$17^{th}$ *International Conference on Environmental Science and Technology*

*Athens, Greece, 1 to 4 September 2021*

# Mechanism of tolerance and adaptive strategies developed by Moringa oleifera (L) during its growth under a salt constraint in hydroponics

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**Abstract** *Moringa oleifera* (L) is a promising species to be used in reforestations of dry areas. A question that remains to be investigated is its resistance to salinity conditions. This work's major goal was to investigate the response of this species in increasing salinity conditions, in order to increase our understanding on the mechanisms of tolerance and/or sensitivity and of the morpho-physiological and biochemical responses of *Moringa* plants under a salt constraint. *Moringa* seedlings were subjected to increasing concentrations of NaCl, 0 (control), 50, 100 and 150 mM NaCl. The results suggest that the variations in the responses of the parameters analyzed depend on the concentration and the duration of application of the salt. Vertical growth, relative average growth rate (RGRh), and relative water content showed a great variability of responses, depending on the concentration and the duration of application of NaCl. Leaf area (SF) was affected by salinity whereas leaf succulence index (ISF) and photosynthetic pigments (chlorophylls (a), (b), were significantly improved by such stress. The results suggest a strong resistance of the species to high doses of NaCl and in particular those greater than 50 Mm. Salinity conditions cause the accumulation of organic solutes (proline and soluble sugars), as a physiological adaptation to prolonged salinity.

**Keywords:** dry areas, salt constraint, tolerance mechanism, Tunisia

## 1. Introduction

*Moringa oleifera* is a fast growing, multiuse plant grown in tropical, subtropical and temperate climates. *Moringa* is native from north-western India and well distributed in southeast Asia, Africa, Saudi Arabia and south America (Olson and Fahey 2011, Severino and Auld 2013). It can develop in arid and semi-arid environments, in regions with low rainfall and soils with high salinity (Lim 2015). It can tolerate prolonged periods of drought (Tian et al. 2015), which allows it to be found in very arid areas such as the Sahara. The various parts of *Moringa* such as immature leaves, roots, seed, bark, fruit, flowers and pods are used in traditional medicine in various developing countries and in modern medicine (Sharma et al. 2011). Its richness in mineral elements, vitamins and proteins allows them to have a capital interest in human nutrition and fodder for animals, in particular in developing countries. Its properties increase the interest for its cultivation and further scientific research.

## 2. Materials and Method

Young *M. oleifera* seedlings (36 days old) were subjected to increasing salt concentrations for 20 days. The seedlings were developed in a Hoagland's solution (Hoagland and Arnon 1950). Each treatment was replicated three times with 12 plants (3 tubs x 4 plants) on the nutrient medium as control, 36 plants were maintained in the presence of the nutrient medium, added at increasing concentrations of NaCl. The treatments were 0 (control), 50, 100 and 150 mM of NaCl, applied gradually (25 mM / day until reaching the concentration sought). The seedlings were kept in controlled room concerning temperature and humidity. The parameters evaluated included growth (vertical growth, and relative growth rate RGRh (in day-1, as a function of the height of the main stem, a reliable indicator of the degree of sensitivity of the plant. It reflects the cumulative effect of damaging and inhibiting the physiological functions of the plant), measurement of the leaf area ($mm^2$), tissue hydration is evaluated by the TRE parameter, expressed as % of the fresh material, physiological (Succulence index $g.cm^{-2}$), determination of photosynthetic pigments, determination of soluble sugars and proline. The chlorophyll content is the most used criterion to quantify the general photosynthetic state of the plant, it is an excellent bio-indicator of stress (Lemzeri, 2007). The significance of the treatments applied to the various parameters studied, was evaluated by analyzes of variance (ANOVA) with a single classification criterion at the 0.001 threshold. Means were compared by the Duncan test at $\alpha = 5$ %. The statistical analyses were conducted by the XL STAT 2014.5 software. 04.

## 3. Results - Discussion

As a general observation, the plants growing in the hydroponic solution (control or on increasing concentrations of NaCl), showed similar development to those growing in the soil, with the only exception that the hydroponic plants develop roots that showed more branching (filamentous) rather than creating a tap root as the other ones. This renders them more sensitive to drought (Nouman et al. 2012). Although a reduction was detected in the various growth parameters measured, different parts of the *Moringa oleifera* plant reacted differently to the application of salt. This implies that a long-lasting salinity could be decisive for the growth of the young plants. The applied salt stress has no significant effect on the general appearance of the plants neither on the morphological aspect of the leaf nor on the color. Also, no chlorosis was noted except from a limited number of leaves growing at the lower level of 150 Mm NaCl who showed some chlorosis.

