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# Germination Stage Screening of Mutants of Cowpea (*Vigna unguiculata* L. Walp) to Salinity Tolerance

***Ndeye Fatou Deme***
Laboratoire de Biotechnologies des Champignons, Département de Biologie Végétale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, Dakar-Fann, Sénégal
***Mouhamadou Moussa Diangar***
Institut Sénégalais de Recherches Agricoles (ISRA), ISRA CNRA de Bambey, Member of the Center of Excellence of CERAAS, Thies, Senegal
***Mohd Yusuf Rafii***
Laboratory of Climate-Smart Food Crop Production, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
***Mame Arama Fall-Ndiaye***
***Tahir Abdoulaye Diop***
Laboratoire de Biotechnologies des Champignons, Département de Biologie Végétale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, Dakar-Fann, Sénégal

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## Abstract

To test the tolerance of cowpea mutants to salinity, cowpea wilds and mutants were subjected to 50, 100, 150, 200, and 250 mM NaCl to test for tolerance to salinity. Genotype and salt concentration interaction were significant. GxS explained mostly the variation observed. More informative salt concentrations were found in 50 mM (99.08) and C100 mM (72.50) against 26.80 in the control environment. High salt concentrations had the lowest germination rates. Seed germination rate of cowpea genotypes decreased from 56.46 to 20.58 with a mean of 36.28 and a variance of 99.08. Despite strong correlations observed between indices, very weak ones were

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found between AD and STI, -0.02, -0.44, -0.7, -0.79 and -0.84 respectively at salt concentration of 50, 100, 150, 200 and 250. Mouride wild types were most tolerant to salt with a germination rate of 43 % at 50 mM versus 48 and 551 % for respectively Melakh and Yacine. Six (6) mutants were more tolerant to the weakest checks performance which was the 9th best performance.

**Keywords:** Cowpea mutants, NaCl tolerance, Germination

## Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) also called niebe in francophone countries in Africa is a leguminous crop with high protein content belonging to Fabaceae family (Verdcourt, 1970). Cowpea grows in various agro-ecological zones and plays an important role in human and animal feeding and in the improvement of soil fertility by fixing atmospheric nitrogen (Ndiaye, 2015; Erana Kebede & Zelalem Bekeko, 2020).

Sixty-four percent of the world production occurs in Nigeria, while Senegal is among the least countries producing cowpea in West Africa (Timko et *al*., 2007; Olufajo, 2012). Despite its importance in tropical zones, cowpea cultivation is often affected by various biotic and abiotic stresses among which salinity is a major constraint. In fact, salinity is a growing thread to various crops including cowpea and causing losses up to US$12 billion in world agricultural production (Zhang et *al*., 2012; Bose et *al*., 2013; Gupta et *al*., 2014). It affects germination, growth, and development of cowpea limiting its productivity by destroying various molecular, physiological, and biochemical pathways (Parida et *al*., 2004; Ashraf & Foolad, 2007; Kendirli et *al*., 2005; Tiwari et *al*., 2010; Etesami & Noori, 2019). As a result, photosynthesis process is reduced which then affects transpiration and enzymatic activity.

Various methods have been used so far to improve salt tolerance of existing cowpea cultivars. Mutation induced by Gamma-rays is one of the methods used to enhance genetic variability. Advantages of gamma rays include their easy application, good penetration, reproducibility, high mutation frequency, and less disposal problems. According to International Atomic Energy Agency (IAEA 2004), two hundred gamma cells have been used worldwide and are mostly used as emitters to induce mutation in plants compared to chemicals mutagens. Their radiation source is mainly Cobalt-60 (60Co) and Caesium-137 (137Cs) isotopes reported by Food and Agriculture Organization (FAO) and IAEA (2018). Despite possibilities that mutation breeding offers, few mutants' resources are available. In fact, FAO (2014b) stated that Asia production is about 60 % of plant mutants followed by Europe 30%, North America 6%, Africa 2%, Latin America and the Caribbean (2%). According to IAEA Mutants Varieties Database (FAO/IAEA, 2018), there are at least 3275 mutants in more than 220 plant species. Considering production lines,

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maximum mutant species are cereals (48%), flowers (20%), legumes and pulses (14%), and vegetables, forge, edible oil plants and trees (3% portions) (Mba, 2013). Fortunately in Senegal, the collaboration between IAEA, Institut Sénégalais de Recherches Agricoles (ISRA), and Université Cheikh Anta Diop de Dakar (UCAD) has led to developing mutant lines using Mouride, Melakh and Yacine, which are broadly cultivated cowpea lines in Senegal. However, utilization of those cowpea lines in production areas is hindered by high soil salt content. Therefore, the aim of this study is to test the responses of cowpea mutant lines to varying concentrations of NaCl at the germination stage.

