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Full Length Research Paper

Growth and development of date palm (*Phœnix dactylifera* L.) seedlings under drought and salinity stresses

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The present investigation has been performed to evaluate date palm (*Phœnix dactylifera* L.) tolerance to osmotic stress induced by polyethylene glycol (PEG) or NaCl during the early stages of plant development. Two varieties Nakhla hamra (NHH) and Tijib widely cultivated in Mauritania were tested. NHH showed increasing of epicotyl length, primary root length, secondary root number and proline content when water deficit was induced by PEG. In contrast, on the basis of the same developmental and biochemical characters, the Tijib cultivar was more tolerant in salinity stress. This difference of cultivars' behavior according the growth conditions is discussed.

Key words: Date palm, water stress, salinity stress, proline content.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) 2n = 36) is a dioecious perennial monocotyledon fruit tree domesticated for at least 5 000 years. It is believed to be a native of the Arabian Gulf region, possibly in Southern Iraq (Wrigley, 1995). This tree, with annual world production about 3.7 million tons (Anonymous, 1992) mainly cultivated in arid regions in the Middle East, was introduced in early time into Northern India, North Africa and Southern Spain and to a small extent in California. In several tropical countries, date palm plays an important social, environmental and economic role because it constitutes the principal financial resources and food sources of oasis cultivators and contributes to the development of subjacent cultures (alfalfa, fig trees, pepper, tomato, saffron, etc.) (Ould Sidina, 1999). Despite its outstanding agronomic and socio-economic significance, attempts to use date palm biodiversity have been limited and are becoming an urgent priority. In particular, date plantations in Mauritania are in danger of being destroyed by drought and salinity, which are reducing its genetic biodiversity. Drought or salinity tolerant cultivars have been identified in many crops (Cherian and Reddy, 2003; Badiane et al., 2004) and in few trees (Tahir et al., 2003) by developing strategies based on the use polyethylene glycol (PEG) or NaCl. PEG widely used to induce water stress, is a non-ionic water polymer, which is not expected to penetrate into plant tissue rapidly (Nepomuceno et al., 1998). In contrast, the ions Na^+ and Cl^- penetrate into plant cells and can be accumulated in the vacuole for the tolerant plants or in the cytoplasm for sensitive cultivars (Kefu et al., 2003).

The objective of this study is to test the effect of PEG and NaCl on date palm seedlings for screening drought

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Abbreviations: ANOVA, Analysis of variance; MANOVA, multiple analyses of variance; NHH, Nakhla hamra; PEG, polyethylene glycol.

or salinity tolerant cultivar during the early stages of plant development.

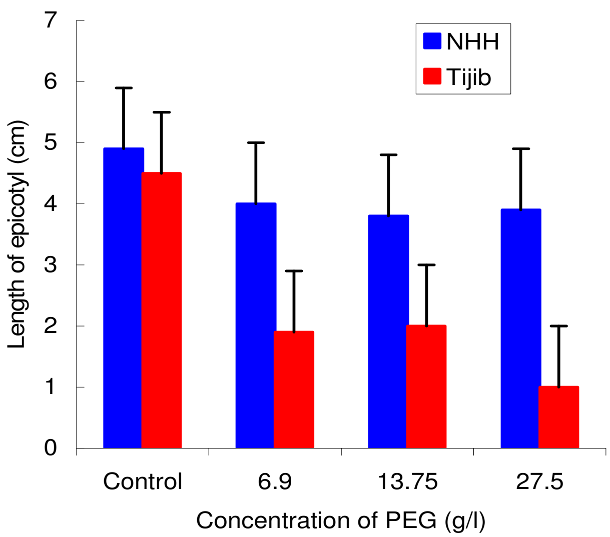

Figure 1. Effect of PEG on elongation of seedling epicotyl 15 days after sowing. Bar = ± standard deviation Each value corresponds to the mean of 24 repetitions.

