World Applied Sciences Journal 17 (3): 292-295, 2012
ISSN 1818-4952

Effects of Salinity on *In vitro* Shoot Proliferation and Rooting of Apple Rootstock MM.106

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Abstract: In the present study, the *in vitro* response of MM.106 apple rootstock to increasing concentrations of NaCl (0, 20, 40, 80, 100 and 120 mM) in the Murashige and Skoog culture medium was analyzed. Explant growth was seriously affected by salinity treatments. Elevated salinity from 20 (control) to 40, 80, 100 and 120 mM NaCl resulted in reduction in shoot growth (shoot number, length and fresh weight) and rooting (rooting percentage, root number and length). At 20 mM NaCl the shoot length, fresh weight and root length was increased significantly as compared with the control. At 120 mM NaCl the shoot length were adversely effected and only half length of that in the 20 mM NaCl, whereas the shoot number had slightly decreased.

Key words: Apple · Proliferation · Rooting · NaCl

INTRODUCTION

Salinity at present is one of the most serious environmental stresses influencing crop growth and productivity [1, 2]. Salinity has a two-fold effect on plants: the salt in the soil solution decreases the availability of water to the roots (osmotic stress) and the salt taken up by the plant can accumulate to toxic levels in certain tissues (ionic stress) [3].

The MM.106 apple rootstock has been extensively used in many countries to produce semi-dwarf trees [4, 5]. Therefore, *in vitro* micropropagation is very important for commercial practices.

Plant tissue culture provides useful information to elucidate plant response to salt stress [6]. Such system offers greater control than *in vivo* growth conditions and has the advantage of small scale with clear visibility for monitoring shoot and root responses in the presence of imposed stress [6]. The *in vitro* response of tomato genotypes during the regeneration phase to salinity was studied by El-Anany [7] and Sancho-Carrascosa *et al*. [8]. Bhivare and Nimbalkar [9] stated that plant species differ in their response to salinity with respect to chlorophyll contents. Abed Alrahman *et al.* [10] report that the Cucumber cultivar Q.S.1034 could tolerate salinity up to 75 mM NaCl, therefore it can be planted in slightly saline soils. El-Sharabasy *et al.* [11] has been reported effect of salinity stress on some date palm cultivars during proliferation stage *in vitro*, but the effect of salinity on proliferation and rooting of apple rootstock MM. 106 has not yet been investigated, Therefore, in the present study proliferation and rooting stages *in vitro* of apple rootstock MM. 106 were evaluated for salinity tolerance represented by NaCl levels in culture medium.

MATERIALS AND METHODS

Plant Material and Culture Condition: The explants employed were shoots of the apple (Malus domestica Borkh) rootstock MM106 of about 25 mm in length preserved from previous *in vitro* cultures and maintained in growth room. Shoots of apple rootstock MM106 subcultured monthly in 250 ml polypropylene containers containing 40 ml of the MS [12] culture medium with 0.5 µM IBA, 4.43 µM BAP, 0.6% agar and 90 mM sorbitol [13]. The pH of the media was adjusted to 5.8 before autoclaving at 121°C for 15 min. shoots were illuminated by cool-white florescent light (50 µMol m-2. S-1 per 16-h photoperiod) at 25±1°C.

Application of Salinity Treatments: For the proliferation treatments, salinity stress was achieved by using shoot tips (1-1.5 cmlong), from the proliferation medium, which were placed in the standard proliferation medium with six

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different concentrations of NaCl (0, 20, 40, 80, 100 and 120 mM). Each treatment consisted of six replicates (jars) with 5 shoots per jar.The explants were transferred to fresh medium every 4 weeks. Each experiment was conducted and repeated twice After 6 weeks of stress treatment, shoot multiplication was evaluated by counting the number and length of shoots per explants and the fresh weights.

For the rooting treatments, Shoots (10 mm long) were transferred to 25 ×100 mm tubes containing 40 ml of half-strength MS media with 5 µM IBA, 0.6% (W/V) powder agar (Becton and Dickinson granulated), 90 mM sucrose [14] and six different concentrations of NaCl (0, 20, 40, 80,100 and 120 mM). Shoots were incubated in the dark for five days. After 5 days, the shoots were transferred to auxin-free medium and to the light. Rooting frequency, root number and root length were recorded after 4 weeks.

Analysis of variance (ANOVA) was performed on all measurements. Significant differences between means were determined using the Duncan's multiple range test (DMRT) at the P # 0.05 (n=5) level. All statistical analysis were performed using facility of computer and SAS software package [15].

RESULTS AND DISCUSSION

After 6 weeks in the proliferation culture media, the growth parameters (shoot number, length and fresh weight) were significantly affected by the NaCl treatments. As shown in Table 1, the shoot number decreased with increasing of NaCl concentrations from 20 to 120 mM. Among the different NaCl concentrations applied, 20 mM NaCl did not significantly reduce the shoot number of the explants and produced significant increases of the shoot length with respect to 0 mM NaCl. At 120 mM NaCl the shoot length were adversely effected and only half length of that in the 20 mM NaCl, whereas the shoot number had slightly decreased (Table 1).

The effect of salinity indicated that fresh weight increased significantly at 20 mM concentration as compared to all other treatments (Table 1). Afterwards, a significant gradual decrease in fresh weight took place at 40, 80, 100 and 120 mM where the mean fresh weight generally decreased with the increase in NaCl level in the medium, from 233 mg at 20 mM NaCl to 86.25 mg at 120 mM NaCl.

