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Effects of salt stress on physiological and agronomic characteristics of three tropical cucurbit species

Victor Desire Taffouo[1*], Norbert Lidovic Djiotie[1], Martin Kenné[2], Ndongo Din[1], Jules Richard Priso[1], Siegfried Dibong[1] and Amougou Akoa[3]

[1*]Department of Botany & [2]Department of Animal Biology, Faculty of Science, University of Douala, P.O. BOX 24157 Douala, Cameroon; [3]Department of Biology and Plant Physiology, Faculty of Science, University of Yaoundé I, P.O. BOX 812 Yaoundé, Cameroon.

*Corresponding author e-mail: dtaffouo@yahoo.com

ABSTRACT
Objectives: To study the effects of varying NaCl concentrations on some tropical cucurbits and identify species able to grow on saline medium.
Methodology and results: Three Cucurbite species, *Cucurbita lanatus*, *C. moshata* and *Lagenaria siceraria* were studied under 0, 50, 100 and 200 mM of NaCl under laboratory and field conditions. In the laboratory, seedling growth, water content and mineral elements partitioning were tested. In the field, the agronomic parameters evaluated during the vegetative and harvesting phases were chlorophyll content, time of flowering, weight of the mature fruit, number of seeds per fruit and yield. Low concentrations of NaCl had a negative effect on agronomic parameters and limited the growth of plants, as well as the Na^+ and K^+ content in the shoots of *C. lanatus* and *C. moshata*. Salt injury symptoms, e.g. chlorosis were clearly visible in the plants at high NaCl concentration. The growth and agronomic parameters of *Lagenaria siceraria* were not significantly affected by varying salt concentrations. This species absorbs and transports sodium and potassium in its aerial parts.
Conclusion and application of findings: *Citrullus lanatus* and *Cucurbita moschata* presented characteristics of sensitivity to salt stress while *L. siceraria* seemed to be tolerant. The absorption of Na^+ and K^+ significantly increased the water content in the aerial parts for *L. siceraria*. *L. siceraria* could be cultivated in environments with varying salinity.

Key words: Cameroon, cucurbits, salinity, tolerance, sudanian zone

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INTRODUCTION
Salinity is a serious constraint to agricultural productivity in the arid and semi-arid zones of tropical Africa. In these areas, salt tends to accumulate in the upper layers of the soil profile, where leaching is insufficient due to inadequate precipitation (Munns, 2002). Cultivation of salt-tolerant species, especially N_2-fixing species, would be advantageous towards rehabilitating regions with salinity problems (Oba *et al*., 2001). There are a great number of plant species which are regarded as salt tolerant, the most competitive being those that are able to become established, grow to maturity and survive until they are able to reproduce (Turan *et al*., 2007). The issue of salinity and its effects on biomass production has been considered by numerous

authors (Mehari *et al*., 2005; Al-Khateeb, 2007; Turan *et al.*, 2007; Zadeh & Nacini, 2007).

In order to improve African traditional food crops, agronomists recommend the culture of local plant species including cucurbits, because they offer an effective way of diversification and improving livelihoods of peasants in Africa (Nyabyenda, 2006). Cucurbite species are important sources of lipids and proteins. For example, seeds of *Citrullus lanatus* and *Cucurbita moschata* can be transformed into pastes for kitchen sauces or for oil extraction. The dried shells of *Lagenaria siceraria* fruits have numerous uses, e.g. for artistic works, containers for water and floats for fishing.

In the soudanosahelian agro ecological area, the soil has a ferruginous nature. This zone covers a large part of northern Cameroon and is considered to be fragile, with low levels of soil fertility due to very sandy texture. The most important processes of degradation are changes in salinity due to irrigation with brackish water. High salt concentrations disturb several physiological processes of plants, reducing plant growth and development (Taffouo *et al.,* 2004). On the basis of the differential ability of plants to grow on more or less saline medium, plants can be grouped into two principal categories, i.e. halophytes species that tolerate high salt concentrations and glycophytes species that tolerate low salt concentrations. This adaptation of halophytes enables them to absorb high quantities of ions in order to maintain cellular turgidity without succumbing to their toxicity. The osmotic balance of the cytoplasm is ensured by an active synthesis of organic and soluble compounds (Trinchant *et al.,* 2004; Le Rudulier, 2005). Sensitive glycophytes restrict the transport of Na^+ in the aerial parts and maintain relatively low salt levels in photosynthetic tissues (Taffouo *et al.*, 2004). Glycophytes, e.g. cotton and barley transport and accumulate high quantities of Na^+ in their leaves (Heller, 1995).

