Global NEST Journal, Vol 23, No 1, pp 152-161

Tolerance and adaptation mechanisms developed by *Moringa oleifera* (L) seeds under oxidative stress induced by salt stress during *in vitro* germination

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Received: 10/02/2020, Accepted: 24/11/2020, Available online: 23/05/2021
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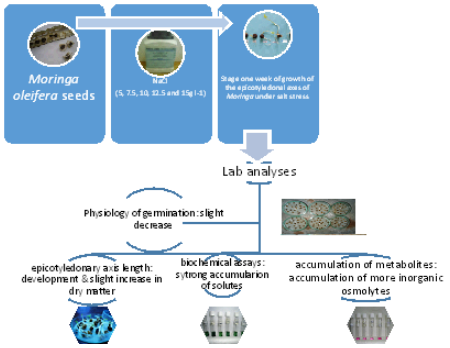
https://doi.org/10.30955/gnj.003292

Graphical abstract



Abstract

The current study explores the capacity of *Moringa oleifera* (L), to overcome and/or tolerate oxidative stress induced by a saline substrate as a constraining factor. For this, *Moringa* seeds have been subjected, for 15 days after germination *in vitro*, with increasing concentrations of NaCl, and specifically at 0 (control), 5, 7.5, 10, 12.5 and 15g l^{-1}. Both morpho-physiological and biochemical aspects were evaluated which were considered as probable indicators of tolerance or sensitivity to this stress. Our results reveal an increased synthesis of proline, lipids, proteins, CAT, GPX, APX, flavonoids, condensed tannins and a decrease in total polyphenols (-29.97%), mainly due to their high carbon cost. The degradation state of the cell membranes was evaluated by the MDA assay, which increased by 91.25%. On the contrary, the oxidative damage caused by Reactive Oxidative Species (ROS), detected by H_2O_2, decreased by 37.89%. The germinative ability of the seeds was affected indicating moderate resistance to stress as it was evaluated by the development of epicotyledonary axes, even under 15 g l^{-1} of NaCl without showing symptoms of stress or sensitivity. This result supports our hypothesis on the ability of the species to maintain or even improve its oxidative status.

Key words: Antioxidant, osmoregulatory activity, adaptive strategy.

1. Introduction

The salinity of the arid and semi-arid regions of the Mediterranean basin is a major problem affecting the quality of the soil and, therefore, agricultural production. This is particularly evidenced in the Maghreb region where both coastal and continental areas are influenced by this situation. For example, in Tunisia more than 25% of the irrigated soils are affected by this problem (Douaoui and Hartani, 2008).

Salt stress in plants produces Reactive Oxygen Species (ROS) which cause oxidative stress and, therefore, damages plant components (Ahmad *et al.,* 2014; Latef and Chaoxing, 2014). This renders this subject as one of the priorities of scientific research, aiming to better understanding the phenomenon and being able to select more tolerant and/or resistant species, capable of rehabilitating such areas and minimizing the depressive effects of oxidative stress on agricultural yields (Arbaoui *et al.,* 2000). This represents a crucial issue for agricultural production in the coming decades under the changing climate (Selma *et al.,* 2015).

Under the framework of the above research quests, we investigated the germination *Moringa oleifera*, a widely distributed species in the Mediterranean region (Olson and Carlquist, 2001). It can develop in areas with low rainfall and high salinity (Lim, 2015; Pandey *et al.,* 2011; Tian *et al.,* 2015), and has significant antioxidant potential under severe stress (Tesfay *et al.,* 2016). There are only a few studies focusing on the effect of oxidative stress to the germination of *Moringa*, which is an essential issue for the survival of the plant. Our study aims to clarify the capacity of antioxidant activities (enzymatic and non-enzymatic) and certain organic osmolytes, in the defense mechanisms and strategies and/or resistance of *Moringa* seeds to oxidative stress. This will enhance our knowledge on the possibility of using such a species in arid areas affected by salinity.

2. Materials and methods

2.1. Plant material and experimentation

Moringa oleifera (L), belongs to the Moringaceae family (Laleye *et al.,* 2015). It is native to India, in tropical and subtropical regions (Leone *et al.,* 2015), where it was described as early as 2000 B.C. (Oluduro *et al.,* 2016). All parts of this species have a variety of uses such as for consumption and medical purposes (Leone *et al.,* 2015; Sharma *et al.,* 2011). Similarly, the seeds have multi-functional roles (Anwar *et al.,* 2007) for human nutrition and the production of high-quality biofuels and oil (Osman and Abohassan, 2012) as well as for water purification (Yusuf *et al.,* 2015).

