Original Research Article

Genotypic evaluation of cowpea germplasm for salinity tolerance at germination and during seedling growth

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Abstract

Soil salinity represents a major constraint limiting crop production in arid and semi-arid countries. The effect of salinity induced by sodium chloride (NaCl) at five levels (0, 50, 100, 150 and 200 mM) was investigated on four germination traits and thirteen seedling growth characteristics in twenty cowpea [*Vigna unguiculata* (L.) Walp.] genotypes (ET11, KEB-CP004, KEB-CP006, KEB-CP009, KEB-CP 010, KEB-CP020, KEB-CP033, KEB-CP038, KEB-CP039, KEB-CP045, KEB-CP051, KEB-CP054, KEB-CP057, KEB-CP060, KEB-CP067, KEB-CP068, KEB-CP118, MTA22, NO74 and NO1036). The germination tests were carried out on Petri dishes in the laboratory while seedling growth experiments continued in plastic pots in the greenhouse, both setting up using a randomised complete block design with three replications. Genotypic responses were significant for all germination traits ($p < 0.001$). Germination percentage, germination rate index, and coefficient of velocity of germination were all decreased by salt stress. However, the mean germination time increased with increasing saline conditions. Significant differences were found between genotypes for most growth attributes. Growth rate (centimeter increased in height per week) decreased significantly with increasing salinity, starting at 100 mM NaCl (24.20% reduction, 2.66 cm/week) with maximum reduction (38.58%) corresponding to 2.16 cm/week observed at 200 mM NaCl, compared to control (3.51 cm/week growth rate). Also, significant decline in shoot weights, number of functional leaves and dry matter production were observed under salinity. Salinity also reduced water content in shoot and root and did not affect root weights. Under salinity, significant correlations were found between all germination variables ($p < 0.001$). Growth rate was significantly associated with ten out of the twelve other seedling growth traits. Also, the dry matter production under salinity was significantly associated with all other seedling growth characteristics with the exception of root water content. Given the effect of salt stress, cowpea genotypes, namely NO1036, KEB-CP004, KEB-CP038 and KEB-CP051, were the most tolerant while KEB-CP068 and ET11 were the most sensitive ones. The results confirm substantial genetic variation in salt stress tolerance among the studied genotypes. The most tolerant genotypes should be further explored in genetic improvement programs and should be promoted for culture in regions affected by salinity.

Keywords: Cowpea genotype; genetic improvement; growth characteristics, salt tolerance, seed germination

INTRODUCTION

Agriculture plays a pioneering role in economic development of many emerging countries in sub-Saharan Africa like Cameroon. Cowpea [*Vigna unguiculata* (L.) Walp.] is an important crop in tropical and subtropical regions worldwide. The maximum distribution area of the crop is centered in tropical regions and includes sub-Saharan Africa, Asia, Central and South America (Fatokun et al., 2018). The importance of this crop relies on its high protein content (20–25%) and, therefore, is referred to as the "meat of the poor man" according to Hamid et al. (2016). Cowpea is also known to feed people, their livestock and is a source of cash income (Kebede and Bekeko, 2020). In Cameroon, the far north region is the largest contributor to the national production of cowpea, producing 300 to 500 kg/ha in farmer field and 1200–2000 kg/ha in research stations (Dugie et al., 2009). The western region also produces significant quantities. The national production is estimated to about 110,000 tons from a cultivated area of 105,000 ha according to Bidima (2012). It is well recognised that the best way to significantly address the present food security issue in Africa is the exploitation of local plant genetic resources. There is need to feed the fast-growing population in tropical and sub-tropical regions and, for that, genomic preservation and full exploitation of the diverse range of crop resources is required, in order to suit all the various land and soil characteristics offered. Using plants resources available in the diverse ecosystems existing are therefore dominant considerations in crop production today.

Soil salinity is an ever-increasing problem limiting significantly crop production in arid and semi-arid regions according to Shanon (1986). These regions from estimations represent around 40% of the earth areas (Fisher and Turner, 1978). Rengasamy (2010) estimated 800 million hectares of cultivated land worldwide that are affected by salinity, stress which day after day, is gaining more importance worldwide because of climate changes. Soil salinity is defined as the accumulation of salt in soils (Shrivastava and Kumar, 2015). Several different salts are responsible for salinity. Generally, sodium, calcium and magnesium combined with chloride, sulfate and carbonate to form salt (National Research Council, 1993). The most common salt causing salinity is sodium chloride (NaCl) (Tavakkoli et al., 2010). High salinity is due to the high concentration of soluble salts in soils, inefficient soil drainage or a high rate of evaporation caused by the high temperatures (Ruprecht and Dogramaci, 2005). In plants, a high level of salinity was reported to be responsible for inhibition and the delay of seed germination, seedling development and growth (Almansouri et al. 2001).

Several studies on annual crops which screen individual genotypes for salinity tolerance and using distinctive approaches have been carried out. These studies include some of the following crops: cowpea (Kouam et al., 2017a), common bean (Kouam et al., 2017b; Torche et al., 2018), barley (Askari et al., 2016), wheat (Hamam and Negim, 2014), rice (Hakim et al., 2010). Breeding salt tolerant cultivar is difficult without utilizing the diversity of genetic resources available. In light of this knowledge, our research objectives were to study the effect of salt stress, using different concentrations of sodium chloride, on the germination and seedling growth of twenty cultivated *Vigna unguiculata* genotypes and determine the extent of its tolerance to salinity with the identification of salt tolerant genotypes.

MATERIAL AND METHODS

Plant material and experimental site

Plant material consisted of seeds of a total of 20 cowpea genotypes consisting of cultivars from Cameroon Agricultural Research Institute (IRAD) and landraces collected from farmers' farms and local markets. Codes and origin of the studied cowpea genotypes are presented in Table 1. The experiment was set up out in the laboratory and at the experimental genetics greenhouse of the research and teaching farm of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, located in the west region of Cameroon, latitude of 5°20' North, longitude of 10°05' East and altitude of 1407 m above sea level.

Salt stress treatments and experimental design

The different sodium chloride solutions to be used for the experiments were prepared at concentrations of 0.0 (Control), 50, 100, 150 and 200 mM by dissolving adequate amount of solid NaCl in appropriate quantity of distilled water. These prepared solutions were used during seed germination test in the lab and seedling growth trial in the greenhouse. The present study was carried out as a factorial experiment with two factors (salinity stress and cowpea genotype). The first factor was salinity stress at five different levels and the second factor was the cowpea genotypes.

The germination experiment was carried out in a 120 mm diameter sterilised Petri dishes in the laboratory. Three hundred Petri dishes were used for the laboratory experiment. The Petri dishes were arranged in a completely randomised block design

Table 1. Codes and origin of the studied cowpea genotypes

No	Genotype	Type	Location/District	Division	Region, Country
1	ET11	Local landrace	Market	Harar	Eastern, Ethiopia
2	KEB-CP045	Local landrace	Balembo	Haut-Nkam	Western, Cameroon
3	KEB-CP057	Local landrace	Bangou	Haut-Plateaux	Western, Cameroon
4	KEB-CP060	Local landrace	Bangou	Haut-Plateaux	Western, Cameroon
5	KEB-CP009	Local landrace	Bandjoun	Koung-Khi	Western, Cameroon
6	KEB-CP010	Local landrace	Bandjoun	Koung-Khi	Western, Cameroon
7	KEB-CP051	Local landrace	Bandjoun	Koung-Khi	Western, Cameroon
8	KEB-CP054	Local landrace	Bandjoun	Koung-Khi	Western, Cameroon
9	NO1036	Local landrace	Logone-Birni	Logone-et-Chari	Far North, Cameroon
10	NO74	Local landrace	Gobo	Mayo-Danay	Far North, Cameroon
11	KEB-CP118	Local landrace	Bafia Market	Mbam-et-Inougou	Central, Cameroon
12	KEB-CP039	Local landrace	Fondenera	Menoua	Western, Cameroon
13	KEB-CP004	Local landrace	Bafoussam	Mifi	Western, Cameroon
14	KEB-CP020	Local landrace	Bafoussam	Mifi	Western, Cameroon
15	KEB-CP006	Local landrace	Bafoussam	Mifi	Western, Cameroon
16	KEB-CP067	Local landrace	Bangangte	Ndé	Western, Cameroon
17	KEB-CP068	Local landrace	Bazou	Ndé	Western, Cameroon
18	KEB-CP038	Local landrace	Foumbot	Noun	Western, Cameroon
19	KEB-CP033	Local landrace	Kouoptamo	Noun	Western, Cameroon
20	MTA-22	Breeding line	IRAD-Foumbot	Noun	Western, Cameroon

with three replications. A total of of thirty seeds was placed in each Petri dish on double-layer Whatman paper. Then, 10 cm^3 of appropriate solution was added to each Petri dish. Seeds were imbibed in the different solutions for 24 hours at room temperature. Seeds were then drained, rinsed twice with distilled water, and were allowed to continue germination on a new moist double layer Whatman paper. The seed counting process started 24 hours after seeds were moistened for the first time and the process was repeated every day at the same hour. Every day, the germinated seeds were counted, recorded and removed from the Petri dishes. Seed germination was validated when a 5 mm radicle had emerged from the seed coat as was validated in wheat by Sayar et al. (2010). The experiment was concluded after 21 days.

