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Effect of reduced humidity and antitranspirants in acclimatizing micropropogated *Citrus* plantlets

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Abstract

A study was made to find out the effect of reduced humidity and antitranspirants on *in vitro* hardening and *ex vitro* survival of micropropagated *Citrus* plantlets. Relative humidity inside the culture vessel was reduced with silica gel and antitranspirants used were Alar and 8-hydroxyquinoline (8 HQ). Results indicated that plants under silica gel treatment exhibited reduced shoot growth *in vitro* and lower *ex vitro* survival. 8 HQ (2 mg/l) was found effective in controlling excessive water loss which helped in more *ex vitro* survival as compared to control.

Key words: Antitranspirants, micropropagation, *Citrus,* acclimatization

Introduction

The aseptic culture of plant tissue by *in vitro* methods lead inevitably to the saturation with water vapour of the atmosphere within the culture vessel which result in various abnormalities including water relation, reduced photosynthetic capability and inhibition of surface wax production (Fuchigami *et al.,* 1981). When transferred to the greenhouse or field, *ex vitro* plantlets are subjected to desiccation due to rapid water loss and may die soon. To minimise the effect of transplantation stress, the *ex vitro* plantlets should be hardened off before being exposed to harsh field condition. The ability to grow plantlets *in vitro* at reduced level of humidity may induce development of normal amount of wax and thereby enhance the rate of survival after transfer of the plantlets to greenhouse or field conditions. Wardle *et al.* (1983) were able to induce wax development and the formation of stomata with narrow apertures by culturing chrysanthemum plantlets *in vitro* under low humidity conditions. Using substances that reduce transpiration rates by forming a semi permeable membrane on the leaf surface recently has been reported for limiting water use by peach trees (Steinberg *et al.*, 1990). Voyiatzis and McGranahan (1994) found more survival rate in tissue cultured walnut plants using latex polymer as an antitranspirants. However, the beneficial effect on the rate of water loss was offset by the deleterious effect on plantlets growth of some *in vitro* grown flowers by covering the leaf surface with antitranspirants (Sutter and Hutzell, 1984).

In this study we have attempted to harden *in vitro* grown *Citrus* plantlets with reduced humidity and antitranspirants without agar based medium.

Materials and methods

Microshoots of about 2.0-2.5 cm long were harvested from *in vitro* proliferating culture of four different *Citrus* speices *viz., Citrus retuculata* Blanco (KM), *C. nobilis* x *C. deliciosa* Tenore (KIN), *C. volkameriana* Ten and Pasq (CV) and *C. reshini* Tanaka (CLM) were aseptically cultured in tissue culture bottle (450 ml capacity) with soilrite as a carrier. Bottles were filled upto one third with soilrite and one fourth strength MS salt solution were added depending on its capacity of absorption before autoclaving at 15 lb for 20 min. Three to four microshoots were placed in each bottle and 30 bottles were used for each treatment and placed in culture room with culture condition of $25 \pm 1^{o}C$, 16 hrs photoperiod under 2000 lux.

Reduced humidity treatment was given with silica gel (8 mg) and antitranspirants used were alar (1 mg/l and 2 mg/l) and 8 Hydroxy Quinolin (1 mg/1 and 2 mg/1). Silica gel was tied with perforated cloth and suspended inside the bottle with cotton thread. Antitranspirants were spread over plants at two days interval. Observations were recorded after four weeks. Then they were transferred to mist house in polythene bag containing 1:1 soil and FYM and after one week survival percent was recorded. The relative water content (RWC) was estimated following the methods of Barrs and Weatherly (1962) and stomatal index (SI) of abaxial surface was estimated following the method of Dhawan and Bhojwani (1987).

