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Research Article

Salinity tolerance at germination of some main cultivated cowpea (*Vigna unguiculata*) genotypes from Western Cameroon

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Abstract: This study investigates the effect of salt (NaCl) on the germination of seeds of 10 genotypes of cowpea (*Vigna unguiculata*). The experiment carried out on petri dishes was done using a completely randomized design comprising three replications. The germination of the cultivars was studied using distilled water as control and under osmotic potentials of 50, 100, 150 and 200mM NaCl. The results indicated that the Mean Germination Time (MGT), Time to 50% Germination (T_{50}), Germination Index (GI), Coefficient of Velocity of Germination (CVG), and Germination Percentage (GP), varied between 2.33 and 5.51 days; 2.83 and 14.87 days; 16.66 and 1.63; 0.53 and 0.11; 87.11 and 27.98% respectively. The variables GI, CVG and GP tended to decrease with increasing NaCl concentration while MGT and T_{50} tended to increase with increasing salt concentrations. For all the germination variables, significant differences at 0.001 probability levels were found among salinity treatments, cowpea genotypes and their interaction. Four genotypes (KEB-CP118, OU59A, OU100 and ICV12) were tolerant to salt stress and performed well under salinity conditions. Four other genotypes were found to be moderately tolerant (ET11, NO1036, MTA22 and KEB-CP009) and two genotypes (KEB-CP098 and NO193) were susceptible to salt stress and were most affected by salinity. At 0.001 probability level, positive correlations were observed between GP and GI ($r = 0.792$); GP and CVG ($r = 0.774$); GI and CVG ($r = 0.955$) and negative correlations were observed between GP and T_{50} ($r = - 0.845$); GI and MGT ($r = - 0.894$); CVG and MGT ($r = - 0.895$).

Keywords: Cameroon; Correlation; Germination; NaCl; Salinity tolerance; *Vigna unguiculata*

Introduction

Cowpea (*Vigna unguiculata*, (L.) Walp.) is a tropical leguminous plant species belonging to the *Fabaceae* family. The importance of this crop species in the Sub-Saharan regions cannot be overemphasized. Dry or fresh seeds contain 23–32% of protein and 64% of carbohydrate; the immature pods and leaves are used for human and animal consumption (Mahamadou *et al.,* 2013). In addition, hay from cowpea is used for animal feeding during the dry season (Chinma *et al.,* 2008). Cowpea appear then as a valuable source of income for farmers and grain traders in many African countries (Langyintuo *et al.,* 2003; Timko *et al.,* 2007). The market value in Sub-Saharan Africa is estimated at US$ 662 / ton (Akibode, 2011). Cowpea production in Sub-Saharan Africa amounts to be 4.93 million tons of dry seed per year on 11.03 million ha and making an average yield of 0.45 ton / ha (Akibode, 2011). African production accounts for more than 70% of the world production and occupies 80% of the world's surface area for cowpea (Tengomo, 2011).

Salinity is one of the main constraints for agricultural productivity affecting almost 80 million hectares of arable lands worldwide (Yamaguchi and Blumwald, 2005). A soil is considered salty when the electrical conductivity is greater or equal to 4 dS·m−1, making approximately 40mM NaCl (Munns and Tester, 2008). Salt stress is induced by a wide range of dissolved salts, but NaCl is the most widespread one (Munns and Tester, 2008; Rengasamy, 2002). To enhance understanding of the mechanisms of tolerance in high salinity conditions, several studies have been carried out during the last three decades on plants collected worldwide. These investigations led to the conclusions that saline soils present unfavorable conditions for seed germination, plant growth and production (Mahamadou *et al.,* 2013). Irrigation induces accumulation of salt at soil surface that negatively affect seed germination, plant vegetative development and productivity (Dantas *et al.,* 2007; Tawfik, 2008). At some worst cases, salinity may cause plant death (Dantas *et al.,* 2007; Tawfik, 2008). Salinity reduces the ability of plants to utilize water and causes a reduction in growth, as well as changes in plant metabolic processes (Munns, 1993, 2002). The impact of the salt will depend on the plant species, salinity levels, and the ionic composition (Yadav *et al.,* 2010). According to Asana and Kale (1965) and Maas and Hoffman (1977), germination aptitude of seeds under salinity stress will differ from one crop to another and significant variation can be observed among

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different genotypes of the same crop as well. It appears important to identify the sensitivity and acceptance level of a variety or a genotype at germination stage for successful crop production in a saline milieu. Therefore, this study was undertaken to identify the salt-tolerance of some cowpea genotypes at germination level.