In the greenhouse, the soil used for the experiment was collected from the ploughed field close to the university site. The soils' characteristics are presented in Table 2. Plastic pots of 210 mm × 300 mm dimensions each were filled with 7 kg of soil. These pots had no drainage hole at the bottom. In each plastic pot filled with soil, eight seeds of each genotype were planted. Thinning followed two weeks later, leaving only four plants in each pot. We used a randomised complete block design with three replications for the experiment. Salinity treatments were applied as NaCl solutions at the same five molarities as for the germination trial. Pots were irrigated with 200 cm^3 appropriate saline solution every three days from two weeks after planting up to six weeks. Growth parameters were then measured.

Table 2. Chemical and physical characteristics of the soil used (0–20 cm depth)

Element	Content
Clay (%)	9.00
Silt (%)	10.00
Sand (%)	81.00
Exchangeable potassium (mg kg^{-1})	237.90
Exchangeable sodium (mg kg^{-1})	200.10
Exchangeable calcium (mg kg^{-1})	416.00
Exchangeable magnesium (mg kg^{-1})	136.08
Assimilable phosphorus (mg kg^{-1})	0.89
Nitrogen (%)	0.10
Organic carbon (%)	4.20
Organic matter (%)	7.23
C/N ratio	42.00
pH-water	6.80
pH KCl	5.30
ΔpH	–1.50
Electric Conductivity (μs/cm)	60.00

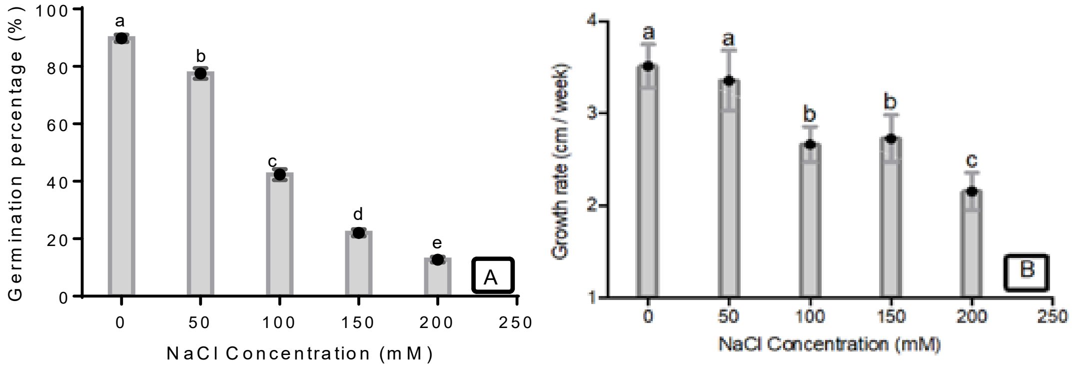

Figure 1. Effect of salinity (NaCl) on germination percentage (A) and growth rate (B) of cowpea genotypes. For each variable, values followed by a same letter indicate no significant difference (Tukey multiple range test at p = 0.050 probability level).

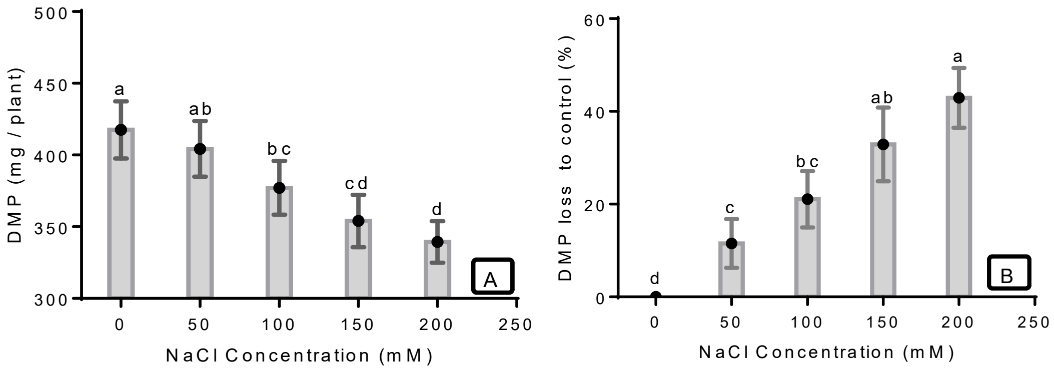

Figure 2. Effect of salinity (NaCl) on dry matter production (A) and dry matter production losst o control (B) of cowpea genotypes. For each variable, values followed by a same letter indicate no significant difference (Tukey multiple range test at p = 0.050 probability level).

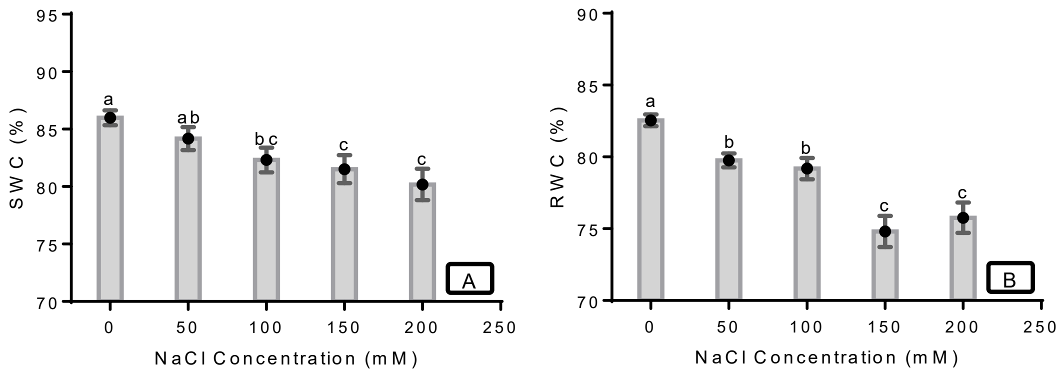

Figure 3. Effect of salinity (NaCl) on the shoot (A) and root (B) water content of cowpea genotypes. For each variable, values followed by a same letter indicate no significant difference (Tukey multiple range test at p = 0.050 probability level).

Measured traits and classification of genotypes

Four germination traits were measured and included: (1) Germination percentage (GP = 100 × n/N), obtained by dividing the number of germinated seeds in each Petri dish (n) by the total number of seeds tested (N), multiplied by 100 (Cokkizgin and Cokkizgin, 2010). (2) The mean germination time (MGT), calculated to assess the rate of germination (Hu et al. 2005) as follows: $MGT = \Sigma(n_i \times d_i) / \Sigma n_i$. where n_i = number of the newly germinated seeds and d_i equals day number. (3) Germination Rate Index (GRI), calculated as described by the Association of Official Seed Analysts (AOSA, 1983): $GRI = \Sigma\ Gt / Dt$. where Gt is the number of seeds germinated in t days; Dt is the number of corresponding germination days; (4) Coefficient of velocity of germination (CVG) evaluated according to Maguire (1962) as follows: $CVG = (G_1 + G_2 + ... + G_n) / (1xG_1 + 2xG_2 + ... + nxG_n)$ where G is the number of germinated seeds and n is the last day of germination.

Thirteen seedling growth characteristics were recorded. Seedling length was measured weekly on two plants in each replication using metric ruler.

