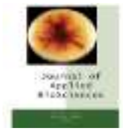

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Early detection of salinity tolerance level of five groundnut genotypes during seed germination.

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ABSTRACT

Objectives: The aim of this study was to evaluate the effect of salt stress on seed germination of five groundnut varieties, with the view to determine their level of tolerance to salinity during this early developmental phase of the plant.

Methodology and results: The study was carried out at Farakoba research station. Calibrated kernels of five groundnut varieties were submitted to six levels of salt treatments, using a split plot design with 3 replications. Sixteen (16) seeds per treatment were placed in Petri dishes containing blotting papers, soaked with different concentrations of sodium chloride (NaCl): 0 mM, 25 mM, 75 mM, 125 mM, 175 mM and 225 mM. Seeds treated in this way were incubated in an oven at 25°C for a week.

Conclusion and application of results: The study showed that salt not only delayed germination, but also considerably reduced the germination rate of groundnuts. However, this effect was dependant on both the stress intensity and the groundnut genotype. The variety SH470P was the most tolerant to salt stress with a germination rate of 40% under the most severe stress condition (225 mM), while variety *Séreba* 1 showed the highest sensitivity with a germination rate of 4% under the same conditions. Groundnut response to salinity is variety dependent. The possibility to detect groundnut response to salt stress at an early stage as the seed germination phase saves time for breeders during varietal trials.

Key words: Salt stress, *Arachis hypogaea* L., adaptation, NaCl

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important source of agricultural income and makes a significant contribution to human and animal nutrition. Cultivated throughout the world in all hot regions, it is a highly valued legume because of its health, environmental and economic benefits. It prevents physiological diseases such as diabetes and cardiovascular disorders due to its low glycemic index (Mahdavi and Ahmad, 2016). The nutritional composition of groundnuts (Hama-Ba *et al.* 2017) makes them a very important food for combating malnutrition. From an agro-ecological perspective, growing groundnuts improves soil health through their ability to fix atmospheric nitrogen due to their nodules. This symbiotic nitrogen-fixing capacity is particularly beneficial for the environment by reducing the use of synthetic nitrogen fertilisers, which production increases greenhouse gas emissions (Mahdavi and Ahmad 2016). Despite its advantages, groundnut production faces various biotic and abiotic constraints that impact the crop productivity. One of the major stresses hampering agricultural production worldwide is soil salinity (Latef, 2010, Gouveitcha *et al.* 2021), which affects more than 7% lands around the world. It is a growing phenomenon that could affect previously unsuspected areas such as Burkina Faso (Gouveitcha *et al.* 2021). Salinity is the presence of excess salt, generally Na^+ and Cl^- ions, in the soil (Parida and Das, 2005). Excess salt in the rhizosphere and in irrigation water is harmful to plants, generally reducing crop yields and quality (Khales and Baaziz , 2006). As a result, Salinity is a major environmental concern and a serious problem for agriculture, as it reduces arable land and threatens food security (Zaman-Allah *et al.* 2009). In such a context of rising salinity and climate change, it will be necessary to be able to produce groundnuts in saline environments if the sector is to survive and remain prosperous in future. Although little is known about the salinity issue in Burkina Faso, it is clear that the country has not been spared. The progressive salinization of irrigated lands in Burkina Faso has been reported previously (Gorine, 2021), suggesting the need to anticipate the development of salt-tolerant cultivars and other mitigation strategies , even before the problem becomes alarming. Research for salinity-tolerant varieties is a recommendable approach for future exploitation of saline soils (Cherifi *et al.* 2017). With this in mind, it appears as appropriate to identify, upstream, sources of 'halo tolerance' trait among groundnut varieties grown in Burkina Faso. The aim of this work is to assess the impact of salt stress on germination, which is the most sensitive phase during plant development (Li *et al.* 2010). Five groundnut varieties will be assayed, to determine their level of salinity tolerance and, possibly, identify varieties tolerant to saline environments.