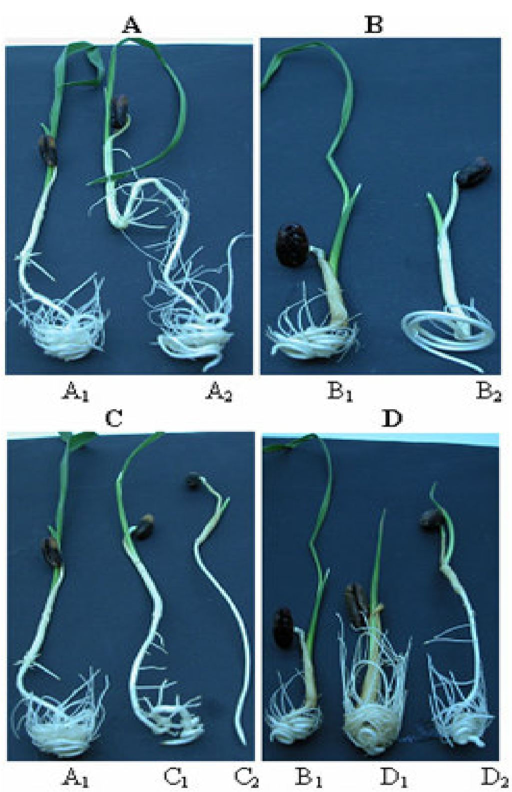

Figure 2. NHH and Tijib cultivars 3 months after culture under different concentrations and NaCl. A_1: NHH control; A_2: NHH stressed at 27.5 g/l of PEG; B_1: Tijib control; B_2: Tijib stressed at 27.5 g/l of PEG; C_1: NHH stressed at 8 g/l of NaCl; C_2: NHH stressed at 16 g/l of NaCl; D_1: Tijib stressed at 8 g/l of NaCl; D_2: Tijib stressed at 16 g/l of NaCl.

MATERIALS AND METHODS

Plant materials

Seeds were collected from date palm tree located in Kseir Torchane at 20 km in North of Atar in Mauritania. Two varieties have been chosen for their early maturity, the first belongs to Nakhla hamra (NHH) cultivar with black fruit and the second to Tijib cultivar with light red fruit. Seeds were scarified with sulfuric acid (96%) for 5 min and washed 5 times with sterile distilled water. They were then sterilized with mercuric acid 1% for 3 min, washed 5 times with sterile distilled water and imbibed for 48 h. The seeds were sterilized a second time with calcium hypochlorite (5%) for 4 min, washed 4 times with sterile distilled water.

Plant growth conditions

Seeds were cultivated in Murashige and Skoog (1962) medium (macro and microelements) supplemented with Nitsch and Nitsch vitamins (1965), 30 g/l of sucrose, complemented with 0.2 mg/l of glutamine at pH 5.7 and solidified with 8 g/l of Agar (Difco Agar). Plants were grown under 80 $\mu E.s^{-1}.m^{-2}$ and different conditions of PEG 6000 concentrations (6.9; 13.75 and 27.5 g/l) or NaCl (4, 8 and 16 g/l) with a photoperiod of 12 h at 30°C.

Proline extraction

Proline was extracted from 100 mg of fresh leaves and measured by colorimetry method as described by Monnevaux and Nemmar (1986). The amount of proline was determined according to a calibration curve prepared with a series of standard proline solutions.

Statistical analysis

Each treatment consisted of 24 test tubes with 1 plant per tube. Position of the tubes were randomized to minimize the position affects in the growth chamber. Analysis of variance was conducted in control, PEG and NaCl treatments by using General Linear Model ANOVA/MANOVA of STATISTICA [data analysis software system version 6. Statsoft, Inc. (2001). www. Statsoft.com]. Differences between means were analyzed using the Newman and Keuls' test and significance was determined at 95% confidence limits.

RESULTS

Effects of PEG on growth and seedlings development

The statistical analysis showed that there is significant ($F = 22.82$; $P = 0.000$) difference of the epicotyl elongation between the two cultivars NHH and Tijib when they are both cultivated in normal conditions. In water deficit conditions induced by PEG at 27.5 g/l Tijib variety showed significant decrease of epicotyl elongation ($F = 32.12$; $P = 0.000$) by 4 fold compared to the control plant (Figure 1). Water deficit sensitivity started at 6.9 g/l of PEG. However, NHH variety was not affected so much at 15 days after sowing. The total weight of the aboveground part was constant 3 months after growing at 27.5 g/l of PEG (Figures 2A and 2B). In contrast,

Table 1. Influence of PEG and NaCl on number, elongation and biomass of secondary roots in NHH and Tijib cultivar seedlings 3 months after sowing.