Table 3. Mean Comparison of the effect of Genotype × Salinity interaction on growth and germination variables in cowpea at 6 weeks' growth

Genotype											Growth variables			Germination variables		
	[NaCl] concentration	RFW (mg/ plant)	RDW (mg/ plant)	SFW (mg/ plant)	SDW (mg/ plant)	RL (cm)	SL (cm)	NFL	RL/SL	RDW/ SDW	Growth (cm/week)	RWC (%)	SWC (%)	MGT (days)	CVG	GRI
ET11	0 mM	160.00	24.67	843.33	77.33	11.40	15.00	3.00	0.76	0.50	1.08	84.62	91.22	6.4415	0.1631	7.7528
	50 mM	153.33	29.33	730.00	242.00	11.33	18.00	1.67	0.71	0.28	1.75	81.36	70.56	5.2520	0.1974	4.3351
	100 mM	176.67	33.67	676.67	82.00	13.50	16.00	2.00	0.91	0.52	1.25	80.75	87.73	12.0185	0.0832	0.8232
	150 mM	243.33	19.33	2056.67	51.67	5.13	20.00	0.33	0.40	0.38	1.75	87.31	96.44	8.4222	0.1202	0.6793
	200 mM	170.00	42.33	626.67	131.67	8.60	15.67	1.33	0.58	0.32	0.83	75.68	64.89	5.5000	0.1944	0.3810
KEB-CP045	0 mM	300.00	51.00	2213.33	283.00	7.70	23.00	3.00	0.36	0.17	3.00	83.23	85.87	3.8201	0.2631	9.0718
	50 mM	473.33	111.00	3366.67	462.33	11.97	42.67	3.00	0.32	0.24	6.25	75.64	86.20	5.3219	0.1883	5.9298
	100 mM	416.67	85.00	1963.33	345.67	10.47	30.67	2.67	0.33	0.24	3.50	79.73	72.30	3.9307	0.2628	2.2316
	150 mM	460.00	102.67	2330.00	335.00	13.07	27.67	2.67	0.52	0.32	2.83	77.46	85.97	4.7500	0.2109	1.6816
	200 mM	370.00	80.00	2353.33	313.67	8.37	24.00	1.33	0.34	0.26	2.17	78.77	86.72	4.9111	0.2255	1.0650
KEB-CP057	0 mM	306.67	31.67	2763.33	210.00	10.00	23.33	3.67	0.48	0.16	2.83	88.14	89.68	2.1905	0.4586	13.5833
	50 mM	326.67	56.67	2533.33	285.33	9.63	40.33	4.00	0.32	1.49	3.17	82.38	91.00	2.5277	0.3989	11.7847
	100 mM	433.33	55.33	1463.33	197.67	9.33	24.00	1.67	0.57	0.39	2.83	83.61	74.40	4.2472	0.2443	5.8930
	150 mM	266.67	80.67	816.67	104.33	10.50	19.33	1.67	0.56	1.17	1.83	67.52	70.03	11.0111	0.0908	0.4577
	200 mM	196.67	46.33	1210.00	169.33	9.97	21.00	2.33	0.46	0.27	1.67	74.18	75.63	10.9444	0.0918	0.2201
KEB-CP060	0 mM	223.33	49.00	1450.00	236.00	10.53	24.67	3.67	0.45	0.21	2.25	77.04	81.98	2.4333	0.4199	13.3333
	50 mM	210.00	43.67	863.33	124.67	9.50	22.33	2.33	0.46	0.38	1.83	79.17	85.35	3.0889	0.3308	12.1906
	100 mM	196.67	37.00	713.33	147.33	8.93	17.33	0.67	0.50	0.31	1.08	81.16	76.28	5.9400	0.1686	4.8148
	150 mM	310.00	67.00	1326.67	200.33	10.33	25.00	3.33	0.40	0.32	3.25	78.89	85.03	9.0595	0.1120	0.9158
	200 mM	190.00	42.67	253.33	95.00	7.33	16.33	1.00	0.46	0.45	0.58	76.18	50.33	9.5905	0.1091	0.5123
KEB-CP009	0 mM	190.00	34.67	1450.00	193.00	8.33	24.67	5.00	0.35	0.19	2.33	81.17	86.25	2.2232	0.4515	12.4833
	50 mM	216.67	53.33	1713.33	240.00	8.27	33.67	4.00	0.27	0.23	3.00	75.01	85.60	3.1689	0.3174	11.2750
	100 mM	326.67	70.33	1503.33	259.67	9.87	21.00	2.00	0.47	0.30	2.00	78.96	69.36	4.1090	0.2541	7.2150
	150 mM	150.00	45.67	513.33	151.33	10.83	18.00	1.67	0.62	0.33	0.67	68.76	48.28	10.1905	0.0989	0.6045
	200 mM	146.67	40.00	660.00	145.00	7.83	19.67	0.67	0.39	0.33	1.50	69.82	58.81	11.6667	0.0859	0.1439
KEB-CP-010	0 mM	343.33	64.33	2016.67	260.00	10.63	18.67	3.67	0.56	0.24	1.42	82.49	86.79	2.7857	0.3752	10.6144
	50 mM	500.00	62.33	1263.33	191.00	10.70	16.67	2.33	0.64	0.35	1.08	81.22	84.58	4.0609	0.2511	6.4232
	100 mM	330.00	56.67	1003.33	148.00	7.33	15.33	1.33	0.49	0.40	1.00	83.05	81.69	3.4722	0.3072	4.0005
	150 mM	286.67	74.33	1516.67	251.00	9.67	18.33	2.00	0.56	0.30	0.92	73.51	83.21	9.5833	0.1065	0.3854
	200 mM	396.67	83.00	1910.00	272.67	11.00	17.00	2.00	0.64	0.31	1.50	78.19	85.41	7.3333	0.2340	0.4377

Genotype												Growth variables			Germination variables	
	[NaCl] concentration	RFW (mg/ plant)	RDW (mg/ plant)	SFW (mg/ plant)	SDW (mg/ plant)	RL (cm)	SL (cm)	NFL	RL/SL	RDW/ SDW	Growth (cm/week)	RWC (%)	SWC (%)	MGT (days)	CVG	GRI
KEB-CP051	0 mM	636.67	107.67	5760.00	495.00	11.97	49.67	5.00	0.25	0.29	8.08	81.92	91.38	4.2264	0.2410	4.4206
	50 mM	493.33	86.33	3146.67	394.67	11.63	45.33	4.00	0.29	0.21	7.67	82.29	87.10	5.4504	0.1841	2.5696
	100 mM	353.33	74.67	2153.33	302.33	9.37	29.67	2.67	0.40	0.24	3.67	79.40	86.03	5.3316	0.1898	2.1368
	150 mM	446.67	115.67	1766.67	413.33	13.67	55.33	3.33	0.26	0.56	9.92	65.37	73.38	8.6508	0.1227	0.8693
	200 mM	503.33	109.33	2753.33	384.33	13.53	31.00	3.00	0.52	0.29	3.67	77.26	85.99	8.0000	0.1270	0.2193
KEB-CP-054	0 mM	486.67	91.67	4023.33	473.00	9.00	17.33	3.33	0.53	0.20	1.67	81.18	88.18	3.8778	0.2634	9.6808
	50 mM	610.00	110.33	3776.67	456.67	12.67	18.33	3.33	0.69	0.25	1.33	81.73	88.08	4.2944	0.2368	9.3843
	100 mM	546.67	110.33	4363.33	537.67	13.53	22.00	3.33	0.61	0.22	2.08	79.80	87.45	5.0383	0.2011	3.7345
	150 mM	306.67	74.33	2253.33	297.00	12.10	21.33	1.67	0.63	0.28	2.42	67.20	86.81	9.2722	0.1096	1.3293
	200 mM	503.33	112.33	3100.00	399.67	12.57	16.33	2.67	0.79	0.28	1.33	77.51	87.02	7.5397	0.1396	0.9938
NO1036	0 mM	696.67	113.33	3640.00	494.67	13.57	49.67	3.33	0.27	0.23	8.33	83.50	86.39	4.8454	0.2071	6.7406
	50 mM	643.33	127.00	2746.67	391.67	16.00	43.33	3.00	0.71	0.34	7.17	80.23	85.85	7.4502	0.1344	3.7979
	100 mM	660.00	125.33	3473.33	454.33	16.57	45.00	2.00	0.37	0.27	7.25	81.30	86.11	8.2499	0.1213	2.4812
	150 mM	636.67	112.67	3186.67	416.67	16.20	46.67	2.67	0.39	0.32	7.50	82.42	87.21	8.7857	0.1145	1.6539
	200 mM	500.00	119.33	3433.33	452.33	13.00	56.67	3.00	0.23	0.27	10.33	76.01	86.65	8.7423	0.1156	1.4640
NO74	0 mM	220.00	47.33	1616.67	211.67	7.57	14.00	2.67	0.67	0.45	2.33	78.70	80.26	2.5652	0.3904	12.5310
	50 mM	220.00	50.00	1343.33	173.67	11.17	15.67	3.67	0.71	0.28	1.17	77.60	86.96	3.5653	0.2983	9.5886
	100 mM	230.00	52.00	1256.67	153.33	8.60	12.00	2.00	0.72	0.38	0.58	76.24	87.67	9.0536	0.1108	1.5796
	150 mM	193.33	50.67	496.67	76.67	13.07	13.33	0.67	0.95	0.65	0.92	73.85	79.20	10.0000	0.1000	0.2680
	200 mM	220.00	55.33	1413.33	194.67	9.50	15.00	1.00	0.64	0.28	1.33	74.69	86.15	10.1667	0.0984	0.1939
KEB-CP118	0 mM	370.00	70.33	2200.00	306.33	11.93	28.67	3.33	0.41	0.23	3.00	81.05	85.94	3.1444	0.3195	10.8278
	50 mM	460.00	87.33	2556.67	348.00	7.50	28.00	2.67	0.27	0.27	2.42	80.26	85.57	4.3094	0.2643	9.6846
	100 mM	390.00	84.67	2633.33	386.67	13.57	30.67	2.67	0.47	0.23	2.75	78.19	85.38	9.9921	0.1001	1.1944
	150 mM	266.67	76.67	1273.33	194.67	16.43	23.67	3.00	0.72	0.60	1.67	66.91	84.42	10.0370	0.0998	0.8601
	200 mM	390.00	92.67	1596.67	235.67	10.03	20.00	2.00	0.51	0.40	1.17	75.53	85.04	7.3333	0.1815	0.2148
KEB-CP039	0 mM	533.33	58.00	2090.00	147.67	9.50	23.67	3.67	0.40	0.99	2.00	85.57	93.13	3.3252	0.3036	8.1873
	50 mM	306.67	56.33	1823.33	264.67	9.30	26.67	2.33	0.37	0.21	2.75	82.88	82.41	4.8272	0.2181	6.6657
	100 mM	280.00	66.33	1800.00	281.33	11.23	19.67	2.67	0.60	0.24	1.42	76.26	84.25	5.6828	0.1811	5.0511
	150 mM	213.33	57.00	813.33	134.67	8.33	16.67	0.67	0.49	0.47	0.83	72.94	75.18	6.5707	0.1593	3.1384
	200 mM	263.33	108.33	903.33	145.67	7.57	18.67	1.33	0.45	1.00	0.75	53.73	75.88	6.8355	0.1516	1.5733