In order to overcome the adverse effects of salinity on yield, some agronomists have proposed the use of mineral fertilizers. However, the success of this method is largely influenced by the degree of salinity of soils. Identifying crop varieties that are able to grow and develop in saline medium is one of the possible ways to address this constraint.

Although the effect of salinity on plants been studied for long, leading to remarkable results, a lot is yet to be learned about the function of salinity caused by NaCl to plants, especially to tropical cucurbits that are of strategic importance and are increasingly being cultivated in Cameroon. This study aimed to determine the effect of salinity on some physiological and agronomic characteristics of some cucurbit species under laboratory and field conditions.

MATERIALS AND METHODS

Physiological parameters: Seeds of *Citrullus lanatus*, *Cucurbita moschata* and *Lagenaria siceraria* were first disinfected using sodium hypochlorite 3% for 1h and rinsed using distilled water. Germination occurred in 3 days after which the seedlings obtained were separated into 4 groups of 15 individuals each. For each group, 5 seedlings were randomly selected and planted together in one pot containing sand. Pots were kept in laboratory and supplied with nutrient solution after every three days. The composition of the nutrient solution was 0.4 mN of KNO_3, 0.2 mN of KH_2PO_4, 1.0 mN of Ca_2NO_3 and 0.4 mN of $MgSO_4$. Temperature was 26 ± 3°C, light 5000 lux for 12 hour alternating periods and relative humidity of 51 - 70%) (Taffouo, 2005).

Treatments: The control group of seedlings was fed using a nutritive solution (pH = 6) without salt (0 mM of NaCl). The three experimental groups were supplied daily with salt concentrations of 50, 100 and 200 mM of NaCl, respectively (Taffouo *et al.,* 2004). After four weeks, the dry weight of aerial parts and roots was obtained on 5 randomly sampled plants. The cations (Na^+ and K^+) contents of vegetal organs (leaves, stems and roots) were determined by Flame photometer (JENWAY) on another 5 randomly sampled plants. Leaf chlorophyll content was determined from four plants per pot. Leaf tissues samples (2 g) were crushed in a motar and tissues were homogenised using 80% acetone. The resulting mixture was decanted through a filter paper into a 100ml volumetric flask. The resulting solution (5 ml) was pipeted into a 50 ml volumetric flask and made up to volume with 80% acetone. The absorbance of the extract was then measured at 645 and 663 nm wavelengths for chlorophyll a and b, respectively. Chlorophyll content (mg g^{-1} fresh leaf weight) was calculated using the following formula: total chlorophyll = (20.2 x D_{645} + 8.02 x D_{663}) x (50/1000) x (100/5)x 1/2 where D = absorbance.

Parameters assessed under field conditions were time to flowering, weight of the mature fruit, number of seeds per fruit, chlorophyll content and grain.

Site description: The trials were conducted at the University of Douala research farm (4°01 N, 9°44 E, 13 m above sea level, with total annual rainfall of 3597 mm and average temperature of 27°C). The physical and chemical characteristics of the soil at the site are given in table 1. The experiment was arranged in a randomised complete block design with three replicates. Two treatments were investigated on 10x10 m plots: (1) the control plot was fed using distilled water (0 mM of NaCl); (2) the experimental plots were supplied with solution of 4 g L^{-1} NaCl. The trials were carried out in pure culture without fertilization. Water was applied once per week at a rate of 20 l m^{-2}.

Statistical analysis: Multiple comparison of means by ANOVA and pairwise analysis was set up using the Student-Newman-Keuls procedure when the normality and equal variance conditions passed. Multiple comparisons of data noted in experimental groups *versus* those recorded in the single control group were set up using the Dunnett's procedure (SigmaStat sofware).

Table 1: Physical and chemical characteristic of soil (0-20 cm of depth) at the Douala University Research Farm, Cameroon.

