



(RESEARCH ARTICLE)



Effect of salinity on the physiology of peanut variety 73-33 (*Arachis hypogaea* L.)

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Abstract

Peanut (*Arachis hypogaea*, L.) is an annual papilionaceous oilseed legume cultivated on nearly 25 million hectares in tropical and temperate zones due to its remarkable plasticity to temperature and water requirements. In Senegal, peanut is produced in rural areas where they are the main source of agricultural income. Variety 73-33 is cultivated in the river valley and the groundnut basin is subject to the influence of salinization affecting four of Senegal's ecogeographic zones.

Studies conducted with randomized full-block experimental set-up in the laboratory and under semi-controlled greenhouse conditions have shown the effect of different concentrations of NaCl on the peanut variety 73-33: 0mM; 25mM; 50mM; 100mM; 200mM; 300mM; 400mM; 500mM for germination and 0mM; 25mM; 50mM; 100mM for growth and chlorophyll production. The parameters which were measured are: the germination rate (ten days after sowing), the number of nodules, the number of gynophores, the dry biomass and the production of chlorophyll at twenty, forty and seventy-five days after sowing.

Germination is significantly negatively affected from 400mM.

Twenty days after sowing there is a significant difference between the control and the other treatments for the production of chlorophyll a (Chla) only.

Forty days after sowing, the production of chlorophyll a and total chlorophyll shows a very highly significant difference between all the treatments. The dry biomass only shows a significant difference from 100mM.

Seventy-five days after sowing, the dry biomass and the number of gynophores are significantly reduced by the salinity from 25mM.

Keywords: Peanut; Chlorophyll; Growth; Germination; NaCl

1. Introduction

Groundnut or ground pistachio (*Arachis hypogaea*, L.) is an annual papilionaceous oilseed legume that grows in hot countries [1]. It is cultivated over an area of nearly 25 million hectares throughout the tropics and in temperate zones up to 40° C North latitude thanks to its remarkable plasticity in the face of temperatures and water requirements. Africa

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provides 25% of its production mainly from Nigeria, Senegal and Sudan. In Senegal, it is cultivated on nearly one million hectares [2]. Produced for its agronomic and food interests, it is the main source of income, especially economic in rural areas ([2]; [3]). Many varieties are cultivated in Senegal [4]. Among these, variety 73-33 of type Virginia, resistant to drought, to *Aspergillus flavus* (aflatoxin), plastic and slightly dormant. Its cycle is 105 days [5]. This variety is one of the best among the main groundnut varieties grown in West Africa. It can be used as a peanut for food or in confectionery; its thousand-seed weight is greater than 50 g [6].

It is used in rainfed and irrigated crops in the river valley and the groundnut basin [5] and subject to the influence of salinization affecting four of the ecogeographic zones of Senegal [7].

Salinization is a brake on their agricultural development [8], and one of the causes of the drop in agricultural production [9] which affects more than one million hectares of Senegambian lands. It is caused by various types of salt (Na_2SO_4 , MgSO_4 , etc.), but especially by sodium chloride (NaCl), one of the main abiotic stresses that inhibit plant growth and reduce their productivity [10].

Indeed, when salty soils if they contain 40 mM of NaCl or if their electrical conductivity is greater than 4 dS m^{-1} or if their osmotic potential is less than 0.117 MPa, they have a degraded structure, a low porosity and a pH high (> 8) ([11]; [12]). The plants that develop on these soils are subjected to physiological drought and have a very low root osmotic potential (around -100 bars) [11] leading to an inhibition of their growth and a reduction in their productivity [10].

In Senegal, the salinity is estimated at 9% in the Niayes, 20% in the groundnut basin, between 26 and 37% in eastern Senegal, between 36 and 53% in Casamance to reach a maximum of 66% in the River region [13].

In this study our objective is to know under semi-controlled conditions the effect of the salinity due to NaCl on the peanut variety 73-33.