Genotype	[NaCl] concentration	RFW (mg/plant)	RDW (mg/plant)	SFW (mg/plant)	SDW (mg/plant)	RL (cm)	SL (cm)	NFL	RL/SL	RDW/SDW	Growth variables: Growth (cm/week)	Growth variables: RWC (%)	Growth variables: SWC (%)	Germination variables: MGT (days)	Germination variables: CVG	Germination variables: GRI
KEB-CP004	0 mM	580.00	99.67	2553.33	343.00	10.33	30.33	3.00	0.39	0.32	3.58	82.97	86.64	4.3031	0.2324	7.6325
	50 mM	496.67	114.33	2520.00	359.67	14.37	30.67	2.67	0.47	0.32	3.25	77.14	84.40	5.0874	0.1975	6.2737
	100 mM	570.00	81.67	2300.00	337.67	15.13	31.00	2.00	0.52	0.28	3.08	83.70	85.18	8.2389	0.1225	1.3863
	150 mM	596.67	135.00	2090.00	318.33	12.27	39.33	2.00	0.35	0.50	5.33	77.55	84.82	8.6995	0.1168	1.0212
	200 mM	466.67	100.33	1803.33	208.67	10.40	24.33	0.33	0.43	0.50	1.67	77.79	86.86	8.5500	0.1185	0.8693
KEB-CP020	0 mM	620.00	107.67	3820.00	495.00	11.87	38.33	3.67	0.33	0.21	4.50	83.30	86.83	2.5033	0.4023	10.8175
	50 mM	570.00	122.00	2890.00	447.00	12.30	34.33	3.00	0.38	0.28	3.92	77.49	84.62	3.2754	0.3077	8.9564
	100 mM	580.00	71.67	3253.33	485.00	13.70	72.00	2.67	0.33	0.16	4.42	87.01	85.10	4.0524	0.2679	2.8365
	150 mM	313.33	65.67	2163.33	243.67	10.03	24.00	1.33	0.45	0.35	2.17	70.74	89.01	2.0000	0.5000	0.5000
	200 mM	423.33	86.67	1916.67	335.00	8.83	25.33	2.67	0.39	0.26	3.08	79.56	78.36	2.0000	0.5000	0.6667
KEB-CP006	0 mM	406.67	61.00	4790.00	405.00	7.90	46.67	4.67	0.17	0.32	7.92	84.37	91.64	3.3554	0.3014	10.8021
	50 mM	586.67	123.33	5100.00	500.67	11.17	30.33	4.00	0.40	0.44	8.00	78.82	89.68	3.4938	0.2983	9.0053
	100 mM	390.00	84.67	3866.67	531.00	9.60	32.33	3.67	0.29	0.17	3.83	77.89	85.63	4.7510	0.2183	3.1572
	150 mM	480.00	110.67	4100.00	595.67	12.63	41.67	3.00	0.41	0.19	2.83	76.95	85.43	4.9048	0.2254	1.6239
	200 mM	473.33	113.00	2720.00	360.67	13.50	26.00	3.00	0.52	0.32	2.50	75.19	86.40	2.0000	0.5000	0.5000
KEB-CP067	0 mM	406.67	80.33	4716.67	758.00	12.53	29.67	5.33	0.45	0.11	3.42	79.86	79.48	3.3092	0.3076	11.1563
	50 mM	396.67	92.00	3773.33	545.00	13.37	39.67	4.67	0.43	0.17	1.43	76.81	85.40	4.0086	0.2510	9.5065
	100 mM	293.33	76.67	3166.67	454.33	9.90	25.33	4.00	0.39	0.17	2.33	74.15	85.54	7.3869	0.1386	1.3825
	150 mM	436.67	86.67	2193.33	300.33	9.00	21.33	2.67	0.46	0.29	1.67	78.95	86.27	10.6333	0.0941	0.4091
	200 mM	460.00	117.00	3030.00	417.00	9.90	35.00	2.67	0.38	0.29	1.32	74.67	86.08	11.3333	0.0889	0.3402
KEB-CP068	0 mM	160.00	17.33	1180.00	306.67	7.50	19.33	2.67	0.39	0.06	2.00	89.03	71.80	3.3894	0.3043	9.0683
	50 mM	104.00	23.33	376.67	71.33	8.83	11.00	2.00	0.76	0.34	0.42	77.72	69.23	4.8299	0.2122	4.1239
	100 mM	210.00	44.33	1010.00	178.33	10.70	15.00	2.00	0.77	0.27	1.25	78.71	70.60	6.6019	0.1557	1.2999
	150 mM	250.00	79.33	676.67	151.67	7.23	14.00	0.67	0.59	1.40	0.83	68.08	75.24	6.7500	0.1528	0.9851
	200 mM	186.67	19.33	853.33	72.00	6.43	10.33	1.00	0.66	0.33	0.42	79.52	79.16	7.0667	0.1430	0.7073
KEB-CP038	0 mM	426.67	88.33	3293.33	433.00	10.80	39.00	4.00	0.28	0.21	4.75	79.41	86.82	4.3230	0.2320	8.5388
	50 mM	553.33	62.33	3606.67	285.67	10.43	56.00	3.33	0.27	0.21	5.92	88.95	91.56	7.8268	0.1286	3.2928
	100 mM	550.00	118.67	2626.67	346.00	11.03	37.00	2.00	0.34	0.34	4.67	78.41	86.90	8.8056	0.1167	1.3552
	150 mM	553.33	109.67	2920.00	407.00	11.80	55.67	2.67	0.27	0.26	4.08	80.67	85.67	10.7429	0.0934	0.4934
	200 mM	493.33	122.67	2433.33	334.33	12.27	30.00	2.33	0.43	0.39	2.33	74.76	86.36	11.6667	0.0859	0.1414

