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Tolerance mechanisms and adaptive strategies of *Moringa oleifera* (L) during germination and precocity seedling growth under hydroponics water stress

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Abstract In order to highlight the potential tolerance and/or resistance of *Moringa oleifera* (L), to drought during two weeks of germination *in vitro*. Osmotic water stress was induced under semi-controlled conditions. The plantlets water potential (Ψ_H) was reduced by adding the polyethylene glycol (PEG-6000) solution. Four water potentials were measured: 0 (control), -2; -4 and -6 MPa. Results showed different forms of such variations in a range of physiological traits were explored: A slight decrease in the germination speed, accompanied by an increase in the latency time, as the Ψ_H decreases. However, compared to controls, significant reductions ($p<0.05$) of 27.8% and 77.5%, respectively were recorded for growth of radicles and epicotyls of young *M. oleifera* seedlings after two weeks. Similarly, compared to controls high accumulation of compatible solutes such proline and proteins was observed with an increase rates estimated to 55%, and over 100%. In addition, by comparing cationic ions profiles of control seeds to the treated ones, we registered that the latter have the possibility of more cations accumulating such as K^+, Ca^{2+} and Mg^{2+} which would have roles of osmoregulation. Moreover, *M. oleifera* (L) had the capacity to maintain or even improve its oxidative status by increasing total polyphenols activities, total antioxidant capacity, catalase and ascorbate peroxidase.

Keywords: *In vitro* germination, growth, osmoregulatory and antioxidant activity

1. Introduction

Moringa oleifera (L), is a fast growing, multiuse plant grown in tropical, subtropical and temperate climates, 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 was also introduced to arid and semi-arid bio-climates where regions are characterized with low rainfall and sometimes soils are affected by high salinity (Lim 2015). Likewise, this plant species tolerates extended drought periods (Tian et al. 2015), qualifying its occurrence within large and extremely dry land. The whole plant parts (leaves, roots, seed, bark, fruit, flowers and pods) have been used as a primary source of traditional and modern treatment (Sharma et al. 2011). Furthermore, this plant species is ridiculous in human consumption and livestock (Fodder) explaining the rise demand for its plantation.

During this research study an investigation of the water stress on seeds germination of *Moringa oleifera* (L), under semi-controlled conditions is assumed.

2. Material and Methods

Egyptian seeds of *M. oleifera* (L) seeds were used during the whole experimental design of the present study. Within the INRGREF's cold room (temperature 4 ± 1°C) species seeds were retained. Then, seeds were assumed with an embryo viability test with tetrazolium (Moore 1966) before the following experimental steps.

First, water stress was induced by PEG-6000. The different water potentials (Ψ_H) tested, in mega pascal (MPa), were imposed through increasing concentrations of PEG-6000 (in $g.l^{-1}$), using the equation of Michel and Kaufmann (1973). Thus, water stress was applied in gradients of - 2 MPa: T_0 (control): 0 MPa with 0 $g.l^{-1}$ PEG-6000; T_1: – 2 MPa with 119,4 $g.l^{-1}$ PEG-6000; T_2: - 4 MPa with 178,34 $g.l^{-1}$ PEG-6000, and T_3: -6 MPa with 223,66 $g.l^{-1}$ PEG-6000.

Second, seeds were randomly grouped into 4 sets of 80 seeds (4 replicates) for each treatment. During a couple of week, sterilized seeds were retained in glass Petri dishes by applying an increasing PEG-6000 solutions (hydric treatments). The whole seeds sets were in an INRGREF's grow room of 3 m^3 volume (temperature of 26 ± 2 °C, relative humidity of 65 %) with a total darkness. The success of germination was linked to radicle emergence length of 1 mm (Rachidai et al. 1994).

