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IN VITRO PROPAGATION OF CARNATION (*DIANTHUS CARYOPHYLLUS* L.) UNDER SALT STRESS

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ABSTRACT

Herbaceous micro-cuttings of Carnation (*Dianthus caryophyllus* L., cv Yellow Liberty and Crimson Tempo) were rooted on half-strength Murashige and Skoog medium (MS/2) supplemented with different concentrations of NaCl (50, 100 and 150mM). Cultures were maintained for eight weeks with monthly subculture. On NaCl deprived medium (Control), the rooting rate was better for the cultivar Crimson Tempo (94.1%) than for Yellow Liberty (86.3%). Salinity affected significantly the rooting of cuttings. In fact, the rooting rate and the length of the main root were clearly reduced in presence of NaCl. Crimson Tempo was more tolerant to NaCl than Yellow Liberty. Rooting rate of its cuttings at 150mM NaCl was 36.2%. No roots were obtained with the cv Yellow Liberty. Vegetative growth of cuttings was also reduced by adding 100 and 50mM NaCl to the culture medium for Crimson Tempo and Yellow Liberty, respectively.

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) is a largely cultivated species as bedding plants and for cut flowers production. In Tunisia, the area reserved for carnation cut flowers is limited (2.17 ha) and could be developed in the coastal region which offer a favourable climate for this species (Haouala 2005). However, two major constraints limit its development; the availability of plant material and the quality of irrigation water. Salinity affects actually 25% of irrigated areas over the world (Levigneron *et al.*, 1995). This important constraint reduces plant growth and productivity of the most important ornamental species (Mass 1986). Carnation is classified as a sensitive plant species, the use of irrigation water having an electrical conductivity higher than $1.2 dS.m^{-1}$ reduces its growth and production (Sonneveld and Voogt 1983, Haouala 2002). Genetic improvement using biotechnological tools may offer the possibility to obtain tolerant varieties. As first stage, we have to determine the degree of tolerance of each variety before undertaking an improvement program. Therefore, the objective of the current work was to study the response of Carnation to salinity during *in vitro* propagation phase using herbaceous micro-cuttings. The evaluation of the plant material performances was based on rooting and vegetative growth of cuttings.

MATERIALS AND METHODS

Plant material: Herbaceous micro-cuttings of Carnation (*D. caryophyllus* L.) cultivars (Yellow Liberty and Crimson Tempo) having one node and 12 mm length were used. They were sterilized by alcohol at 90°. After washing them in sterile distilled water

($d.H_2O$),these microcuttings were soaked in calcium hypochlorite solution at 5% for 20 min, then rinsed three times with sterile $d.H_2O$ and used for inoculation.

Culture medium and experimental conditions*:* The culture medium used was that of Murashige and Skoog (1962) half-strength (MS/2) for macro and microelements supplemented with 0.5 mgl^{-1} IBA (Palet *et al.*, 1991, Haouala 1999), 2.0% Sucrose and 8.0% agar (Bacto Difco). The pH was adjusted to 5.8. Micro cuttings were inoculated individually in tubes of 24 mm diameter and 150 mm length, containing 15 ml of medium. Cultures were conducted in a growth chamber at 24±1°C, with a 16-hr photoperiod under cool white light at 35 $\mu mol.m^{-2}.s^{-1}$ supplied by white fluorescent tubes.

Micro-cutting rooting in presence of NaCl: The culture medium was added with NaCl at different concentrations (50, 100 and 150mM). The control medium was deprived of NaCl. Each NaCl concentration considered as a treatment. For each cultivar, the number of micro-cuttings per treatment was 48. Cultures were maintained for eight weeks with monthly subculture. Analysis of variance was performed for all data. Statistically significant differences between means were evaluated using LSD test. Analysed parameters were: rooting rate, number of roots and shoot length.

RESULTS

Rooting rate: Rooting rate of Carnation micro-cuttings was the highest on the control medium (Table 1), which showed 94.1 and 86.3% after eight weeks, respectively for Crimson Tempo and Yellow Liberty. The presence of NaCl in the culture medium delayed the rooting and reduced its rate. In fact, Carnation micro-cuttings rooting began after two weeks for the control and after three or four weeks, according to the cultivar, on medium containing NaCl 100mM. Yellow Liberty seems to be more sensitive to salt than Crimson Tempo. Thus, on all media supplemented with NaCl, its rooting began one or two weeks later than Crimson Tempo. Moreover, rooting rate of cuttings on all used media was always the highest for Crimson Tempo. In presence of NaCl 150mM, there was no rooting for Yellow Liberty.

Table-1: Carnation micro-cutting rooting (%), cultivars Crimson Tempo (V1) and Yellow Liberty (V2), obtained at different NaCl. Culture conditions: rooting medium: MS/2 + 0.5 $mg.l^{-1}$ IBA; 16-hr photoperiod; light intensity 35 $\mu mol.m^{-2}.s^{-1}$ and temperature 24 ± 1°C.