Genotype	[NaCl] concentration	RFW (mg/ plant)	RDW (mg/ plant)	SFW (mg/ plant)	SDW (mg/ plant)	RL (cm)	SL (cm)	NFL	RL/SL	RDW/ SDW	Growth (cm/week)	RWC (%)	SWC (%)	MGT (days)	CVG	GRI
												Growth variables			Germination variables	
KEB-CP033	0 mM	520.00	98.67	2903.33	476.67	12.63	29.00	3.67	0.48	0.21	3.67	81.24	82.87	4.0014	0.2500	6.3024
	50 mM	543.33	109.33	2680.00	412.00	9.20	28.67	3.00	0.33	0.28	2.83	79.86	70.04	4.7965	0.2123	4.3191
	100 mM	476.67	90.67	1946.67	222.33	9.80	24.00	4.00	0.42	1.19	2.00	78.93	80.88	8.1439	0.1244	1.0866
	150 mM	446.67	83.33	2876.67	444.67	8.87	24.33	2.00	0.39	0.19	1.83	81.16	84.54	8.3254	0.1220	0.8042
	200 mM	273.33	74.33	1663.33	253.67	10.00	20.00	2.00	0.62	0.31	3.25	72.52	84.91	8.2905	0.1220	0.5708
MTA22	0 mM	446.67	79.67	2780.00	369.33	10.77	25.33	3.33	0.43	0.22	2.08	82.16	86.59	4.0939	0.2445	8.2702
	50 mM	426.67	81.00	2980.00	288.67	12.17	21.67	3.33	0.56	0.29	1.75	78.58	89.19	4.5263	0.2209	5.6491
	100 mM	283.33	108.67	1426.67	163.67	11.37	23.00	2.00	0.50	0.74	2.25	66.43	87.86	7.8000	0.1309	2.4430
	150 mM	536.67	107.00	2236.67	339.00	14.00	20.67	2.67	0.69	0.33	1.33	79.88	84.14	8.3106	0.1212	1.3916
	200 mM	410.00	25.67	2113.33	277.00	11.50	22.67	2.67	0.51	0.12	1.75	93.61	87.06	9.4259	0.1066	0.7456
All genotypes	0 mM	401.66 a	68.82 a	2805.17 a	348.72 a	10.32 a	28.50 ab	3.68 a	0.42 a	0.27 a	3.51 a	82.85 a	85.99 a	3.56 d	0.31 a	9.59 a
	50 mM	414.53 a	80.07 a	2489.50 ab	324.23 a	11.08 a	30.17 a	3.12 b	0.47 a	0.34 a	3.35 a	79.76 b	84.17 ab	4.56 c	0.24 b	7.24 b
	100 mM	384.67 a	76.82 a	2130.00 bc	300.72 ab	11.18 a	27.15 ab	2.40 c	0.50 a	0.35 a	2.66 b	79.19 b	82.32 bc	6.64 b	0.18 c	2.81 c
	150 mM	369.67 a	82.70 a	1880.33 c	271.35 b	11.26 a	27.32 ab	2.03 d	0.51 a	0.46 a	2.73 b	74.81 c	81.51 c	8.34 a	0.14 d	1.01 d
	200 mM	351.83 a	79.53 a	1837.33 c	259.90 b	10.11 a	23.25 b	1.92 d	0.49 a	0.35 a	2.16 c	75.76 c	80.19 c	8.45 a	0.13 d	0.60 e

RFW = Root fresh weight; RDW = Root dry weight; SFW = Shoot fresh weight; SDW = Shoot dry weight; RL = Root length; SL = Seedling length; NFL = Number of functional leaves; RL/SL = Root length – Seedling length ratio; RDW/SDW = Root dry weight – Shoot dry weight ratio, MGT = Mean germination time; GRI = Germination rate index; CVG = Coefficient of velocity of germination. For each genotype, means followed by a same letter in the same column are not significantly different (Tukey multiple range test at p = 0.050 probability level)

After completing the growth experiment at six weeks, the measured variables included: (1) Root fresh weight, (2) Root dry weight, (3) Shoot fresh weight, (4) Shoot dry weight, (5) Root length, (6) Seedling length, (7) Number of functional leaves (green leaves, not senescent), (8) Root length / Seedling length ratio, (9) Root dry weight / Shoot dry weight ratio, (10) Growth rate, (11) Dry matter production, (12) Root water content, (13) Shoot water content. Dry weights were measured after drying plants at 70°C for 48 hours. Root water content (RWC) and shoot water content (SWC) were determined as follows: RWC = (Root fresh weight – Root dry weight) / Root fresh weight. SWC = (Shoot fresh weight – Shoot dry weight) / Shoot fresh weight.

Cowpea genotypes were classified for salinity tolerance. The classification was based on the deficit in the total dry weight of the plant (shoot + root) at each level of salinity compared to controls as suggested by Fageria (1985). Genotypes classified as Tolerant (T) had a total dry matter deficit of equal or less than 20%. Moderately tolerant (MT) genotypes had dry matter deficits between 21% and 40% and, in moderately susceptible (MS) genotypes, the deficit dry weight varied from 41% to 60%. In susceptible (S) genotypes, the deficit dry weight was larger than 60%.

Statistical analysis

Software packages named XLSTAT 2014 and GraphPadPism 6.0 were used to analyse the data obtained from germination and seedling growth variables of cowpea genotypes under salinity. Data were analysed using two-way Analysis of Variance (ANOVA) with three sets of assumptions that are: (1) mean values of each variable among salinity treatments are equal, (2) mean values of each variable among the different genotypes are equal, and (3) there is no interaction between salinity treatment and genotype for each tested variable. Differences were declared significant at $p < 0.050$ probability levels by the Fisher test. Where the ANOVA test showed significant differences among means, Tukey's multiple range test of XLSTAT 2014 software was performed at the 0.050 level of probability to separate means. Pearson correlation coefficients were used to assess the relationship between the different variables under salinity.

RESULTS

Germination traits

This study revealed a significant effect of salinity on germination traits of cultivated *Vigna unguiculata* genotypes (Figure 1A, Table 3 and 4). In overall, salt stress negatively affected germination of cowpea genotypes. The effect of salinity varied significantly between the different genotypes tested. Under control conditions, germination percentage (GP) ranged from 58.67 [KEB-CP051] to 100% [KEB-CP054, KEB-CP060 and KEB-CP118] with the mean at 89.82% and from 3.33 [KEB-CP006] to 34.44% [NO1036] with a mean at 12.73% under maximum salt stress conditions (200 mM NaCl). At 50, 100, and 150 mM salinity stress conditions, the mean GP ranges from 40 [KEB-CP068] to 100% [KEB-CP118], 23.33 [KEB-CP045] to 73.33% [KEB-CP009] and 4 [KEB-CP020] to 50% [KEB-CP039] with a mean over genotype of 77.55, 42.67 and 22.06%, respectively (Figure 1A, Table 5). The general tendency is that as the NaCl concentration augmented, cowpea genotypes showed reducing GP (Figure 1A). Like that of germination percentage, the germination rate index (GRI) and the coefficient of velocity of germination (CVG) had the same change movement with values decreasing as the level of salinity increasing (Table 3). The highest GRI and CVG were observed in the controls, 9.521 ± 0.341 and 0.307 ± 0.012, respectively, and the lowest values observed at maximum salt stress treatment (200 mM NaCl) and were 0.598 ± 0.066 for GRI and 0.129 ± 0.017 for CVG (Table 3). Salinity induced by NaCl significantly affected also the mean germination time (MGT) (Table 3). In contrary of GP, GRI and CVG that decreased with increasing salinity level, the MGT increased with increasing salt stress concentration. Over genotype, significantly longer MGT (8.448 ± 0.401) was obtained with treatment at 200 mM NaCl as compared with controls (MGT = 3.558 ± 0.144) (Table 3). A two-way Analysis of Variance showed a significant individual effect of salinity, genotype and their interaction in affecting all the studied germination traits in cultivated *Vigna unguiculata* (Table 4).