## Material and Methods

### 1. Plant Material and its Preparation

Cowpea varieties, namely Mouride, Melakh and Yacine, released by Institut Sénégalais de Recherches Agricoles (ISRA) and derived mutants were used in this study. Mutants ($M^4$) included 12 lines from Mouride, 12 from Melakh, and 11 from Yacine giving a total of 35 genotypes tested.

Prior to this work, uniform $M^0$ cowpea seeds from varieties Melakh, Yacine and Mouride were exposed to gamma radiation dose range of 280, 300 and 340 Gy through a cobalt source (60Co) at the Plant Breeding Laboratory of the International Atomic Energy Agency (IAEA) in Seibersdorf (Austria). Cl Seeds from the $M^4$ generation having uniform size were chosen for laboratory screening to salt tolerance. Seeds were sterilized with 1% sodium hypochlorite solution (NaClO). Petri dish of 9 cm in diameter, used for the germination, was sterilized using bleach 2% and ethanol 75% to prevent contamination. Thirty (30) seeds from each line were placed in Petri dishes on paper filter Whatman and filled with distilled water or NaCl solution. Prior to screening, the incubator was sterilized with 75% ethanol solution to avoid microbial expansion.

### 2. Screening Procedure

12 ml of distilled water and 12 ml of NaCl solution was added to each dish as respectively controls and treatments. Five concentrations of NaCl including 50, 100, 150, 200, and 250 mM NaCl were used to test cowpea mutants lines tolerance to salinity. All Petri dishes were placed in a plant growth chamber (GC-101C; Daeyang ETS, Hwasung-si, Kyunggi-do, South Korea) at $28^0$ for 48 hours. Calculation of the concentration and the optimal temperature for cowpea germination has been previously determined by Ravelombola's method (2017). The number of germinate seed were obtained 72 hours after incubating Petri dishes. Germination is considered effective when the radicle reached a third of the length of the seed (Ravelombola, 2017).

## 3. Germination Parameters

Five different parameters were considered to assess germination under salinity stress such as Seed Germination Rate (SGR), Absolute Decrease (AD), Inhibition Index (II), Relative Salt Tolerance (RST), and Salt Tolerance Index (STI).

- **Seed Germination Rate** was calculated by dividing the number of germinated seeds to the total number of seeds. The result was expressed as percentage (Benidire et *al*., 2015; Batabyal et *al.*, 2014).
  SGR %= (Number of germinated seeds/Total number of seeds)*100
  SGR %= NGS/TNS*100
- **Absolute Decrease** indicated the decrease of germination rate between saline and non-saline condition expressed by the difference between Seed germination rate without salt stress and Seed germination rate under salt stress (Rosielle & Hamblin, 1981; Ravelombola, 2017).
  $AD = SGR_C - SGR_S$
- **Inhibition Index** was calculated according to modified González (1996) formula.
  $II = 100 * (SGR_C - SGR_S) / (SGR_C)$
- **Relative Salt Tolerance** was calculated by the ratio of Seed germination rate under salt stress dived by Seed germination rate without salt stress (Saad et *al*., 2014; Ravelombola, 2017).
  $RST= SGR_S / SGR_C$
- **Salt Tolerance Index** was expressed by the following formula (Saad et *al*., 2014; Ravelombola, 2017):
  $STI = (SGR_S * SGR_C) / (SGR_{AV})^2$
  With $SGR_C$= Seed germination rate without salt stress
  $SGR_S$= Seed germination under salt stress
  $SGC_{AV}$= Average of seed germination rate of all cowpea mutants.

## 4. Experimental Design and Statistical Analysis

The experimental design used in this study was a randomized complete block design (RCBD) with two factors namely genotype and salt concentration. Treatments were repeated trice. The *genotype-by-environment* data structure was subjected to Additive Main-effect and Multiplicative Interaction (AMMI) analysis following the linear model (Rodrigues et al., 2015).