In this study three hundred and sixty Moringa seeds (360) were used. The seeds were obtained from an Egyptian origin of *Moringa*, collected at the end of 2016 and kept in a cold room with a temperature of 4 ± 1°C. In vitro germination was conducted at the Forest Ecology Laboratory (LEF) of the National Institute for Research in Rural Water and Forests Engineering (INRGREF) of Tunis (Tunisia).

The seeds were initially imbibed for 48 hours in distilled water (change of water in the bath every 24 hours) and subsequently disinfected by Betadine (80% vol/vol) for 5min. After an abundant rinsing with distilled water, they were further disinfected with 70% (vol/vol) sodium hypochlorite (NaOCl) 70% (vol/vol) for additional 5 min, and finally, thoroughly rinsed again with distilled water. Batches of 20 seeds were germinated in *Petri* glass dishes (14×14 cm^2), lined with filter paper (Wattman N°4) soaked in distilled water (control) and/or increasing concentrations of NaCl (5, 7.5, 10, 12.5 and 15g l^{-1}). These *Petri* glass dishes were placed in a culture chamber at a temperature of 26 ± 1°C and a relative humidity of 60% in total darkness (Quashie and Tchezoum, 2009; ISTA, 2014). After the emergence of the radical (Rachidai *et al.,* 1994), the sprouts of each treatment were counted daily.

2.2. Parameters studied and analyses performed

These included:

2.2.1. Physiology of germination (n = 4)

The curve that represented the evolution of the percentle of cumulative germination as a function of time. This parameter is used to enhance the understanding of the physiological significance of the germination behavior of seeds under salt stress (Hajlaoui *et al.,* 2007).

2.2.2. Growth in epicotyledonary axis length (n = 4)

It was evaluated by measuring epicotyledonary axis lengths (every day) during the whole germination phase, taking into account the curvatures of the radicle and/or the epicotyl, measured by a graduated ruler (in mm).

2.2.3. Biochemical assays

The phenolic compounds, malondialdehyde (MDA) and H_2O_2 were evaluated in the developed cotyledon of each seed (kernels). In specific, the *Phenolic compounds* by *i.* Total polyphenols (TP), according to the method of Dewanto *et al.* (2002), ii. Flavonoids (Flav), according to a colorimetric method described by Dewanto *et al.* (2002), and iii. Dose of condensed tannins (CT), according to the method of Sun *et al.* (1998). The *Malondialdehyde (MDA)*, according to the method of Hernandez *et al.* (2002). The *Hydrogen peroxide* (H_2O_2), based on the protocol of Novillo *et al.* (2014) slightly modified. The *Antioxidant capacity (AAT)* was measured according to Prieto *et al.* (1999). The antioxidant enzymes, proteins, lipids and proline have been measured at the epicotyledonal axes of the seeds, stored at -80 °C, (n = 3), and in specific the *Antioxidant enzymatic activities* by i. Catalase (CAT. EC 1.11.1.6) produced according to the protocol of Novillo *et al.* (2014), ii. Guaiacol peroxidase (GPX. EC 1.11.1.7) measured according to Nagalakshmi and Prasad (2001), and iii. Ascorbate peroxidase (APX. EC 1.11.1.11) measured according to Nakano and Asada (1981).

The *Accumulation of metabolites* was evaluated by the total protein (TP), that was measured according to Bradford (1976), total lipids (TL), was estimated by Goldsworthy *et al.* (1972) and proline (Pro), was measured according to Troll and Lindsley (1955) modified by Dreier and Goring (1974).

2.2.4. Statistical analysis

The statistical analyzes were carried out using the software XL STAT 2014.5.04. The results are given in terms of mean ± standard deviation (SD). The simultaneous comparison of several means was conducted by the ANOVA parametric procedure (Duncan's F test/Analysis of the differences between the modalities with a 95% confidence interval), when the conditions of normality and equality of the variances are verified.