MATERIAL AND METHODS

Five groundnut varieties (Table1), popular in Burkina Faso, were used for this evaluation. Among these, three were known to be drought tolerant (Fleur 11, ICGV 86015, TS 32-1). A split-plot experimental design was used to evaluate the effect of six different concentrations of sodium chloride (NaCl) on the five groundnut genotypes, with three replicates per genotype and per concentration. The experimental units were 9 cm diameter Petri dishes containing 16 at 17 seeds each. Salt treatments included a negative control (0 mM NaCl); 25 mM; 75 mM; 125 mM; 175 mM and 225 mM NaCl (Annex).

Table 1: List of groundnut varieties tested for early salinity tolerance in this study

N°	Variety	Response to drought
1	Fleur 11	Drought tolerant
2	ICGV 86015	Drought tolerant
3	Séréba -1	Unknown
4	SH470P	Unknown
5	TS 32 -1	Drought tolerant

The choice of these concentrations was inspired by previous research (Taffouo *et al.* 2008, Ly *et al.* 2014) studying the effect of salt stress on some legume crops germination rate and growth parameters. Matured seeds were used for this test, and were sorted and calibrated to have kernels of similar size, and so minimise physiological variability. They were disinfected with bleach (3%) and rinsed with sterile distilled water twice for one minute each time. The disinfected seeds were then germinated in sterile Petri dishes containing blotting paper soaked in 10 ml solution of each of the salt treatments. The Petri dishes were placed in an oven at 25°C (Figure 1). The seed is considered to have germinated when the radicle appears and elongates by at least 5 mm (Akman 2009).

Figure 1: Salt-treated groundnut seeds in Petri dishes germinating in an oven at 25°C.

Observations were made daily for all treatments over six consecutive days, and the following variables were measured:

Radicle length: measurement of the length of the radicle every day from its appearance until the end of the study.

Germination rate (GR): this variable was calculated as the ratio of the number of germinated seeds (n) on day 6 over the total number of seeds tested (N).

$$GR = \frac{n}{N} * 100$$

Germination kinetics (GK): this represents the evolution of cumulative germination percentages as a function of time and was recorded daily.

Salt effect: this estimate was computed by making the difference between the value of the salt treated seeds and that of non-treated ones.

Salt effect was measured on quantitative variables.

The data collected were subjected to an analysis of variance (ANOVA) using XLSTAT software 2017 version. The analysis was complemented by the Newman & Keuls test when the variation was significant at the 5% error threshold. Comparisons of means were then performed using Dunnet's test.

RESULTS

Effect of salt stress on the germination rate of groundnut seeds: The low concentrations of NaCl (25 and 75 mM) enhanced the germination rate of some varieties. Thus, positive effects of salt were recorded with TS 32-1 (25 and 75 mM) and ICGV 86015 (25 mM). There was no effect of low salt concentrations on the germination rates of varieties Fleur 11 (25 and 75 mM) and SH470P (25 mM). However, three varieties (Sereba 1, ICGV 86015 and SH 470P) presented negative effects of salt treatments on their germination rates, irrespective of the salt level (Figure 2). With these varieties, salt effects were proportional to its concentration level. High salt concentrations (125, 175 and 225 mM) resulted in negative effects on germination rates for all groundnut varieties. The magnitude of these effects is roughly proportional to the salt concentration and also variety dependent (Figure 2). Sereba 1 appeared as the most salt sensitive variety with the highest salt level inhibiting the germination up to 76%. Conversely, ICGV 86015 was the most salt tolerant, showing the lowest salt-induced inhibition of the germination (-32%) at 225 mM NaCl (Figure 2).