2. Material and methods

2.1. Plant material

The plant material consists of seeds of the peanut variety 73-33. The seeds are disinfected with 5% sodium hypochlorite for 2 minutes and then rinsed three times with sterile distilled water. They are sown in Petri dishes on tissue paper moistened with 10 ml of the corresponding solution. A seed is considered germinated when there has been emergence of the radicle [14].

2.2. Experimental apparatus

A first experimental device randomized to an abiotic constrained factor (NaCl) with eight modalities (0 mM; 25 mM; 50 mM; 100 mM; 200 mM; 300 mM; 400 mM and 500 mM) is used to evaluate the effect in vitro of salinity on peanut germination.

The second device, a randomized full-block experimental set-up with a four-modality ($T_0 = 0$ mM; $T_1 = 25$ mM; $T_2 = 50$ mM; $T_3 = 100$ mM) abiotic stress factor (NaCl), is used to determine the effect of salinity on plant growth and chlorophyll production.

In both devices, each treatment is repeated three times.

2.3. Soil sample

The experimental unit was represented by a pot containing 1.5 kg of substrate. The substrate used is the soil of the botanical garden of the Faculty of Sciences and Techniques of the University Cheikh Anta Diop in Dakar, the characteristics of which are defined (Table 1).

Table 1 Physicochemical characteristics of the soil used

pH eau 1 / 1,25	7.4
CE 1 / 10 $\mu\text{s}/\text{cm}$	65
% C	2.37
% MO	4.086
% N	0.21
C / N	11
Ca meq / 100 g	6.9
Mg meq / 100 g	0.525
Na meq / 100 g	0.0425
K meq / 100 g	0.139
P ppm	48
S meq / 100 g	7.606
CEC meq / 100 g	15
T %	51
PSE %	0.3
A %	10.75
LF %	2.5
LG %	1.29
SF %	48.3
SM %	36.66
SG %	0.485

T: rate of base saturation; PSE: Percentage of Exchangeable Sodium; A: clay; LF: fine silt; LG: coarse silt; SF: fine sand; SM: medium sand; SG: coarse sand.

2.4. Culture mode

Three seeds are sown in each pot. The pots are sprayed with tap water for up to 10 days after emergence.

For each pot the best plant was kept. The saline treatment is applied from the tenth after the emergence of the seedlings. Watering to capacity in the field [15] is done every 3 days with the corresponding solutions. Three harvests were made, the first at the end of the growth phase 20 days after sowing (20 DAS), the second at the end of the flowering phase 40 days after sowing (40 DAS) and the last at 75 days after sowing (75 DAS) corresponding to the start of maturity for the earliest varieties [16].

2.5. Parameters measured

The final germination rate is evaluated after 10 days for laboratory experimentation.

At the third harvest (75 days after sowing), the number of gynophores and the number of nodules per plant were counted.

The mass of dry matter obtained after drying in an oven at 70 ° C for 48 hours ([17]; [15]) is measured with a precision balance ($400 \pm 0.001\text{g}$). The number of leaves on the main stem and the number of branches were counted for each plant.

The chlorophyll content is estimated based on the Arnon method [18] ; a mass of 100 mg of fresh plant material (leaves) was ground with 10 ml of 80% acetone, the ground material was centrifuged at 4000 revolutions / minute for five minutes. The supernatant which contains the pigments is recovered. Optical densities are read at wavelengths 645 nm and 663 nm. The chlorophyll content is determined according to the Arnon [18] equations:

$$\text{Chl a (mg/gFW)} = 12.7 \times \text{DO (663)} - 2.69 \times \text{DO (645)} \text{ (mg/gFW) (Equation 1)}$$

$$\text{Chl b (mg/gFW)} = 22.9 \times \text{DO (645)} - 4.68 \times \text{DO (663)} \text{ (mg/gFW) (Equation 2)}$$

$$\text{Chl (mg/gFW)} = \text{Chla} + \text{Chlb} = 20.2 \times \text{DO (645)} + 8.02 \times \text{DO (663)} \text{ (mg/gFW) (Equation 3)}$$

2.6. Statistical analyzes

Statistical analyzes were performed with R software version 3.6.3 (2020-02-29). All data is subjected to the Shapiro-Wilk normality test. Statistical processing of normally distributed data is performed by adopting a parametric approach with analysis of variance (ANOVA). For data with non-normal distribution, a non-parametric approach is applied with an analysis of variance on the ranks of the means. The Tukey test at the 5% probability threshold is performed in order to compare and rank the means or ranks on the means of the variables evaluated.