3. Results and discussion

3.1. Physiology of germination

A single-factor variance analysis on early germination, at the $P \leq 0{,}001$ threshold, showed that salt stress has a very highly significant effect on this parameter. Indeed, the seeds, germinated in distilled water (control), have early germination, from the second day, at a rate of 36.25% whereas the increasing concentrations of NaCl, cause a decrease in germination, reaching 96% for 15g l^{-1}, accompanied by a delay of one day.

The response of *M. oleifera* seeds to oxidative stress induced by salt stress was very variable. Indeed, our results in Figure 1, suggest that there is a partial and very highly significant inhibition ($P \leq 0.05$) of germination which is reflected by the reduction of the cumulative germination rates as a function of time. The evolution of germination as a function of time is a typical sigmoidal curve. It is noteworthy that the control seeds show a best germination rate of 90% ± 1.31. A similar behavior, not statistically different, was detected for the treated by 5g l^{-1} NaCl solution, which reached a germination rate of 82.5% ± 1.22. The opposite phenomenon is observed for the levels of treatments ≥ 7.5g l^{-1} with those treated with 15g l^{-1} displaying a rate of 48.75% ± 0.18, corresponding to a 45.83% drop in germination compared to the controls.

Similar results are reported by Hussein and Abou-Baker (2014), Hegazi (2015) and Tesfay *et al.* (2016) on seeds of the same species, treated with increasing concentrations of diluted sea water (in %) and by the work of Bafeel *et al.* (2018) on the seeds of *Moringa peregrina* and *M. oleifera* similarly treated by increasing concentrations of sea water. In this context, Xia *et al.* (2010) report that salt stress triggers oxidative stress in plants as a consequence of the excessive production of ROS responsible for cell damage, metabolic disorders and the senescence process. Bafeel *et al.* (2018) assume that this stress decreases the rate of water absorption, the enzymatic activation and disturbs the metabolism of the seeds. Similarly, El-Dabh *et al.* (2011) report that the absorption of water by *Moringa* seeds is slowed with increasing levels of salinity and, as a result, seeds' germination is inhibited.

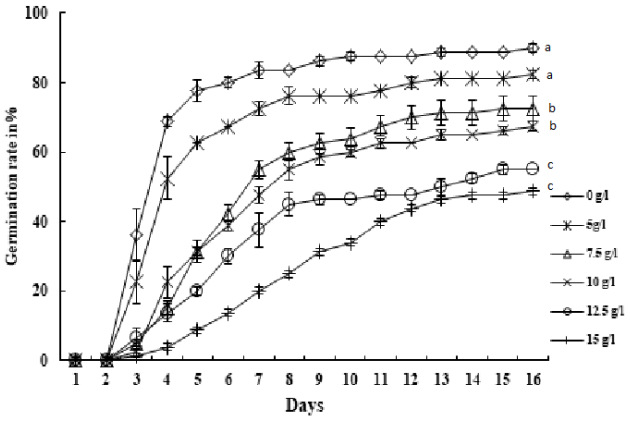


Figure 1. Variation in the cumulative average of final germination rates (%) of *Moringa* grains in the presence of increasing concentrations of NaCl (g l^{-1}). Df = 5; Pr = 0.0001< 0.05 very highly significant. The lines followed by different letters are significantly different (P ≤ 0.001) according to Duncan's test. The treatments are control (0 g.l^{-1}), low stress (5 g.l^{-1}), average stress (7.5 and 10 g.l^{-1}) and strong stress (12.5 and 15 g.l^{-1})

According to Rejili *et al.* (2006) this phenomenon may be linked to a cellular accumulation of salt. This disrupts the enzymes involved in the physiology of the germinating seeds so that it disturbs the dormancy of the embryo and leads to a decrease in the germination capacity. As for Prado *et al.* (2000), an osmotic dormancy process developed under these stress conditions can also explain this disturbance in germination. Alternatively, this could be due to the alteration of enzymes and hormones present in the seed. Based on the same authors, this phenomenon thus represents an adaptation strategy to face such constraints.