Early growth characteristics

Over genotype, growth rate that was expressed in terms of seedling height gained per week in centimetres (cm/week) decreased significantly with increasing salinity (Figure 1B, Table 3). With increasing NaCl concentrations, seedling length decreased significantly. Seedling length was affected by salinity only at 200 mM NaCl. Statistical analysis did not show significant differences in root weights and length as exposed to different salinity level, revealing that they were not affected by salinity (Table 3, Table 4). Results presented in Table 3 showed that greater levels of salinity reduced the number of functional leaves during the experiment. In treated plants, significant reduction was observed even at the lowest salt concentration (50 mM NaCl) and the reduction continued at 100 and 150 mM NaCl and

Table 4. Analyses of variance results (Fractions) and Fisher test on germination and growth variables for the plant genotypes, salinity level and their interaction

	Genotype (df = 19)			Salinity (df = 4)			Genotype × Salinity (df = 76)		
	F	P	Sign.	F	P	Sign.	F	P	Sign.
Germination variables									
GP (%)	10.817	0.000	***	584.455	0.000	***	3.384	0.000	***
MGT (day)	21.692	0.000	***	200.159	0.000	***	6.898	0.000	***
GRI	18.972	0.000	***	742.507	0.000	***	6.251	0.000	***
CVG (day^{-1})	26.323	0.000	***	116.697	0.000	***	6.382	0.000	***
Growth variables									
RFW (mg/plant)	13.983	0.000	***	2.013	0.094	NS	1.034	0.420	NS
RDW (mg/plant)	9.970	0.000	***	1.779	0.134	NS	1.061	0.366	NS
SFW (mg/plant)	13.889	0.000	***	10.006	0.000	***	0.970	0.551	NS
SDW (mg/plant)	11.498	0.000	***	4.373	0.002	**	0.952	0.589	NS
RL (cm)	3.206	0.000	***	1.508	0.201	NS	0.939	0.618	NS
SL (cm)	9.206	0.000	***	2.393	0.048	*	0.935	0.625	NS
NFL (No)	4.622	0.000	***	28.223	0.000	***	1.047	0.394	NS
RL/SL (ratio)	4.603	0.000	***	1.592	0.178	NS	0.815	0.848	NS
RDW/SDW (ratio)	1.345	0.159	NS	1.649	0.163	NS	1.040	0.408	NS
Growth rate (cm/week)	12.126	0.000	***	4.107	0.003	**	0.864	0.766	NS
DMP (mg/plant)	13.225	0.000	***	2.830	0.026	*	0.931	0.634	NS
RWC (%)	0.732	0.784	NS	7.335	0.000	***	0.955	0.584	NS
SWC (%)	2.979	0.000	***	2.587	0.038	*	1.208	0.152	NS

Level of significance: *** $p < 0.001$; ** $p < 0.010$; * $p < 0.050$; NS = Not significant
df = Degrees of freedom; GP = Germination percentage; GRI = Germination rate index; MGT = Mean germination time; CVG = Coefficient of velocity of germination; RFW = Root fresh weight; RDW = Root dry weight SFW = Shoot fresh weight; SDW = Shoot dry weight; RL = Root length; SL = Seedling length; NFL = Number of functional leaves; RL/SL = Root length / Seedling length; RDW / SDW = Root dry weight / Shoot dry weight; DMP = Dry matter production; RWC = Root water content; SWC = Shoot water content

did not change for 150 and 200 mM NaCl salinity stress. The general tendency reflects a gradual decrease in the number of plant functional leaves with the increase of salt concentration in the soil. Under salinity, significant differences were observed for shoot weights, dry matter production (Table 3, Figure 2A). Dry matter production loss compared to control increased with increasing salinity (Figure 2B). Over genotype, shoot had significantly more water content compared to root (Figure 3). Salinity significantly reduced water content in shoots and roots (Figure 3, Table 4). A two-way Analysis of Variance showed a significant effect of genotype and salinity for most growth attributes in cultivated cowpea. The genotype × salinity interaction did not affect significantly any of the studied growth attributes (Table 4). Generally, most of early growth traits were inhibited by salt stress with variables significantly higher in controls compared to treated plants (Table 3).

Classification of genotypes and correlation between traits

Among the twenty studied cowpea genotypes, different responses for salinity tolerance at any soil salinity level were observed. At the lowest salinity concentration (50 mM NaCl), fifteen out of the twenty studied genotypes were tolerant (T) and the remaining five were moderately tolerant (MT). When increasing the salinity level to 100 mM NaCl, eleven genotypes among the fifteen tolerant at 50 mM NaCl continued to be tolerant, eight were moderately tolerant and one moderately susceptible (ET11). Moving to the salinity level of 150 mM NaCl, four genotypes continued to be tolerant (KEB-CP038, KEB-CP051, KEB-CP004 etNO1036), ten were moderately tolerant (MT), four were moderately susceptible and two were susceptible (KEB-CP068 and ET11). At the highest soil salinity level, one genotype continued to be tolerant (T) (NO1036), seven were moderately tolerant (KEB-CP118, KEB-CP038, KEB-CP 010, KEB-CP051, KEB-CP004, KEB-CP006 and MTA22), ten were moderately susceptible and two susceptible (KEB-CP068 and ET11). The general tendency is that as salinity increases, most genotypes will remain or shift from their previous ranking according to salinity tolerance to the next lower rank of salinity tolerance. Combining germination test and early growth trial, NO1036, KEB-CP004,

Table 5. Mean Comparison of the effect of Genotype × Salinity interaction on germination and dry matter production in cowpea at 6 weeks' growth and their classification according to salinity. DMP = Dry matter production; T = Tolerant; MT = Moderately tolerant; MS = Moderately susceptible; S = Susceptible.

Genotype	[NaCl] concentration	Germination percentage (%)	DMP mg/Plant	DMP loss to Control (%)	Salinity tolerance
ET11	0 mM	96.67 ab	271.33 bcdef		
	50 mM	54.44 efghij	174.00 bcdef	35.87 cde	MT
	100 mM	25.56 opqrst	115.66 cdef	57.37 ab	MS
	150 mM	15.56 vwxxyz	102.00 def	62.41 ab	S
	200 mM	5.55 z	71.00 f	73.83 a	S
KEB-CP045	0 mM	92.22 abcde	573.33 abcdef		
	50 mM	76.67 abcdefgh	439.67 abcdef	23.27 cde	MT
	100 mM	23.33 qrstuvwxy	437.67 abcdef	23.62 cde	MT
	150 mM	16.67 uvwxyz	393.66 abcdef	31.30 cde	MT
	200 mM	15.56 vwxyz	334.00 abcdef	41.71 bcd	MS
KEB-CP057	0 mM	95.56 abc	342.00 abcdef		
	50 mM	87.78 abcdef	241.67 bcdef	29.34 cde	MT
	100 mM	56.67 defghij	253.00 bcdef	26.02 cde	MT
	150 mM	16.67 uvwxyz	215.67 bcdef	36.94 cde	MT
	200 mM	7.78 xxyz	185.00 bcdef	45.71 bcd	MS
KEB-CP060	0 mM	100.00 a	285.00 bcdef		
	50 mM	100.00 a	267.33 bcdef	6.20 f	T
	100 mM	72.22 abcdefgh	184.33 bcdef	35.12 cde	MT
	150 mM	25.55 opqrstuvw	137.67cdef	51.69 ab	MS
	200 mM	15.56 vwxxyz	168.33 bcdef	40.94 bcd	MS
KEB-CP009	0 mM	88.89 abcde	330.00 abcdef		
	50 mM	94.44 abcd	293.33 bcdef	11.11 edf	T
	100 mM	73.33 abcdefg	227.67 bcdef	31.01 cde	MT
	150 mM	20.00 stuvwxy	197.00 bcdef	40.30 bcd	MS
	200 mM	5.55 z	185.00 bcdef	43.94 bcd	
KEB-CP-010	0 mM	82.22 abcdefgh	324.33 abcdef		
	50 mM	63.33 abcdefghij	325.33 abcdef	–0.31 f	T
	100 mM	33.33 nopqrst	255.67 abcdef	21.17 cde	MT
	150 mM	11.11 wxyz	253.33 bcdef	21.89 cde	MT
	200 mM	5.55 z	202.66 bcdef	36.39 bcd	MT
KEB-CP051	0 mM	58.67 bcdefg	602.67 abcdef		
	50 mM	46.67 ghijklm	529.00 abcdef	12.22 edf	T
	100 mM	42.67 ijklmnop	493.67 abcdef	18.09 cde	T
	150 mM	25.33 opqrstuv	483.00 abcdef	19.90 cde	T
	200 mM	5.33 z	377.00 abcdef	37.45 bcd	MT
KEB-CP054	0 mM	100 a	564.67 abcdef		
	50 mM	96.67 ab	567.00 abcdef	–0.41 f	T
	100 mM	45.56 ghijklm	548.00 abc	2.92 f	T
	150 mM	30.00 opqrstu	371.33 abcdef	34.34 cde	MT
	200 mM	17.78 uvwxyz	512.00 abcdef	44.75 bcd	MS
NO1036	0 mM	96.67 ab	608.00 abcdef		
	50 mM	83.33 abcdefg	518.67 abcdef	14.69 def	T
	100 mM	60.00 bcdefghij	579.67 abcdef	4.66 ef	T
	150 mM	40.00 jklmnopq	571.66 abcdef	5.98 ef	T
	200 mM	35.44 mnopqrst	529.33 abcdef	12.94 def	T

