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Physiology and adaptation strategy of sesame (*Sesamum indicum L.)* to salinity

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Abstract

Salinization is a global environmental problem. It is particularly prevalent in Africa in areas with a low rainfall trend such as the Senegalese groundnut basin where 20% of the land is affected. It reduces global food production by more than 10%.

In Senegal, sesame (*Sesamum indicum* L.) moderately tolerant to drought and salinity is increasingly cultivated. It is an alternative to fight poverty in rural areas and allows the revaluation of salty land.

The objective of this work is to evaluate at the early stage of reproduction the effect of salinity on the chlorophyll and ion (Na^+ and Cl^-) contents of four African varieties of sesame.

The experimental device consists of randomized blocks with two factors and three repetitions. The sesame variety factor consists of four modalities (AS09, AS14, AS15 and AS25). The salinity factor or abiotic stress (NaCl) includes three modalities (0 mM, 17 mM and 34 mM).

The parameters evaluated are the contents of Na^+ and Cl^- ions and of chlorophylls (Chl a, Chl b and total Chl).

The results showed that the contents of chlorophylls (Chl a, Chl b and Chl) and Cl^- and Na^+ ions of sesame leaves increased with NaCl at 44 days after sowing. The Cl^- contents of sesame leaves are higher than those of Na^+ in all treatments.

Chlorophyll increased with sesame varieties and salinity. Variety AS15 produced the greatest amounts of chlorophyll. Sesame, an inclusive-type plant, compartmentalizes Cl^- and Na^+ ions at the leaf level in vacuoles. He developed a tissue tolerance to salinity.

Keywords: Salinity; Chlorophyll; Sesame; Na+; Cl^-

1. Introduction

Land salinization is a major global problem and is the primary environmental constraint in Africa ([1]; [2]). It is especially widespread in arid and semi-arid areas [3]. It leads to a decrease in the productivity of the affected land.

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Excessive salinization affects the rhizosphere and limits the distribution of plants in their natural habitat. At the plant level, sodium (Na^+) and chloride (Cl^-) ions enter through the roots, and are transported by the xylem sap to the stems and leaves. These ions are either stored in the vacuoles (inclusive plants), or on the contrary very little retained and mobilized by the phloemic sap to the roots (exclusive plants). Ion exclusion is a process of transport of Na^+ and Cl^- ions in the roots reducing their accumulation in the leaves. Tissue tolerance is manifested by high salt concentrations in the leaves, but the ions (Na^+ and Cl^-) are compartmentalized in the vacuole [4]. This concentration of mineral ions in vacuoles allows plants to achieve osmotic adjustment and avoid physiological drought caused by salinity [5]. These mechanisms allow plants to adapt to salinity.

In Senegal, saline land has increased by an average of 1,000 hectares per year since 1994. It has affected 1,700,000 hectares since 1997, or 45% of the country's total cultivable area [2]. It affects practically all regions of Senegal to varying degrees [6]. It is higher in the groundnut basin [2] where it is estimated at 20% [7].

Salinization is likely to reduce global food production by about 10% [8]. Moderate to high salinity reduces the yield of most grains and oilseeds by at least 50% [9]. Crop adaptation to salinization is one of the greatest challenges of modern agriculture [10]. However, in the Senegalese eco-geographical zones, the diversification of cultures occupies a place of choice. In addition to traditional crops such as millet and peanuts, other crops such as sesame (*Sesamum indicum* L.) are increasingly developed.

In Senegal, the cultivation of sesame is relatively old since its practice in Casamance dates back to the colonial era. However, after independence, sesame had practically disappeared in favor of peanuts and remained only as a vestige for the needs of traditional pharmacopoeia. Sesame was reintroduced to Senegal in 1985 from Gambia extensively and informally [11]. This reintroduction took place in a context of crop rotation, crop diversification, increased income for rural populations and the fight against food insecurity ([11]; [12]). However, like other crops, sesame is confronted with the effects of biotic and abiotic stresses; its productivity is limited by drought and salinity ([13]; [14]). Sesame, sensitive at the seedling and germination stages, is moderately tolerant to drought and salinity [15].

This work aims to evaluate at the early stage of reproduction the effect of salinity on the chlorophyll and ion (Na^+ and Cl^-) contents of four African varieties of sesame.

2. Material and methods

2.1. Plant material and growing medium

The plant material consists of the seeds of four varieties of sesame from Mali (AS09), Cameroon (AS14), Sudan (AS15) and Togo (AS25) obtained after NaCl screening of 13 African varieties.

The substrate used is sandy soil from the botanical garden of the Faculty of Sciences and Techniques of the Cheikh Anta Diop University of Dakar [16].

