

Screening of thirteen salt-tolerant cowpea (*Vigna unguiculata* (L.) Walp) accessions during germination

Christiane Fokoua Djocne

University of Yaoundé I

Edwige Teufack Lekeumo

University of Yaoundé I

Nono Carine Temegne

`carine.temegne@facsciences-uy1.cm`

University of Yaoundé I

Michaël Kenne Fomekong

University of Yaoundé I

Laura Scottie Tchinda Ngouana

University of Yaoundé I

Leslie Sodje Ngayakal Cheye

University of Yaoundé I

Odile Lili Ndong Evoung

University of Yaoundé I

Bibiche Dorice Donke Mbumbia

University of Yaoundé I

Esaïe Tsoata

University of Yaoundé I

Emmanuel Youmbi

University of Yaoundé I

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Abstract

Soil salinity is one of the most important production constraints. Many studies have attempted to find a solution to this problem. The general objective of this study was to evaluate the influence of salinity on cowpea seed germination. Two salt concentrations (0 control and 150 μM NaCl) and thirteen varieties were tested in the laboratory following randomized complete block design with three replicates per treatment. The number of germinated seeds was recorded every until the eighth day, when the germination parameters were evaluated. The results indicate that the germination percentage (GP), the germination index, the seed vigour index, the germination vigour index, the velocity coefficient and the emergence rate decreased significantly ($p \leq 0.05$) in the saline treatment, while the latency time, the 50% germination time (T) and mean germination time increased. Thus, on day 8, the PG of V1(IR15M02) was 83.33% and 43.33% for 0 and 150 μM respectively. The T increased by 55.67% to 150 μM compared to 0 μM in V11(LORI). Seedling length decreased from 8.30 cm in control to 2.00 cm at 150 μM in V10(LOCAL3GG). The classification of varieties resulted in four groups: group 1 consisting of susceptible V1(IR15M02), V6(IR15MA33), V10(LOCAL3GG), V11(LORI) and V12(MTA22); group 2 corresponding to moderately susceptible V2(IR16MAK), V4(TILIGRE), V8(LOCAL3PG) and V9(BRI); group 3 comprising moderately tolerant V7(LOCAL3fht) and group 4 including V3(WIBALI), V5(IR16MAP) and V13(KOMCALLE), which are tolerant to salinity at the germination stage. The varieties in groups 3 and 4 can therefore be recommended for their tolerance to salinity during germination.

1 Introduction

According to the United Nations Organisation publication, approximately 720 million people suffered from hunger in 2024, representing 8.8% of the world's population. Although this represents a slight decrease compared to previous years, the situation remains worrying, especially in Africa, where hunger is on the rise due to wars, climate change and economic difficulties [1]. In addition, the world's population is constantly growing. However, this population increase far outstrips that of agricultural production. Thus, the challenge for agricultural research is to work to increase crop and soil production while protecting the environment. In this regard, cowpea (*Vigna unguiculata* (L.) Walp) could play an important role.

Indeed, grain legumes, particularly cowpea, play a key socio-economic role in tropical Africa, where they are a traditional part of the local diet [2, 3]. They are introduced into rotation and crop rotation systems with grasses [4, 5]. Cowpeas are rich in protein (26%), fibre (42%), magnesium (37%), iron (30%), B vitamins, etc. [6]. However, despite consumption needs, there has been a decline in legume production in Cameroon since the 1980s. The low production of cowpea is linked to biotic (diseases and pests) and abiotic (nutritional stress, water stress, salinity, etc.) constraints [7, 8].

In addition, a gradual decline in arable land in coastal, arid and semi-arid areas caused by salt accumulation due to insufficient rainfall, proximity to the sea, poor drainage and prolonged droughts is being observed globally and in Cameroon in particular. Furthermore, in 2024, 1.4 billion hectares of land (~ 10% of the total land area of the globe) are already affected by salinisation [9]. Salinity negatively affects the physical, chemical and biological properties of cultivated soils and, in turn, the agronomic potential of the soil.

In Cameroon, the South-West, Littoral, South, Adamaoua, North and Far North regions have high salt concentrations [10, 11, 12]. Several studies show that by 2050, this salinity due to sodium chloride will affect more than 50% of arable land [13]. Effective management of saline cultivated land requires the restoration of fertility through amendments; the elimination of excess salts; the suppression of the source of NaCl; and the use of salt-tolerant

cultivars. The use of tolerant cultivars is an essential feature of most breeding programmes and a reliable control tool that takes ecological and economic factors into account. Variety selection is therefore one of the most appropriate solutions for solving the problem of salinity in this work. In addition, the identification of salt-tolerant varieties would be more economically efficient for the exploitation of soils affected by salt stress.

Apart from the work of [14], no study has been conducted on the selection of cowpea varieties tolerant to salinity during germination in Cameroon. Furthermore, these authors used ten cowpea accessions that were different from those tested in this study. The overall objective of this study is therefore to assess the effect of salinity on cowpea seed germination in the laboratory.

2 Material and methods

2.1 Biological material

The biological material consisted of seeds from thirteen (13) varieties of cowpea from the accession collection of the IRAD (Agricultural Research Institute for Development) in Foumbot, Maroua, and from women farmers in the West (Fig. 1, Table 1). These seeds were provided by IRAD and did not need a license for usage by customers (farmers, producers, researchers, etc.) and are free to utilize.

Table 1
Visual characteristics of the accessions used. Var: variety

Var	Names	Seed color	Appearance seed	Shape seed	Seed size	Eye color
V1	IR15M02	White	Rough	oval	Large	Black-white
V2	IR16MAK	White	Rough	oval	Large	Black-white
V3	WIBALI	White	Rough	oval	Large	Red - white
V4	TILIGRE	White	Rough	oval	Large	Black-white
V5	IR16MAP	White	Rough	oval	Average	White-black
V6	IR15MA33	White	Rough	oval	Large	White-black
V7	LOCAL3fbt	Brown	Smooth	oval	Small	Black-white
V8	LOCAL3PG	Light brown	Smooth	oval	Very small	Black-white
V9	BRI	White	Rough	oval	Large	Black-white
V10	LOCAL3GG	Brown	Smooth	oval	Small	Black-white
V11	LORI	White	Rough	oval	Large	Black-white
V12	MTA22	Brown	Smooth speckled	oval	Small	Black-white
V13	KOMCALLE	White	Rough	oval	Average	Black-white

3 Methods

3.1 Evaluation of the effect of salt stress on seed germination parameters

The saline solution was prepared by dissolving 8.77 g of NaCl in one liter of distilled water [15, 16]. The solutions prepared correspond respectively to:

- 0 mM NaCl consisting of distilled water used as a control and
- 150 mM NaCl used to induce salt stress.

The cowpea varieties were selected by sorting the seeds to ensure they were vigorous, without malformations or perforations. They were then disinfected with a 70° alcohol solution for 1 minute and rinsed three times with distilled water. The sterilized seeds were then placed in Petri dishes that have been labeled and lined with filter paper to allow for good distribution of the solution and air, at a rate of 10 seeds per dish. Each treatment was repeated three times. A total of 78 Petri dishes were used, at a rate of 39 per treatment, for a total of 780 seeds, or 390 seeds for each treatment. Five (5) mL of each prepared solution were taken using a Pasteur pipette and used to moisten the Petri dishes containing the disinfected seeds as needed. These dishes were then incubated in the laboratory at $25 \pm 3^{\circ}\text{C}$ under a 12/12 photoperiod using a randomized complete block design with three factors: NaCl concentrations (0 and 150 mM), varieties (V1, V2, V3, V4, V5, V6, V7, V8, V9, V10, V11, V12, and V13) and blocks.