The germination rate and germination kinetics describing advantages of cumulative germination percentile during the two weeks of experimental period. Growth of seedling length, protein and proline amount were predicted by colorimetric method of Bradford (1976), and the method

labelled by Troll and Lindsley (1955) and later improved by Dreier and Goring (1974)), respectively. Cations elements such K^+ and Ca^{2+} were valued by flame emission photometry to Glenn et al. (1994). The assessment of non-enzymatic antioxidant activities is valued by measuring total polyphenols agreeing the method given in Dewanto et al. (2002). The prediction of total antioxidant capacity is done according Prieto et al. (1999)). The enzymatic activities such catalase activity is measured according to Aebi (1984) and ascorbate peroxidase activity via the method of Nakano and Asada (1981).

3. Results

3.1. *Kinetics of germination*

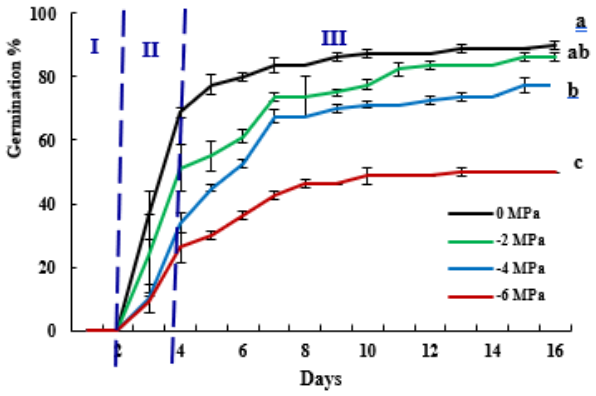

Figure 1: kinetics germination of *M. oleifera* (L) seeds, under four water treatments: Control (0MPa), -2 MPa (low), -4 MPa (medium) and -6 MPa (high), differences are connected by different letters, DDL=3; Pr =0.0001< 0.05: Very highly significant. Values are denoted by mean ± SE (n = 4).

The graphical representation registered in Figure 1, show a decreasing germination rate by reducing Ψ_H intensity. However, assumed seeds under -6 MPa offered the lowest significant germination rate of 50 ± 0.52% compared to seeds under controls treatments (90 ± 0.65%). Similarly, the three stressed treatments showed after a couple week a below germination rate level in comparison to the control seeds. Likewise, three phases were observed for any germination treatments.

Phase I: This first step starts with the uptake of seeds by water after two days. The radicle swelling after seeds imbibition showing that germination has begun and it is similar for the four Ψ_H treatments.

Phase II: This medium and second step is characterized by quick and significant difference which was observed for cumulative germination rate during two additional days after seeds imbibition. Furthermore, control and -2MPa treatments revealed the highest recorded germination rate (more than 50%) during four days. In contrast, -4MPa and -6MPa showed a germination rate lower than 30% during the same periods (4 days).

Phase III: This step is presenting the final seeds germination rate under the 4 water stress treatments (Ψ_H). Seeds under -6MPa has shown the lowest emergency in comparison to the three others treatments.

3.2. *Growth in length of the epicotyledonary axis (mm)[1] Epicotyl length (mm)[2]*

Significant reductions were observed in rootlet length by comparing radicles under control treatments and the others three water stress treatments (-2MPa, -4MPa and -6MPa). By opposite, significant increases were observed for epicotyl (Table 1).

Table 1: *Moringa oleifera* (L) rootlet length and epicotyl growth by applying four water stress treatments (Control (0MPa), -2MPa, -4MPa, -6MPa during a couple of week.

Treatments (MPa)	Rootlet length (mm)[1]	Epicotyl (mm)[2]
0	78.8 ± 6.6^a	115.62 ± 2.1^a
- 2	67.4 ± 1.4^b	65.08 ± 2.8^b
- 4	64.6 ± 1.1^b	37.51 ± 4.6^c
- 6	56.9 ± 5.0^c	26.0 ± 3.5^c

3.3. *Organic and inorganic osmolytes accumulation*

Results showed an increase of proteins, proline and metabolite contents (Ca^{2+} and K^+) by decreasing Ψ_H as registered in Table 2. Severe water stress (-6MPa) is coupled with the highest mean values recorded for any organic and inorganic osmolytes accumulations. Control treatments (0MPa) registered the lowest mean values for any analyzed components.