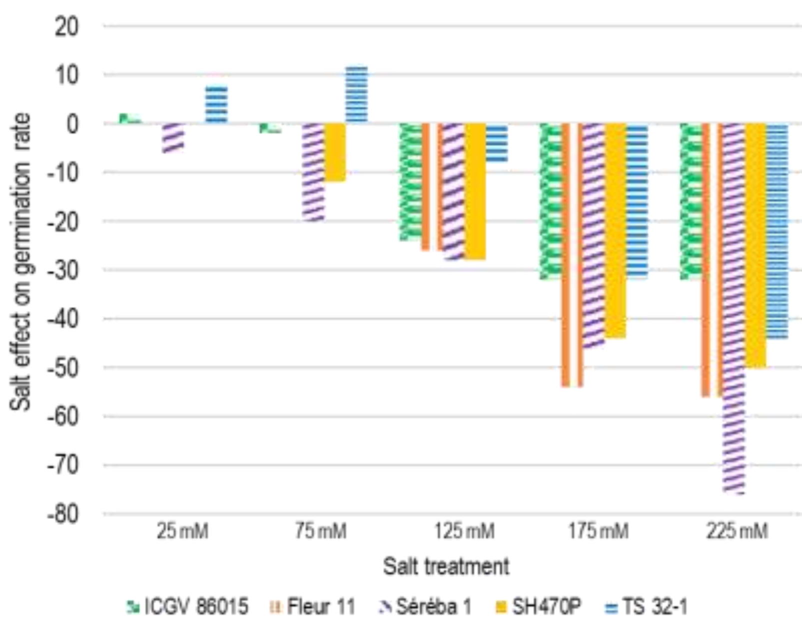

Figure 2: Effect of salt treatments on the germination rate of five groundnut varieties. The effect is computed by subtracting the measure of the control from that of salt treatment.

Seed germination kinetics under salt stress: The germination rate evolved faster in seeds treated with low concentrations of NaCl (0 mM, 25 mM and 75 mM), independently of the varieties (Figure 3). Not only is germination slowed with increasing NaCl concentration, but generally, maximum germination rates at the end of the incubation period are inversely proportional to salt concentration. However, although germination was strongly affected by the 225 mM NaCl concentration (Figure 3.f), seed germination kinetics at this level remained increasing for most varieties, with the exception of Sereba 1 which reached its maximum rate as early as 72 hours of incubation.