Media (g/l)	Mean number of secondary roots/plant		Mean elongation of secondary roots/plant (cm)		Mean weight of fresh roots' matter (mg)	
	NHH	Tijib	NHH	Tijib	NHH	Tijib
Control (0)	36[a]	40[a]	05[a]	07[a]	426[a]	567[a]
PEG 6.9	45[a]	20[b]	12[a]	02[b]	430[a]	364[a]
PEG 13.75	60[a]	19[b]	10[a]	2,5[b]	528[a]	359[a]
PEG 27.5	89[a]	07[b]	08[a]	1,5[b]	766[a]	214[b]
NaCl 4	30[b]	70[a]	02[b]	07[a]	392[b]	1048[a]
NaCl 8	20[b]	175[a]	02[b]	10[a]	383[b]	1208[a]
NaCl 16	08[b]	09[a]	1,5[b]	3,8[a]	314[a]	288[a]

Mean number and elongation of secondary roots were determined using 24 repetitions for each cultivar.
In the same line and for the same variable, the values followed by the same letter do not differ significantly between cultivars at 5% level (Mean comparison by Newman et Keuls' test).

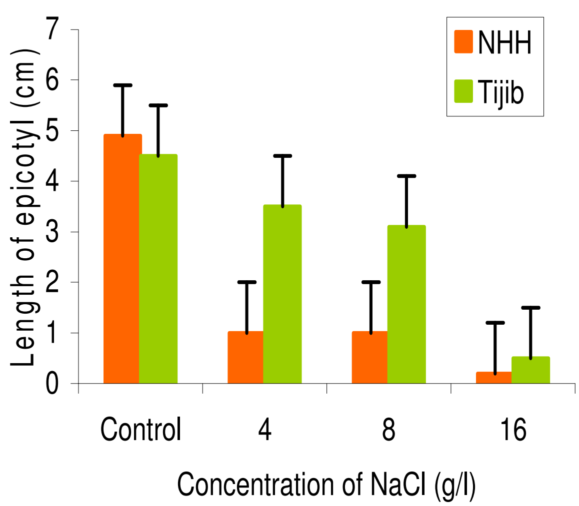

Figure 3. Effect of NaCl on elongation of seedling epicotyl 15 days after sowing. Bar = ± standard deviation Each value corresponds to the mean of 24 repetitions.

significant decrease of 2.4 fold was observed with Tijib variety from 990 mg to 400 mg when cultivated at 27.5 g/l of PEG. A significant difference (F = 9.64; P = 0.003) was observed between NHH and Tijib cultivars in term of secondary root number and length resulted from the specific effects of the variety under drought stress (Table 1). NHH developed more secondary roots than Tijib. Root biomass decreased for Tijib cultivar and increased for NHH (F = 3.08; P = 0.000). At 27.5 g/l of PEG, NHH had 12 times more roots than Tijib cultivar.

Impact of salinity on growth and seedlings development

Epicotyl elongation was affected by salinity in NHH cultivar and the effect was highly significant and had been started at 4 g/l NaCl (F = 23.40; P = 0.000). In contrast NaCl at 4 or 8 g/l had no effect on Tijib cultivar, which was very much affected at 16 g/l (Figure 3). The total weight of above ground part was also measured 3 months after sowing under salinity conditions. Significant increase of the aboveground part was described in Tijib cultivar at 4 or 8 g/l of NaCl but in the same time, it was decreasing for NHH. The root biomass increases for Tijib cultivar when the concentration of NaCl is between 4 and 8 g/l and the number of lateral roots increase respectively by 2 to 4 fold (Table 1). The cultivar NHH root biomass and secondary root length and number were affected negatively at any concentration of NaCl (Figures 2C and 2D).