3. Results

3.1. Effect of salinity on germination of peanut

The germination rate (TR) of peanut variety 73-33 is not affected by salinity up to 200 mM NaCl and is 100%. Between 200 and 300 mM, a slight decrease is observed without this translating into a significant difference. From 300 mM the level decreases to cancel out at 500 mM (Figure 1).

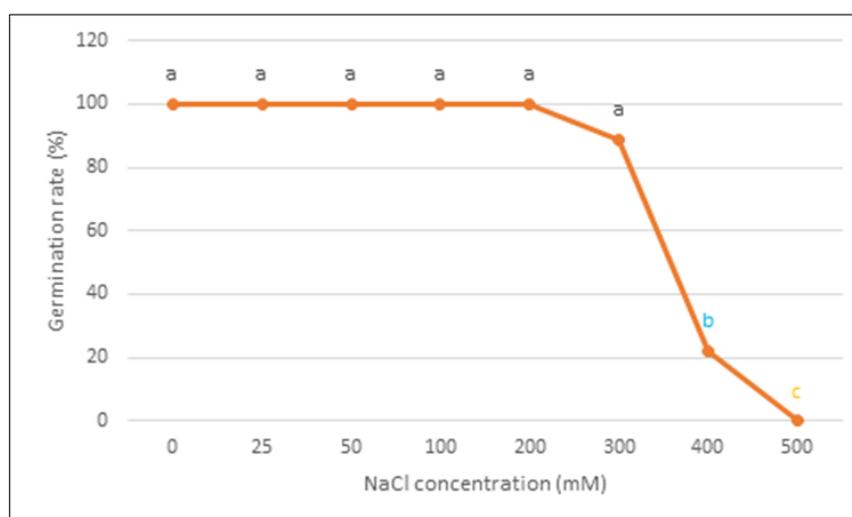


Figure 1 Effect of salinity on the germination rate of peanut variety 73-33

The same letter indicates that there is no significant difference at the 5% level on the Student-Newman-Keuls test (SNK).

3.2. Effect of salinity on dry biomass

The mass of dry matter decreases with salinity, especially at 75 DAS. No significant difference was observed for this mass of dry matter at twenty days after sowing (20 DAS) unlike the 75th DAS where a difference was observed between all the concentrations. A significant difference is noted forty days after sowing (40 DAS) between the biomass at 100 mM and that of the other treatments (Figure 2).

The mass of dry matter of the control (0 mM) is greater and that of the treatment with the greatest concentration of NaCl (100 mM) is the lowest whatever the harvest date. The dry biomass (DB) of peanut variety 73-33 decreased with increasing salt (NaCl) content (Figure 2).