$$\boldsymbol{\gamma ij} = \mu + \boldsymbol{\alpha_i} + \boldsymbol{e_j} + \sum_{k=1}^{n} \boldsymbol{\lambda_k}\, \boldsymbol{\alpha_{ik}}\, \boldsymbol{\gamma_k}\, \boldsymbol{\varepsilon_{ij}}$$

where $\boldsymbol{\gamma_{ij}}$ is the Seed Germination Rate of the $\boldsymbol{i}^{\mathbf{th}}$ genotype in the $\boldsymbol{j}^{\mathbf{th}}$ salt concentration, $\boldsymbol{\mu}$ is the grand mean, $\boldsymbol{g_i}$ is the deviation of the $\boldsymbol{i}^{\mathbf{th}}$ genotype from $\boldsymbol{\mu}$, $\boldsymbol{e_j}$ is the deviation of the $\boldsymbol{j}^{\mathbf{th}}$ salt concentration from $\boldsymbol{\mu}$, $\boldsymbol{\lambda_k}$ is the square root of the eigenvalue of **G x E** interaction principal components PCA axis **K**, $\boldsymbol{\gamma_{ik}}$

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and $\alpha_{jk}$ are respectively the eigenvalue of vectors of the $i^{th}$ genotype and the $j^{th}$ salt concentration in the $k^{th}$ IPCA axis and $\varepsilon_{ij}$ is the residual.

## Results

**Analysis of Variance**: The analysis of variance of seed germination rate for AMMI model (Table 1) showed significant effects of genotype, salt concentration, and genotype by salt concentration interaction (GxE). Salt concentrations, genotypes, and GxE explained respectively 95.49%, 2.92% and 1.36% of the total germination rate variation observed. First two interaction principal components accounted for 82.29% of the GxE.

**Table 1.** Analysis of variance of seed germination rate for AMMI model

| Source | d.f. | s.s. | m.s. | F pr | Effect % |
|---|---|---|---|---|---|
| Genotypes | 37 | 6873 | 186 | <0.001 | 2.92 |
| Salt concentrations | 5 | 225020 | 45004 | <0.001 | 95.49 |
| Interactions | 185 | 3198 | 17 | | 1.36 |
| IPCA 1 | 41 | 2006 | 49 | <0.001 | 62.71 |
| IPCA 2 | 39 | 626 | 16 | <0.001 | 19.58 |
| Residuals | 105 | 566 | 5 | | 0.24 |

d.f= degrees of freedom; s.s= sum of squares ; m.s= mean of squares; F pr= p value

**Ranking using GGE Analysis**: The seed germination rate among 38 cowpea genotypes under the non-salt stress condition varied from 99.79% to 73.40%, with a mean of 94.16% and a variance of 26.80% (Table 2). In saline conditions, seed germination rate of cowpea genotypes decreased from 56.46 to 20.58 with a mean of 36.28 and a variance of 99.08 for C50; 43.91 to 9.93 with a mean of 22.23 and a variance of 72.50 for C100; 37.08 to 4.79 with a mean of 12.51 and a variance of 48.28 for C150; 18.20 to 0.14 with a mean of 5.06 and a variance of 19.62 for C200; and 7.36 to 0.08 with a mean of 1.94 and a variance of 5.91 for C250. These results showed that the seed germination rate dropped down after salt treatment. In fact, high salt concentrations had the lowest germination rates. Mo495 had the highest germination rate in condition of non-salt stress (99.96) followed by Mo502 (99.90), Mo488 (98.78), Me611 (98.48), and Melakh732 (98.22).

Y706 (74.35), Y783 (87.23), Mo348 (88.77), Me261 (88.89), and Y176 (88.94) had the lowest germination rate. Under salt stress, the average germination rate varied from 36.28 at C50 to 1.94 at C250 for all genotypes. Considering the averages, Y706 (19.71) was found as the most sensitive line followed by Me261 (20.79), Y557 (22.19), Y632 (22.26), and Y701 (22.80). Results indicated that more informative salt concentrations were found in C50 (99.08) and C100 (72.50) against 26.80 in the control environment.

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**Table 2.** Summary statistics of seed germination rate and GGE mega environments

| Salt concentrations | Mega environments | Mean | Min | Max | Variance |
|---|---|---|---|---|---|
| C0 | 1 | 94.16 | 73.40 | 99.79 | 26.80 |
| C50 | 2 | 36.28 | 20.58 | 56.46 | 99.08 |
| C100 | 2 | 22.23 | 9.93 | 43.91 | 72.50 |
| C150 | 2 | 12.51 | 4.79 | 37.08 | 48.28 |
| C200 | 2 | 5.06 | 0.14 | 18.20 | 19.62 |
| C250 | 2 | 1.94 | 0.08 | 7.36 | 5.91 |

The GGE biplot showed two (2) mega environments where all salt concentrations were grouped into one mega-environment except the control environment. Optionally, GGE without the control gave similar results. This suggests that characterization of genotypes used in this study can be done on C50 and C0 by the ranking of genotypes based on salt effect on seed germination.