Materials and Methods

Plant materials

Plant materials were cowpea seeds of ten accessions: Eight from Cameroon (MTA22, KEB-CP009, KEB-CP098, KEB-CP118, NO193, NO1036, OU59A, OU100) one from Kenya (ICV12) and one from Ethiopia (ET11). The study was conducted under controlled environmental conditions in the laboratory of genetics at the Faculty of Agronomy and Agricultural Sciences of the University of Dschang in Cameroon during April and May 2016.

NaCl Solutions and seed treatments

Healthy and uniform cowpea seeds of each accession were surface-sterilized with 7% sodium hypochlorite (NaOCl) for 20 min (Sauer and Burroughs, 1986) and washed twice with distilled water for 10 min. Seeds were then germinated in 90 mm diameter sterilized Petri dishes. All Petri dishes were washed with tap water and rinsed with distilled water. NaCl solutions were prepared at a concentration of 0.0 (Control), 50, 100, 150 and 200mM (Saline (NaCl) conditions). The Petri dishes were arranged in a completely randomized block design with three replications. A total of 30 seeds were placed in each Petri dish on double-layer Whatman paper N° 1. 10 ml of appropriate solution was added to each Petri dish (Asgharipour and Rafiei, 2011). Seeds were imbibed in NaCl solutions for 24 H at room temperature. Seeds were then drained, rinsed twice with distilled water, and were allowed to continue germination on new moist double-layer Whatman paper. The seed counting process started 24 hours after seeds were moistened for the first time. That process was repeated every day at the same hour. After sowing every day, the germinated seeds were counted, recorded and removed from the Petri dishes. Germination was counted when a 5-mm radicle had emerged from the seed coat (Sayar *et al.,* 2010). The study was concluded after 21 days.

Variables studied

Germination percentage (GP)

The number of germinated seeds was recorded every 24 h (AOSA, 1990). After 21 days, the germination percentage (GP) was obtained by dividing the number of germinated seeds in any Petri dishes by the total number of seeds tested, multiplied by 100 (Cokkizgin and Cokkizgin, 2010; Tanveer *et al.,* 2010) as described below

$$GP = \frac{Total\ number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ tested} X\ 100$$

Mean germination time (MGT)

The mean germination time (MGT) was calculated to assess the rate of germination (Hu *et al.,* 2005) as follows:

$$MGT = \frac{\sum T_i N_i}{\sum N_i}$$

Were Ni = number of the newly germinated seeds in times of Ti (day)

Time to 50% germination (T_{50})

This is the time at the end of which 50% of the tested seeds have germinated (Côme *et al.,* 1970). It was calculated using this formula below:

$$T_{50} = T_1 + \frac{(0.5 - G_1)}{(G_2 - G_1)} \times (T_2 - T_1)$$

With:

G_1= cumulative percentage of germinated seeds whose value is closest to 50% by lower value.

G_2= cumulative percentage of germinated seeds whose value is closest to 50% by higher value.

T_1= time whose cumulative percentage of germinated seeds is closer to 50% by lower value.

T_2 = time whose cumulative percentage of germinated seeds is closer to 50% by higher value.

Germination index (GI)

Germination Index (GI) was calculated as described by the Association of Official Seed Analysts (AOSA, 1983):

$$GI = \sum \left(\frac{G_t}{T_t}\right) = \frac{G.\ first\ count}{Days\ to\ first\ count} + \cdots + \frac{G.\ last\ count}{days\ to\ last\ count}$$

With G = Number of germinated seeds

Coefficient of velocity of germination (CVG)

Coefficient of velocity of germination (CVG) was evaluated according to Maguire (1962) as follows:

$$CVG = \frac{G_1 + G_2 + \cdots + G_n}{1 \times G_1 + 2 \times G_2 + \cdots + n \times G_n}$$

Where *G* is the number of germinated seeds and *n* is the last day of germination.