Germination was noted when the radicle emerges from the seed coat and was at least 2 mm long. Germinated seeds were counted every day for 8 days. Then, the length of the radicle, the length of the stem, and the number of secondary roots were evaluated randomly in each treatment (3 replicates/treatment) on the 8th day, allowing the germination parameters to be calculated.

3.2 Evaluation of seed germination parameters

The germination percentage (GP) is the ratio between the number of germinated seeds and the total number of germinated seeds multiplied by one hundred. It was obtained using the following formula [17]:

$$\text{GP (\%)} = \frac{(\text{number of sprouted seeds})}{(\text{number of seeds germinated})} \times 100.$$

The germination index (GI) is the weighted sum of the daily number of germinated seeds. [18].

$$\text{GI} = \left[\frac{N^{\circ} \text{ of germinated seeds at first count}}{\text{day of first count}} \right] + \dots + \left[\frac{N^{\circ} \text{ of sprouts at final count}}{\text{final count day}} \right]$$

This is the time (TL) required (in days) to observe the first germinated seed [19].

The 50% germination time (T_{50}) corresponds to the time required to obtain 50% of the germination capacity of the batch [19].

Germination duration (GD) is the time (in days) between the first germination of the experimental batch and the last germination of that batch [20].

The velocity coefficient (VC) [21] defines the speed of germination as a function of time.

$$\text{VC} = \frac{(G_1 + G_2 + \dots + G_n)}{(1 \times G_1 + 2 \times G_2 + \dots + n \times G_n)} \times 100$$

Where: G = Number of germinated seeds and n = Last day of germination.

The average (mean) germination time (MGT) is the sum (from 1 to n) of the products of the number of seeds germinated and the day on which they germinated, divided by the total number of seeds germinated at the end of the test [22].

$$\text{MGT} = \frac{\sum (Dn)}{\sum n} \text{ Where: } n = \text{the number of seeds germinated each day and } D = \text{the day of counting.}$$

The seed vigor index (SVI) was determined according to [23].

$$\text{SVI} = \text{Seedling length (cm)} \times \text{GP (germination percentage)}$$

The germination vigor index (GVI) was determined as follows [24]:

$$\text{GVI} = \text{Root length (cm)} \times \text{GI (germination index)}$$

The emergence rate (EV) was determined by the formula [25]:

$$\text{EV} = \frac{\text{Number of germinated seed at the first day}}{\text{Number of germinated seed at the last day}}$$

4 Data analysis

The collected data was subjected to ANOVA using R Studio software version 4.0.5. The mean comparison test was performed using Duncan's test at a 5% threshold. Data that did not meet the ANOVA assumptions (normality and homogeneity of variance) were analysed using the non-parametric Chi-square (X^2) test. Principal component analysis (representations of individuals) and dendrograms were performed using R Studio software. Graphical representations were created using Microsoft Excel.

5 Results

5.1 Effect of salt stress on seed germination parameters

NaCl significantly reduced ($p < 0.001$) the germination percentage of cowpea seeds (Fig. 2, Table 2). Thus, on day 6, it decreased by 48% compared to the control at V1.

The germination index decreased significantly ($p \leq 0.001$) with salt treatment except at V5, V8, V9 and V10 (Fig. 3). Thus, at V1, from 0 to 150 μM , it decreased from 14.51 to 7.1

Table 2

Effect of salinity and variety on the germination percentage of cowpea seeds over time. Var.: varieties, V1 = IR15M02, V2 = IR16MAK, V3 = WIBALI, V4 = TILIGRE, V5 = IR16MAP, V6 = IR15MA33, V7 = LOCAL3ft, V8 = LOCAL3PG, V9 = BRI, V10 = LOCAL3GG, V11 = LORI, V12 = MTA22, V13 = KOMCALLE. **Treatment effect:** for each day, by variety, the means $\pm$ standard deviations with the same letters (uppercase) are not significantly different at the 5% threshold. **Variety effect:** for each day and for each salt concentration, the means $\pm$ standard deviations with the same letters (lowercase) are not significantly different at the 5% threshold of Duncan's test