2.2. Experimental apparatus

The experimental device consists of randomized blocks with two factors and three repetitions. The sesame variety factor consists of four modalities (AS09, AS14, AS15 and AS25). The salinity factor or abiotic stress (NaCl) includes three modalities (0 mM, 17 mM and 34 mM). The experimental unit consisted of a pot containing 1 kg of the culture substrate. In each pot, five seeds of one variety were sown. Seven days after sowing, we thinned out, keeping two plants in each pot. The saline constraint is applied by watering from one week after germination until data collection at 44 days after sowing [17]. Watering at field capacity was carried out daily with the corresponding solution (non-saline for the control and saline for the other treatments) in order to avoid any water deficit.

2.3. Parameters measured

In each pot, one of the plants was used to determine the following parameters: the Na^+ and Cl^- ion contents and the chlorophyll contents (Chl a, Chl b and total Chl). Chlorophyll contents were estimated based on Arnon's method [18]. A mass gain of 100 mg of fresh plant material (leaves) was ground with 10 ml of 80% acetone, the ground material is centrifuged at 4000 rpm for five minutes. The supernatant which contains the pigments is recovered. Optical densities are read at 645 nm and 663 nm wavelengths.

The chlorophyll contents are determined according to Arnon's [18] equations:

- Chl a (mg/gFW) = 12.7 x DO (663) – 2.69 x DO (645) (mg/g FW) ....(Equation 1)
- Chl b (mg/gFW) = 22.9 x DO (645) – 4.68 x DO (663) (mg/g FW) .... (Equation 2)
- Chl (mg/gFW) = Chla + Chlb = 20.2 x DO (645) + 8.02 x DO (663) (mg/g FW) .... (Equation 3)

The Na^+ and Cl^- ion contents in the leaves harvested 44 days after sowing were measured at the Analysis and Testing Laboratory of the Ecole Supérieure Polytechnique de Dakar (ESP).

The Na^+ is measured in the ashes by ion chromatography (ICS1100 DIONEX, Methanesulfonic acid, 1 ml/min, 59 mA, conductivity) according to the method used by Giuffrida *et al.* [19].

The Cl^- was determined by colorimetry according to the method used by Khaled *et al.* [20].

2.4. Statistical analyzes

Statistical analyzes were performed with R software version 3.6.3 (2020-02-29). All data are subjected to the Shapiro-Wilk normality test. Statistical processing of normally distributed data are 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 NaCl on chlorophyll contents of sesame leaves

Table 1 shows the results of the statistical analyzes of the Tukey test for chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Chl) of the four African varieties of sesame at 44 days after sowing (44 DAS). The differences are statistically very significant within the variety and between varieties (Chl a and Chl: p-value $<2^{e-16}$ *** and Chl b: p-value = 4.65^{e-15} ***). In the absence of salinity, the lowest amounts of chlorophyll are produced by the AS25 sesame variety and the highest by the AS15 variety. In the presence of NaCl, the highest chlorophyll contents are obtained with the AS15 sesame variety at 34 mM NaCl and the lowest with the AS14 variety at 17 mM. The amounts of chlorophyll obtained at 17 mM NaCl decreased for sesame varieties AS14 and AS15 compared to those of their controls. The chlorophyll contents (Chl a, Chl b, Chl) increased with salinity for the four African varieties of sesame (Table 1).

Table 1 Amount of chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Chl) of four African varieties of sesame as a function of salinity at 44 days after sowing