Salt tolerance index (STI)

Salt tolerance index (STI) was calculated as according to Zeng *et al.,* (2002)

$$STI = \frac{Germination\ under\ salinity}{Germination\ in\ control}$$

Statistical analysis

Data of the germination percentage, germination index, mean germination time, time to 50% germination and the coefficient of velocity of germination were analyzed using XLSTAT 2014 and GraphPad Pism 6.0 softwares packages. Analysis of variance (ANOVA) was conducted to test the significance of differences among salinity treatment, among accessions and their interaction. Differences were declared very significant at $P <$ 0.001 and significant $P < 0.01$ probability levels by the *F* test. Pearson correlation coefficients were used to assess all the germinations variables under salinity. Relationships between NaCl concentrations and germination percentage, germination index, mean germination time, time to 50% germination and the coefficient of velocity of germination data of the genotypes were performed using r^2 goodness-of-fits of the linear regression from GraphPad Prism 6.0 computer program

Results

This study revealed a significant effect of salinity on germination traits of cowpea genotypes (Table 1). The germination percentage under control conditions fluctuated from 39 (*NO193*) to 100% (*OU100; OU59A* and *ICV12*). Under salinity stress at 100mM and 200mM NaCl, the germination percentage ranges from 1.00 (*NO193*) to 98.33 (*ICV12*) and 0.00 (*NO193*) to 68.33 (*ICV12*) respectively. As the NaCl concentration increased, all the ten cowpea genotypes showed a decreasing trend of germination percentage (Figure 1A). For example, the germination percentage for the genotype NO1036 at 0, 50, 100, 150 and 200mM NaCl treatment were 94, 94, 88, 44 and 2 % respectively. This shows that salinity stress unfavorably affected the germination of cowpea cultivars. Though the germination of cowpea seeds was strongly inhibited when subjected to salinity stress, the degree of inhibition differed markedly between genotypes. The ten genotypes studied here could be classified into three groups depending on their ability to germinate in saline medium. The most salt tolerant genotypes with the smallest degree of salt inhibition. These genotypes (*OU59A, OU100, ICV12 and KEB-CP118*) had higher salinity tolerance index: > 0.8 at 150mM NaCl and still had a STI more the 0.6 at the highest salinity condition (200mM NaCl) (Table 1). Four genotypes were found to be moderately tolerant (*ET11, NO1036, MTA22* and *KEB-CP009*) with their STI more than 0.8 at 100mM Nacl and less than 0.5 at 200mM NaCl (Table 1). The least tolerant (susceptible genotypes) were *NO193* and *KEB-CP098*. This group had a STI less than 0.5 at 100mM NaCl and STI = 0 at 200mM NaCl (Table 1). A two-way Analysis of Variance showed a significant individual effect of salinity, genotype and their interaction in affecting germination variables in cultivated *V. unguiculata* (Table 3). The germination index (GI) and the coefficient of velocity of germination (CVG) had the same change trend with that of germination percentage (Table 1). The highest GI (16.66) and CVG (0.53) were observed in the control and the lowest values were observed at 200mM NaCl treatment (1.63 for GI and 0.11 for CVG)). Fig. 1B and 1E shows the change in GI and CVG respectively with different NaCl osmotic potentials. The different levels of NaCl also significantly affected the mean germination time (MGT) and time to 50% germination (T_{50}) (Table 1, Table 2 and Table 3). For all genotypes, significant greater number of days for MGT (5.11) and T_{50} (14.87) was obtained from treatments in 200mM NaCl as compared with controls (MGT = 2.33 days and T_{50} = 2.83 days) (Table 2). Fig. 1C and 1D shows the variation of MGT and T50 as affected by NaCl osmotic potentials of cowpea seeds. The interaction between salinity level and genotypes were significant for all the germination parameters studied (Table 3). Significant positive and negative Pearson correlations were found between all the germination variables studied (Table 4).