Table 2: *Moringa oleifera* (L) cotyledons metabolite contents, proteins and proline after a growing period of fifteen days under four water stress treatments (Control 0MPa, -2MPa, -4MPa, -6MPa).

Treatments (MPa)	Proteins (µg.l^{-1})	Proline (µg/g DM)	Ca^{2+} (mg.l^{-1})	K^+ (mg.l^{-1})
0	0.5±0.0^c	6.2±0.0^d	23.7±0.3^d	1.7± 0.1^b
-2	1.5±0.0^c	7.2±0.0^c	26.6± 0.3^c	18± 0.1^a
-4	11.2±0.0^b	8.4±0.0^b	30.4± 0.3^b	1.8± 0.0^b
-6	21.4±0.0^a	9.7±0.0^a	32.6±0.4^a	2.3± 0.1^b

3. 4. *Evaluation of antioxidant activities*

Table 3: *Moringa oleifera* (L) cotyledons analyses of antioxidant activities in addition to catalase and ascorbate peroxidase mean values under four water stress treatments (0MPa, -2MPa, -4MPa, -6MPa) during fifteen days of growing.

Trmt (MPa)	Total polyphenols[1]	Total antioxidant capacity[2]	Catal-ase[3]	Ascorbate peroxidase[4]
0	5,86± 0,07^c	173,4±2,38^c	22,77± 0,00^c	0,53± 0,01^c
-2	6,3± 0,04^b	263,33± 2,87^b	26,57± 1,46^{bc}	0,64± 0,01^b
-4	6,62± 0,024^{ab}	269,46±4,61^{ab}	32,26± 2,92^b	0,67± 0,01^b
-6	6,68± 0,12^a	279,46± 1,02^a	43,65± 1,46^a	1,10± 0,01^a

* Units of expression: (mg EAG/g DM) 1 and 2, (U/g MF) 3 and 4.

During a couple of week growing period, the species cotyledons showed an active impact by applying the four water stress treatments during this short growing period. However, under control treatment (0MPa), the lowest mean values were observed with a total polyphenol mean value equal to 5.8 ± 0.1mgEAG/gDM, total antioxidant capacity of 173.4 ± 2.4 mgEAG/gDM, catalase of 22.8 ± 0.0 U/gMF and ascorbate peroxidase of 0.5 ± 0.0 U/GFM. Severe water stress (-6MPa) showed the highest mean values for all the aforementioned parameters

Discussion

During two successive weeks, the effect of decreasing Ψ_H on kinetics germination has shown an inhibition seeds emergency up to 48 % under severe stress intensity (-6MPa), in comparison to control treatments which showing an inhibition rate less than 12% (Figure 1). This is also confirmed by Popoola et al. (2016) in *M. oleifera* (L), by Steinitz et al. (2018) in *M. stenopetala* and *M. peregrine*. Previous published work of Prado et al. (2000) shown an important decreasing germination rates under high water stress intensity explained by the dormancy of embryo under severe circumstances as an avoidance strategy against climatic restrictions. All these research authors indicated that decrease in germination rates after applying water stress could be due to enzymes and hormones alteration touching seeds imbibition. Likewise, similar published results were deduced in *Pistacia vera* by Benmahioul et al. (2017) showing a germination and survival decreasing rate by applying severe water stress amplified by PEG6000.