Parameter	Unit
Clay (%)	14.2
Coarse sand (%)	27.9
Fine sand (%)	25.6
Coarse silt (%)	26
Fine silt (%)	5.4
Carbon (%)	0.28
Nitrogen (%)	0.80
Ratio C/N	0.35
Assimilable phosphorous (ppm)	4.83
Exchangeable potassium (meq $100g^{-1}$)	0.23
Exchangeable sodium (meq $100g^{-1}$)	0.006
CEC (meq $100g^{-1}$)	7.55
Exchangeable calcium (meq $100g^{-1}$)	2.01
pH- Water	5.72

RESULTS

Seedling's growth: The variation in the dry matter of roots and shoots of plants enabled the assessment of the effect of salt on growth of *Citrullus lanatus* and *Cucurbita moschata* compared to the control group (Fig. 1). In contrast, the presence of salt in the nutritive solution did not significantly affect (P >0.001) the dry matter of aerial parts and roots of *Lagenaria siceraria* (Fig. 1)

Ion uptake: In all cucurbit species, plants from experimental plots showed high accumulations of sodium ions in plant organs (Fig. 2). Potassium ions were more accumulated in organs of treated *C. lanatus* and *C. moschata,* than in the control plants (Fig. 3). Pairwise comparison between the control and the three experimental groups of plants showed that the presence of salt in the nutritive solution significantly increases potassium ions in organs of *L. siceraria* (Fig. 3).

Water content: The supply of NaCl in the medium caused a significant decrease in the water content in organs of *C. lanatus* and *C. moschata* (table 2). However, salt presence did not affect water content in organs of *L. siceraria* (P >0.001).

Agronomic parameters: The presence of NaCl caused reduction of time to flowering and a significant decrease in weight of the mature fruit, the number of seeds per fruit, the chlorophyll content and grain yield of *C. lanatus* and *C. moschata* (table 3). The depressive effect of salt was less marked on the agronomic parameters of *Lagenaria siceraria* (P >0.001).

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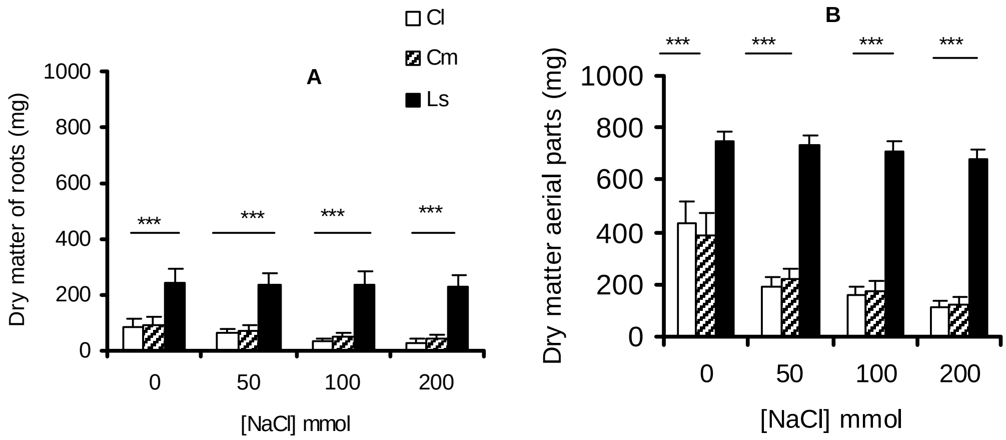

Figure 1: Effect of NaCl concentration on root biomass (A) and shoot biomass (B) production by three tropical curcubits. n = 7 for each species; Cl = *Citrullus lanatus; Cm = Cucurbita moshata; Ls = Lagenaria siceraria* ** = P < 0,01; *** = P<0,001.