PEG	Var.	Time (day)							
		1	2	3	4	5	6	7	8
T0	V1	3.3 $\pm$ 5.8 ^{Ab}	4.30 $\pm$ 32.4 ^{Abcd}	70.0 $\pm$ 26.4 ^{Aabc}	83.3 $\pm$ 15.2 ^{Aa}	83.3 $\pm$ 15.3 ^{Aa}	83.3 $\pm$ 15.3 ^{Abc}	83.3 $\pm$ 15.3 ^{Abc}	83.3 $\pm$ 15.3 ^{Abc}
	V2	6.7 $\pm$ 5.80 ^{Ab}	56.7 $\pm$ 25.1 ^{Aabcd}	83.3 $\pm$ 20.8 ^{Aabc}	90.0 $\pm$ 17.3 ^{Aa}	93.3 $\pm$ 11.5 ^{Aa}	93.3 $\pm$ 11.5 ^{Aab}	93.3 $\pm$ 11.5 ^{Aab}	93.3 $\pm$ 11.5 ^{Aab}
	V3	10.0 $\pm$ 10.0 ^{Ab}	86.7 $\pm$ 15.3 ^{Aab}	93.3 $\pm$ 11.5 ^{Aab}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}
	V4	0.0 $\pm$ 0.0 ^{Ab}	80.0 $\pm$ 0.0 ^{Aabc}	90.0 $\pm$ 0.0 ^{Aab}	90.0 $\pm$ 0.0 ^{Aa}	90.0 $\pm$ 0.0 ^{Aa}	90.0 $\pm$ 0.0 ^{Aabc}	90.0 $\pm$ 0.0 ^{Aabc}	90.0 $\pm$ 0.0 ^{Aabc}
	V5	0.0 $\pm$ 0.0 ^{Ab}	90.0 $\pm$ 17.3 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}
	V6	10.0 $\pm$ 17.3 ^{Ab}	66.6 $\pm$ 15.3 ^{Aabcd}	83.3 $\pm$ 5.8 ^{Aabc}	93.4 $\pm$ 5.8 ^{Aa}	93.3 $\pm$ 5.8 ^{Aa}	93.3 $\pm$ 5.8 ^{Aab}	93.3 $\pm$ 5.8 ^{Aab}	93.3 $\pm$ 5.8 ^{Aab}
	V7	20.0 $\pm$ 17.3 ^{Ab}	80.0 $\pm$ 10.0 ^{Aabc}	80.0 $\pm$ 0.0 ^{Aabc}	96.7 $\pm$ 5.8 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}
	V8	0.0 $\pm$ 0.0 ^{Ab}	30.0 $\pm$ 34.6 ^{Ad}	53.3 $\pm$ 40.4 ^{Ac}	60.0 $\pm$ 34.7 ^{Ab}	60.0 $\pm$ 34.6 ^{Ac}	86.7 $\pm$ 34.7 ^{Aabc}	86.7 $\pm$ 34.7 ^{Aabc}	86.7 $\pm$ 34.7 ^{Aabc}
	V9	10.0 $\pm$ 17.3 ^{Ab}	90.0 $\pm$ 100.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 5.8 ^{Aa}	100.0 $\pm$ 5.8 ^{Aa}	100.0 $\pm$ 5.8 ^{Aa}	100.0 $\pm$ 5.8 ^{Aa}	100.0 $\pm$ 5.8 ^{Aa}
	V10	3.3 $\pm$ 5.8 ^{Ab}	40.0 $\pm$ 34.6 ^{Ac}	63.3 $\pm$ 28.9 ^{ABC}	76.7 $\pm$ 15.3 ^{Aab}	76.7 $\pm$ 15.3 ^{Aab}	76.6 $\pm$ 15.3 ^{Ac}	76.6 $\pm$ 15.3 ^{Ac}	76.6 $\pm$ 15.3 ^{Ac}
	V11	6.7 $\pm$ 5.8 ^{Ab}	15.5 $\pm$ 15.3 ^{Aab}	93.3 $\pm$ 11.5 ^{Aab}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}
	V12	56.7 $\pm$ 49.3 ^{Aa}	83.3 $\pm$ 28.9 ^{Aabc}	96.7 $\pm$ 5.8 ^{Aab}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}	100.0 $\pm$ 0.0 ^{Aa}
	V13	6.7 $\pm$ 5.8 ^{Ab}	53.3 $\pm$ 25.1 ^{Aabcd}	90.0 $\pm$ 10.0 ^{Aab}	90.0 $\pm$ 10.0 ^{Aa}	93.3 $\pm$ 11.5 ^{Aa}	93.3 $\pm$ 11.5 ^{Aab}	93.3 $\pm$ 11.5 ^{Aab}	93.3 $\pm$ 11.5 ^{Aab}
T1	V1	0.0 $\pm$ 0.0 ^{Ab}	0.0 $\pm$ 0.0 ^{Ac}	30.0 $\pm$ 0.0 ^{Abcd}	36.7 $\pm$ 11.5 ^{Bd}	43.3 $\pm$ 11.5 ^{Bcde}	43.3 $\pm$ 11.5 ^{Bcd}	43.3 $\pm$ 11.5 ^{Bcd}	43.3 $\pm$ 11.5 ^{Bcd}
	V2	10.0 $\pm$ 10.0 ^{Aa}	43.3 $\pm$ 11.5 ^{Aa}	46.7 $\pm$ 5.8 ^{Babcd}	46.7 $\pm$ 5.8 ^{Bcd}	46.7 $\pm$ 5.8 ^{Bcde}	46.7 $\pm$ 5.8 ^{Bcd}	46.7 $\pm$ 5.8 ^{Bcd}	46.7 $\pm$ 5.8 ^{Bcd}
	V3	0.0 $\pm$ 0.0 ^{Ab}	26.7 $\pm$ 15.3 ^{Babc}	60.0 $\pm$ 5.8 ^{Aabc}	80.0 $\pm$ 5.77 ^{Aa}	80.0 $\pm$ 5.8 ^{Aab}	80.0 $\pm$ 5.8 ^{Aab}	80.0 $\pm$ 5.8 ^{Aab}	80.0 $\pm$ 5.8 ^{Aab}

V4	0.0 ± 0.0 ^{Ab}	26.7 ± 15.3 ^{Babc}	53.3 ± 5.8 ^{Babcd}	60.0 ± 10.0 ^{Babcd}	63.3 ± 5.8 ^{Babcd}	63.3 ± 5.8 ^{Babcd}	63.3 ± 5.8 ^{Babcd}	63.3 ± 5.8 ^{Babcd}
V5	0.0 ± 0.0 ^{Ab}	40.0 ± 40.0 ^{Aab}	70.0 ± 26.5 ^{Aa}	83.3 ± 15.3 ^{Aa}	86.7 ± 11.5 ^{Aa}	86.7 ± 11.5 ^{Aa}	86.7 ± 11.5 ^{Aa}	86.7 ± 11.5 ^{Aa}
V6	0.0 ± 0.0 ^{Ab}	10.0 ± 17.3 ^{Babc}	26.7 ± 11.5 ^{Bcd}	33.3 ± 11.5 ^{Bd}	33.3 ± 5.8 ^{Be}	33.3 ± 5.8 ^{Bd}	33.3 ± 5.8 ^{Bd}	33.3 ± 5.8 ^{Bd}
V7	0.0 ± 0.0 ^{Ab}	33.3 ± 30.6 ^{Aabc}	63.3 ± 28.9 ^{Aab}	76.7 ± 5.8 ^{Bab}	83.3 ± 5.8 ^{Ba}	86.7 ± 5.8 ^{Ba}	86.7 ± 5.8 ^{Ba}	86.7 ± 5.8 ^{Ba}
V8	3.3 ± 5.8 ^{Aab}	6.7 ± 11.5 ^{Abc}	46.7 ± 23.1 ^{Aabcd}	60.0 ± 26.5 ^{Aabcd}	63.3 ± 28.8 ^{Aabcd}	63.3 ± 28.9 ^{Aabcd}	63.3 ± 28.9 ^{Aabcd}	63.3 ± 28.9 ^{Aabcd}
V9	6.7 ± 11.5 ^{Aab}	30.0 ± 20.0 ^{Babc}	60.0 ± 26.5 ^{Aabc}	66.7 ± 25.2 ^{Aabc}	70.0 ± 20.0 ^{Aabc}	73.3 ± 25.16 ^{Aabc}	73.3 ± 25.16 ^{Aabc}	73.3 ± 25.16 ^{Aabc}
V10	0.0 ± 0.0 ^{Ab}	20.0 ± 17.3 ^{Aabc}	30.0 ± 0.0 ^{Abcd}	36.7 ± 5.8 ^{Ad}	36.7 ± 5.8 ^{Ade}	36.7 ± 5.8 ^{Ad}	36.7 ± 5.8 ^{Ad}	36.7 ± 5.8 ^{Ad}
V11	0.0 ± 0.0 ^{Ab}	10.0 ± 10.0 ^{Bc}	23.3 ± 20.8 ^{Bd}	43.3 ± 5.8 ^{Bcd}	53.3 ± 5.8 ^{Bbcde}	53.3 ± 5.8 ^{Bbcd}	53.3 ± 5.8 ^{Bbcd}	53.3 ± 5.8 ^{Bbcd}
V12	0.0 ± 0.0 ^{Ab}	10.0 ± 10.0 ^{Babc}	36.7 ± 5.8 ^{Babcd}	50.0 ± 17.3 ^{Bbcd}	53.3 ± 20.8 ^{Bbcde}	53.3 ± 20.9 ^{Bbcd}	53.3 ± 20.9 ^{Bbcd}	53.3 ± 20.9 ^{Bbcd}
V13	0.0 ± 0.0 ^{Ab}	3.3 ± 5.8 ^{Babc}	50.0 ± 0.0 ^{Babcd}	60.0 ± 10.0 ^{Babcd}	60.0 ± 10.0 ^{Babcde}	60.0 ± 10.0 ^{Babcd}	60.0 ± 10.0 ^{Babcd}	60.0 ± 10.0 ^{Babcd}

5.2 Time to 50% germination

Overall, NaCl significantly increased ($p < 0.001$) the time to 50% germination of cowpea seeds (Fig. 3). However, the interaction between NaCl concentration and variety was significant ($p \leq 0.01$). Thus, at 150 μM , in V11, it increased by 55.67% to 150 μM compared to 0 μM . However, salt did not significantly affect T50 in V2. It was not reached in V6 and V10.