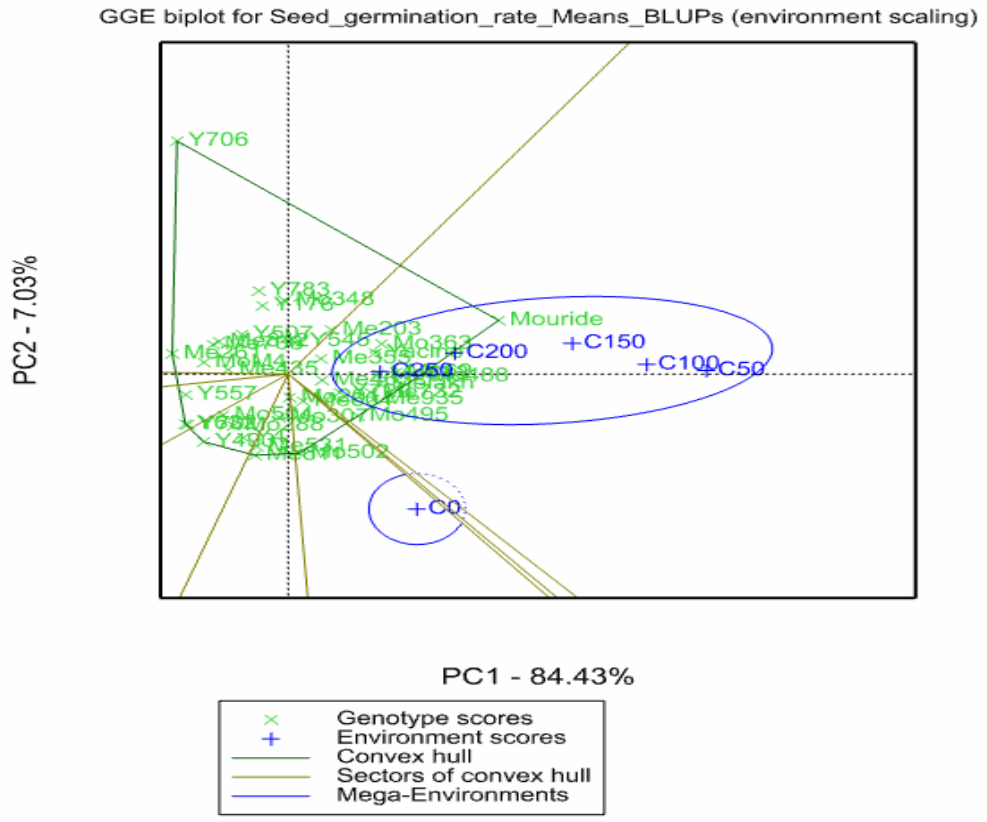


**Figure 1.** Environmemt scaling GGE biplot for Seed germination rate

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**Ranking using stress indices:**