Drought and salinity affect proline content in date palm seedlings

Our results showed that proline content increase progressively and significantly in the drought tolerant cultivar NHH when the amount of PEG increases in the growth medium. Proline content was 279 to 651 nmoles/g of fresh matter respectively at 6.9 to 27.5 g/l of PEG (Figure 4). In contrast, for the non tolerant cultivar Tijib, proline increased quickly and significantly from 200 to 1000 nmoles/g of fresh matter respectively for the control and stressed plants.

Figure 5 showed that NaCl treatment promptly increases proline accumulation in the tissues of non tolerant cultivar NHH at 4 to 8 g/l compared to the control and the tolerant cultivar, Tijib. At the same concentrations, proline content increases from 702 to 869 nmoles/g of fresh matter for NHH and from 536 to 809nmoles/g of fresh matter for Tijib. In the tolerant cultivar, our results showed that proline content increase progressively with the different concentrations of NaCl tested.

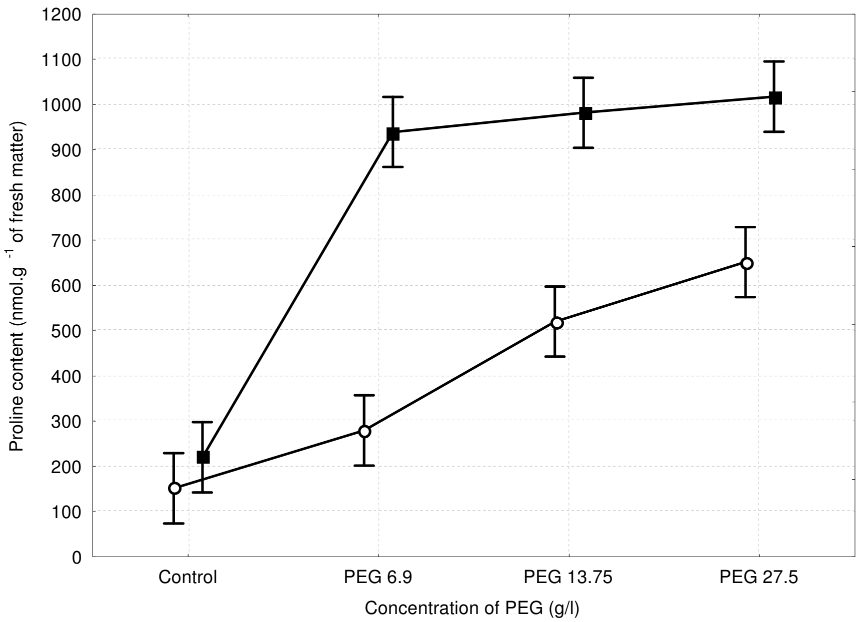

Figure 4. Effect of PEG on proline accumulation in NHH (○) and Tijib (■) cultivars 3 months after sowing. Bar = ± interval of confidence at 95%. Each value corresponds to the mean of 3 repetitions.