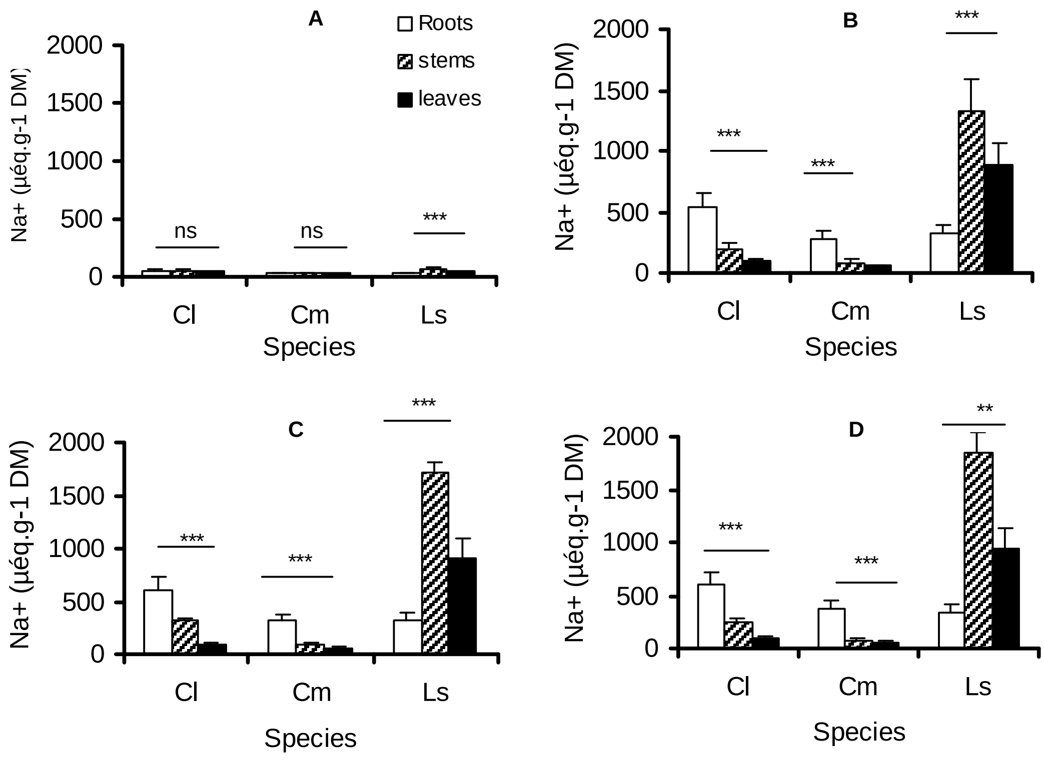

Figure 2: Distribution of Na^+ in roots, stem and shoots of three cucurbits grown in media with varying salt (NaCl) concentrations. Chart A= control medium (0 mM of NaCl); B=: 50 mM; C=: 100 mM; D=: 200 mM. n=7 for each speciies; Cl = *Citrullus lanatus, Cm = Cucurbita moshata, Ls = Lagenaria siceraria,* ns = P > 0,05; * = P < 0,05; *** = *P<0,001*

Table 2: Effect of salt on the water content in roots, stem and leaves of three tropical cucurbits (n = 5). Significant probabilities are in bold. n = 5 ; *ns = P>0,05 ; *** = P<0,001*

Organs	Species	Salinity (mmol of NaCl)				ANOVA
		0	50	100	200	$F_{(3, 16)}$
Roots	*C. lanatus*	92.6 ± 0.2	86.9 ± 0.9 *	76.8 ± 1.2 *	68.4 ± 2.8 *	26.44 ***
	C. moshata	94.1 ± 2.4	90.8 ± 3.1	77.9± 0.2 *	63.6 ± 1.8 *	14.28 ***
	L.siceraria	72.5 ± 0.5	68.9 ± 3.2	70.3 ± 2.1	69.9 ± 1.9	1.21 ns
Stem	*C. lanatus*	92.1 ± 2.1	79.6 ± 0.3 *	68.3 ± 1.2 *	51.6 ± 0.4 *	410.52 ***
	C. moshata	94.9 ± 0.4	89.0 ± 0.2*	70.2 ± 1.1 *	47.1 ± 0.5 *	376.32 ***
	L.siceraria	79.2 ± 0.7	77.8 ± 1.4	78.2 ± 0.7	76.1 ± 2.1	0.21 ns
Leaves	*C. lanatus*	91.8 ± 0.3	73.3 ± 4.4 *	57.3 ± 2.6 *	37.2 ± 4.1 *	251.66 ***
	C. moshata	92.6 ± 0.2	86.5 ± 0.4	62.6 ± 1.9 *	38.2 ± 1.3 *	860.87 ***
	L siceraria	89.6 ± 0.4	85.2 ± 4.1	88.1 ± 0.7	87.3 ± 1.2	1.51 ns