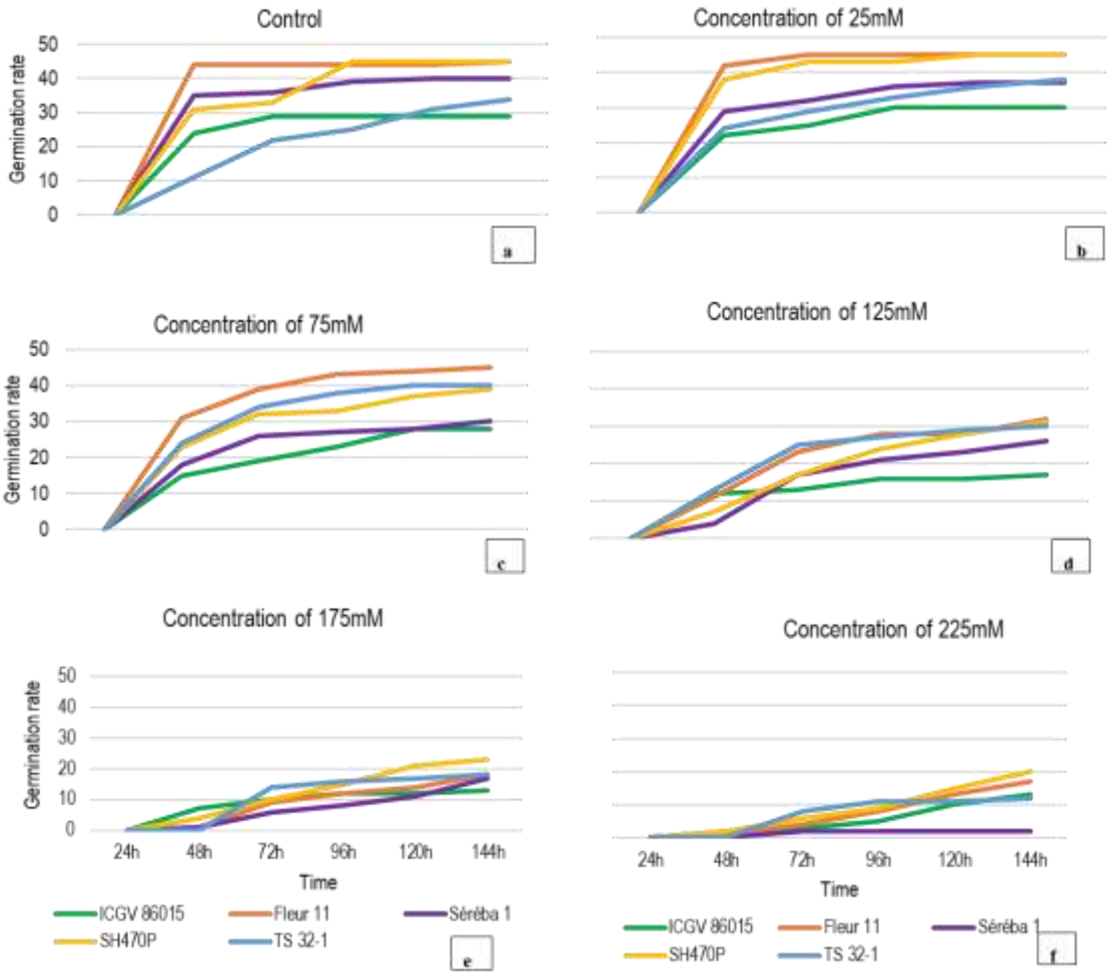

Figure 3: Germination kinetics over six days of five groundnut varieties subjected to different concentrations of NaCl. a: Control 0 mM, b: treatment 25 mM; c: treatment 75 mM; d: treatment 125 mM; e: treatment 175 mM et f: treatment 225 mM.

Effect of salt on groundnut root development: The 25 mM salt treatments had a positive effect on root elongation of some genotypes such as Séréba 1 (Figure 4.a) throughout the experiment, TS 32-1 from 72h and SH470P from 96h to the end of the experiment (Figure 4.a). Conversely, ICGV 86015 and Fleur 11 were negatively affected throughout the study. Additionally, significant differences were observed between the varieties 48 h and 72 h after germination. From 96 h onwards, there was no more significant difference between varieties for the radicle length (Table 2). The 75 mM salt treatment presented a significant effect on radicle development for all the varieties throughout the experiment (Table 2). However, this effect was positive in only two varieties, TS 32-1 at 72h and ICGV 86015 from 120h (Figure 4.b). Apart from TS 32-1 which showed some positive effect at 72 h, the 125 mM salt treatment significantly slowed down radicle growth in all the varieties studied (Figure 4.c). Thus, significant differences were observed between varieties at 48h, 72h and 144h incubation in oven. However, at 96h and 120h, non-significant differences were noted (Table 2). The 175 mM salt treatment had a negative effect on radicle growth in all the varieties studied (Figure 4.d). Significant differences were observed between the varieties, except at 96 h, when the differences between the varieties were not significant (Table 2). The 225 mM salt treatment had a negative effect on radicle growth in all the varieties studied (Figure 4.e). Significant differences were observed between the varieties except at 96h and 120h where the varieties showed non-significant differences (Table 2).

