes and mung bean responsible during amelioration of aluminum toxicity with organic matter. Plant, 1992. 140: p. 183–186.
19. Iroh, L., Plant growth, nutritional status and final yield in cowpea genotypes grown on an acid mineral soil with different levels of phosphorus supply. Master's Thesis, Stuttgart, Germany: Universit at Hohenheim, 2004.
20. Akinrinde, E.A., et al., Response of cowpea varieties to phosphorus supply on an acidic alumi – haplic – acrisol from Brazil. Nigerian Journal of Soil Science, 2005. 16: p. 1.
21. Akinrinde, E.A., et al., Differential expression of aluminium tolerance mechanisms in cowpea genotypes under phosphorus limitation. Journal of Applied Sciences, 2006. 6 (4): p. 854–859.
22. Kushwala, J.K., et al., Screening of cowpea (*Vigna unguiculata* L. Walp] for aluminium tolerance in relation to growth, yield and related traits. Legume Research, 2017. 40 (3): p. 434–438.
23. Kenechukwu, N.E., M.O. Adewale, and A.A. Ezekiel, Aluminium influence on performance of some cowpea (*Vigna unguiculata* L. Walp) varieties on a Nigerian Alfisol. World Journal of Agricultural Sciences, 2007. 3 (4): p. 517–522.
24. Ryan, P.R., J.M. Ditomaso, and L.V. Kochian, Aluminum toxicity in roots: An investigation of spatial sensitivity and the role of the root cap. Journal of Experimental Botany, 1993. 44: p. 437–446.
25. Blancaflor, E.B., D.L. Jones, and S. Gilroy, Alterations in the cytoskeleton accompany aluminum – induced growth inhibition and morphological changes in primary roots of maize. Plant Physiology, 1998. 118: p. 159–172.
26. Pellet, D.M., D.L. Grunes, and L.V. Kochian, Organic acid exuda- scanning microscopy. Plant Physiology, 1995. 124: p. 545–552.
27. Kamh, M., P. Roppel, and W.J. Horst, Exudation of organic acid anions by different maize cultivars as affected by phosphorus deficiency and aluminium toxicity, in Plant Nutrition-Food Security and Sustainability of Agro-Ecosystems, W.J. Horst, Editor. 2001, Kluwer Academic Publishers. Dordrecht: Dordrecht. p. 490–491.
28. Richard, C., et al., Genotypic variation in seedling tolerance to aluminum toxicity in historical maize inbred lines of Zambia. Agronomy, 2015. 5: p. 200–219.
29. Tang, Y., et al., Physiological genetics of aluminum tolerance in the wheat cultivar Atlass66. Crop Science, 2002. 42: p. 1541–1546.
30. Jamal, S.N., M.Z. Iqbal, and M. Ather, Effect of aluminum and chromium on the germination and growth of two *Vigna* species. International Journal of Environmental Science and Technology, 2006. 3 (1): p. 53–58.
31. Pan, X.B., C. Zhu, and C. Chang, Assessment of techniques for screening alfalfa cultivars for aluminum tolerance. Euphytica, 2008. 164: p. 541–549.
32. Roy, B. and S. Bhadra, Effect of toxic levels of aluminium on seedling parameters of rice (*Oryza sativa* L.) under hydroponic culture. Rice Science, 2014. 21 (4): p. 217–223.
33. Ojo, G.O.S., L.L. Bello, and S.A. Ayuba, Genetic variation for seedling traits in hydroponics and correlated response with mature plant traits on acid soil field. International Journal of Plant and Soil Science, 2016. 10 (6): p. 1–8.
34. Leon, V., et al., Effects of three nickel salts on germinating seeds of *Grevillea exul* var. *rubiginosa*, an endemic serpentine Proteaceae. Annals of Botany, 2005. 95: p. 609–618.
35. Pan, X-B., C. Zhu, and C. Cheng, Assessment of techniques for screening alfalfa cultivars for aluminum tolerance. Euphytica, 2008. 164: p. 541–549.
36. SPSS, Statistical package for social science, 2017. SPSS Inc., Chicago IL: USA.
37. Iqbal, M.D.A., Study of drought tolerance indices in some bread and durum wheat cultivars. Jordan Journal of Agricultural Sciences, 2016. 12 (4): p. 1125–1139.

38. Hammer, O., D.A.T. Harper, D.A.T., and P.D. Ryan, PAST: Paleontological Statistical Software Package for Education and Data Analysis. Palaeontologia Electronica, 2001. 4 (1): p. 9.
39. PB Tools, Plant breeding tools, Biometrics and breeding informatics, 2014. 1.4., Los Banos: Laguna.
40. Alamgir, A.N.M. and S. Akhter, Effects of aluminum on seed germination and seedling growth of wheat (*Triticum aestivum* L.). Bangladesh Journal of Botany, 2009. 38 (1): p. 1–6.
41. Neogy, M., et al., Studies on phytotoxic effect of aluminum on growth and some morphological parameters of *Vigna radiata* L. Wilcz. Journal of Environmental Biology, 2000. 23: p. 411–416.
42. Masole, H., An assessment of maize development in Zambia. African Crop Science Society, 1997, 3: 747–753.
43. Hosseini, S.J., et al., Estimation of heritability and genetic advance for screening some rice genotypes at salt stress conditions. International Journal of Agronomy and Plant Production, 2012. 3: p. 475–482.