5.3 Latency time

Salinity significantly increased ($p < 0.001$) latency time. Thus, it increased from 1 day 7 hours 55 minutes 12 seconds at 0 μM to 2 days 16 hours 4 minutes 48 seconds at 150 μM in V13 (Fig. 3).

5.4 Emergence rate

NaCl significantly influenced ($p \leq 0.01$) the emergence rate of cowpea seedlings. Similarly, the [NaCl]*Varieties interaction was significant ($p \leq 0.05$). Thus, NaCl did not significantly influence ($p > 0.05$) the emergence rate of seeds of varieties V1, V2, V3, V4, V5, V6, V7, V8, V9, V10, V11 and V13 (Fig. 3). However, it significantly reduced ($p \leq 0.01$) the emergence rate of seeds of variety V12. Thus, from 0 to 150 μM , a decrease in seed emergence rate was observed in V12 (100%).

5.5 Seed vigour index

The vigor index of cowpea seeds failed significantly ($p < 0.001$) with salt treatment (Fig. 4). Thus, it was 9.84 times higher in control compared to 150 μM in V11.

5.6 Germination vigour index

The germination vigour index of cowpea varied significantly ($p < 0.001$) in the presence of salt in the medium. Although this decrease was significant in V2, V4, V6, V7, V9, V10, V11, V12 and V13; it was not significant in V1, V3, V5 and V8 (Fig. 4). Thus, compared to 0 μM , it was 6 times smaller at 150 μM in V2.

5.7 Germination time

NaCl did not significantly influence ($p > 0.05$) the germination time of cowpea seeds. Furthermore, the interaction between [NaCl] and varieties was not significant ($p > 0.05$).

5.8 Velocity coefficient

NaCl significantly influenced ($p \leq 0.001$) the velocity coefficient of cowpea seeds (Fig. 5). Similarly, the interaction between NaCl concentration and varieties was significant ($p \leq 0.05$). Thus, NaCl significantly reduced ($p \leq 0.001$) the velocity coefficient in V4 and V11. However, it did not affect the velocity coefficient of varieties V1, V2, V3, V5, V6, V7, V8, V9, V10, V12 and V13. Thus, in V4 and V11, the velocity coefficient of the saline treatment decreased by 5.94% and 12.86%, respectively, compared to the control.

5.9 Average germination time

NaCl significantly influenced ($p \leq 0.001$) the average germination time. Similarly, the interaction between [NaCl]*Varieties was significant ($p \leq 0.05$). Thus, NaCl did not significantly influence ($p > 0.05$) the average germination time of seeds of varieties V1, V2, V3, V5, V6, V7, V8, V9, V10, V12 and V13 (Fig. 5). However, it significantly increased ($p \leq 0.05$) the average germination time of seeds of varieties V4 and V11. Thus, from 0 to 150 μM , an increase in average germination time was observed in V4 (5.94%) and V11 (13.10%).

5.10 Effect of variety on seed germination components

The germination percentage of cowpea seeds increased over time in all varieties. It varied according to variety (Fig. 2, Table 2). Varieties V5 (86.67%), V7 (86.67%) and V3 (80.00%) had the highest values and V6 (36.67%) and V10 (33.33%) the lowest at T1 from day 6 to day 8.

The germination index varied significantly ($p < 0.001$) according to variety. Thus, at 150 μM , varieties V5 (11.92), V7 (11.13), V9 (10.42), V3 (10.41), V2 (8.85), V4 (8.63), V8 (7.74) and V13 (7.14) had the highest germination indices, while varieties V12 (6.36), V11 (5.74), V10 (5.24), V1 (4.67) and V6 (4.34) had the lowest (Fig. 3).

The time to 50% germination of cowpea seeds varied significantly ($p < 0.001$) according to variety (Fig. 3). Thus, varieties V1, V11 and V12 had the longest times to 50% germination at T1.

Variety did not significantly influence ($p > 0.05$) the latency time and the germination duration of cowpea seeds. In addition, the [NaCl]*Varieties interaction were not significant ($p > 0.05$).

The variety significantly influenced ($p \leq 0.05$) the emergence rate of cowpea seeds. Similarly, the [NaCl]*Varieties interaction was significant ($p \leq 0.05$). At 150 μM , V12 recorded the highest seed emergence rate compared to other varieties (Fig. 3).

Variety significantly influenced ($p \leq 0.001$) the vigour index of cowpea seeds (Fig. 4). Similarly, the interaction between [NaCl]*Varieties was significant ($p \leq 0.001$). This index varied significantly according to variety at 150 μM .

Thus, varieties V7 (389.97), V13 (259.00), V5 (219.54) and V3 (202.00) had the highest vigour indices. They were followed by varieties V9 (158.88), V8 (158.88), V4 (137.22) and V2 (115.10). The varieties V6 (107.77), V11 (85.33), V1 (82.33), V12 (74.67) and V10 (73.32) had the lowest vigour indices for seeds at 150 μ M.

The variety significantly influenced ($p \leq 0.001$) the germination vigour index of cowpea (Fig. 4) similarly, the interaction [NaCl]*Varieties was significant ($p \leq 0.001$). This index varied significantly according to varieties treated with salt. Thus, at 150 μ M, varieties V7 (19.66), V5 (12.71), V8 (10.84), V3 (10.6), V2 (6.49), V13 (5.95), V9 (5.20) and V4 (4.31) had the highest germination vigour indices, while the other varieties had the lowest indices (V6: 4.04, V10: 3.67, V11: 3.44, V12: 2.97 and V1: 0.78).

Variety significantly influenced ($p \leq 0.05$) the germination velocity coefficient of cowpea. Similarly, the [NaCl]*Varieties interaction was significant ($p \leq 0.05$). At 150 μ M, V2 and V11 had the highest coefficients (Fig. 5).

The variety significantly influenced ($p \leq 0.05$) the average germination time of cowpea seeds. Similarly, the interaction between [NaCl]*Varieties was significant ($p \leq 0.05$). At 150 μ M, V2 had the shortest average germination time compared to the other varieties (Fig. 5).

5.11 Correlation between germination parameters

Analysis of the correlations between the different parameters (Fig. 6) showed a detrimental effect of salt stress on the germination parameters of cowpea varieties.