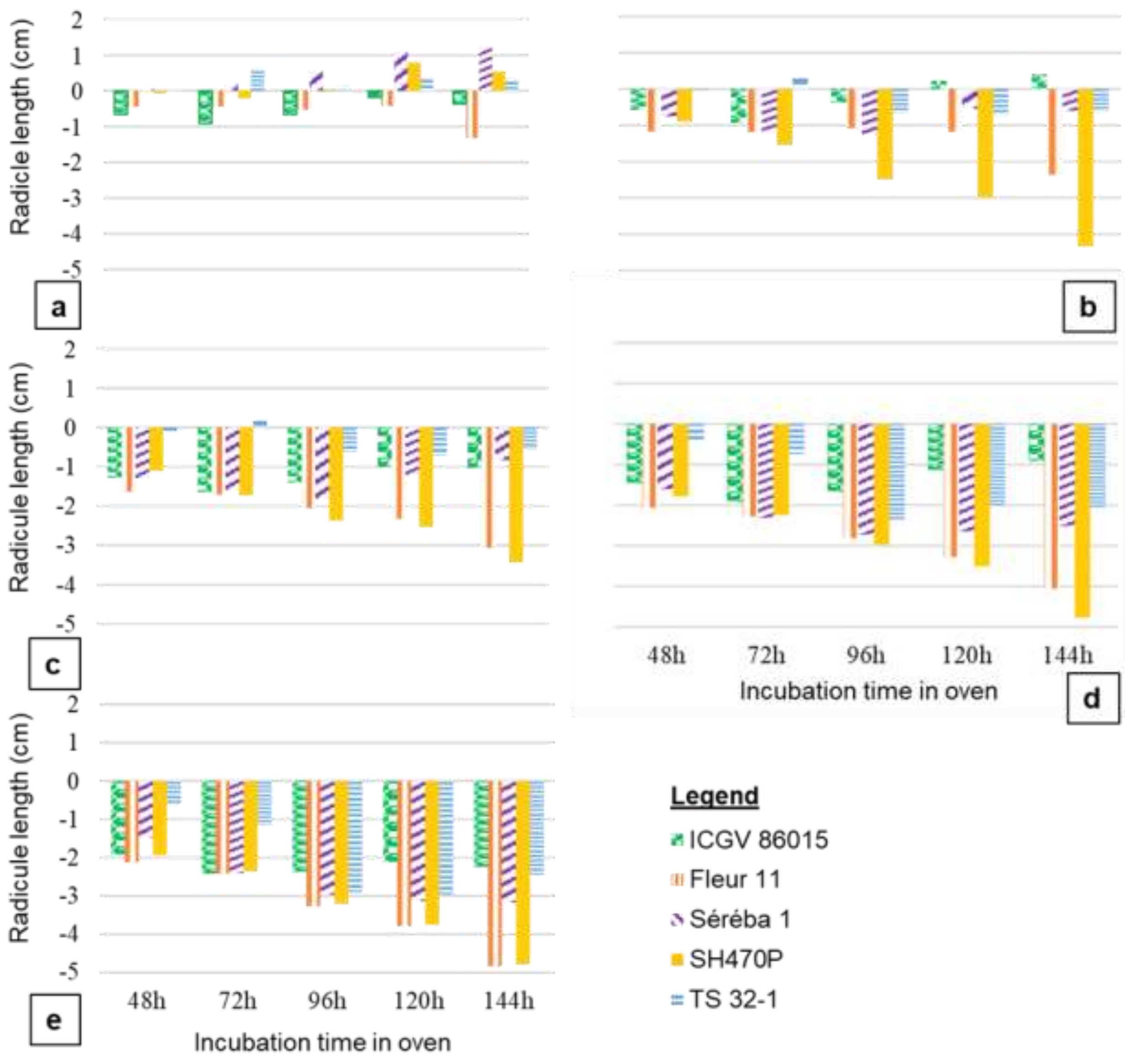

Figure 4: Effect of salt treatments on radicle length compared with the control. a; b; c; d and e: treatments 25 mM; 75 mM; 125 mM; 175 mM and 225 mM, respectively.

Table 2: Analysis of variance of the differences in radicle length between salt (25; 75; 125; 175 and 225 mM) and control (0 mM) treatments of five groundnut varieties (ICGV 86015; Fleur 11; Séréba 1; SH470P and TS 32-1), depending on the incubation time in oven.