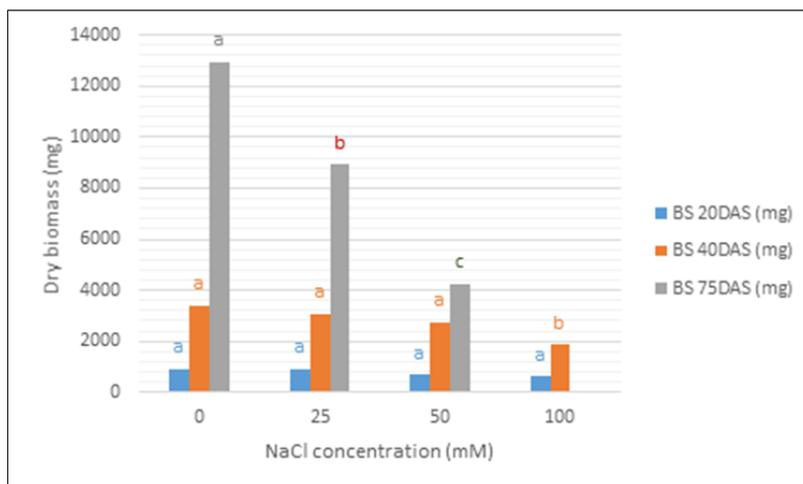


Figure 2 Effect of salinity on dry biomass in peanut variety 73-33 at 20 DAS, 40 DAS and 75 DAS
Dry biomass (DB), twenty days after sowing (20DAS), forty days after sowing (40DAS), seventy-five days after sowing (75DAS).
The bars followed by the same letter are not significantly different at the 5% level on the Student-Newman-Keuls test (SNK).

3.3. Effect of salinity number of gynophores and nodules

The number of gynophores and nodules of the plants of the peanut variety 73-33 decreased respectively from 13 to 1 and 12 to 4 with the increase in the concentration of NaCl without this translating into significant differences for the plants. 25 mM and 50 mM NaCl concentrations relative to the control. For the number of nodules there is no significant difference (figure 3).

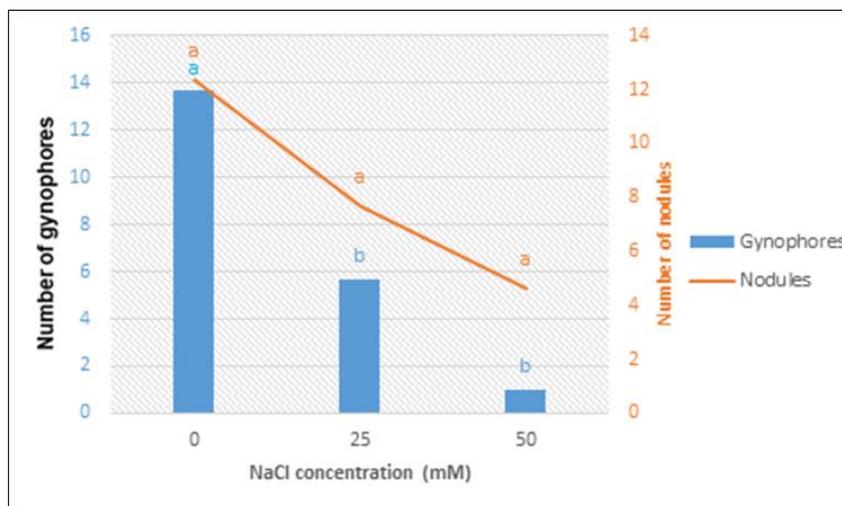


Figure 3 Effect of salinity on the number of gynophores and nodules in the peanut variety 73-33 to 75 DAS
Seventy-five days after sowing (75DAS).

The same letter indicates that there is no significant difference at the 5% level on the Student-Newman-Keuls test (SNK).

3.4. Effect of salinity on chlorophyll

The amount of chlorophyll (Chla, Chlb, Chl) in plants of the peanut variety 73-33 decreased from 0.85 to 0.47 mg / g and 3.38 to 2.49 mg / g with increasing of the NaCl concentration (amount of NaCl in the irrigation water) at 20 JAS and 40 DAS respectively (Figures 4 to 6).

On the other hand at 75 JAS, the quantity of chlorophyll increased from 1.08 to 1.20 mg / g with salinity (Figure. 4). The amount of chlorophyll is lower at 20 JAS but is higher at 40 JAS regardless of the NaCl concentration (Figures 4 to 6).