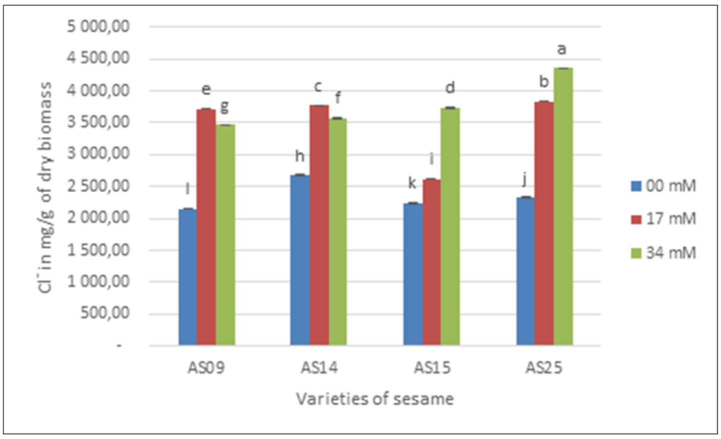
Sesame varieties	NaCl	Chl a (mg/g FW)	Chl b (mg/g FW)	Chl (mg/g FW)
AS09	0 mM	1,35 ± 0,01 fg	0,42 ± 0,01 ef	1,77 ± 0,02 e
	17mM	1,38 ± 0,01 f	0,42 ± 0,0 ef	1,79 ± 0,02 de
	34mM	1,62 ± 0,02 c	0,50 ± 0,03 cd	2,12 ± 0,06 c
AS14	0 mM	1,28 ± 0,01 h	0,39 ± 0,01 efg	1,67 ± 0,02 ef
	17mM	1,17 ± 0,01 ij	0,34 ± 0,01 g	1,50 ± 0,02 g
	34mM	1,48 ± 0,01 e	0,44 ± 0,02 de	1,92 ± 0,03 d
AS15	0 mM	1,84 ± 0,00 b	0,57 ± 0,01 bc	2,41 ± 0,01 b
	17mM	1,31 ± 0,01 gh	0,41 ± 0,02 ef	1,72 ± 0,02 e
	34mM	2,49 ± 0,02 a	0,72 ± 0,02 a	3,21 ± 0,04 a
AS25	0 mM	1,13 ± 0,01 j	0,34 ± 0,01 g	1,46 ± 0,01 g
	17mM	1,20 ± 0,01 i	0,36 ± 0,02 fg	1,56 ± 0,03 fg
	34mM	1,84 ± 0,04 b	0,58 ± 0,05 b	2,41 ± 0,09 b
p-value		$<2^{e-16}$ ***	$4,65^{e-15}$ ***	$<2^{e-16}$ ***

Meaning codes: 0 (very significant) '***'; 0.001 (significant) '**'; 0.01 (not significant) '*'; On the same column, the averages assigned the same letters do not show any significant differences.

3.2. Adaptation strategy of sesame to NaCl

The Cl^- contents of the four varieties of sesame vary significantly depending on the treatments and the varieties. The Cl^- contents are lower for the control treatments. The leaves of the AS09 and AS14 sesame varieties showed higher Cl^- contents at 17 mM NaCl than those of the 34 mM treatments. However, sesame varieties AS15 and AS25 showed a significant increase in Cl^- content in leaves with increasing salinity. The Cl^- levels in the leaves are significantly lower in the controls compared to the saline treatments.

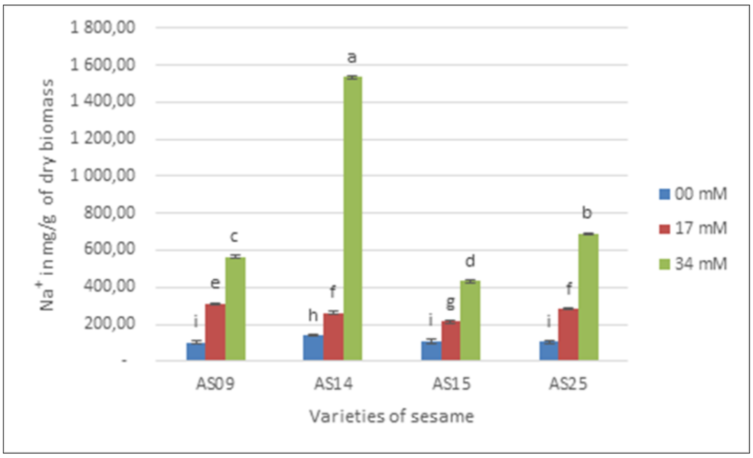
In the absence of salinity, the leaves of the AS14 sesame variety contain more Cl^- (2,671.00 mg/g of dry matter) than those of the other varieties. While in the saline treatments the leaves of the AS25 sesame variety show the highest Cl^- contents which are respectively 3,832.33 mg/g and 4,351.00 mg/g of dry matter with concentrations of 17 mM and 34 mM NaCl (Figure 1).



The bars followed by the same letter are not significantly different at the 5% level on the Student-Newman-Keuls test (SNK)

Figure 1 Cl– content in dry leaves as a function of NaCl concentrations of four African sesame varieties at 44 days after sowing

Figure 2 shows the variation in Na^+ content as a function of salinity for the four varieties of sesame. The Na^+ contents in the leaves are higher in the saline treatments than in the controls. Leaf Na^+ contents increased significantly with salinity for all sesame varieties. In the absence of salinity, the leaves of the AS14 sesame variety contain more Na^+, which is equal to 138.00 mg per gram of dry matter. At 17 mM NaCl, the leaves of the AS09 sesame variety have the highest Na^+ content (311.00 mg/g dry matter). While at 34 mM, the leaves of the AS14 sesame variety contain the high Na^+ content, it is 1534.67 mg/g of dry matter (Figure 2).