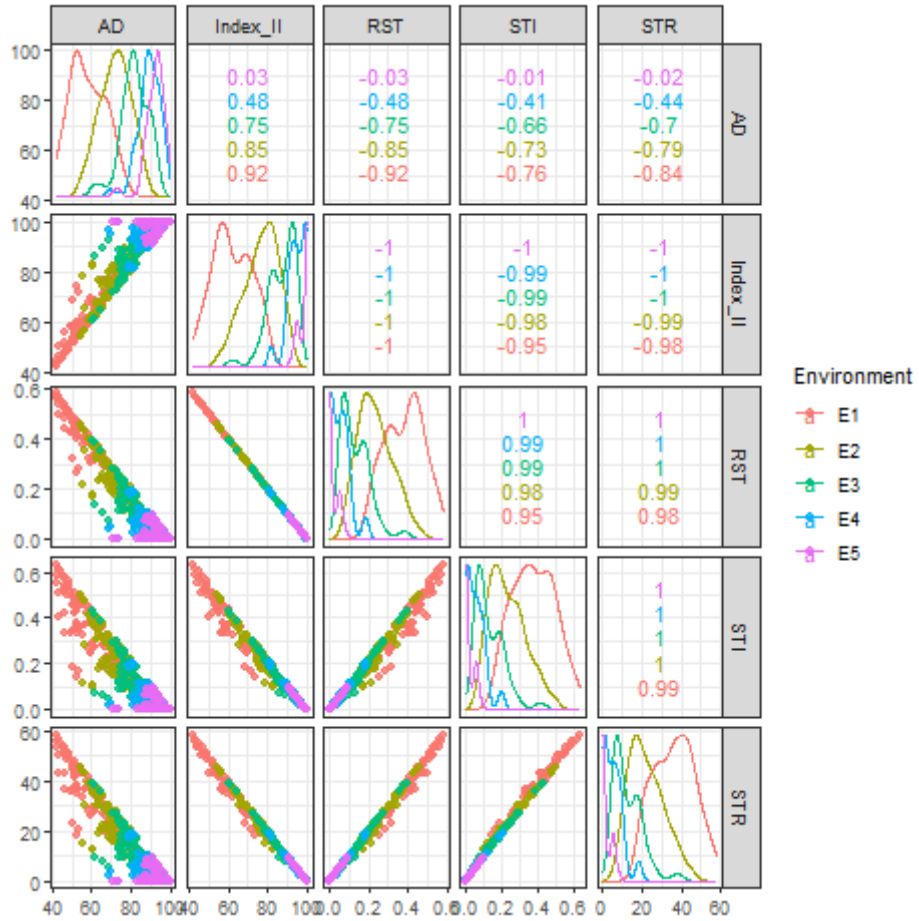


**Figure 2.** Correlation between indices in salt concentrations
*E: salt concentration; 1 = 50; 2 = 100; 3 = 150; 4 = 200; 5 = 250*

In all environments except the control, symmetrical similarities were observed for Index II and RST (r = -1); Index II and STI (r < -0.9); Index II and SGR (r < -0.9); RST and STI (r > 0.9); RST and SGR (r.> 0.9); and STI and SGR (r.> 0.9). In opposite, the weakest correlation was found between AD and STI where r was -0.02, -0.44, -0.7, -0.79, -0.84 respectively at salt concentration of 50, 100, 150, 200 and 250.

**Genotype Performance**: Results indicated that salt concentration of 50 is suitable for ranking performances of genotype for their tolerance to saline stress (Table 1). Also, both indices AD and STI were adequate to discriminate genotypes (Figure 1). At concentration of 50, the line with the least AD value (best) was MoTemoin which is the variety Mouride (43.18) followed by Mo488 (44.20), Mo533 (46.16), and MeTemoin (48.77).

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## Discussion

This study investigated the effect of salt stress on mutants of cowpea lines using varying levels of NaCl concentration at germination stage. It was found that increasing salinity levels induced a lower seed germination rate. Our findings corroborated various studies carried out on crops tolerance to salt stress (Praxedes et *al*., 2014; da Silva Sa et *al*., 2016; Tsague et *al*., 2017). Likely, the salt treatment negatively affected seed physiology and germination mechanism by increasing the salt osmotic potential as mentioned by Kaymakanova et al. (2009cu), Abdel-Haleem and El-Shaieny (2015), Ravelombola (2017), and Dangue et *al.* (2020). The increased osmotic pressure is a consequence of high concentrations of Na+ and Cl-. Changes in cells yield toxicity and inevitably dehydration of seeds that will cancel any chances of germination (Panuccio et *al*., 2014; Wu et *al*., 2015; Munns & Tester, 2008; Liang et al., 2018; Miransari & Smith, 2019). Ashraf et *al*. (2010) and Farissi et *al.* (2013) reported that excessive NaCl promote hormonal and cellular disorders, increase the reactive oxygen species (ROS), and delay cell division and cell elongation which ultimately slow down germination process, plant growth and development. However, these findings are in agreement with our results. However, various methods of resilience are used by plants to cope with salinity stress through mechanisms leading to osmotic adjustment. Taffouo et *al*. (2009) reported that seed germination rate can be used as a first criterion for the screening of legumes populations and species tolerant to salt stress.

In this study, high correlations were observed between AD and other stress indices except STI. In addition, AD has shown high values leading to low germination rate. In rice, high correlations were obtained between AD and other stress indices (González, 1996). Also, in cowpea, genotypes with high AD were considered very susceptible to salt (Ravelombola, 2017).

## Conclusion

Consequently, this study demonstrated that salt stress significantly affected seed germination with a large variation of responses among the cowpea genotypes. The results showed that genotypes with least value of AD had the highest seed germination rate like variety Mouride, Mo488, Mo533, and Melakh.

This study could be enhanced by using molecular tools (markers) to investigate salt stress tolerance mechanisms which lead to fast-track developing salt-tolerant cultivars.

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**Authors Contributions**

D. N. F. (Deme Ndeye Fatou) designed and coordinated the study, was involved in data collection, data analysis and drafted the manuscript.

D. M. (Diangar Moussa), data analysis, software, review and editing. Ndiaye, Rafii, and Diop; Formal analysis. Deme and Diangar; Methodology. Deme; Resources. Rafii, Diop and Ndiaye; Supervision. Rafii and Diop; funding, OWSD.

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**Conflicts of Interest:** the authors declare no conflict of interest.

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