The parameters velocity coefficient (VC), germination index (GI), mean germination time (MGT), germination time (GT), germination vigour index (GVI), seed vigour index (SVI), dormancy time (LT), 50% germination time (T_{50}) and emergence rate (EV) were strongly correlated with each other. VC was strongly correlated with GI ($r = 0.77$) and negatively correlated with MGT ($r = -1.00$), GT ($r = -0.74$), LT ($r = -0.73$) and T_{50} ($r = -0.71$). A significant correlation was observed between GT and MGT ($r = 0.74$). GI was correlated with VC ($r = 0.77$) and SVI ($r = 0.73$) and negatively correlated with T_{50} ($r = -0.79$) and MGT ($r = -0.76$). GVI was strongly correlated with SVI ($r = 0.84$). There was a positive correlation between LT and MGT ($r = 0.73$).

5.12 Principal component analysis and clustering

Factor analysis (Fig. 7A) and hierarchical classification or clustering (Fig. 7B) were used to classify the varieties into four groups with almost identical characteristics based on the variables studied.

Class 1 (cluster 1) consists of varieties V1, V6, V10, V11 and V12, which are sensitive to salinity. Class 2 mainly corresponds to varieties V2, V4, V8 and V9, which are moderately sensitive to salinity. Class 3 includes variety V7, which is moderately tolerant to salinity. Class 4 consists of varieties V3, V5 and V13, which are tolerant to salinity at the germination stage.

6. Discussion

6.1. Effect of salt stress on seed germination parameters

Seed germination marks the beginning of plant growth, development and reproduction. Germinating seeds initially undergo water imbibition, followed by cell elongation and an increase in cell number [26]. Several factors such as salinity, temperature, light, water and plant hormones can modulate seed germination [27, 28]. Among these factors, salinity can significantly affect seed germination and seedling establishment.

The germination percentage (GP), germination index (GI), seed vigour index (SVI), germination vigour index (GVI) and velocity coefficient (VC at V4 and V11) of cowpea seeds decreased significantly with salt treatment. However, the latency time (LT), the time to 50% germination (T_{50}), and the MGT (at V4 and V11) increased significantly with salt treatment.

This result could be explained by the fact that at high salt concentrations, germination is affected by increased levels of abscisic acid (ABA) and reduced levels of seed germination-stimulating hormones such as gibberellins (GAs) [29]. In addition, high salt concentrations lead to impaired membrane permeability due to metabolic defects [30]. Salinity negatively affects seed germination, either osmotically through reduced water absorption [31–33], or ionically through the accumulation of Na^+ and Cl^- , leading to an imbalance in nutrient absorption [34] and a toxic effect affecting the embryo's protoplasm [35]. Pronounced salt stress due to salts affects germination by altering seed imbibition due to low potential. Osmotic environment, disrupting hormonal balance and reducing the use of seed reserves [36, 37]. According [38], the reduced ability of cowpea seeds to germinate under conditions of soil salinity is possibly caused by a reversible osmotic effect that induced dormancy due to salinity.

Similar results were published by [24, 39–41] on PG, GI, MGT, GVI, SVI and VC of beans. These results are like those of [42] on PG, GI, MGT and SVI of squash. [43] Point out that increased salinity in the environment delays the start of germination and increases MGT and T_{50} .

6.2 Salt stress tolerant varieties

The varieties are classified into two groups by the hierarchical classification tree produced by the R software: Class 1 consists of varieties V1, V6, V10, V11 and V12, which are sensitive to salinity. Class 2 comprises varieties V2, V4, V8 and V9, which are moderately sensitive to salinity. Class 3 consists of variety V7, which is moderately tolerant to salinity. Class 4 comprises varieties V3, V5 and V13, which are tolerant to salinity at the germination stage. These different classes can also be obtained by structuring the averages of the seed germination components (percentage of germination, germination index, time to 50% germination, latency time and seed vigour index, etc.).

The varieties tested differ significantly depending on the parameters tested. Varieties V3, V5, V13 and V7 have the best GP, GI, T_{50} , TL, SVI and GVI, while varieties V1, V6, V10, V11 and V12 have the lowest. Furthermore, the results of the work by [24] show that IG and SVI are more sensitive indices for assessing the salt stress tolerance of legumes. Indeed, [44] found that GI is the parameter that best describes the PG/speed relationship; GP and MGT alone are not sufficient to represent a batch of seeds in terms of germination activity over a given period. The variability of cultivars in terms of germination and other growth characteristics is thought to be due to the heterogeneity of their genetic makeup [45–51, 52].

7 Conclusion

With a view to colonising soils degraded by salinity, the overall objective of this study was to assess the effect of salinity on the germination of cowpea seeds. The results revealed that the germination percentage (GP), germination index (GI), seed vigour indices (SVI), germination vigour index, velocity coefficient and emergence rate decreased significantly with saline treatment. Thus, on the sixth day of germination, NaCl reduced the GP of seeds compared to the control (0 μM) by 48.00% at V1(IR15M02), 50.00% at V2(IR16MAK), 20.00% at V3(WIBALI), 29.63% at V4(TILIGRE), 13.33% at V5(IR16MAP), 64.29% at V6(IR15MA33), 13.33% at V7(LOCAL3fbt), 36.67% at V8(LOCAL3PG), 26.67% at V9(BRI), 52.17% at V10(LOCAL3GG), 46.67% at V11(LORI), 46.67% at V12(MTA22) and 35.71% at V13(KOMCALLE). Nevertheless, the average germination time, latency time and 50% germination time

(T_{50}) increased significantly with the saline treatment. However, salt did not significantly affect T_{50} in V2(IR16MAK) and was not reached in V6(IR15MA33), and V10(LOCAL3GG). Furthermore, the classification of averages, the representation of individuals in the principal component analysis and the dendrogram showed that the varieties tested differ significantly according to the parameters evaluated. Groups 3 [V7(LOCAL3ft)] and 4 [(V3(WIBALI), V5(IR16MAP) and V13 KOMCALLE)] had the best GI, SVI and T_{50} , while group 1 [V1(IR15M02), V6(IR15MA33), V10(LOCAL3GG), V11(LORI) and V12(MTA22)] and group 2 [V2(IR16MAK), V4(TILIGRE), V8(LOCAL3PG) and V9(BRI)] had the lowest levels. Variety groups 3 and 4 are therefore recommended to farmers for their tolerance to high salinity levels in the environment during germination.

Declarations

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Plant reproducibility

The germination experimentation under salt stress conditions was done on 13 varieties of cowpea, mainly provided by IRAD (Agricultural Research Institute for Development), Cameroon, accession collection in Foumbot and Maroua. The exhaustive protocol (substrate, growth conditions, etc.) is provided in the Methodology part to guarantee whole reproducibility of the test.

Author contributions

Conceptualization: CNT, ET, EY, CFD; Methodology: CNT, ETL, CFD; Formal analysis and investigation: CNT, ETL, MKF, OLNE, LSNC; Writing-original draft preparation: CFD, ETL, LSTG, BDDM; Writing—review and editing: CNT, LSTG, MKF; Supervision: CNT

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Data availability

The raw datasets used and/or analyzed throughout the present research are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This research did not require ethical approval for instance it exclusively involved samples of healthy cowpea seeds and crops and did not involve human or animal subjects. The management of cowpea seeds in this research conformed to institutional principles and national guiding principle (Code de l'Environnement, Article L412-1). No safe or unlawfully collected species were utilized in this research. This research did not involve human participants.

Consent to Publish

Not applicable. There were no human participants in this research and for the images/photographs, the authors confirm authorship of this data and did not need any mandatory permission to make known the images/photographs being in the manuscript.