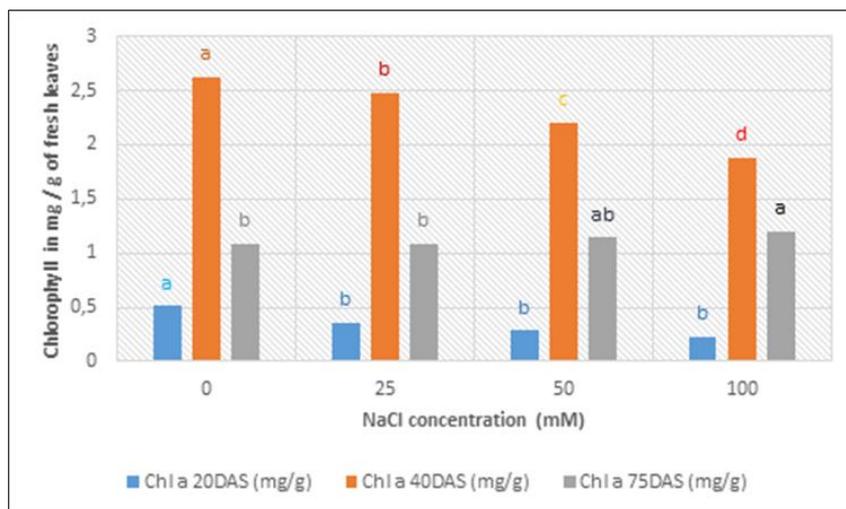


Figure 4 Effect of salinity on the amount of chlorophyll a in the peanut variety 73-33 at 20 DAS, 40 DAS and 75 DAS Chlorophyll a (Chla), twenty days after sowing (20DAS), forty days after sowing (40DAS), seventy-five days after sowing (75DAS). The bars followed by the same letter are not significantly different at the 5% level on the Student-Newman-Keuls test (SNK).

The quantity of chlorophyll a (Chla) is greater than that of chlorophyll b (Chlb) regardless of the date of harvest (Figures 4 and 5). Statistical analysis does not show a significant difference for the total chlorophyll (Chl) and chlorophyll b (Chlb) of the plants of the different treatments at each of the harvests on dates 20 JAS and 75 JAS (Figures 4 and 5). However, chlorophyll a (Chla) shows significant differences between the control and the other treatments at 20 DAS and between all the treatments except the control and the lowest concentration (25 mM NaCl) at 75 DAS (Figure 4).

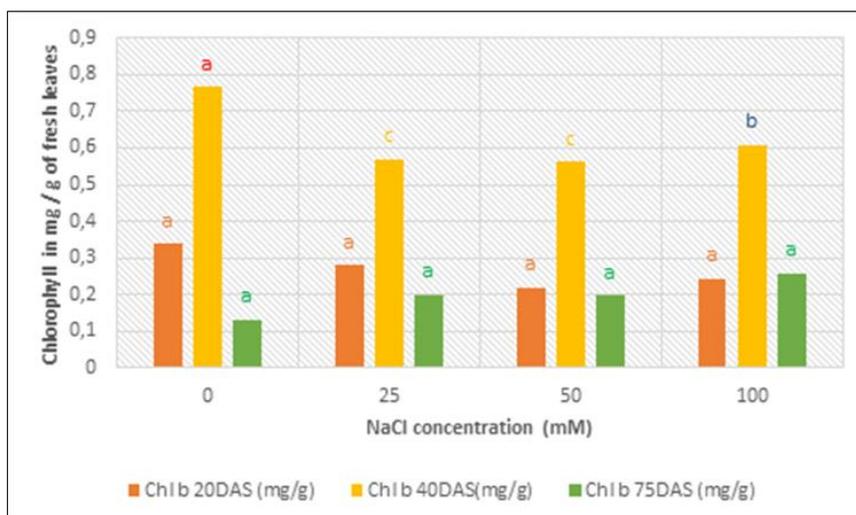


Figure 5 Effect of salinity on the amount of chlorophyll b in the peanut variety 73-33 at 20 DAS, 40 DAS and 75 DAS Chlorophyll b (Chlb), twenty days after sowing (20DAS), forty days after sowing (40DAS), seventy-five days after sowing (75DAS). The bars followed by the same letter are not significantly different at the 5% level on the Student-Newman-Keuls test (SNK).