Therefore, high drought intensity could diminish the growth length of radicle and epicotyl either for *M. oleifera* (Table 1) or for *P. vera* as it was previously published by Tazi et al. (2003). Consequently, under sever climatic circumstance Chaves (2002) concluded the activation of regulatory mechanism by increasing osmotic pressor as a strategy developed by the plant species to avoid water deficit. This severe condition stimulates plant to reduce, or to break water absorption by its root system. Similar deductions were earlier published by Bois (2005) and Zhu (2004) recording a suffering plant growth as an adaptive strategy for limited survival during abiotic stress fitting. Subsequently, under deficit circumstances, plants avoid it by storing resources to contest stress effect before installation of permanent damage. Similar published research work was also registered with Benmahioul et al. (2017) for *P. vera* (L) reporting that plant aerial party is more considerate to water stress than the root party. This later deduction was also underlined by Bachtarzi and Bensaad (2015) concluding that water deficit seems persuading better biomass distribution to plant root party.

Moreover, severe and intense water deficit stimulate high accumulation inside the cell cytoplasm of solute products such organic and inorganic osmolyte (Le Rudulier, 2005), without disturbing intracellular biochemistry (Parida and Das, 2005). Furthermore, results shown in Table 2, debate *M.oleifera* (L) ability to improve its osmoprotective / osmoregulatory status, occurred by an increased synthesis of proteins, proline, Ca^{2+} and K^{+}. Likewise, Rebeca et al. (2013) and Tesfay et al. (2016) has been confirmed previous observed deductions showing clear proteins productions and its accumulation under drought stress conditions. However, solutes compounds are integrated in directive seedlings metabolites (primary and secondary) to avoid drought deficit. Former revelations published by Sánchez-Díaz and Tapia (2007) and by Cattivelli et al (2008) clarifying the impact of water deficit on the accumulation of new proteins synthesis diminishing the growth and proteins synthesis. Equally, cell metabolic accumulation advantage is to maintain plant physiological status and the timing of cellular growth. Similarly, the research work of proline accumulation in-side cells could increase the osmotic potential which conserve water in vacuoles and ensure cellular homeostasis (Garavillon-Tournayre, 2017).

However, the osmotic potential increases can be realized by Ca^{2+} and K^{+} accumulation in side plant cell. Thus, the accumulation of K^{+} ions at the seed level of *M. oleifera*, is considered a major mechanism of adaptation to water stress (Munns et al., 2006). Consequently, K^{+} ions plays a feed-back role for cell turgor (Sairam and Tyagi., 2004) contributing to diminish osmotic potential by facilitating solute flux (Houala et al. 2007). The same tendency is observed for Ca^{2+} playing role to build cell walls and membranes structure (Ding et al., 2010). Both chemical elements are crucial for plant protection against oxidative stress (White and Broadley, 2003).

Nevertheless, the mechanisms avoidance of *M.oleifera* (L), to water stress during the germination period is also sustained by antioxidant activity (total antioxidant capacity, total polyphenols, catalase and ascorbate peroxidase) as registered in Table 3. Indeed, the high accumulation of total polyphenols and total antioxidant was observed mainly during the growth of vegetative organs (Tesfay et al. 2016), in order to ensure their protections and stimulate secondary metabolic pathways as a strategy to combat dehydration of the plant (Selma et al. 2015). Work published by Bibata et al. (2017) explains this by a slowing of the degradation of nutrients contained in the seed endosperm.

Current results argue, for an accumulation in a broad array of antioxidant enzymes such as ascorbate peroxidase (APX) and catalase (CAT). This finding was previously confirmed by Tesfay et al. (2016) and by Joseph and Jini (2011) in the same species. The latter authors, suggest that the accumulation at the cellular level of this enzymatic activity in order to ensure their detoxification and fixation to palliate the damage of germination (Gimeno et al. 2009) and growth (Roxas et al. 2000) under water stress.

Finally, the whole recorded parameters conclude that *M. oleifera*, is talented to germinate and grow under sever abiotic stress. This behavior derives from avoidance mechanisms under deficit environmental conditions. Indeed, Gill and Tuteja (2010), demonstrated that GPX are enhanced by the overexpression of APX, which is at the origin of a better tolerance to this type of stress.

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