3.2. Variation in the growth in length of the epicotyledon axes and in the MDA activity of H_2O_2

Figure 2. Stage one week of growth of the epicotyledonal axes of Moringa under salt stress

Our results (Table 1) reveal a significant negative correlation ($P \leq 0.05$), between the growth in length of the epicotyledon axes and the increasing salt concentrations, with the reduction reaching 88% in radicles (Rd) and 94% in the epicotyls (Ep) under the 15g l^{-1} of NaCl compared to the control. The same fate is observed at the level of hydrogen peroxide (H_2O_2) which is manifested by a decrease of 37.89%, under the same dose of salt. In addition, the MDA activity has developed variable responses, under the effect of different salt treatments. Indeed, under concentrations ≤ 10g l^{-1}, it is gradually increasing until reaching 91.25%. However, it decreases as the concentration of NaCl increases, for a marked fall of 17.87% under 15g l^{-1} (Figure 2).

Table 1. Variation in the growth in length of the epicotyledonal axes and in the MDA activity of H_2O_2 under the different NaCl concentrations

Treatment in g l^{-1}	Length of the Rd in mm^{-1} (*)	Length of the Ep in mm^{-1} (**)	H_2O_2 in µmol g^{-1} (***)	MDA in µmol gFM^{-1} (****)
0	78.77 ± 6.64^{a}	115.62 ± 2.11^{a}	7.98 ± 5.10^{-2a}	$85.10^{-4} \pm 11.10^{-5d}$
5	65.13 ± 6.5^{a}	73.88 ± 1.88^{b}	6.69 ± 9.10^{-2b}	$88.10^{-4} \pm 24.10^{-5cd}$
7,5	32.11 ± 4.25^{b}	40.53 ± 4.44^{c}	6.0 ± 11.10^{-2c}	$10.10^{-3} \pm 23.10^{-5b}$
10	25.46 ± 0.57^{bc}	24.84 ± 1.95^{d}	6.13 ± 35.10^{-2c}	$16.10^{-3} \pm 13.10^{-5a}$
12,5	11.96 ± 0.56^{cd}	12.52 ± 0.93^{e}	5.57 ± 17.10^{-2d}	$9.10^{-3} \pm 2.10^{-4c}$
15	9.19 ± 0.35^{d}	6.50 ± 3.25^{e}	4.95 ± 49.10^{-2e}	$7.10^{-3} \pm 15.10^{-5e}$

* Df = 5; Pr = 0.0001 <0.05 Very highly significant, ** Df = 5; Pr = 0.0001 <0.05 Very highly significant, ***Df = 5; Pr = 0, 0001< 0.05 Very highly significant, ****Df = 5; Pr = 0.0001< 0.05 Very highly significant, FM: fresh material

The reduction of growth in the length of the epicotyledonal axes is consistent with that of Hussein and Abou-Baker (2014), Hegazi (2015), Silva *et al.* (2017), Noreen *et al.* (2018) on the same species, treated with increasing concentrations of diluted sea water (in %). They are also consistent with those of Bafeel *et al.* (2018) on *Moringa*

pérégrina and *M. oleifera*. These latter authors suggest that salinity induces oxidative stress in plants, which disrupts cellular metabolism (McCord, 2000). Dos Santos *et al.* (2010), report that this phenomenon is probably associated with metabolic changes and the uncontrolled cellular process and so creates that physio-logical disorder (Hussein and Abou-Baker, 2014).

According to Munns (2008), the harmful effect of NaCl on growth can be expressed in three ways: i. reduction of the water available in the root zone leading to a water deficit, ii. phytotoxicity of ions (Na^+ and Cl^-) and iii. a nutrient imbalance reducing the absorption and transport of nutrients and the competition of Na^+, K^+ for binding sites essential to cellular function. As to Houle *et al.* (2001), this is a survival strategy. It allows the plant to accumulate energy and resources to fight stress, before the imbalance between the inside and the outside part of the body reaches a threshold point where the damage will be irreversible (Bois, 2005; Zhu, 2004).