Competing interests

The authors declare no competing interests.

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Figures



Figure 1

Cowpea varieties used as plant material. V1= IR15M02, V2= IR16MAK, V3=WIBALI, V4=TILIGRE, V5=IR16MAP, V6=IR15MA33, V7=LOCAL3ft, V8=LOCAL3PG, V9=BRI, V10=LOCAL3GG, V11=LORI, V12=MTA22, V13=KOMCALLE

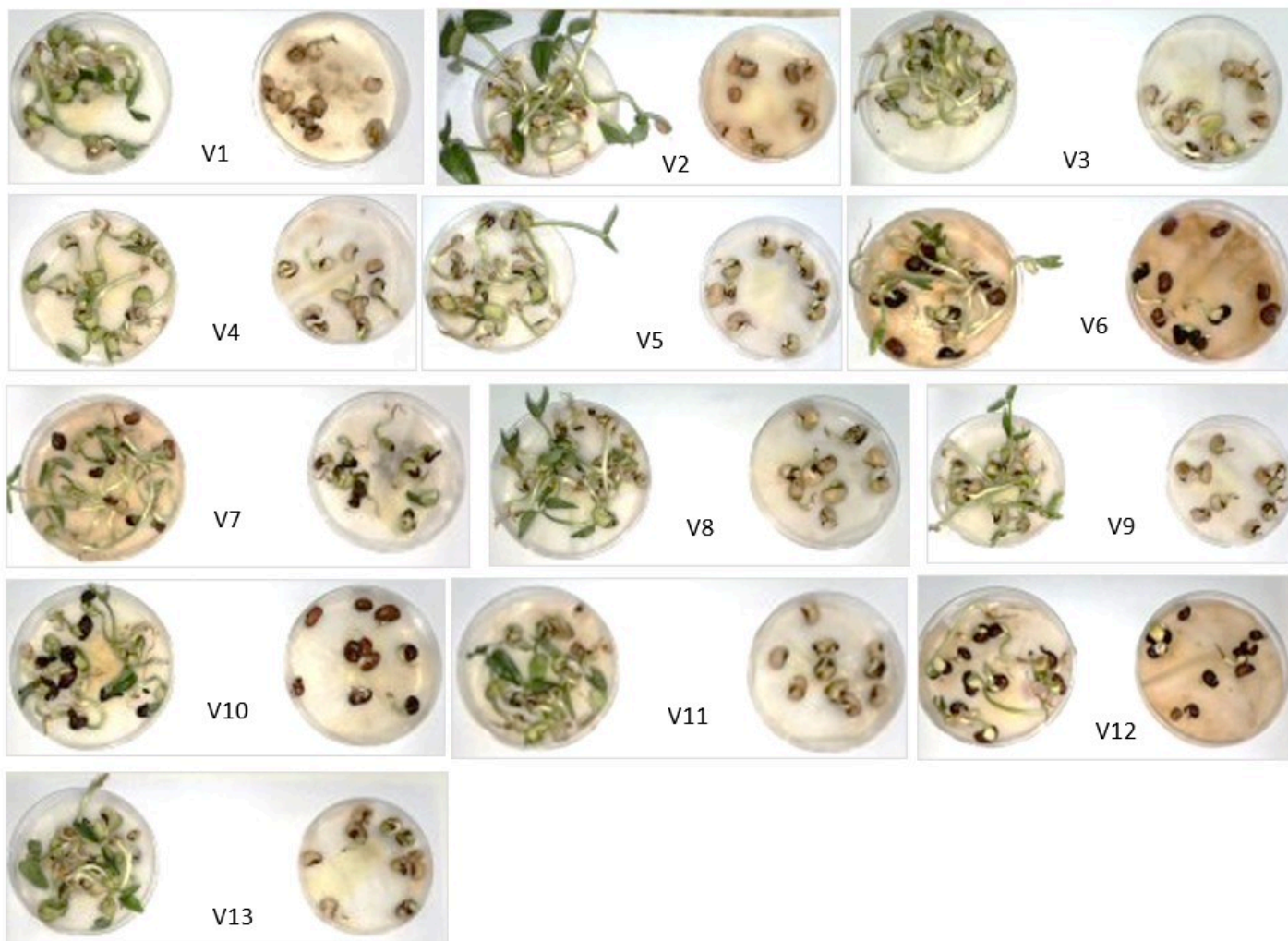


Figure 2

Sprouted seeds of cowpea varieties according to treatment. Left: T0, right: T1

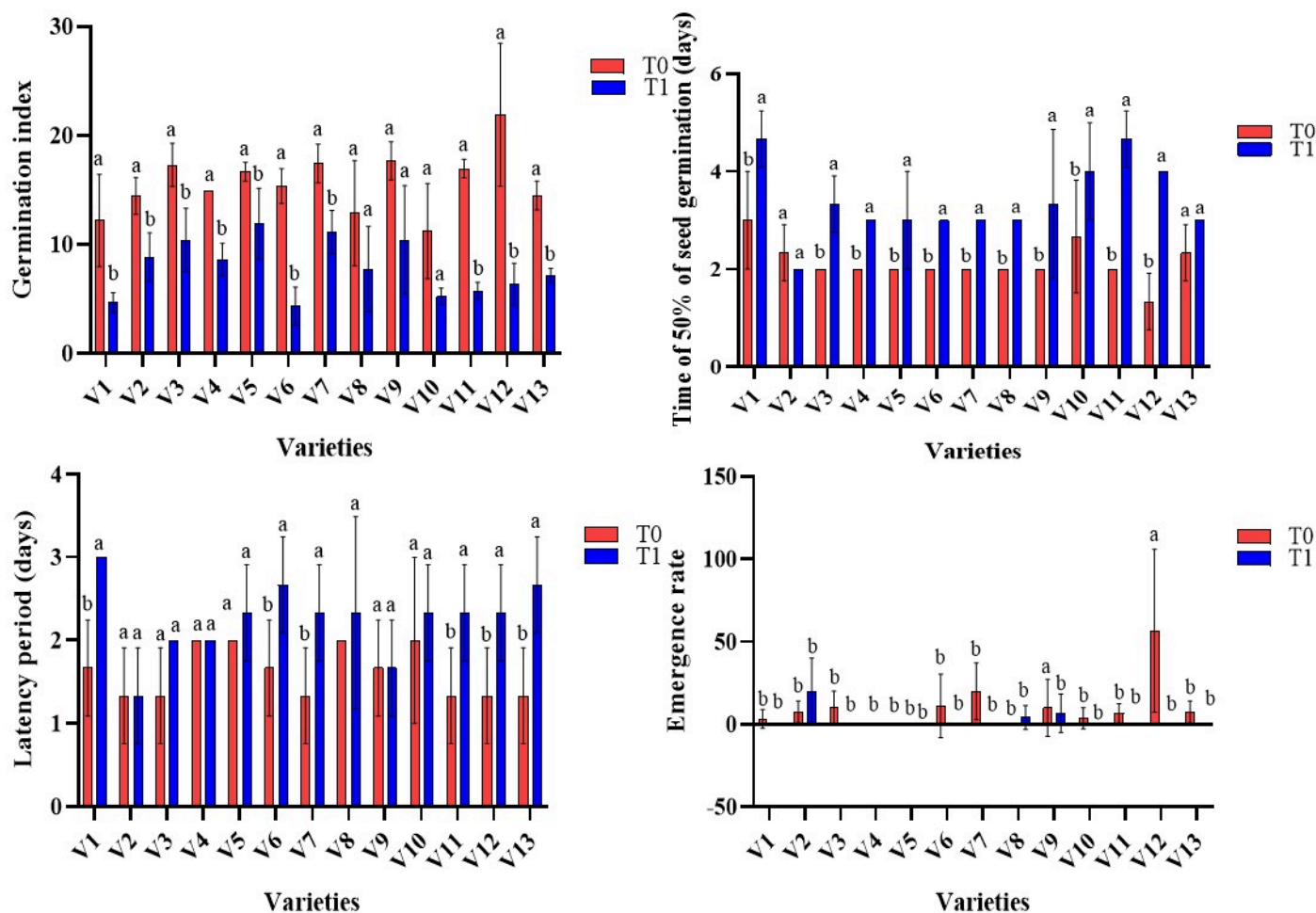


Figure 3

Effect of salinity on the germination index, time to 50% germination, latency time and emergence rate according to treatment of cowpea seeds. V1=IR15M02, V2=IR16MAK, V3=WIBALI, V4=TILIGRE, V5=IR16MAP, V6=IR15MA33, V7=LOCAL3ft, V8=LOCAL3PG, V9=BRI, V10=LOCAL3GG, V11=LORI, V12=MTA22, V13=KOMCALLE. For each parameter and for each variety, the means of the histograms with different letters are significantly different at the 5% level of Duncan's test