Difference with the control		Incubation				
		48h	**72h**	**96h**	**120h**	**144h**
25 mM	ICGV 86015	-0,656 b	-0,911 c	-0,667 a	-0,189 a	-0,367 ab
	Fleur 11	-0,444 b	-0,444 bc	-0,533 a	-0,422 a	-1,322 b
	Séréba 1	0,056 a	0,189 ab	0,544 a	1,078 a	1,211 a
	SH470P	-0,067 a	-0,211 abc	0,044 a	0,800 a	0,544 ab
	TS 32-1	-0,022 a	0,622 a	0,178 a	0,400 a	0,333 ab
	Pr > F	0,001	0,003	0,216	0,192	0,052
	Significant	Yes	Yes	No	No	No
75 mM	ICGV 86015	-0,578 ab	-0,933 b	-0,378 a	0,222 a	0,400 a
	Fleur 11	-1,178 b	-1,189 b	-1,089 ab	-1,178 a	-2,367 b
	Séréba 1	-0,756 b	-1,189 b	-1,278 ab	-0,544 a	-0,600 ab
	SH470P	-0,900 b	-1,544 b	-2,489 b	-2,967 b	-4,333 c
	TS 32-1	-0,022 a	0,289 a	-0,667 a	-0,733 a	-0,633 ab
	Pr > F	0,005	0,001	0,017	0,002	0,000
	Significant	Yes	Yes	Yes	Yes	Yes
125 mM	ICGV 86015	-1,278 b	-1,644 b	-1,400 a	-1,000 a	-1,033 a
	Fleur 11	-1,633 b	-1,711 b	-2,056 a	-2,333 a	-3,067 a
	Séréba 1	-1,289 b	-1,589 b	-1,811 a	-1,211 a	-0,833 a
	SH470P	-1,100 b	-1,722 b	-2,367 a	-2,533 a	-3,433 a
	TS 32-1	-0,089 a	0,167 a	-0,622 a	-0,711 a	-0,544 a
	Pr > F	0 ;000	0,000	0,111	0,061	0,016
	Significant	Yes	Yes	No	No	Yes
175 Mm	ICGV 86015	-1,467 b	-1,922 b	-1,678 a	-1,144 a	-0,911 a
	Fleur 11	-2,067 b	-2,278 b	-2,811 a	-3,278 b	-4,056 bc
	Séréba 1	-1,600 b	-2,311 b	-2,722 a	-2,644 ab	-2,511 ab
	SH470P	-1,778 b	-2,244 b	-2,967 a	-3,511 b	-4,767 c
	TS 32-1	-0,400 a	-0,733 a	-2,367 a	-2,022 ab	-2,056 ab
	Pr > F	0	0	0,176	0,003	0,001
	Significant	Yes	Yes	No	Yes	Yes
225 mM	ICGV 86015	-1,911 bc	-2,433 b	-2,389 a	-2,111 a	-2,256 a
	Fleur 11	-2,122 c	-2,422 b	-3,267 a	-3,789 a	-4,833 b
	Séréba 1	-1,478 b	-2,411 b	-2,967 a	-3,133 a	-3,167 ab
	SH470P	-1,933 bc	-2,356 b	-3,211 a	-3,744 a	-4,789 b
	TS 32-1	-0,633 a	-1,144 a	-2,900 a	-2,956 a	-2,444 ab
	Pr > F	0	0,001	0,581	0,114	0,009
	Significatif	Yes	Yes	No	No	Yes

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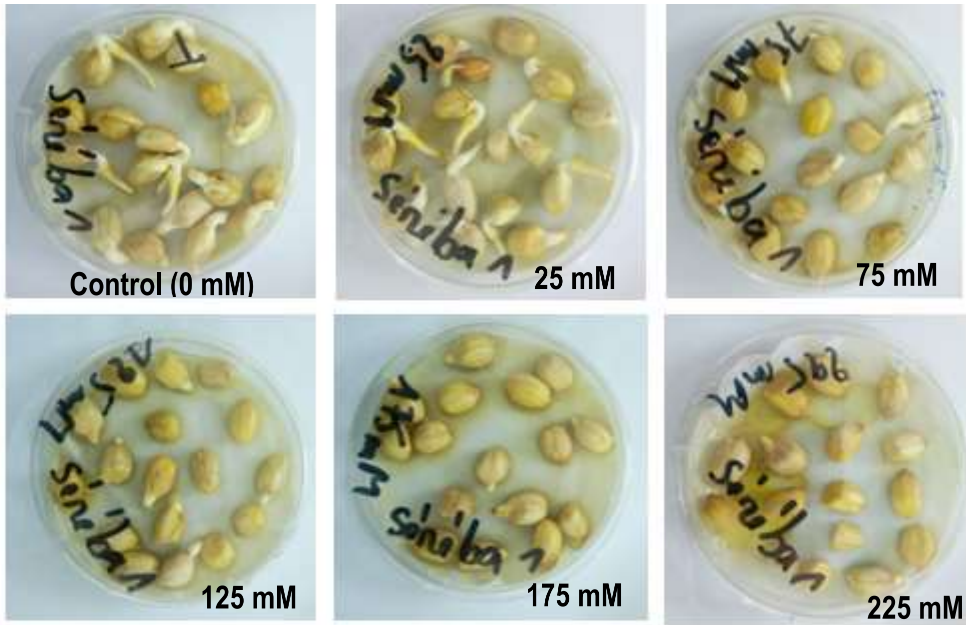

Figure 5: Evolution of the radicle at 96h for Séréba 1 in different concentrations of saline solution