The reduction in H_2O_2 production (Table 1) during germination is consistent with the work of Rivas *et al.* (2013) and Tesfay *et al.* (2016) on *Moringa* seeds. In addition, H_2O_2 plays an important role at the cellular level by participating in programmed cell death or with a hormonal effect (Parent *et al.,* 2008). This active process is found throughout the life of plants and is involved in their germination (aleurone layer) and growth (Dat *et al.,* 2003; Van Breusegem *et al.,* 2006). Thus, we can attribute the disturbances observed during our study at the level of germination and length growth of *Moringa* to this ROS. However, H_2O_2 has the capacity to diffuse far from its pro-duction site and can cross membranes using aqueous channels (aquaporins), thanks to its great chemical simi-larity with H_2O (Bienert *et al.,* 2006; 2007). However, the Fenton and Haber–Weiss reactions, causing lipid peroxidetion, protein breakdown and DNA damage (Parent *et al.,* 2008) can also convert it to the hydroxyl radical. For Sudhakar *et al.* (2001), these ROS damage the cellular components of proteins, membrane lipids and nucleic acids.

Concerning the danger that H_2O_2 represents as a ROS for plant cells, its concentration can be regulated by enzymes, such as APX, CAT or GPX (Parent *et al.,* 2008). This may explain our results, which highlighted a negative correla-tion between the H_2O_2 content and the concen-trations of the different antioxidant enzymes analyzed (Table 3). This explanation is confirmed by Yang and Poovaiah (2002) who claim that the CAT sometimes eliminates H_2O_2 with the help of phenolic compounds.

Our results are in agreement to the work of Zineb *et al.* (2015) and Bafeel *et al.* (2016), who suggest that MDA provides information on the state of degradation of cell membranes. According to Hernández *et al.* (2000), MDA can be considered as a good indicator of the tolerance of plants to different abiotic constraints and in particular to saline stress. It has also been shown that plants with high levels of MDA without showing symptoms can withstand the effect of oxidative stress (Arbona *et al.,* 2007). On the other hand, Da Costa *et al.* (2005), argues that lipid peroxidation is not a universal marker for the tolerance of all plant species to saline stress. This is also true for Ashrafi *et al.* (2014), who have shown by their work on *Medicago sativa*, that the MDA content was lower in salt tolerance cultivars compared to susceptible cultivars.

3.3. Study of the activity of phenolic compounds in the shelled seeds of Moringa (kernels)

Our results (Table 2) have highlighted the presence of various types of phenolic compounds (TP, Flav and CT) in the ethanolic extracts of the husks of *M. oleifera* seeds. This is in total agreement with the data from Singh *et al.* (2013) and Olagbemide and Alikwe (2014) in the same species. These results suggest that the contents of these compounds show highly significant variations ($P \leq 0.05$) as a function of increasing NaCl concentrations. Indeed, the TP content was initially positively correlated with increasing salt concentrations until reaching an increase of 19.53% under 7.5g l^{-1} compared to the control. However, this content decreased as the concentration of NaCl increased, falling by 29.97% under 15g l^{-1}.

On the other hand, our results reveal an increased synthesis of Flav and CT. The Flav content increased by 97.76% and CT by 22.78% under 15g l^{-1}, compared to the controls. The decrease in the synthesis of TP under concentrations higher than 7.5g l^{-1} agree with those of Karoune (2016), on the seeds of two species of *Acacia* (*A. albida* and *A. raddiana*). To explain this phenomenon Gallé *et al.* (2007), hypothesize that the stress is too intense, limiting the availability of carbon resources (basic substances for the production of new phenols). Sakihama *et al.* (2002) suppose that it is possible, under certain restrictive conditions, such substances convert to pro-oxidants by ester hydrolysis and bioreductive metabolism, which limits their biosynthesis by plants. However, the increase in Flav levels in *Moringa* seeds is confirmed by the work of Gao *et al.* (2015), Mendoza-Sánchez *et al.* (2016) and Karoune (2016). Løvdal *et al.* (2010), assume that these compounds act as antioxidant molecules, ensure the fixation of ROS produced during stress and thus neutralize their effects before the manifestation of oxidative damage at the cellular level. As for Ben Sekerifa and Khellafi (2018), they suggest that these are potentially antioxidant substances having the capacity to trap radical species and ROS. In the same context, Nijveldt *et al.* (2001) report that Flav prevent lipid oxidation, as they mainly act as scavengers for free radicals such as DPPH and superoxide.

The synthesis of CT by *Moringa* seeds is supported by the work of Nouman *et al.* (2012) on the same species but also by Abd EL-Azim and Ahmed (2009) and Guardado-Félix *et al.* (2017) on the seeds of *Pisum sativum.* According to Aguilera-Carbo *et al.* (2008), CT are generally amorphous compounds located in vacuoles. Thus, they are very effective in preventing lipid peroxidation. They also have scavenger activity of the radical DPPH and the superoxide anion (Okuda, 2005). Spranger *et al.* (2008), indicate that these polymeric molecules are responsible for a strong antioxidant activity and therefore for the protection of the plant against environmental constraints.