The bars followed by the same letter are not significantly different at the 5% level on the Student-Newman-Keuls test (SNK).

Figure 2 Na^+ content in dry leaves as a function of NaCl concentrations of four African sesame varieties at 44 days after sowing

4. Discussion

Statistical analyzes using Tukey's test showed very significant differences at 44 days after sowing for all treatments.

The chlorophyll contents (Chl a, Chl b, Chl) of the four African sesame varieties increased with salinity at 44 days after sowing. The AS15 variety has the highest amounts of chlorophyll (Table 1). The work of Hussein *et al.* [21] showed an increase proportional to the salinity of the quantities of chlorophylls a, b and total in pepper. Bazrafshan and Ehsanzadeh [22] obtained higher chlorophyll contents at 30 mM NaCl than controls in seven sesame (*S. indicum* L.) genotypes. These results agree with those of Sivasankaramoorthy [23] who observed an increase in photosynthetic pigments (chlorophylls a, b, total and carotenoids) in pepper at 5 and 10 mM NaCl concentrations. These results corroborate those of Thouraya *et al.* [24] who showed an increase in chlorophyll a and total in a variety of pepper at 50 mM compared to 25 mM. Similar results were obtained in vetiver (*Vetiveria zizanioides* stapf.) [25], in *Brassica juncea* [26]. The increase in the amounts of chlorophyll is due to the inhibition of the activity of chlorophyllase since its decrease results from the stimulation of the activity of this enzyme ([25]; [27]). The increase in chlorophyll can also be explained by a plant's tolerance to salinity ([16]; [28]).

However, Kanagaraj and Desingh [29] showed in seven varieties of sesame a reduction in chlorophyll accompanied by a decrease in photosynthesis and the activity of photosynthetic enzymes following an increase in salinity. This drop in chlorophyll is due to the suppression of the enzymes responsible for its synthesis [30]. It can also be due to destruction by salinity of chloroplast membranes or their disorganization by Cl^- and Na^+ ions which are toxic and/or promote osmotic imbalance [31]. Salinity induces nutritional disorders causing damage to macromolecules such as chlorophyll due to the loss of integrity and photosynthetic activity of the membrane [32]. Chlorophyll biosynthesis is a crucial factor for photosynthesis. The reduction in chlorophyll is explained by the stimulation of the enzymatic activity of chlorophyllase by salinity ([16]; [27]; [28]). It may be due to the destruction of chlorophyll precursors, to the conversion of the latter into other pigments or to the reduction 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 [23]. This reduction in chlorophyll may also be linked to the sensitivity of its biosynthesis to NaCl [33].

The Cl^- contents vary significantly depending on the treatments and the varieties at 44 days after sowing (Figure 1). Hota *et al.* [34] obtained similar results in ten sesame genotypes. The Cl^- contents in the leaves of the four varieties of sesame (*S. indicum* L.) increased with salinity (Figure 1). Fall *et al.* [35] showed an increase with salinity of Cl^- ions in the leaves of *Senegalia senegal* (L.) Britton, *Vachellia seyal* (Delile) P. Hurter and *Prosopis juliflora* (Swartz) DC plants. These results corroborate those of Turan *et al.* [36] in maize who show an increase in Cl^- levels with increasing NaCl concentrations. Giuffrida *et al.* [19] obtained similar results in tomato (*Solanum lycopersicum* L. cv. Durinta).

The Na^+ contents in the leaves were higher in the saline treatments than in the controls at 44 days after sowing (Figure 2). Similar results were obtained in sweet pepper (*Capsicum annuum* L.) [33]. Leaf Na^+ levels increased significantly with salinity for all sesame varieties (Figure 2). Tariq and Shahbaz [37] noted the significant increase in Na^+ content in the leaves of two varieties of sesame seedlings. Indeed, under saline conditions, sodium ions (Na^+) compete with other major macronutrients such as potassium ions (K^+) and cause nutritional and metabolic disturbances causing the death of plant cells ([37]; [38]). In general, the increase in the Na^+ content is accompanied by a decrease in that of K^+ because the two ions compete for the same site ([38]; [39]; [40]) due to the similarity of their nature's physico-chemical [39].

Khalid *et al.* [20] showed in durum wheat (*Triticum durum* Desf. “Massa”) that the high accumulation of sodium ions in aerial organs is one of the causes that limit plant growth.