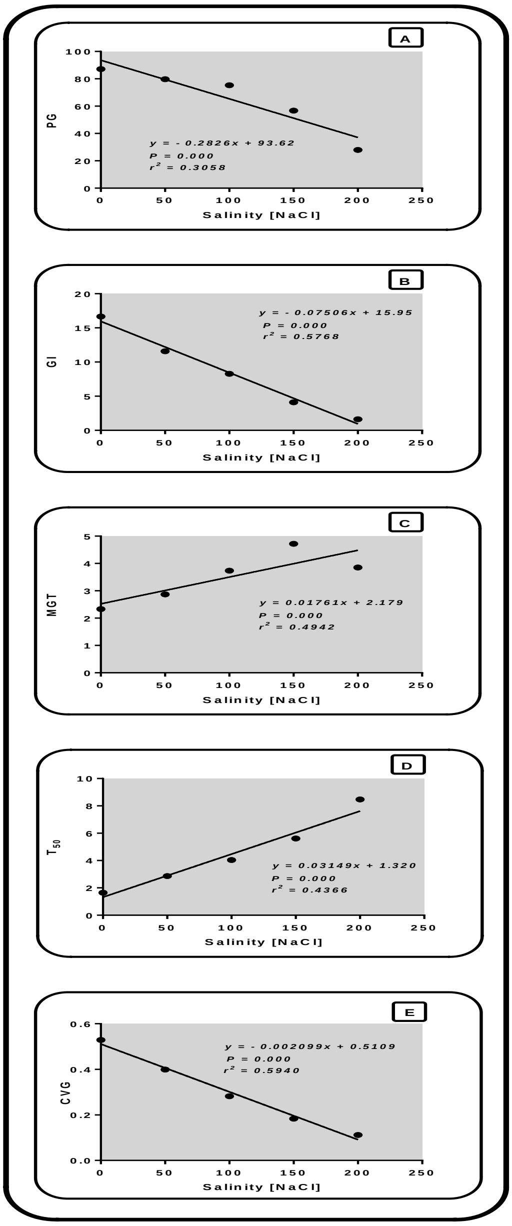

Figure 1: Relationship between germination percentage (GP), germination index (GI), mean germination time (MGT), time to 50% germination (T50), coefficient of velocity of germination (CVG) and salinity (NaCl) level in V*igna unguiculata* seeds.

Table 1: Effects of different salinity levels on Germination percentage, Germination index, Mean germination time, Time of 50% germination, Coefficient of velocity of germination and the Salt tolerance index of some cowpea genotypes