There was a negative correlation between salt stress degree and length of the main stem. Indeed, the height of the rod goes from 140.54 mm ± 1.35 in the controls, to 105.6 mm ± 1.4 in the treated with 150 mM NaCl, with an estimated decrease of 24.86 %. The analysis of the results show that the effect of salt is accentuated with the increase in the NaCl concentration. Thus, our results agree with the work of Nouman et al. al. (2012) in the same species, cultivated in greenhouse under increasing concentrations of salt. These authors reported that all growth parameters of the seedlings were significantly affected by salt stress, and concluded that *Moringa* shoots could tolerate salinity of up to 8 dSm-1 (≈ 85 mM). Hussein and Abou-Baker (2014), cultivated the same species in a greenhouse, irrigated by increasing concentrations in dilute seawater (2000 and 4000 ppm). They suggest as well, that salinity affects plant growth through several physiological and biochemical means. Karoune et al. (2017) assume that growth reduction is an adaptive capacity necessary for the survival of a plant exposed to abiotic stress. Lobato et al. (2009), attributes this to cell division and lower production of ethylene in species subjected to this type of stress. Kausar et al., (2014), attributes this to the reduction of water available in the root zone causing a water deficit, to the phytotoxicity of ions such as Na+ and Cl- and nutrient imbalance which decreases the absorption and transport of nutrients and Na+, K+ for binding sites essential for cell function (Farissi et al. 2013).

There is a negative correlation between the NaCl concentration and the production of root biomass. This is true for all the treated and in particular with 150 mM of NaCl, which shows a reduction of 52.97 %, compared to the controls, showing a higher sensitivity of the roots to the depressive effect of salt than the aerial part. Noreen et al. (2018) observed the same phenomenon in *M. oleifera*, grown in nurseries. This confirms that the roots of *M. oleifera* are the most affected by salt in agreement with similar work (Hegazi, 2015; Noreen et al., 2018, Bafeel et al., 2018). Belfakih et al., (2013) suggests that the plant seems to adapt to salt stress by first reducing its root system and preserving the aerial part in order to maintain and ensure photosynthetic functioning.

Concerning RGRh of *Moringa* plants, it appears that salt significantly reduces the growth rate of the plants and this reduction becomes significant (α = 5% threshold), beyond 50 mM. For the 100 mM treatment, the RGRh is estimated at 0.014 /day ± 44.10-5, i.e. a delay of 0.021 days compared to the control (0.035 /day ± 82.10-5). The same fate for the treated with 150 mM, which commenced at 0.007 /day ± 44.10-5, ie a delay of 0.028 days compared to the controls. It appears that the variations of the RGRh evolve downwards and that, despite the high concentrations applied which reach 150 mM of NaCl, the plants of *Moringa* continue to survive. Therefore, our results largely agree with those of (Karoune et al. 2017) in *Acacia albida*. It is a survival strategy, allowing the plant to accumulate energy and resources to fight against stress, before the imbalance between the inside and outside the body reaches a threshold where the damage will be irreversible (Bois 2005, Zhu 2004). Sengupta and Majumder (2009), suggest that salinity limits plant development and triggers resistance mechanisms such as osmotic adjustment.

Relative water content (TRE) analysis does not indicate any significant effect of the salt concentration tested, at the α = 5 % threshold. As a result, the water status of *Moringa* plants is independent of salt stress. Some authors consider that the relative water content is a widely used indicator to highlight the state of the water balance of a plant. Our results are in agreement with the work of Karoune et al. (2017) in *Acacia albida* and those of Bennani (2013) in *Trifolium isthmocarpum*. This is considered to be salt tolerance (Bafeel et al. 2016), and that salt affects the activity of hydrolytic enzymes affecting the rate of water uptake (Lobato et al. 2009).

*Moringa* leaves, are significantly reduced in stressed plants. Thus, the differences recorded, compared to the controls, were accentuated with the duration of the treatments and the intensity of the stress. Thus, leaf surface (SF) goes from 452.35 mm2 ± 9.05 at the level of the controls, respectively to 356.44 mm2 ± 3.3, 319.79 mm2 ± 2 and 289.73 mm2 ± 5.04 at the level of treated by 50; 100 and 150 mM, with respective reductions of 21.2 %; 29.3 % and 35.95 %. These results are commonly reported by the work of (Hegazi, 2015), reporting a decrease in SF estimated at 52.32% under 50 % seawater. Hussein and Abou-Baker (2014) report for the same species a decrease in SF of around 17.7% below 4000 ppm. Nouman et al. (2012) also observed a negative correlation between SF in green *Moringa* leaves and increasing salt concentrations. This suggests that the leaves are the most sensitive tissues of the plant to excessive salinity (Benmahioul et al. 2009), and that the effects of salt are more marked in old leaves than in young leaves. Kulaeva and Prokoptseva (2004) report that Na+ and Cl- alter the metabolism and reception sites of the hormones involved in cell division and expansion. This accelerates senescence and limits the formation of new photosynthetically active leaves. At the chloroplast scale (Sudhir and Murthy 2004) have shown that it is the processes of carboxylation, and not photophosphorylation, that are most affected by salt stress. Beddiar and Benkachrouda (2013), indicated that the decrease in the growth of aerial organs by salt is manifested by a reduction in SF controlled by the number and size of cells.