Results and discussion

It was observed that the plant treated with antitranspirants had significant effect on growth of *Citrus* plantlets (Table 1). Shoot - growth was lowest in silica gel treatment while control recorded the highest shoot and root growth except stomatal index. However, alar and 8 HQ showed *at par* result in most of the parameter studied. This might be due to restricted growth inside the bottle in dry environment in silica gel treatment which influence on reducing plant water status thereby decreasing the RWC of leaves which is evident from the lowest RWC (15.93%) in this treatment. Wardle *et al.* (1983) also found similar findings with silica gel treatment in chrysanthemum and cauliflower. They found that antitranspirant treatments were effective in reducing humidity and inducing epicuticular wax deposition in cauliflower but proved detrimental for plant growth. Mean value for different

Table 1. Effect of silica gel and antitranspirants on morphogenetic parameters in citrus

Treatment	Plant wt. (mg)	Shoot length (cm)	Leaf No.	Leaf wt. (mg)	Root length (cm)	Root wt. (mg)	RWC (%)	S.I. (%)
Control	313.92	4.10	6.25	38.92	4.38	13.08	29.16	10.81
Silica gel	264.83	2.80	5.45	24.18	3.39	8.29	15.93	11.77
Alar 1 mg/l	288.79	2.90	5.55	26.65	3.11	7.60	27.66	11.36
Alar 2 mg/l	285.52	2.90	5.85	25.90	3.00	7.07	28.86	11.37
8HQ 1 mg/l	284.98	3.00	5.90	27.17	3.05	6.80	27.84	10.87
8HQ 2 mg/1	291.33	2.80	5.90	27.20	2.93	7.81	29.27	11.00
SEm±	4.06	0.05	1.13	0.61	0.08	0.31	0.66	0.34
CD (p = 0.05)	11.41	0.14	0.37	1.70	0.23	0.87	1.84	NS

Table 2. Mean response of citrus microshoots under silica gel and antitranspirant treatment

Species	Plant wt. (mg)	Shoot length (cm)	Leaf No.	Leaf wt. (mg)	Root length (cm)	Root wt. (mg)	RWC (%)	S.I. (%
KM	288.92	3.2	5.77	28.93	3.23	7.89	25.30	11.89
KIN	293.53	3.1	6.00	26.65	3.58	8.65	27.17	10.49
CV	290.00	3.2	5.63	30.46	2.88	8.45	26.68	11.03
CLM	280.45	2.8	5.87	27.40	3.55	8.77	26.66	11.38
Sem±	3.02	0.4	0.11	4.95	0.07	2.53	0.54	0.28
CD (p = 0.05)	9.32	1.2	NS	13.89	0.19	NS	NS	0.78

Table 3. Interaction between Citrus species and antitranspirants

Species	Treatment	Plant wt. (mg)	Shoot length (cm)	Leaf No. (cm)	Leaf wt. (mg)	Root length (cm)	Root wt. (mg)	RWC (%)	S.I. (%)
KM	Control	303.0	4.1	6.4	38.54	4.28	10.38	26.20	12.06
	Silica gel	235.5	2.7	5.4	20.30	3.20	7.46	17.34	11.24
	Alar 1 mg	297.2	3.0	5.4	28.24	3.00	7.86	26.19	13.63
	Alar 2 mg	308.5	3.3	5.8	27.42	2.96	7.36	27.21	11.01
	8HQ 1 mg	289.7	3.2	5.6	30.16	3.04	7.32	25.05	11.32
	8HQ 2 mg	299.2	3.0	6.0	28.90	2.92	6.96	29.54	12.05
KIN	Control	316.8	4.4	6.6	38.24	5.22	15.80	32.53	10.56
	Silica gel	283.8	2.7	5.4	23.76	3.54	7.40	15.43	11.18
	Alar 1 mg	293.4	3.1	5.6	24.90	3.38	7.60	26.95	9.58
	Alar 2 mg	297.1	2.7	5.8	24.54	3.10	6.50	29.54	11.18
	8HQ 1 mg	285.7	2.8	6.2	24.36	3.32	6.62	28.06	10.75
	8HQ 2 mg	284.4	2.9	6.4	23.54	2.90	8.80	30.54	9.73
CV	Control	352.4	4.5	5.8	40.80	3.38	12.40	28.95	11.17
	Silica gel	277.4	3.2	5.2	29.30	3.42	9.54	14.84	12.41
	Alar 1 mg	280.4	2.9	5.6	29.30	2.50	7.46	28.19	9.15
	Alar 2 mg	259.2	2.0	6.0	5.80	26.14	2.42	6.56	29.91
	8HQ 1 mg	278.7	3.0	5.8	27.78	2.46	6.38	29.06	11.64
	8HQ 2 mg	291.7	2.8	5.6	29.46	3.08	8.34	29.13	9.93
CLM	Control	283.2	3.4	6.2	38.10	4.62	13.74	28.97	9.48
	Silica gel	262.6	2.6	5.8	23.38	3.40	8.76	15.81	12.26
	Alar 1 mg	284.1	2.7	5.6	24.16	3.54	7.46	29.32	13.10
	Alar 2 mg	277.2	2.8	6.0	25.50	3.54	7.84	28.78	11.45
	8HQ 1 mg	285.7	2.8	6.0	26.38	3.36	6.88	29.19	9.76
	8HQ 2 mg	289.9	2.7	5.6	26.90	2.82	7.92	27.86	12.27
SEm±		8.1	0.1	0.3	1.21	0.16	0.62	1.31	0.68
CD(p = 0.05)		22.8	0.3	NS	3.40	0.46	1.74	NS	1.91