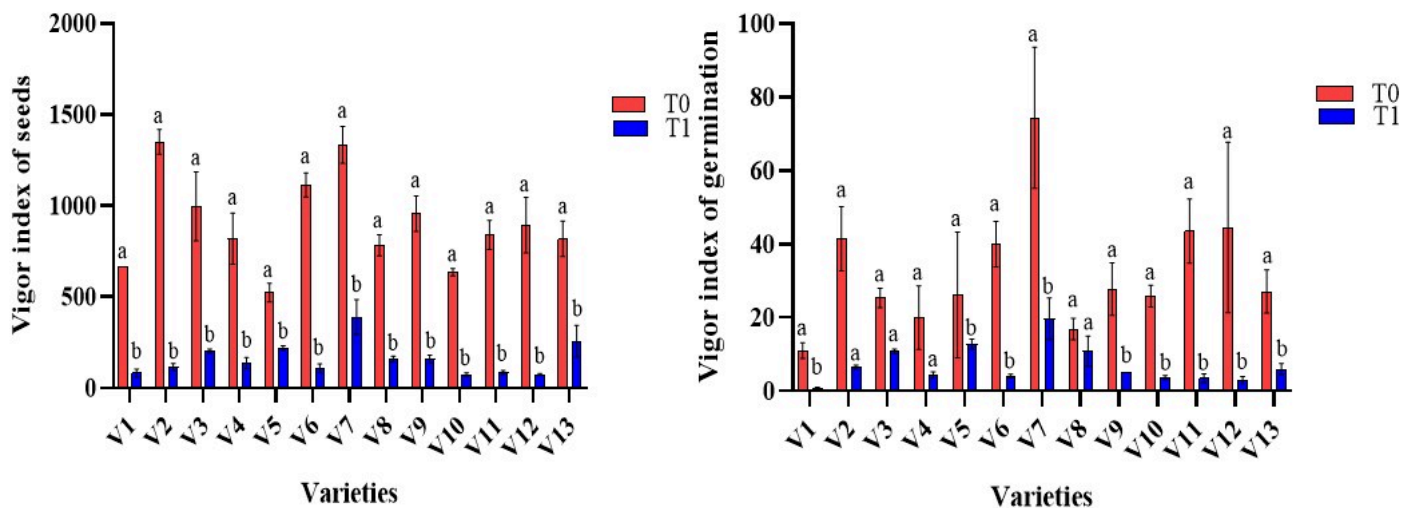


Figure 4

Vigour indexes according to treatment. V1=IR15M02, V2=IR16MAK, V3=WIBALI, V4=TILIGRE, V5=IR16MAP, V6=IR15MA33, V7=LOCAL3fbt, V8=LOCAL3PG, V9=BRI, V10=LOCAL3GG, V11=LORI, V12=MTA22, V13=KOMCALLE. For each parameter and for each variety, the averages of the histograms bearing the same letters are not significantly different at the 5% threshold of Duncan's test

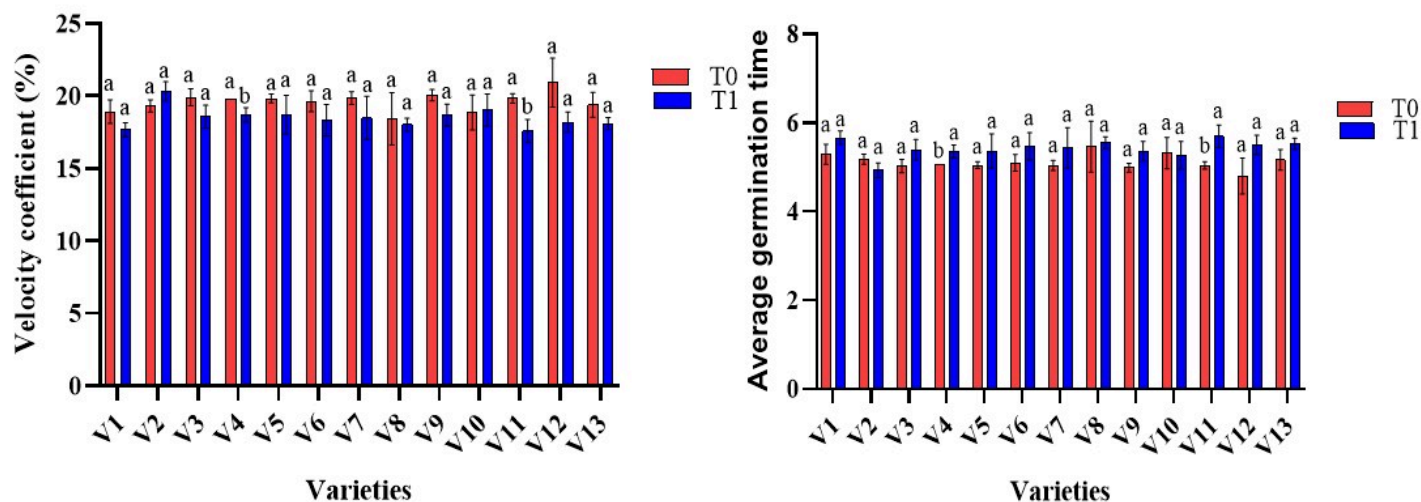


Figure 5

Effect of salinity on velocity coefficient and average germination time of cowpea seeds. V1=IR15M02, V2=IR16MAK, V3=WIBALI, V4=TILIGRE, V5=IR16MAP, V6=IR15MA33, V7=LOCAL3fbt, V8=LOCAL3PG, V9=BRI, V10=LOCAL3GG, V11=LORI, V12=MTA22, V13=KOMCALLE. For each parameter and for each variety, means $\pm$ standard deviation with the same letters are not significantly different at the 5% level of Duncan's test.