There was a positive correlation between the increasing concentrations of NaCl and the contents of Chl.a. The same result was observed in Chl.b. Thus, the results suggest the existence of a positive correlation between the degree of stress induced by the salt and the studied trait. The treated with 150 mM have the highest contents 0.63 μg/g MF ± 29.10-3, compared with the controls 0.31 μg/g MF ± 69.10-3, thus marking, an increase of more than 100%. However, our results showed that the content of chlorophylls (a), (b) and total (Chl.t) is proportional to the intensity of the salt stress. Hussen et al. (2013), noted that the concentration of

Chl.a, increased as the salt concentration in the irrigation water increased. A similar trend has been observed by Hannani (2011) in *Acacia raddiana*, who points out that this behavior is probably an adaptive strategy of the species. Nouman et al. (2012) observed that *M. oleifera* plants survived up to 8 dS. m-1 (≈ 85 Mm) with a slight reduction in Chl.a., but with a significant decrease in at the 12 dS m-1 treatment (≈120 Mm). In our experiment, the levels of chlorophyll (b) (Chl.b) show a notable stimulation in all the treated, and that the increase becomes significant beyond 50 mM. These results agree with the work of Nouman et al. (2012) in the same species who indicate a positive correlation between Chl.b contents and increasing salt concentrations. According to these authors, the improvement in the content of chlorophyll b, may be due to the imbibition of the seeds of *M. oleifera*, carried out before germination, as we also did in our experiment. Thus, such changes in the concentrations of photosynthetic pigments may be important for maintaining growth and, therefore, be considered as a strategy of tolerance to abiotic stresses (Akça and Samsunlu 2012). Similar results are reported by Hussen et al. (2013) for *Moringa.*

Proline was increased by NaCl concentration with a peak at 50 mM NaCl after which it decreased but was always higher than the controls, testifying that the *Moringa* species manages to better exploit the biochemical properties of this amino acid, as a compatible soluble compound without exerting a toxic effect as in the case of ions (Silva-Ortega et al. 2008). This is true for all the treated and in particular at 150 µm of NaCl, which accumulate 178.73 % compared to the controls. In addition, our results show a very interesting accumulation of proline, at the aerial parts of the shoots of *M. oleifera*. These results align with the work of (Bafeel et al. 2016) in *Simmondisia chinensis* and that of (Da Silva et al. 2017), in the same species. Possibly, the synthesis of proline can explain the potential for tolerance and / or resistance demonstrated by the *Moringa* species, on the scale of the morpho-physiological parameters analyzed. Thus, the retention of water (as osmolyte) in the cytoplasm protects against membrane desiccation and protein denaturation (Ben Ahmed et al. 2010). Salt stress caused a significant increase in the content of soluble sugars in our experiment. These results suggest a close correlation between the high contents of soluble sugars in *M. oleifera* and these strong capacities of tolerance and / or resistance. In addition, this accumulation is considered as an indicator of tolerance to salt stress (Achour 2016), it would be due to a modification of enzymatic activities linked to carbohydrate metabolism (Belfakih et al. 2013). This pleads in favor of the tolerance and / or resistance potential of *Moringa* species, in the face of the applied salt stress. Similar findings are reported for *Simmondsia chinensis* (Jojoba) is to that of Bafeel et al. 2016, Rahim Guealia 2019). These last authors indicate that, the foliar contents of soluble sugars evolve proportionally with respect to the salt stress up to 100 Mm. They therefore concluded that the contents of soluble sugars in the leaves and the roots are positively influenced by the stress saline.

## 3. Conclusions

The study of the response of *Moringa oleifera* to a saline treatment, in order to identify the traits of its tolerance and/or morpho-physiological resistance and mechanisms, revealed that: (i) The species tolerates salt concentrations ≤ 50 mM NaCl and its resistance even extends up to 150 mM, which is perhaps the reason why this plant manages to survive even under this high dose. (ii) The reduction in growth (length of stems and main roots, diameter at the crown, relative average growth, etc.) is an adaptive capacity necessary for the survival of plants exposed to salt stress. (iii) The species seems to adapt to high salt concentrations, firstly by reducing its root system and preserving its aerial part, in order to maintain and ensure photosynthetic functioning, as this was evidenced by the increased synthesis of chlorophyll pigments a, b and total. (iv) Hydration of the tissues of the aerial part was not affected even under high salt concentrations (100 and 150 mM). It is expressed as tolerance to the applied stress, to maintain a moderate relative water content, under salt stress. (v) Compatible solutes involved in osmotic adjustment were accumulated, for the protection of cellular macromolecules

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