Parameter	Salinity	Genotype									
		ET11	NO1036	NO193	KEB-CP118	MTA22	OU100	KEB-CP098	KEB-CP009	OU59A	ICV12
PG	0mM	59.00	94.00	39.00	100.00	92.00	100.00	97.67	98.33	100.00	100.00
	50mM	52.00	94.00	4.00	98.57	92.00	100.00	68.33	91.67	100.00	98.33
	100mM	51.00	88.00	1.00	97.14	86.00	98.00	38.33	86.67	98.00	98.33
	150mM	12.00	44.00	0.00	95.71	74.00	86.00	15.00	68.33	80.00	91.67
	200mM	0.00	2.00	0.00	71.43	32.00	32.00	0.00	10.00	64.00	68.33
GI	0mM	13.53	11.71	3.89	21.33	13.13	17.50	21.91	26.67	17.79	19.17
	50mM	9.61	8.44	0.44	17.25	11.56	13.50	6.21	20.85	12.92	14.92
	100mM	7.32	4.45	0.05	14.92	8.99	10.64	2.06	13.71	8.73	11.81
	150mM	1.30	1.66	0.00	10.43	4.24	4.87	0.66	7.06	4.28	6.66
	200mM	0.00	0.06	0.00	4.58	1.41	1.37	0.00	0.54	4.78	3.52
MGT	0mM	3.37	2.19	6.29	1.77	1.86	1.64	1.62	1.20	1.62	1.73
	50mM	3.74	3.22	4.83	2.16	2.24	1.98	4.71	1.62	2.12	2.07
	100mM	3.80	5.58	5.00	2.51	3.22	2.70	6.05	2.41	3.31	2.76
	150mM	5.38	6.82	/	3.93	5.15	5.19	7.00	3.22	5.72	4.81
	200mM	/	4.00	/	6.12	5.76	5.9	/	6.13	4.60	6.03
T50	0mM	5.28	1.52	13.54	1.34	1.51	1.13	0.96	0.63	1.12	1.3
	50mM	8.95	2.56	/	1.60	1.71	1.49	5.25	1.03	1.62	1.55
	100mM	7.75	5.23	/	1.81	2.41	2.04	11.19	1.75	2.54	1.94
	150mM	35.94	10.21	/	3.22	6.06	5.21	38.57	4.06	6.00	4.55
	200mM	/	/	/	6.6	10.91	11.25	/	46.87	7.00	6.60
CVG	0mM	0.30	0.46	0.16	0.57	0.54	0.61	0.62	0.83	0.62	0.58
	50mM	0.27	0.31	0.21	0.46	0.45	0.51	0.21	0.62	0.47	0.48
	100mM	0.26	0.18	0.05	0.39	0.32	0.37	0.17	0.42	0.30	0.36
	150mM	0.19	0.15	0.00	0.26	0.20	0.19	0.14	0.31	0.18	0.21
	200mM	0.00	0.06	0.00	0.16	0.17	0.17	0.00	0.16	0.23	0.17
STI	50mM	0.88	1.00	0.10	0.98	1.00	1.00	0.70	0.93	1.00	0.98
	100mM	0.86	0.93	0.03	0.97	0.93	0.98	0.39	0.88	0.98	0.98
	150mM	0.20	0.47	0.00	0.95	0.80	0.86	0.15	0.69	0.80	0.92
	200mM	0.00	0.21	0.00	0.71	0.35	0.32	0.00	0.10	0.64	0.68

Table 2: Salinity effects on germination parameters in cowpea (*Vigna unguiculata*)

Salinity level (NaCl)	Germination parametres				
	GP (%)	GI	MGT (Days)	T_{50} (Days)	CVG
0mM	88.00 ± 6.73^{a}	16.66 ± 2.01^{a}	2.33 ± 0.47^{c}	2.83 ± 1.26^{b}	0.53 ± 0.06^{a}
50mM	79.89 ± 9.81^{a}	11.57 ± 1.83^{b}	2.87 ± 0.37^{bc}	2.86 ± 0.87^{b}	0.40 ± 0.04^{b}
100mM	74.25 ± 10.49^{ab}	8.27 ± 1.54^{c}	3.73 ± 0.42^{b}	4.07 ± 1.12^{b}	0.28 ± 0,03^{c}
150mM	56.67 ± 11.40^{b}	4.12 ± 1.05^{d}	5.24 ± 0.40^{a}	12.65 ± 4.70^{a}	0.18 ± 0.02^{d}
200mM	27.98 ± 9.54^{c}	1.63 ± 0.61^{e}	5.51 ± 0.32^{a}	14.87 ± 6.45^{a}	0.11 ± 0.03^{d}

Means followed by the same letter in the same column are not significantly different at p = 0.050 probability level

Table 3: Summary of analysis of variance of genotypes, salinity and genotype - salinity interaction for all the analyzed germination parameters

Source of Variation	df		Germination parameters				
			PG	GI	MGT	T_{50}	CVG
Genotype	9	MS	15549.52	319.59	3.15	8224.36	0.25
		F	334.9	315.05	2.67	178.56	441.13
		P value	0.000	0.000	0.007	0.000	0.000
Salinity	4	MS	22467.76	1425.61	34.36	4262.66	1.11
		F	483.91	1405.33	29.17	92.55	1965.59
		P value	0.000	0.000	0.000	0.000	0.000
Genotype X Salinity	36	MS	862.15	33.01	16.28	2732.25	0.02
		F	18.57	32.53	13.82	59.32	35.29
		P value	0.000	0.000	0.000	0.000	0.000

Table 4: Summary of Pearson's correlation coefficients between the analyzed germination parameters