Results revealed that low level of salinity (20 mM) in culture medium significantly enhanced shoot length and fresh weight. High salinity levels from 40 to 120 mM NaCl caused a decrease in shoot length and number of shoots and also the fresh weight. Similar results have been observed in other woody species [16, 17]. Flowers and Lauchli [18] reported that,at low concentrations, NaCl exerts a significant positive effect on shoot proliferation *in vitro* due to the increased osmolarity. Also, El-Sharabasy *et al.* [11] low level of salinity (4000 p.p.m) in culture medium significantly enhanced shoot length and number for the three date palm cultivars, then decreased significantly at 8000 and 12000 p.p.m NaCl. The same were published by Prajuabmon *et al.* [19] who reported that all three cultivars of rice seedlings grown under high salinity had shoot length, fresh and dry weight of shoot and relative growth rate of shoot decreased.

Table 1: Effect of different NaCl concentrations on the growth parameters of MM.106 apple rootstock explants in the proliferation experiment

NaCl (mM)	Shoot number	Shoot length (cm)	Fresh weight (mg)
0	8.50a	1.95b	207.5b
20	9.25a	2.50a	233.0a
40	6.00b	1.62bc	179.0c
80	5.75bc	1.47c	152.25d
100	5.25bc	1.40c	101.25e
120	5.00c	1.30c	86.25f

Means followed by the same letter within each column are not significantly different at the 0.05 level, according to the Duncan's multiple range test

Salinity also significantly affected the rooting growth parameters. The rooting parameters decreased significantly from 40 mM NaCl and were lowest at 120 mM NaCl (Figs. 1, 2 and 3). Root length at 20 mM NaCl was increased significantly as compared with the control, while further increase in salinity level resulted in significant reduction in root length (Fig. 2). Abed Alrahman *et al.* [10] reported that increasing salinity more than 50 mM NaCl significantly decreased root length of Cucumber microshoot. Similarly, a significant reduction in number of roots per shoot was observed with the increase of NaCl concentration (Fig. 1). Both root length and number decreased with increasing salt concentration in the culture medium. These results are in agreement with results obtained previously, which also indicated roots to be among the first plant organs affected by salt stress and the most sensitive ones [20-22]. The rooting percentage decreased from 89.25% at 0 mM to 43.25% at 100 mM NaCl and no rooting occurred when explants were grown with 120 mM NaCl (Fig. 3). Similar results were observed in grapevine and Citrus macrophylla, in which NaCl concentrations higher than 85 and 100 mM prevented rooting [23, 24]. Reduction in rooting percent with increased salinity was reported in other crops [25-27].

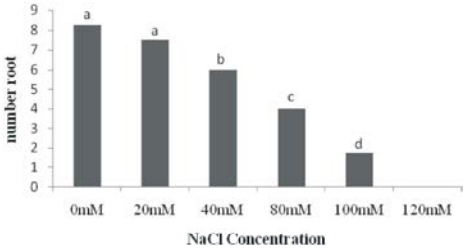

Fig. 1: Influence of different NaCl concentrations on root number of apple rootstock MM106

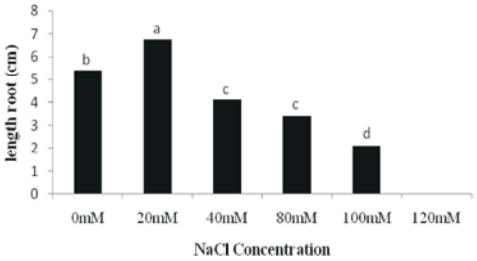

Fig. 2: Influence of different NaCl concentrations on root length (cm) of apple rootstock MM106

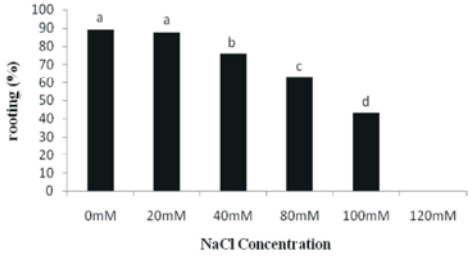

Fig. 3: Influence of different NaCl concentrations on rooting percentage of apple rootstock MM106

In all, the effect of salt stress on the growth parameters may be seen as reduced plant height, stem diam, leaf area, root length, fresh and dry weight and senescence, if salinity increases beyond tolerance limit [28, 29]. Reduction in growth parameters at increasing salinity levels can, in some instances, be attributed to salinity-induced adverse change in leaf water relations reducing photosynthesis, dehydration of proteins and protoplasm to a lower extent. The decreased growth might also be because of osmotic effect of salt on root and toxic effect of accumulated ions in the plant tissue [30, 31]. From the present results, it can be deduced that the reduction growth due to higher NaCl concentration can be attributed to the osmotic effects of salt.

CONCLUSION

In conclusion, we investigated the capacity of MM.106 apple rootstock explants, cultured *in vitro*, to grow under different NaCl concentrations. The growth rate of rootstock explants in both proliferation and rooting experiments decreased at high concentrations of salt. We suggest that the important deleterious effects in the *in vitro* explants of MM.106 apple rootstock grown at elevated NaCl concentrations are due mainly to cellular toxicity of saline ions, mainly Cl^-. The results of this study illustrate the potential of using tissue culture for evaluation of apple salt tolerance, since responses are relatively fast, the generation times are short and the environment is controlled.

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