Duration of culture (weeks)	Control		NaCl concentrations (mM)					
			50		100		150	
	V1	V2	V1	V2	V1	V2	V1	V2
1	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
2	21.1 c	19.8 c	15.4 bc	0 a	0 a	0 a	0 a	0 a
3	42.3 de	38.7 d	34.6 d	0 a	8.4 b	0 a	0 a	0 a
4	65.4 g	60.8 fg	57.1 f	24.8 c	20.7 c	12.5 b	3.6 a	0 a
5	76.4 h	69.4 g	68.9 g	46.2 e	34.2 d	27.9 c	10.8 b	0 a
6	87.8 ij	77.6 hi	77.2 hi	57.9 f	46.8 e	38.6 d	22.5 c	0 a
7	92.7 j	83.7 i	81.5 i	66.8 g	60.6 fg	54.3 f	28.3 cd	0 a
8	94.1 j	86.3 ij	87.4 ij	76.7 hi	72.3 h	65.6 g	36.2 d	0 a

Values followed by the same letter are not significantly different at P=0.05.

Number of roots*:* The number of developed roots per micro-cutting was highest on control medium and more important for Crimson Tempo (Figure 1). It was, after 8 weeks, 6.3 roots against 4.9 for Yellow Liberty. Salinity affected this parameter which is reduced to 2.5 and 0.6 roots on medium containing NaCl 100mM, respectively for Crimson Tempo and Yellow Liberty. Differences between cultivars were significant.

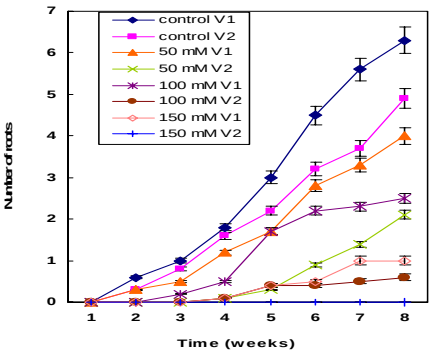

Figure-1:Number of roots for Carnation micro-cuttings, cultivars Crimson Tempo (V1) and Yellow Liberty (V2) on a medium MS/2+0.5 mg.l^{-1} IBA + NaCl (50, 100, 150mM). Culture conditions: 16-hr photo-period;light intensity 35µmol.m^{-2}.s^{-1} and temperature 24 ± 1°C.

Shoot length: Vegetative growth of new plantlets was evaluated by shoot elongation. This parameter was the highest on the control and slightly better for Yellow Liberty (Figure 2). Salinity reduced shoot length and its depressive effect was significant at NaCl 50 or 100mM, respectively for Yellow Liberty and Crimson Tempo. On media containing NaCl 150mM, shoot length did not exceed, after eight weeks of culture, 1.3 and 4 cm, representing 14.1 and 44.4% of control, respectively for Yellow Liberty and Crimson Tempo.

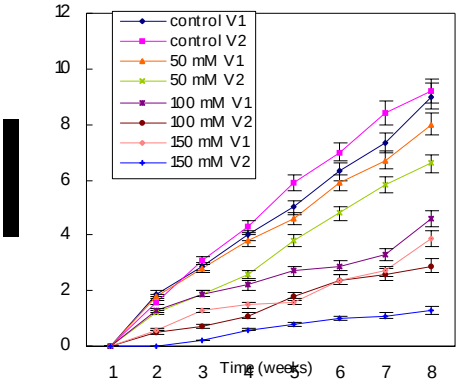

Figure-2: Shoot length for Carnation micro-cuttings, cultivars Crimson Tempo (V1) and Yellow Liberty (V2) on a medium MS/2 + 0.5mg.l^{-1} IBA + NaCl (50, 100, 150mM). Culture conditions: 16-hr photo-period;light intensity 35µmol.m^{-2}.s^{-1} and temperature 24 ± 1°C.

DISCUSSION AND CONCLUSION

Rooting of carnation micro-cuttings was severely affected by salt. This inhibitive effect appeared distinctly on the rooting rate, number of roots and shoot length. This confirms the results obtained by Haouala *et al.*, (2003) for carnation cultivar Legion d'Honneur and by Snapp *et al.*, (1991) for tomato. In our experiments, the cultivar Crimson Tempo presented more tolerance to salinity. Its tolerance is comparable to Legion d'Honneur. For Yellow Liberty, micro-cuttings rooting were totally inhibited in presence of NaCl 150mM. Salt also delayed the rooting for 1 or 2 weeks on NaCl 100mM, as compared to control, respectively for Crimson Tempo and Yellow Liberty. Slowness of rooting

was noted also for tomato (Abriquesta *et al.,* 1991), but it was not reported for Carnation cultivar Legion d'Honneur (Haouala *et al.,* 2003).

Aerial vegetative growth of micro-cuttings estimated by shoot length was clearly reduced on media supplemented with NaCl 50 or 100mM, respectively for Yellow Liberty and Crimson Tempo. The growth rate decreased from 1.1cm per week on control medium to 0.2 at 0.5cm per week on NaCl 150mM, respectively. A similar depressive effect of salt on growth has been reported by Haouala (1999) for Carnation plants cultivated under glasshouse.

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