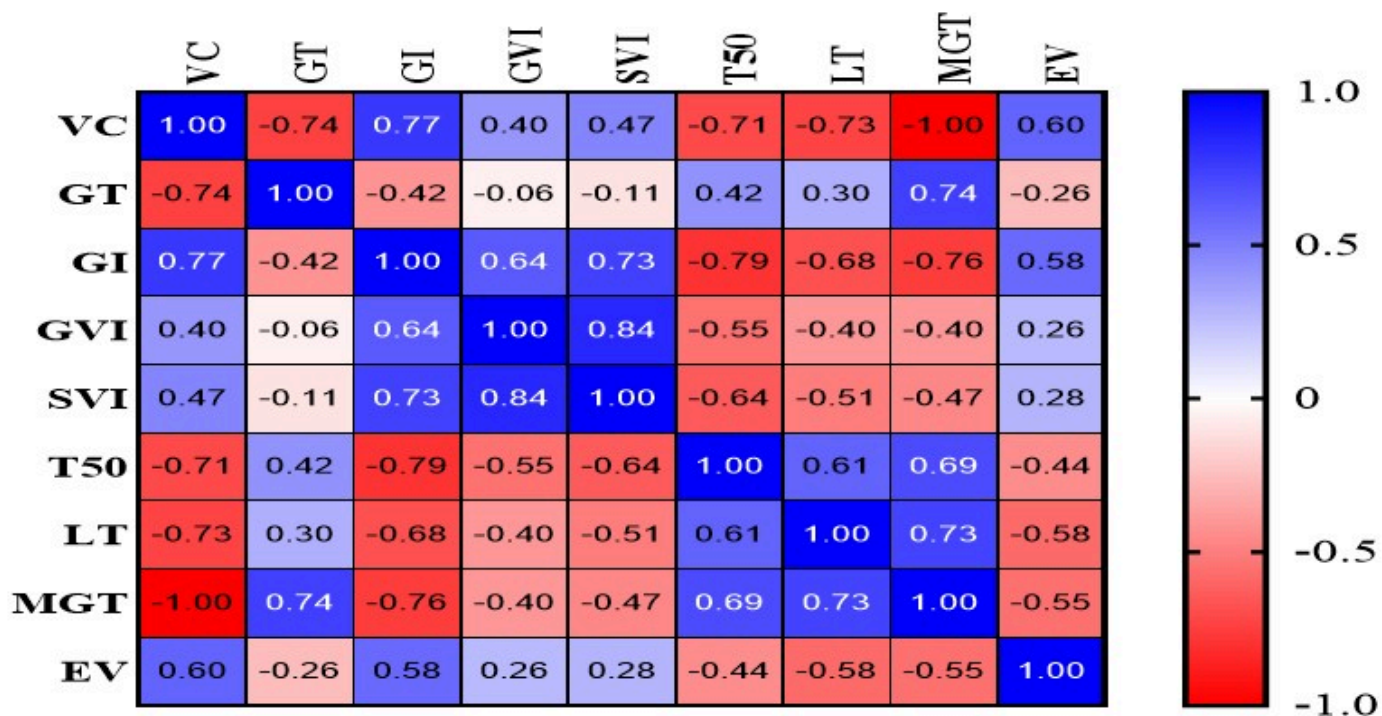


Figure 6

Correlation between cowpea germination parameters (VC: velocity coefficient, GT: germination time, GI: germination index, GVI: vigor index of germination, SVI: vigor index seeds, T₅₀: time of 50% germination, LT: latency time, MGT: mean germination time and EV: emergence rate)

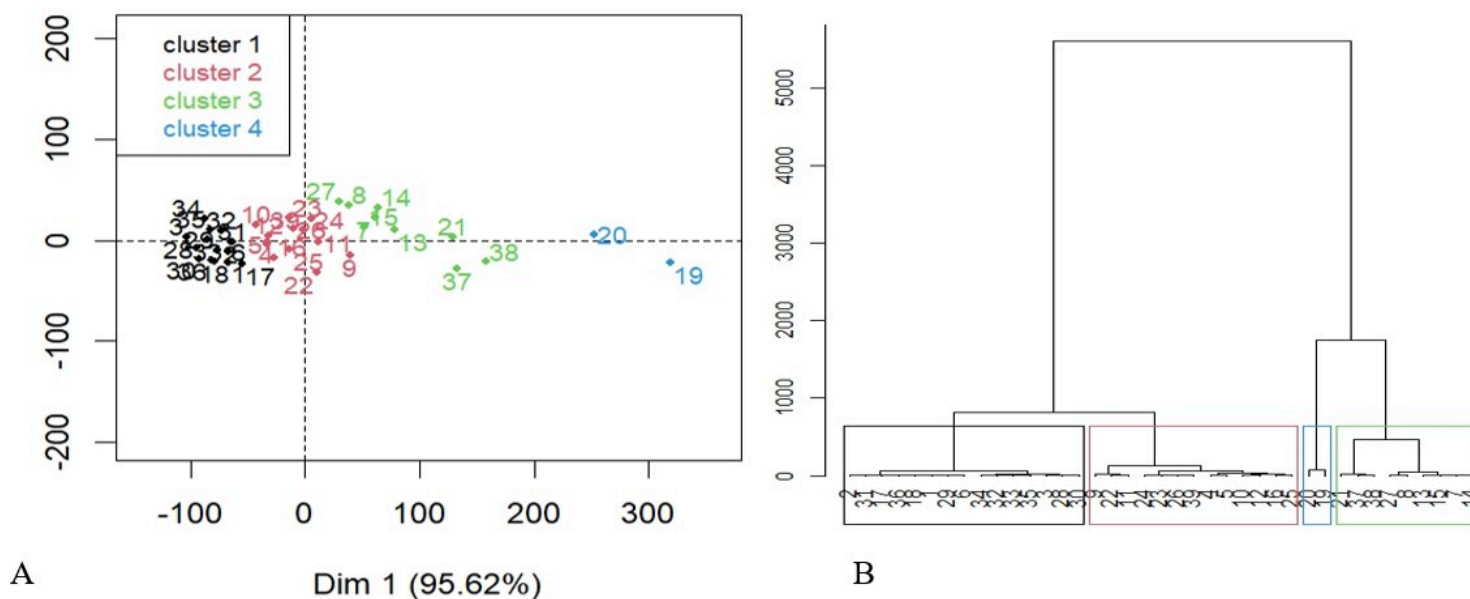


Figure 7

Representation of individuals in the first factorial plane (A) and dendrogram classifying thirteen cowpea accessions (B) in the saline treatment. 1-3=V1 (IR15M02), 4-6=V2(IR16MAK), 7-9=V3(WIBALI), 10-12=V4(TILIGRE), 13-

15=V5(IR16MAP), 16-18=V6(IR15MA33), 19-21=V7(LOCAL3fht), 22-24=V8(LOCAL3PG), 25-27=V9(BRI), 28-30=V10(LOCAL3GG), 31-33=V11(LORI), 34-36=V12(MTA22), 37-39=V13(KOMCALLE)

Supplementary Files

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