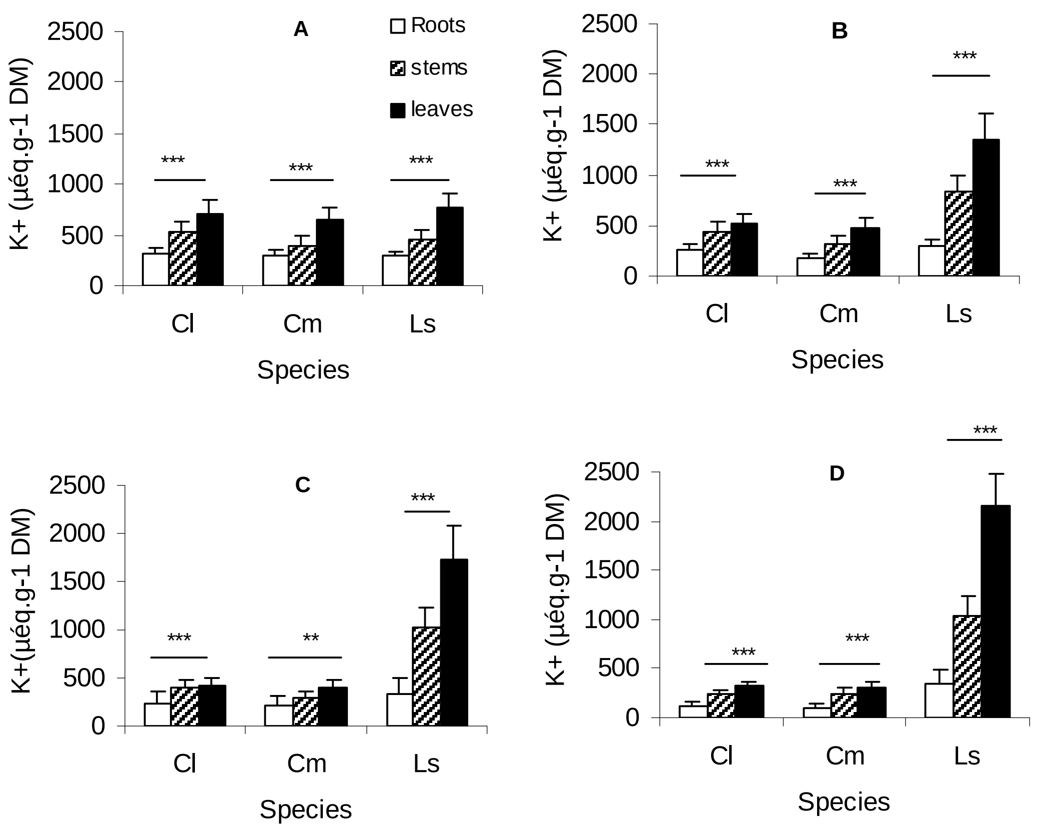

Figure 3: Distribution of K^{+} in roots, stem and leaves of three curcubit species grown in media with varying salt (NaCl) concentrations. Chart A= control medium (0 mM of NaCl); B= 50 mM; C= 100 mM; D= 200 mM. n = 7; Cl = *Citrullus lanatus, Cm = Cucurbita moshata, Ls = Lagenaria siceraria,* ns = P > 0.05; * = P < 0.05 ; *** = P<0.001.

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Table 3: Effect of salt stress on agronomic characteristics of three tropical cucurbits under field conditions (Cl: *Citrullus lanatus*; Cm : *Cucurbita moschata*; Ls : *Lagenaria siceraria*, n = 10 ; *ns = P >0,05 ; * = P<0,05)*

Species	Parameters	Salinity ($g.l^{-1}$ of NaCl)						ANOVA $F_{(3, 16)}$
		0			4			
		Min.	Mean ± SD	Max	Min.	Mean ± SD	Max	
Cl	Time to flowering (j)	31	33.1 ± 1.5	35	23	24.7 ± 1.0*	26	11.7 *
	weight of the mature fruit (g)	500	1017.9 ± 61.2	1525	95	191.3 ± 6.7*	297	66.1 *
	Number of viable seeds per fruit	237	371 ± 26	455	23	37 ± 12*	63	334.5*
	Grain yield ($kg\ ha^{-1}$)	115	129.2 ±12.7	165	26	65.1 ± 8.7*	91	97.6*
	Foliar chlorophyll content ($mg\ g\ MF^{-1}$)	6.4	7.0 ± 1.0	7.5	4.4	5 .1± 0.5*	6.0	10.29 *
Cm	Time to flowering (j)	30	31.4 ± 1.1	33	21	23.6 ± 0.8*	26	27.36 *
	weight of the mature fruit (g)	800	2455.3 ±152.1	6700	156	389.4 ± 53 *	680	17.12 *
	Number of viable seeds per fruit	264	439 ± 22	781	52	105 ± 16*	172	73.40 *
	Grain yield ($kg\ ha^{-1}$)	102	121.7 ± 9.5	172	81	75.1 ± 6.1*	110	15.3*
	Foliar chlorophyll content ($mg\ g\ MF^{-1}$)	6.1	6.6 ± 0.6	7.3	4.1	4.9 ± 0.7*	5.5	12.76 *
Ls	Time to flowering (j)	36	37.2 ± 0.9	38.5	37	38.3 ± 0.9	39.5	4.17 ns
	weight of the mature fruit (g)	450	874.4 ± 51.5	1500	300	668.7 ± 47.3	1100	4.10 ns
	Number of viable seeds per fruit	64	202 ± 25	349	87	161 ± 22	253	2.24 ns
	Grain yield ($kg\ ha^{-1}$)	84	111.1 ± 7.2	185	75	104.9 ± 11.3	175	3.15 ns
	Foliar chlorophyll content ($mg\ g\ MF^{-1}$)	7.5	7.8 ± 0,4	8.0	5.9	7.1 ± 0.8	7.9	1.78 ns