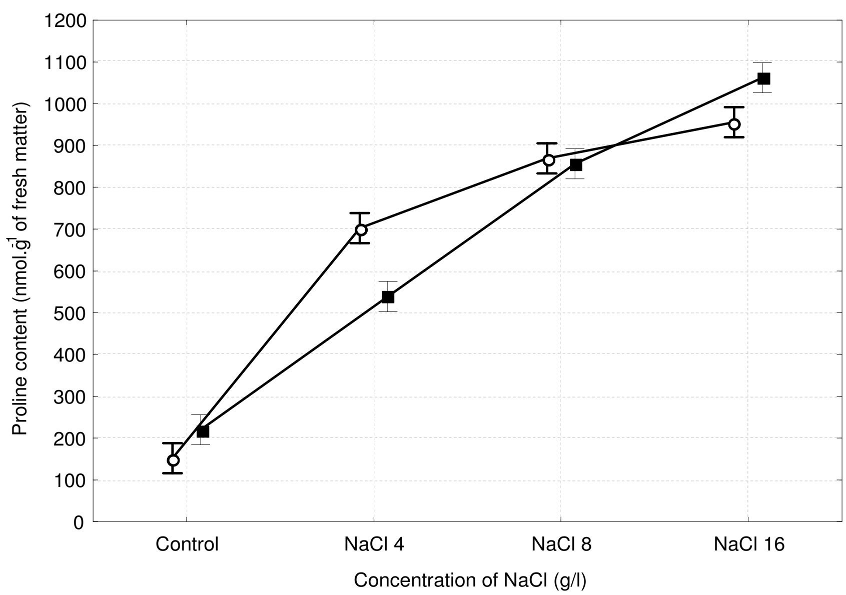

Figure 5. Effect of NaCl on proline accumulation in NHH (○) and Tijib (■) cultivars 3 months after sowing. Bar = ± interval of confidence at 95%. Each value corresponds to the mean of 3 repetitions.

DISCUSSION

Drought and salinity, some of the major constraints limiting agricultural production throughout the world, affect semi-arid and arid regions specially, about 60% of the cultivated lands. They are physiologically related because both induce osmotic stress and most of their metabolic responses in plants are similar (Ramagopal, 1993). Due to their negative impact on plant development and the increasing of the lands affected, it became particularly important to identify and select the best adapted cultivated plants for improving yield in the more sensible area. For these purposes, two decades ago, several investigations had been conducted for understanding drought and salinity tolerance phenomenon, but it was focused mainly on cultivated crops. To our knowledge, this is the first work aimed at selecting drought and salinity tolerant tree varieties by using PEG and NaCl in *in vitro* conditions.

The pattern of selection used in this study, developed in many cultivated crop (Badiane et al., 2004), allows a quick identification of tolerant variety and is less time consuming. Our results showed that water deficit induced by PEG at 27.5 g/l decrease seeds germination by 20% for NHH than for Tijib. However, during the early step of plant development, NHH is more sensitive to salinity than Tijib because their seeds germination decreased by 50 and 30%, respectively (data not shown). Our results showed that when water deficit was induced by PEG at 27.5 g/l, NHH cultivar had the longest epicotyl, more aerial biomass, more lateral roots and longest principal roots. The great development of root system increases water uptake and is also combined with higher proline content in the plant cells for maintaining right osmotic pressure. Previous studies described similar conclusions such as root system increases in mango tree under water stress (Tahir et al., 2003). In contrast, when induced of salt stress by NaCl at 8 g/l, Tijib cultivar showed longest epicotyl length and principal root, more lateral roots and its proline accumulation increased slowly compared to NHH. The environmental stresses such as drought and salinity increase superoxide dismutase and peroxidase activities, which are implicated in cell membrane damage in sensitive species.

Our results showed that some date palm varieties can be drought tolerant or salinity tolerant, suggesting that these two factors are independant. This conclusion is in agreement with previous studies described by Kefu et al. (2003) where *Suaeda salsa* was salinity tolerant and *Kalanchoe claigremontiana* drought tolerant. This physiological behavior was due by the fact that halophyte species can absorb a lot of Na and Cl from the medium, compartmentalize them in the vacuole through Na^+/H^+ antiports in the tonoplast and uptake water to maintain life (Chen and Liu, 2000; Hasegrawa et al., 2000). With PEG alone, it cannot accumulate sufficient ions for osmotic adjustment. The drought tolerant NHH cultivar could maintain Na and Cl ions at a high level in the cytoplasm, which are known to be very toxic to the plant cell by injuring the cytoplasmic enzymes. Proline content increases in the drought tolerant cultivar NHH contributing to the regulation of osmotic pressure in the cells during water stress induced by PEG. Similar results were also described in the tolerant cultivar Tijib in salinity conditions. In contrast, under NaCl stress the contribution of proline in osmotic adjustment is relatively low in NHH. Our results are in agreements with Kefu et al. (2003) who pointed out that the osmotic potential is higher in halophytes than in xerophytes. Our results will be helpful for selecting a drought or salinity tolerant date palm cultivar for sahelian and oasis regions where water availability and salinity are the major constraints.

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