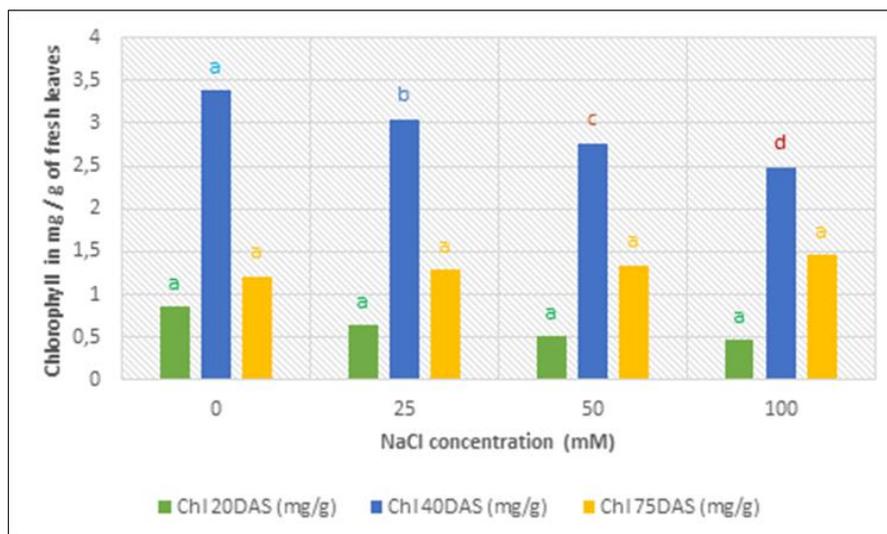


Figure 6 Effect of salinity on the amount of total chlorophyll in the peanut variety 73-33 at 20 dAS, 40 DAS and 75 DAS Total chlorophyll (ChI), twenty days after sowing (20DAS), forty days after sowing (40DAS), seventy-five days after sowing (75DAS). The bars followed by the same letter are not significantly different at the 5% level on the Student-Newman-Keuls test (SNK).

4. Discussion

The germination rate (TR) of peanut variety 73-33 is not affected by salinity for concentrations less than or equal to 200 mM NaCl, it is 100%. From 300 mM, there is a decrease in the germination rate as a function of the salinity (figure 1). These results are in agreement with those of Ndiaye *et al.* [9] who showed a decrease in the final germination percentage in *Gossypium hirsutum* L. with increasing salinity. The germination rates at the concentrations 400 mM and 500 mM show a significant difference between them and also with those of the concentrations less than or equal to 300 mM. These results agree with those of Dangué *et al.* [19] who showed a significant reduction in the germination rate as a function of salinity in sesame (*Sesamum indicum* L.).

The dry biomass (BS) of peanut variety 73-33 decreased with increasing salt (NaCl) content over the harvest date (Figure 2). This negative effect of NaCl on dry biomass has been noted in various species such as roselle [20], wheat [21], chili [22], cowpea (*Phaseolus vulgaris* L.) [23], sesame ([19]; [24]; [25]). This reduction may be related to the toxic effect of Cl^- and Na^+ ions accumulated in the tissues ([26]; [27]). The effects of salinity are manifested mainly by slowing the growth of the vegetative system [15]. This slowing down may explain the decrease in dry biomass with increasing salinity. Bekele *et al.* [28] claim that salinity causes morphological, physiological, biochemical and molecular changes negatively affecting plant growth and metabolism. However, according to these authors crops react differently to salinity and some can be tolerant.

The increased salinity resulted in a reduction in the number of gynophores in plants of the peanut variety 73-33 (Figure 3). Statistical analysis only shows a significant difference in the number of gynophores for the 25 mM and 50 mM NaCl concentrations compared to the control.

These results are consistent with those of Srivastava *et al.* [29], which showed a significant reduction in the number of gynophores due to soil salinity.

The number of nodules at the level of the roots of plants of the peanut variety 73-33 decreases according to the salinity but does not show any significant difference (Figure 3). These results agree with those of Tegger [30] and Saadallah *et al.* [31] who observed, respectively, a decrease in the number of nodules in lentil (*Lens culinaris* L.) and in beans (*Phaseolus vulgaris* L.) proportional to the intensity of the salt stress applied. Similar results are also obtained in beans. According to Saadallah *et al.* [31], the reduction in the number of nodules is linked to a decrease in the potential sites of infection resulting mainly from an inhibition of the emergence and elongation of root hairs and of the growth of the root system in general. Shahid *et al.* [32] showed a depressive effect of salinity on the number of nodules in *Vigna radiata* inoculated with halotolerant bacteria.