Genotype	[NaCl] concentration	Germination percentage (%)	DMP mg/Plant	DMP loss to Control (%)	Salinity tolerance
NO74	0 mM	96.67 ab	259.00 bcdef		
	50 mM	85.56 abcdef	250.00 bcdef	3.47 f	T
	100 mM	38.89 jklmnopqrst	223.66 bcdef	13.64 def	T
	150 mM	8.89 wxyz	205.33 bcdef	20.72 cde	MT
	200 mM	6.67 yz	127.33 cdef	50.84 bc	MS
KEB-CP118	0 mM	100.00 a	471.33 abcdef		
	50 mM	95.56 abc	435.33 abcdef	7.64 ef	T
	100 mM	37.77 klmnopqrst	377.16 abcdef	19.98 cde	T
	150 mM	26.67 opqrstuv	328.33 abcdef	29.34 cde	MT
	200 mM	4.44 z	271.34 bcdef	40.43 bcd	MS
KEB-CP039	0 mM	75.56 abcdefgh	347.66 abcdef		
	50 mM	78.89 abcdefgh	321.00 abcdef	7.67 ef	T
	100 mM	71.11 abcdefgh	254.00 bcdef	26.94 cde	MT
	150 mM	50.00 fghijklm	205.66 bcdef	40.84 bcd	MS
	200 mM	30.00 opqrstuvw	191.66 bcdef	44.87 bcd	MS
KEB-CP004	0 mM	98.89 a	474.00 abcdef		
	50 mM	85.56 abcdef	442.66 abcdef	6.61 ef	T
	100 mM	37.78 klmno	419.33 abcdef	11.53 def	T
	150 mM	30.00 opqrst	453.33 abcdef	4.36 f	T
	200 mM	24.44 pqrst	309.00 abcdef	34.81 cde	MT
KEB-CP020	0 mM	97.33 ab	602.67 abcdef		
	50 mM	92.00 abcde	569.00 abcdef	5.59 ef	T
	100 mM	33.33 nopqrst	556.66 abcdef	7.63 ef	T
	150 mM	4.00 z	421.66 abcdef	26.71 cde	MT
	200 mM	5.33 z	309.33 abcdef	48.67 bcd	MS
KEB-CP006	0 mM	94.44 abcd	624.00 ab		
	50 mM	81.11 abcdef	615.67 abcde	1.33 f	T
	100 mM	36.67 lmnopqrst	506.33 abcde	18.86 cde	T
	150 mM	21.11 rstuvwxxy	466.00 abcdef	25.32 cde	MT
	200 mM	3.33 z	473.66 abcdef	24.09 cde	MT
KEBCP067	0 mM	98.89 a	838.33 a		
	50 mM	96.67ab	637.00 abcd	24.02 cde	MT
	100 mM	30.00 opqrstuvw	531.00 abcdef	36.66 cde	MT
	150 mM	14.44 vwxxyz	534.00 abcdef	36.30 cde	MT
	200 mM	12.22 vwxyz	387.00 abcdef	53.84 ab	MS
KEB-CP068	0 mM	62.50 abcdefghijklm	324.00 abcdef		
	50 mM	40.00 jklmnopqrst	222.66 bcdef	31.27 cde	MT
	100 mM	19.67 tuvwxyz	231.00 bcdef	28.30 cde	MT
	150 mM	14.17 vwxxyz	94.66 ef	70.75 a	S
	200 mM	6.67 z	91.33 ef	71.81 a	S
KEB-CP038	0 mM	98.89 a	521.33 abcdef		
	50 mM	75.56 abcdefgh	516.66 abcdef	0.89 f	T
	100 mM	36.66 lmnopqst	464.66 abcdef	10.87 ef	T
	150 mM	17.77 uvwxy	457.00 abcdef	12.34 def	T
	200 mM	5.56 z	348.00 abcdef	33.25 cde	MT

Genotype	[NaCl] concentration	Germination percentage (%)	DMP mg/Plant	DMP loss to Control (%)	Salinity tolerance
KEB-CP033	0 mM	73.33 abcdefghij	575.33 abcdef		
	50 mM	57.78 cdefghijklm	521.34 abcdef	9.39 ef	T
	100 mM	28.89 opqrstuvw	528.00 abcdef	8.23 ef	T
	150 mM	21.21 rstuvwxxy	313.00 abcdef	45.60 bc	MS
	200 mM	15.55 vwxyz	328.00 abcdef	42.89 bc	MS
MTA22	0 mM	88.89 abcde	449.00 abcdef		
	50 mM	58.89 bcdefgh	446.00 abcdef	0.67 f	T
	100 mM	44.44 hijklmnop	369.66 abcdef	17.67 cde	T
	150 mM	32.22 opqrstuv	272.33 bcdef	39.35 cd	MT
	200 mM	12.22 qrstuvwx	302.66 abcdef	32.59 cde	MT

Within each column, different letters indicate significant differences (Tukey multiple range test at $p = 0.050$ probability level)

Table 6. Relationship between germination variables under salinity determined by Pearson correlation coefficients

Variables	GP	MGT	CVG	GRI
GP	1			
MGT	–0.578***	1		
CVG	0.436***	–0.893***	1	
GRI	0.913***	–0.715***	0.656***	1

Level of significance: ***$p < 0.001$, GP = Germination percentage; MGT = Mean germination time; GRI = Germination rate index; CVG = Coefficient of velocity of germination

KEB-CP038 and KEB-CP051, demonstrated best tolerance to salinity, whereas KEB-CP068 and ET11 were the most sensitive genotypes to salt stress.

Pearson correlation coefficients were determined for each pair of the four germination traits studied (Table 6) and for any couple of the thirteen growth characteristics considered (Table 7). Significant correlations were found between any pair of the germination traits (Table 6). Seventy-eight Pearson correlation coefficients were determined for all pair of the thirteen growth characteristics used for the study. Fifty among the seventy-eight correlations estimated (64.10%) were shown to be significantly positive; fifteen out of seventy-eight (19.23%) were significantly negative and the remaining 13 associations had no significant relationships (Table 7). Shoot fresh weight and the number of functional leaves each was significantly associated with the remaining twelve other growth characteristics. Growth was positively associated with nine other traits and negatively correlated with root length/seedling length ratio. The dry matter production was positively associated with nine other early traits and negatively associated with root dry weight/shoot dry weight ratio and root length/seedling length ratio. The number of functional leaves was positively associated with ten other growth traits and negatively associated with root dry weight/shoot dry weight ratio and root length/seedling length ratio. The shoot water content was positively associated with ten other early growth characteristics (Table 7).

DISCUSSION

In plant breeding, identifying individual specificities among genotypes of a target plant speciesis essential for better utilisation of the genetic resources of that species. This study aimed to explore salt stress tolerance of twenty main cultivated *Vigna unguiculata* genotypes at the germination and early growth stages in order to identify promising genotypes for their better utilisation in agricultural zones affected by salinity. High soil salinity represents a major abiotic stress reducing crop productivity in cultivated regions. Seed germination and seedling establishment are known to be critical processes in a plant's life, especially in the presence of adverse environment factors like salinity (Bohnert et al., 1995). Salt stress is known to cause nutrient imbalances and change of the level of growth regulators in plants. Salt therefore inhibits seed germination, plant's shoots and root growth with the direct result being yield loss in cultivated crops (Hasanuzzaman et al., 2013).

According to the literature, the mechanism of inhibition of seed germination by NaCl is likely to be vastly connected to the insufficiency of water absorption by seed due to salt, or attributed to toxic effects of salt on the embryo (Azza-Mazher et al., 2007). During the study, we found that salinity reduced and

Table 7. Relationship between growth variables under salinity determined by Pearson correlation coefficients

Variables	RFW	RDW	SFW	SDW	RL	SL	NFL	RL/SL	RDW/SDW	Growth rate	DMP	RWC	SWC
RFW	1												
RDW	0.686***	1											
SFW	0.659***	0.519***	1										
SDW	0.593***	0.565***	0.805***	1									
RL	0.368***	0.409***	0.256***	0.329***	1								
SL	0.439***	0.368***	0.496***	0.457***	0.256***	1							
NFL	0.293***	0.146*	0.473***	0.378***	0.157**	0.343***	1						
RL/SL	−0.218***	−0.110^{NS}	−0.299***	−0.252***	0.396***	−0.613***	−0.288***	1					
RDW/SDW	−0.079^{NS}	0.103^{NS}	−0.269***	−0.408***	−0.081^{NS}	−0.123*	−0.116*	0.063^{NS}	1				
Growth rate	0.417***	0.354***	0.484***	0.440***	0.272***	0.782***	0.321***	−0.509***	−0.094^{NS}	1			
DMP	0.653***	0.690***	0.807***	0.987***	0.368***	0.472***	0.360***	−0.243***	−0.338***	0.455***	1		
RWC	0.323***	−0.358***	0.208***	0.079^{NS}	−0.061^{NS}	0.084^{NS}	0.187**	−0.146*	−0.297***	0.071^{NS}	0.001^{NS}	1	
SWC	0.335***	0.196**	0.483***	0.152**	0.218***	0.184**	0.330***	−0.043^{NS}	−0.011^{NS}	0.161**	0.172**	0.261***	1