Based on these facts, it can be assumed that *Moringa*'s ability to overcome the oxidative stress triggered by

salinity, is governed by an adaptive mechanism, based essentially on the biosynthesis of phenolic compounds.

Table 2. Variation of phenolic compounds as a function of different NaCl concentrations

Treatment in g l^{-1}	TP in mgEAG gDM^{-1} (*)	Flav in mgEC gDM^{-1} (**)	CT in mgEC gDM^{-1} (***)
0	$16,4\pm0,21^{b}$	0.18 ± 0.01^{b}	1.34 ± 0.03^{d}
5	$13,9\pm0,13^{c}$	0.19 ± 0.002^{b}	1.64 ± 0.006^{c}
7.5	$19,65\pm0,13^{a}$	0.21 ± 0.003^{b}	1.82 ± 0.03^{b}
10	$11,66\pm0,11^{d}$	0.33 ± 0.002^{a}	1.85 ± 0.02^{a}
12.5	$11,04\pm0,02^{de}$	0.34 ± 0.002^{a}	1.86 ± 0.012^{ab}
15	$11,5\pm0,24^{e}$	0.36 ± 0.003^{a}	1.93 ± 0.012^{a}

*Df = 5; Pr = 0, 0001 < 0,001: Very highly significant, ** Df = 5; Pr = 0.0005< 0.001: Very highly significant, *** Df = 5; Pr = 0.0001< 0.001: Very highly significant, DM: dry matter, mgEAG gDM-1: Mg equivalents Gallic Acid per gram dry matter, mg ECg^{-1}DM: Equivalent Catechin mg per gram of dry matter

Table 3. Evolution of the total antioxidant and enzymatic activity of Moringa under increasing concentrations of Na Cl

Traitement en g l^{-1}	AAT in mgEAG gDM^{-1} (*)	APX in U gFM^{-1} (**)	CAT in U gFM^{-1} (***)	GPX in U gFM^{-1} (****)
0	173.4 ± 2.4^{d}	0.525 ± 0.004^{d}	22.7 ± 0^{b}	0.42 ± 0.014^{b}
5	211 ± 2.08^{c}	0.61 ± 0.007^{c}	18.98 ± 2.9^{b}	0.48 ± 0.013^{b}
7,5	225.7 ± 2.05^{b}	0.71 ± 0.004^{b}	26.6 ± 1.46^{b}	0.62 ± 0.004^{b}
10	228.4 ± 1.54^{ab}	0.84 ± 0.011^{a}	43.65 ± 2.9^{a}	0.61 ± 0.004^{a}
12,5	228.5 ± 1.95^{ab}	0.525 ± 0.009^{d}	45.5 ± 1.09^{a}	0.6 ± 0.012^{a}
15	237.6 ± 2^{a}	0.47 ± 0.004^{e}	41.75 ± 1.8^{a}	$0.62\pm 0,003^{a}$

* Df = 5; Pr = 0.0001< 0.05 Very highly significant, ** Df = 5; Pr = 0.0001< 0.05 Very highly significant, *** Df = 5; Pr =0.0001< 0.05 Very highly significant, **** Df = 5; Pr = 0.0001< 0.05. Very highly significant, DM: dry matter, mgEAG gDM-1: Mg equivalents Gallic Acid per gram dry matter, mg ECg^{-1}DM: Equivalent Catechin mg per gram of dry matter, FM: fresh material

3.4. Involvement of total antioxidant and enzymatic activity in the defense of Moringa against ROS

Our results in Table 3 show a significant accumulation ($P \leq 0.05$) of AAT in shelled *Moringa* seeds, under the different NaCl concentrations. Indeed, it shows an increase of 37% under 15g l^{-1} compared to the control.

Additionally, our results reveal an increased biosynthesis of the antioxidant enzymatic compounds at the level of the epicotyledonal axes of *Moringa*, marked by an 83.92% increase in the CAT and a 47.61% increase in the GPX under 15g l^{-1} of NaCl (the most severe) compared to the controls. On the other hand, the APX represents a disturbance-synthesis which is manifested by an accumulation of 60% under 10g l^{-1} and then a progressive reduction reaching at 10.47% for the 15g l^{-1} level.