DISCUSSION

The two cucurbit species *C. lanatus* and *C. moschata* produced significantly less root and shoot dry matter when exposed to saline medium. These species can therefore be described as "sensitive glycophytes" whose tolerance level ranges from 0 to 50 mM of NaCl (Levitt, 1980). The suppressive effect of salt on plant growth is expressed more on the aerial parts than on the roots of these species. High concentration of NaCl inhibits plant growth by imposing an osmotic stress on the cell and by toxicity of sodium in the cytoplasm, thus reducing the photosynthetic function of the young leaves (Taffouo, 2005). Saadallaha *et al.* (2001) have associated this negative response of plants to salt stress to the genotypic variation between species. In this study, *L. siceraria* withstands salt stress better than *C. lanatus* and *C. moschata*. Similar results were observed with *Ceriops roxburghiana* and with some genotypes of *Lycopersicon esculentum* in saline medium (Agong *et al.*, 2003).

An interesting finding of the present study is that *C. lanatus* and *C. moschata* restrict transportation of Na^+ to the aerial parts of the plants and therefore they maintain relatively low salt levels in the photosynthetic organs. These two species appear to have developed strategies that permit them to avoid the flow of Na^+ and Cl^- from roots to leaves. This first barrier has been reported to function at the level of the endoderm (Munns, 2002). The retention of sodium ions in the roots allows these plants to avoid the invasion of the leaves by toxic elements that are likely to reduce the photosynthetic activity by interfering with the opening of stomates and other metabolic processes (Turan *et al.*, 2007). These findings agree with those obtained for several sensitive glycophytes (Taffouo *et al.,* 2004). On the other hand, the observation that *L. siceraria* tolerates much higher Na^+ levels in the shoots suggests the existence of a good absorption, transport and accumulation mechanism for this ion in the vacuoles of young leaves of this species. These results confirm those obtained with the tropical halophyte *Phaseolus adenanthus* (Taffouo *et al.,* 2004).

The inhibiting effect of salt on the absorption of K^+ of the studied cucurbit species is similar to what has been described in wheat (Turan *et al*., 2007) and the perennial alfalfa (Al-Khateeb, 2007). According to El Haddad & O'Leary (1994), low K^+ levels indicate sensitivity of the plant species or varieties to salinity. In our study, the sensitive *C. lanatus* and *C. moschata* present low contents of K^+ as compared to the tolerant species *L. siceraria*. The reduction in ponderal growth of the organs observed in these two sensitive glycophytes seems to be related to the deficiency of K^+ in the leaves as well as water content, which is caused by NaCl restricting transport of sodium to the leaves (Fig. 2).

Presence of NaCl was also observed to have a depressive effect on the agronomic parameters of *C. moschata* and *C. lanatus*, but not on *L. siceraria*. The decrease in mature fruit weights could be explained by the increase in the number of non viable seeds at 44 and 46% for *C. lanatus and C. moschata*, respectively. Similar results have been reported by Munns & Rawson (1999) on cereals. This unfavourable effect of salt could also be due to the deficiency in calcium, nitrate, phosphate and sulphate (Munns, 2002). The significant decrease in grain yield that was observed under salt stress for *C. lanatus* and *C. moschata* could be partly related to the significant reduction of chlorophyll content (73 and 74%, respectively) since chlorophyll is an essential element to the realization of photosynthesis (Heller, 1995). Our results confirm those noted by El-Iklil *et al.* (2002) & Turan *et al*. (2007) showing that foliar chlorophyll contents are reduced under salt stress.

Considering that the depressive effect of salt was less on growth and agronomic parameters of *Lagenaria siceraria*, it is concluded that this species has ability to absorb and transport large amounts of sodium and potassium to its aerial parts and has mechanisms to tolerate any potential adverse effects of high salt levels. *L. siceraria* is therefore recommended for cultivation in environments where salinity problems are likely to be encountered. This species could also be studied further as a source of genes for salt tolerance that could be exploited in future breeding programs.

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