DISCUSSION

Salinity tolerance is an important trait in crops to adapt to the increasing salinization of agricultural land. Detecting this trait early during seed germination would save time in the process of varietal evaluation of response to salt. It emerged from this study that salinity affects both the germination rate and the germination speed of groundnuts, depending on both the genotype and the NaCl concentration. The negative effect of salt stress on the germination rate of seeds is attributable to dormancy induced by the osmotic tension of the salt in the medium (Lonchamp *et al.* 1983). This response is known as a strategy for adapting to environmental constraints (Daas-Ghrib *et al.* 2011). It is also thought to be due to a difficulty in hydrating the seeds following a high osmotic potential, thus preventing the induction of germination or slowing down the process. These results corroborate previous studies carried out on wheat (Lachhab *et al.* 2013, Wissame and Dehiri 2018), *Anabasis articulata* (Benyahia 2020), and chickpea (Kaya *et al.* 2008), showing that salinity has a significant deleterious effect on the germination rate (Mahmoodzadeh *et al.* 2013, Charushahi *et al.* 2015, Borlu *et al.* 2018) and the germination speed (Benyahia 2020). The highest negative effect was observed with the 225 mM NaCl treatments , indicating that the impact of salt on seed germination is proportional to its concentration in the medium (Lachhab *et al.* 2013). At high concentrations, salt may inhibit germination completely (Lachhab *et al.* 2013), although the inhibitory level can be species- and variety-dependent. For instance, the mild salinity (25 - 75 mM NaCl) was beneficial for groundnut varieties TS 32-1 and ICGV 86015; neutral for Fleur 11 and SH470P; and detrimental for Sereba 1. Although the enhancement of seed germination rate in some varieties was counterintuitive, this has biological foundations, since Na^+ is needed in cellular activity, to ensure osmotic potential and maintain cell turgor (Pardo and Quintero 2002). To this extent, moderate salinity has been reported to be beneficial to plant growth (Pardo and Quintero 2002) and to improve crop biomass and yield (.Hassan *et al.* 1970, Guangyuan *et al.* 2007, Konate *et al.* 2019).

The application of salt stress induced a slowdown of the germination rates in all varieties, but with magnitude dependent on the genotype. Such an effect of salt on the germination process was known in plants, especially in salinity-sensitive species (Bentouati *et al.* 2019, Benyahia 2020). A possible reason for the slowdown response to salinity may be the time needed for the seed to readjust osmotic pressure and take up ambient water for germination. Another reason evoked in previous studies was the modification in the enzymes and growth hormones balance in the seed under salt stress (Daas-Ghrib *et al.* 2011). Germination slowdown was along with a slow growth of groundnut radicles, attributable to their sensitivity to the saline environment (Fellahi *et al.* 2019). The negative influence of salt stress on root length has been extensively reported in crops including peas (Okçu *et al.* 2005), cereals (Atak *et al.* 2006), alfalfa (Lachhab *et al.* 2013) and common beans (Benidire *et al.* 2015). In addition to the deleterious effects of salt on seed germination and plant root development, it has been shown that excess salt damages nucleic acids in root cells (Katsuhara and Kawasaki 1996, Konate *et al.* 2018). Such alterations in the genome, more attributable to Cl- than Na+ ions (Boyko *et al.* 2010), could explain the altered root phenotype and the repression of metaxylem development, which is essential for the conduction of water and nutrients under salinity conditions (Hameed *et al.* 2013). As reported in barley (Katsuhara and Kawasaki 1996), salt-induced DNA degradation might stop or slow down cell division and elongation in roots.

CONCLUSION AND APPLICATION OF RESULTS

To conclude, this study demonstrated that salinity affects groundnut germination either by inhibiting or delaying the process in a genotype-dependent manner. Based on the germination parameters studied here, the SH470P revealed to be the most salt-tolerant variety whereas Séréba 1 was the most salt-sensitive one. However, this study needs to be pursued with genotypes evaluation up to plant maturity to substantiate whether their response to salinity during germination is maintained through developmental stages. This would inform whether the early determination of groundnut response to salinity correlates with salt impact on the yield variables, and so effective for varietal trials.

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