The high accumulation of AAT suggests the involvement of these compounds in its response to salt stress, as also confirmed by Nouman *et al.* (2012) on the same species, by Karoune (2016) on *Acacia albida* and by Guardado-Félix *et al.* (2017) on *Pisum sativum*. Joseph and Jini (2011) explain this phenomenon by the fact that the plant has developed a system of defenses in order to ensure the detoxification and the fixation of ROS. For Bor *et al.* (2003), plants that produce high levels of antioxidants are able to provide better resistance to damage induced by salinity. Mittler (2002) supposes that salt-tolerant species have a better antioxidant system which controls the production of ROS and allows plants to survive under abiotic stress. As a result, *Moringa*'s antioxidant system is relatively stronger than other salinity tolerant species enabling its survival under such high stress conditions (Nouman *et al.,* 2012).

It is evident that salt stress has induced the activity of antioxidant enzymes (CAT, APX and GPX) which constitutes a basic strategy for dealing with ROS. Similar results are reported by Nouman *et al.* (2012) and Silva *et al.* (2017) on the same species and by Bafeel *et al.* (2018) on *Moringa oléiféra* and *M. pérégrina*. Therefore, these enzymes seem to be an indication of salt tolerance for *Moringa* (Ashrafi *et al.,* 2014) since they are considered among the main detoxification enzymes (Gill and Tuteja, 2010), as also confirmed by Ksouri *et al.* (2010), Liu *et al.* (2012) and Nouman *et al.* (2012). This explains why the seedlings used in our experiments did not show symptoms of stress or sensitivity, even under doses ≥10g l^{-1} of NaCl. Also, it demonstrates moderate resistance to stress by the development of epicotyledonal axes even under 15 g l^{-1} of Na Cl. On the other hand, CAT is an intracellular hemoprotein which directly dismutates H_2O_2 into H_2O and oxygen and is responsible for the detoxification of free radicals (Gill and Tuteja, 2011). In the same line of idea, Khalifa *et al.* (2011) note that, sometimes, an increase in the activity of the CAT is perceived even in the absence of an intake of H_2O_2. Compared to CAT, the role of GPX remains regular but secondary in the mobilization of H_2O_2 because of the weak capacity to eliminate ROS (Gondim *et al.,* 2012). Thus, its synthesis is at the origin of a better tolerance to stress (Gill and Tuteja, 2010). As a result, it presents an improvement in germination and growth under saline stress (Roxas *et al.,* 2000), which holds true in our study. Mhadhbi *et al.* (2004, 2008, 2009) suggest it as a potential biochemical marker for tolerance to salt stress. Hence, it is essentially involved in lignification and strengthening of the cell wall and provides better protection of tissue integrity (membrane). In addition, the accumulation of APX in our experiment under concentrations ≤ 10g l^{-1} of NaCl is probably explained by its

key role in eliminating H_2O_2 (Wang and Han, 2009) and by its important role in the protection against oxidative stress induced by salt in higher plants (Li *et al.,* 2012). According to Ben Ahmed *et al.* (2010) and Hakiman and Maziah (2010), APX can reduce H_2O_2 levels by catalyzing its reduction through the use of ascorbate as a co-substrate. Kartashov *et al.* (2008) have shown that plants which exhibit intense activity of APX, exhibit good acclimatization to saline stress conditions. On the other hand, beyond the concentration of 10g l^{-1}, APX activity decreased significantly, which could be due to damage caused by the antioxidant system (Nouman *et al.,* 2012). The capacity for production and accumulation of antioxidant enzymes within the cells is a strategy that *Moringa* is developing to survive under severe saline stress.

3.5. Role of organic solutes in Moringa salt tolerance

The results (Table 4) reveal a positive correlation between the biosynthesis of organic osmolytes and increasing salt concentrations, expressed by a 39.47% increase in proline as well as a large significant accumulation of proteins and lipids which exceed 100% under 15g l^{-1} of NaCl, compared to the control.