Chlorophyll a is more affected by salinity than chlorophyll b in peanut variety 73-33 (Figures 4 and 5). The greatest reduction in chlorophyll a may be related to the sensitivity of one of the steps in its biosynthesis to sodium chloride

[33]. However, Gomes et al. [34] showed greater tolerance to the salinity of chlorophyll a in *Salvinia auriculata* Aubl., An aquatic fern. Thouraya et al. [22] obtained similar results in chili (*Capsicum annum* L). The depressive effect of salinity is more pronounced on chlorophyll a than on chlorophyll b ([35]; [36]). This is explained by a greater sensitivity of the biosynthesis of chlorophyll a to sodium chloride ([35]; [37]). The amounts of chlorophyll in peanut variety 73-33 decreased with increasing salinity (Figures 4, 5, 6). These results are consistent with those of Al-Khaliel [38] who did not observe a significant difference, in peanuts, between the amounts of chlorophyll as a function of salinity. This reduction of chlorophyll by salinity has been obtained in tomato [33], peanuts [38], banana [35], chili [22] and cowpeas (*Phaseolus vulgaris* L.) [23]. Salinity induces nutritional disorders causing damage to macromolecules such as chlorophyll due to loss of membrane integrity and photosynthetic activity [39]. This reduction in chlorophyll is explained by the stimulation of the enzymatic activity of chlorophyllase by salinity [40]. It can be due to the destruction of the precursors of chlorophyll, to the conversion of the latter to other pigments or to the decrease in the accumulation of Mg, an integral component of photosynthetic pigments, to the destruction of the fine structure of chloroplasts or instability of the pigment-protein complex [41]. This reduction in chlorophyll may also be linked to the sensitivity of its biosynthesis to NaCl [37]. Salinity also induces nutritional disorders causing damage to macromolecules such as chlorophyll due to loss of membrane integrity and photosynthetic activity [39]. NaCl also reduces the activity of chlorophyll by inhibiting the synthesis of 5-amino-levulinic acid or by occupying the site of action of nitrogen (N), an essential component of chlorophyll ([22]; [37]).

5. Conclusion

The effect of different concentrations (0 mM, 25 mM, 50 mM, 100 mM, 200 mM, 300 mM, 400 mM and 500 mM) of NaCl on the germination of seeds of the peanut variety 73-33 grown in Senegal has been evaluated in vitro in the laboratory. This variety of peanut is insensitive to salinity up to 200 mM NaCl during its germination. Beyond 300 mM, its germination decreases according to the salinity. From 500 mM NaCl the seeds of this variety can no longer germinate.

The effect of different concentrations (0 mM, 25 mM, 50 mM and 100 mM) of NaCl on the growth of seedlings of peanut variety 73-33 grown in Senegal was determined under semi-controlled greenhouse conditions. The amount of chlorophyll (Chl a, Chl b, Chl) of the plants of the peanut variety 73-33 decreased with the increase in the concentration of NaCl at 20 DAS and 40 DAS, but at 75 DAS the amount of chlorophyll increased with salinity which would be a physiological adaptation.

Dendrometric parameters such as apical growth (length of the main stem), number of leaves and number of branches are negatively influenced by salinity from 25 mM.

The salinity leads to a reduction in the number of nodules at the level of the roots and gynophores.

Dry biomass is the parameter most negatively affected by salinity.

Ultimately, this study shows that the 73-33 peanut variety cultivated in Senegal is sensitive to salinity. This sensitivity can be manifested by a reduction of the parameters studied, a decrease in the quantity of chlorophyll 40 JAS and a lethality or a reduction of the parameters studied, it depends on the stage of development of the plant.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that no conflict of interest exist.

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