	GP	IG	MGT	T_{50}	CVG
PG	1.000				
IG	0.792***	1.000			
MGT	- 0.695***	- 0.894***	1.000		
T_{50}	- 0.845***	- 0.597**	0.628***	1.000	
CVG	0.774***	0.955***	- 0.896***	- 0.520**	1.000

: Significant at 0.01 probability level; *: Significant at 0.001 probability level

Discussion

From the economical point of view, screening crops for salinity tolerance is of great importance for better utilization of zones affected by salt. To explore salt tolerance of 10 genotypes of cultivated *Vigna unguiculata* at the germination stage, four level of salt stress and a control was examined in this study. The experiment revealed that salt stress induced lower germination percentage, germination index and coefficient of velocity of germination and increased time for germination (Mean germination time and time to 50% germination) of the ten genotypes seeds (Tables 1, Table2, Table 3 and Figure 1). For example, four genotypes (*ET11, NO1036, NO193* and *KEB-CP098*) at 200mM NaCl had less than 2% of germinated seeds with their germination indices closes to zero (Table 1). These results indicate that the germination of some genotypes is completely inhibited under high salinity conditions. Similar results were highlighted in *Phaseolus* species (Alihan, 2012; Eroglu, 2007; Bayuelos *et al.,* 2002) and *Crithmum* species (Duros and Magne, 2008). A significant and negative slope was established in the relationship between data for germination index and NaCl concentration (Figure 1B). The higher the NaCl concentration, the lower the germination index of cowpea genotypes (Table 2, Figure 1B). The decrease in ability of cowpea seeds to germinate under salinity conditions is likely due to a reversible osmotic effect that induced dormancy by saline stress (Mehrun-Nisa *et al.,* 2007).

Germination time in cowpea seeds was considerably affected by salinity. The different levels of NaCl significantly affected the mean germination time (MGT) and time to 50% germination (T_{50}) (Table 1). Significant greater number of days for MGT and T_{50} for all genotypes was obtained from treatments in 200mM NaCl. Fig. 1B and 1C shows the relationship between the MGT, T_{50} and NaCl osmotic potentials of cowpea seeds through regression analysis with slopes of these two regressions statistically different from zero. As the increase in salinity decrease the germination, it also delays the germination initiation as MGT and T_{50} increase with the salinity (Hajer *et al.,* 1996). These delay or inhibition in germination under salt stress is due to osmotic effect limiting uptake of water during seed germination (Flowers, 1986; El-Baz *et al.,* 2003). Karagiizel (2003) reported that germination time in several plant species considerably increases with an increase in salt concentration. This delay in seed germination or prolongation of germination time of the genotypes studied has also been observed in *oryza* species by Hakim *et al.,* (2011) and *Limonium* species (Redondo-Gomez *et al.,* (2008) who detected the increase in MGT with the increase in NACl osmotic potentials. The extension of time to 50% germination observed in this study also fits the conclusion of Mrani *et al.,* (2013) on Moroccan wheat varieties.

Conclusion

Considerable variations in salt tolerance were found among the studied cowpea genotypes at the germination level. The relationship between germination variables and NaCl osmotic potentials through regression analysis were significantly different from zero at 0.001 probability level. The percentage of germination (PG), germination index (GI) and the coefficient of velocity of germination (CVG) decreased significantly with increasing salinity while the mean time of germination (MGT) and time to 50% germination (T_{50}) (Figure 1) increased significantly with increasing salinity. Positive significant correlations were found between Germination percentage and Germination index (r = 0.792) and Coefficient of velocity of germination (r = 0.774). Negative significant correlation were observed between germination percentage and Mean germination time (r = -0.695) and time to 50% germination (r = -0.845)). According to the results, genotypes *OU59A, ICV12, KEB-CP118* and *OU100* were identified as tolerant; *ET11, NO1036, MTA22* and *KEB-CP009* were considered as moderately tolerant. The two other genotypes (*NO193* and *KEB-CP098*) were susceptible to salt stress. Further study will be necessary to assess whether these genotypes characterized at the germination level as tolerant based on their responses to salt stress maintains their tolerance at other growth and maturity stages.

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