Level of significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, NS = non-significant, RFW = Root fresh weight; RDW = Root dry weight; SFW = Shoot fresh weight; SDW = Shoot dry weight; RL = Root length; SL = Seedling length; NFL = Number of functional leaves; RL/SL = Root length – Seedling length ratio; RDW/SDW = Root dry weight – Shoot dry weight ratio; DMP = Dry matter production; RWC = Root water content; SWC = Shoot water content

postponed the germination in cowpea, which is a long-standing view on seed germination under saline condition as reported previous studies in cowpea (Thiam et al., 2013; Islam et al., 2019) and related annual self-pollinated plants species like common bean (Kouam et al., 2017b) and rice (Hakim et al., 2010). Similar observations have been made on maize (Aliu et al., 2015). The general trend observed is that seeds germinated more slowly as salinity increases and could not even germinate at higher concentrations of salt. Like germination percentage that was reduced because of salinity, the experiment also revealed that salt stress induced lower germination rate index and coefficient of velocity of germination. Salt stress, however, increased mean germination time of the seeds of the studied genotypes. Salinity showed a significant variability in germination performance between the different cultivars tested. Most cultivars germinate at no (0 mM) or low salt concentrations of NaCl (50 mM). However, medium and high concentrations of NaCl (100, 150, and 200 mM) resulted in a significant reduction in the rate of seed germination for the tested cultivars. This tendency corroborates with previous studies on cowpea by Thiam et al. (2013) and in common bean by Kouam et al. (2017b). Gradual inhibition of germination as salinity increased likely resulted from seed hydration difficulties. This is due to high osmotic potential, with much more time required for seeds to implement mechanisms for adjusting their internal osmotic pressure because of elevated osmotic potential induced by salt that obstruct the emergence of the radicle off as concluded by Gill et al. (2003). At a maximum salt concentration of 200 mM NaCl, eight out of the twenty cowpea genotypes tested had no more than 5% of seeds germinated. This result indicates that the germination of some genotypes is completely inhibited under high salinity environments. Similar results were reported in Common bean (Kouam et al., 2017b). The present results showed that the higher the NaCl concentration, the lower the germination rate index of cowpea genotypes (Table 3, Table 4). This decrease in ability of cowpea seed to germinate under salt stress conditions can be attributed to a reversible osmotic effect inducing seed dormancy as highlighted by Mehrun et al. (2007). Mean germination time of cowpea seeds was considerably affected by salinity. Significantly higher number of days was required for germination of seed treated with 200 mM NaCl (Table 3, Table 4). Salt stress will limit uptake of water during germination and cause delays in seed germination (Kaydan and Yagmur, 2008). This delay in seed germination or prolongation of germination time of the studied seeds due to salinity was previously reported in common bean (Cokkizgin, 2012; Kouam et al., 2017b) and in cowpea (Islam et al., 2019).

Salinity appears to be a major threat to modern agriculture causing inhibition and impairment of crop growth and development (Isayenkov and Maathuis, 2019). Many studies revealed that reduction of plant growth due to salinity differs between plant species and even between cultivars within a same species (Negrão et al., 2016). These divergences observed are a likely link to the variability of salt tolerance among plant species or cultivar germplasms as shown by Ghoulam et al. (2002) and Kouam et al. (2017b). Like seed germination test, the effects of salinity were clearly manifested during the further seedling growth trial in the present study. This impact of salinity on growth was visible in terms of reduction of height, weight and number of functional leaves of plants and plant parts. According to Khalid et al. (2015), salinity is known to cause defection of metabolism in plants such as membrane permeability and, therefore expected to inhibit growth and plant development. This impairment of growth is demonstrated to be because of nutrient imbalances and dysfunction of growth regulators as reported by Fageria et al. (2011). Our results have shown in general that salt has negative effect on growth of cowpea seedlings. Significant disparities in growth characteristics were detected among the numerous genotypes tested. As well, substantial differences of growth behaviours were observed using the various concentrations of sodium chloride tested. Statistical analysis showed that 100 mM [NaCl] significantly slowed a decrease of growth and dry matter production in cowpea genotypes. Several studies similarly reported a significant reduction of dry matter production, growth of roots and shoots for both seedling and adult plants under salt stress (Murillo-Amador et al., 2000; Kouam et al., 2017b). This effect of biomass or dry matter production loss under salt stress was evident in cowpea as it is reported to be a classic response of plants during salinity. In agreement with this finding, biomass loss had been used for the evaluation of the kinetics of the dry matter amount in green bean under sodium chloride stress (Pessarakli, 1991). Salt intake induces a significant reduction in shoot weight of cowpea genotypes compared to controls. This reduction widens as salinity increases with maximum reduction at high salt concentration of 200 mM NaCl. Sánchez-Blanco et al. (1991) reported similar results, demonstrating that decrease in shoot weight of tomato genotypes occurs consecutively to salt stress. Still in agreement with our results, other studies on cowpea testified significant biomass reduction as soil salinity increases (Thiam et al., 2013). Similar depression effects of salt stress in plant biomass were reported in other related self-pollinated crops like common bean (Kouam et al., 2017b), soybean (Amirjani, 2010) wheat (Hamam and Negim, 2014) and rice (Hakim et al., 2010). This depressive effect of salinity mainly occurs in shoots compared to rootsof seedling as also reported by Thiam et al. (2013) on cowpea cultivars.

The twenty genotypes showed considerable variation in salinity tolerance at the germination level at any of low, medium or high salt concentration. A tmaximum concentration of salt (200 mM NaCl), four genotypes NO1036, KEB-CP004, KEB-CP038 and KEB-CP051) expressed considerable germination percentage ranging from 25 to 35%. In seedling growth trial, the results reveal that salinity treatment of 200 mM NaCl strongly predispose to loss of dry matter production of cowpea seedling. The rate of loss differed, however, between the twenty genotypes and at the different level of salinity. These differences indicate variation in the level of salinity tolerance in the studied genotypes as also reported studies using twelve variety of rice (Hakim et al., 2010) and eight genotypes of common beans (Kouam et al., 2017b). Four genotypes demonstrated important salinity tolerance at the low, medium and high stress level and are NO1036, KEB-CP004, KEB-CP038 and KEB-CP051. These genotypes demonstrate to be least affected by salinity. They may be potential sources of gene for salt stress tolerance and are identified as important in plant breeding as demonstrated in common bean by Kouam et al. (2017b) and in tomato by Singh et al. (2012). Correlation analysis generates the understanding of the role of shoot fresh weight, number of functional leaves and the dry matter production. These three growth characteristics appear to be key determinants needed to improve cowpea performance under salinity environments as each correlated significantly with all other growth variables.

CONCLUSION

The present study demonstrated considerable differences among the germination traits and seedling growth characteristics of *Vigna unguiculata* to salinity exposure. Germination in cowpea was significantly delayed with increasing salinity. Growth characteristics were considerably reduced by salinity. The twenty genotypes showed different ranges of tolerance to salinity. Combining germination and seedling growth results, cowpea genotypes, namely NO1036, KEB-CP004, KEB-CP038 and KEB-CP051 were the most tolerant whereas ET11 and KEB-CP068 were the most sensitive ones. These tolerant genotypes can be used as sources of gene of tolerance to salinity in cowpea breeding programs and should be encouraged for culture agricultural lands affected by salinity.

AUTHOR'S CONTRIBUTIONS

Eric Bertrand Kouam collected the plant material, conceived the study, analysed data, interpreted results and wrote the paper

Toscani Ngompe-Deffo performed lab assay and greenhouse trial.

Marie Solange Mandou, Asafor Henry Chotangui, Honoré Beyegue-Djonko, Souleymanou Adamou and Christopher Mubeteneh Tankou reviewed the manuscript prior to submission and provided substantial valuable comments on the interpretation and presentation of results.

CONFLICT OF INTEREST

The authors declared no conflicts of interest with respect to research, authorship and publication of this article.

ETHICAL COMPLIANCE

The authors have followed the ethical standards in conducting the research and preparing the manuscript.

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