The Na^+ contents in the leaves varied significantly depending on the treatments and the sesame varieties (Figure 2). Bazrafshan and Ehsanzadeh [22] obtained concordant results in seven genotypes of sesame (*S. indicum* L.), some of which are tolerant to salinity. This variation in the Na^+ content in the leaves was observed in fifteen sesame varieties grown under saline stress in the greenhouse [17]. Tariq and Shahbaz [37] obtained similar results in two varieties of sesame.

The Cl^- contents of sesame leaves are higher than those of Na^+ in all treatments (Figures 1 and 2). Fall *et al.* [35] obtained similar results with *Senegalia senegal* (L.) Britton, *Vachellia seyal* (Delile) P. Hurter and *Prosopis juliflora* (Swartz) DC in greenhouses. However, our results are contrary to those of Hota *et al.* [34] who observed Na^+ contents higher than those of Cl^- in the leaves of ten sesame varieties (*S. indicum* L.). Khalid *et al.* [20] showed higher Na^+ contents than those of Cl^- independently of the NaCl concentration and the organ in durum wheat (*Triticum durum* Desf. “Massa”).

The Cl^- and Na^+ contents in the leaves of the four sesame varieties increased with salinity at 44 days after sowing (Figures 1 and 2). Similar results were obtained in *Senegalia senegal* (L.) Britton, *Vachellia seyal* (Delile) P. Hurter and *Prosopis juliflora* (Swartz) DC [35]. Khalid *et al.* [20] showed higher levels of these ions (Cl^- and Na^+) in the leaves than in the different parts of the plant. The enrichment of the leaves in Na^+ and Cl^- demonstrates the ability to transport these potentially toxic ions and to effectively compartmentalize them in the vacuoles ([20]; [41]). Plants capable of absorbing, exporting and accumulating Na^+ and Cl^- ions in their aerial parts (by efficiently compartmentalizing them in the vacuoles) are qualified as includer plants or of the inclusive type ([20]; [40]). This exclusion of other parts of the plant and accumulation of Na^+ and Cl^- ions in the leaves constitute a physiological adaptation strategy. It reduces the toxicity of salt inside and outside the root cells so that the plant can survive very high salinity levels. These ions accumulate more, especially in older leaves [42]. In some varieties, the significant storage of these ions in the leaves can disrupt mineral nutrition. It can also promote molecular damage, growth arrest and even plant death due to induced cytoplasmic toxicity. These manifestations are indicative of a particular sensitivity of this variety to salinity at the ionic level ([20]; [41]). Na^+ and Cl^- ions compete with other ions such as (Ca^{2+}, Mg^+, K^+, NO_3^-, PO_4^-) or lead to the modification of photosynthesis [43]. The latter is the main factor determining the growth of green plants [26]. Na^+ and Cl^- ions in the soil reduce the hydromineral supply of plants [44]. Na^+ ions promote a nutritional deficit in macronutrients (N, P, K, Mg, Ca), in micronutrients (Cu, Fe, Mn^{2+}, Zn^{2+}). They limit mineral nutrition, photosynthetic activity, and reduce plant growth and yield ([44]; [45]).

The mechanisms allowing plants to tolerate salinity can be classified into three groups: osmotic tolerance, ion exclusion and tissue tolerance. Osmotic tolerance is regulated by long-range signals that reduce shoot growth and is triggered before the accumulation of Na^+ ions. During ion exclusion the transport processes of Na^+ and Cl^- in the roots reduce the accumulation of these ions in the leaves. However, for tissue tolerance high salt concentrations are found in the leaves but are compartmentalized at the cellular and intracellular level, particularly in the vacuole [4]. This accumulation of mineral ions in the vacuoles allows plants to achieve osmotic adjustment and avoid physiological drought caused by salinity [5]. Our results show significant levels of Na^+ and Cl^- in the leaves (Figures 1 and 2) and plead in favor of tissue tolerance. The salinity tolerance mechanism of plants depends on how they control the transport of salt through their organs. It takes place by minimizing the entry of salt inside the plants or its concentration in the cell cytoplasm [5].

5. Conclusion

The effect of sodium chloride (NaCl) on the physiology of four African sesame varieties was studied in a greenhouse 44 days after sowing. The contents of chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Chl) increased with NaCl concentrations and sesame varieties. Variety AS15 produced the greatest amounts of chlorophyll.

The Cl^- contents of the leaves are higher than those of Na^+ in all the treatments. The contents of these two ions (Cl^- and Na^+) varied with salinity and sesame varieties at 44 days after sowing. The different varieties of sesame behave like the inclusive type species. It compartmentalizes the Cl^- and Na^+ ions at the level of the leaves in the vacuoles. This adaptation strategy is tissue tolerance to salinity.

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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