These results corroborate with those of Silva *et al.* (2017) on the same species. These authors suggest that the production capacity and the accumulation of organic solutes within cells is a strategy that *Moringa* is developing to allow its survival under saline stress. This has been confirmed by Chen and Jiang (2010) and Majumder *et al.* (2010), who point out that this phenomenon is another adaptation strategy to salinity. In the same line of thought, Koyro *et al.* (2012) state that such synthesis is one of the main biochemical responses of plants to abiotic stress. For Kaya *et al.* (2013), the accumulation of compatible solutes often forms a basic strategy for saline stress, increasing the activities of antioxidant enzymes in plants exposed to saline stress. They also add that the oxidative defense in plants stressed by salt had been very effective in countering various ROS produced due to salt stress.

Table 4. Evaluation of the activity of proline, proteins and total lipids under increasing concentrations of Na Cl

Treatment in g.l^{-1}	Pro in µg gFM^{-1} (*)	Pr in µg l^{-1} (**)	TL in µg l^{-1} (***)
0	6.08 ± 0.1^{c}	22.32 ± 1.62^{f}	86.81 ± 0.55^{c}
5	5.96 ± 0.26^{c}	67.06 ± 0.21^{e}	127.5 ± 1.73^{b}
7.5	5.71 ± 0. 1^{d}	71.34 ± 6.0^{d}	203.9 ± 2.76^{b}
10	5.81 ± 0.05^{c}	80.82 ± 00.07^{c}	215.9 ± 5.5a
12.5	8.84 ± 0.07^{b}	95.39 ± 1.67^{b}	239.7 ± 2.9^{a}
15	11.13 ± 0.74^{a}	101.09 ± 0.86^{a}	264.8 ± 5.9^{a}

* Df = 5; Pr = 0.0001< 0.05. Very highly significant, **Df = 5; Pr =0.0001< 0.05 Very highly significant, *** Df = 5; Pr = 0.0001< 0.05 Very highly significant, FM: fresh material.

It has been found that the synthesis of proline gradually increases with increasing NaCl concentrations. This has been reported by a similar work on the same species by Silva *et al.* (2017). Thus, Ashraf and Foolad (2007) assume that proline, in addition to being an osmolyte, is also involved in the elimination of free radicals and protects plant cells against the harmful effects of salt by maintaining osmotic balance. Likewise, it participates as an osmolyte in the retention of water in the cytoplasm as well as it protects against membrane desiccation and denaturation of proteins (Ben Ahmed *et al.,* 2010). According to Chen and Dickman (2005) and Gill and Tuteja (2010), proline is a non-enzymatic osmoprotective, has good inhibitory potential and is considered essential for the plant in order to counteract the effect of ROS under stress conditions. However, Majumder *et al.* (2010) report that the main role of proline is to maintain a low water potential inside the cells in order to generate a suction force for water absorption to improve tolerance to salt stress.

In addition, protein synthesis is in agreement with the work of Silva *et al.* (2017) on *Moringa* and of Parvaiz *et al.* (2014) on *Morus alba* which point out that such a phenomenon often occurs during acclimatization process in stressful environments and is, therefore, involved in the induction of salt tolerance. Similarly, Ben kaddour (2014) have shown that the increase in stress promotes a significant accumulation of total proteins, in contents that are proportional to the concentration of NaCl applied. For Parvaiz *et al.* (2014) this protein accumulation scheme clearly shows that they could be used as potential indicators of tolerance to salinity.

Despite the increasing salt constraint, *Moringa* seeds manage to preserve their lipid status or even involve them in its tolerance mechanism (Mâalem, 2011; Singh *et al.,* 2002). These authors add that lipids also play an important role as constituents of most membrane cells structures. They also have a vital role in tolerance to various physiological stresses.

4. Conclusion

The ability of *Moringa oleifera* to overcome the oxidative stress induced by salt stress during germination is governed by several tolerance strategies. These adaptive mechanisms include changes in physiological and biochemical processes. As a result, *Moringa* adaptation is associated with metabolic adjustments leading to the accumulation of several organic solutes such as proteins, lipids and proline, as well as to strong enzymatic and non-enzymatic antioxidant activities. This makes this species capable of rehabilitating and enhancing the arid and semi – arid zones such as the Tunisian ones. Given its physiological and antioxidant characteristics, this species is undoubtedly a species of the future, especially in arid and semi-arid zones.

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