species to silica gel and antitranspirants (Table 2) revealed the presence of significant difference for most of the characters studied. Highest plant weight (293.53 mg) was recorded in KIN while CLM recorded the lowest plant weight (280.45 mg). Shoot length ranged from 2.8 cm in CLM to 3.2 cm in KM and CV. Highest leaf weight (30.46 mg) was recorded in CV followed by KM (28.93 mg). Interaction among *Citrus* species and antitranspirants are presented in Table 3. Data indicated the significant interactions for most of the characters studied. However leaf number and RWC values were not significant.

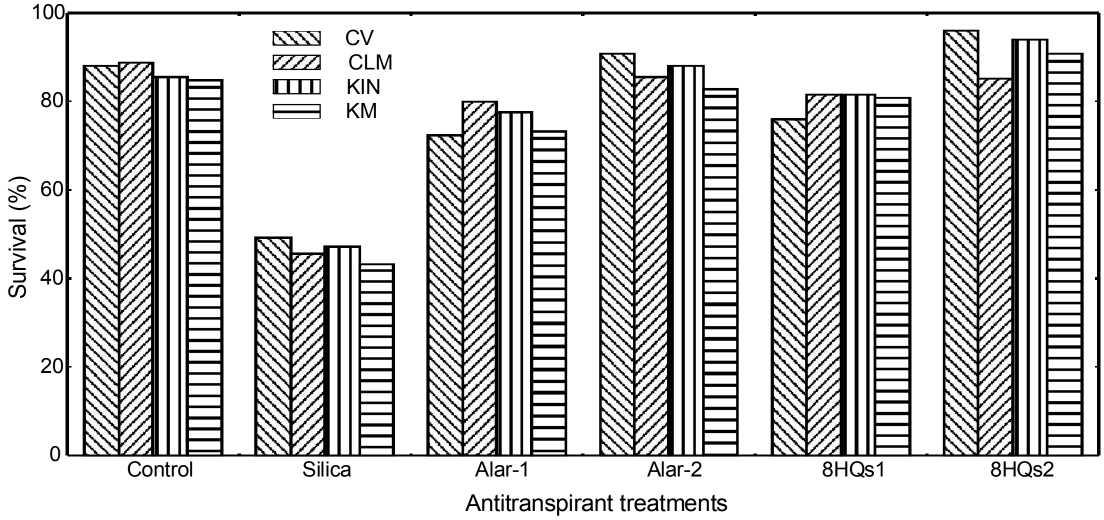

Fig. 1. *Ex vitro* survival as influenced by silica gel and antitranspirants

Significantly reduced leaf weight was recorded with silica gel treatment which might be due to low relative water content in this treatment for all the four *Citrus* species. Ritchie *et al.* (1991) also stated that in abnormal water relations, cultured plants exhibit limited leaf growth.

A perusal of data presented in Fig. 1 shows that lowering the relative humidity with silica gel results lower *ex vitro* survival which might be due to restricted growth in dry environment. On the other hand, 8 HQ (2 mg/1) was found effective in controlling water loss as evident by highest RWC content in this treatment which helped in higher *ex vitro* survival. Higher RWC values with higher stomatal resistance in alachlor and 8 HQ treated plants was also reported by Amaregouda *et al.* (1994) in groundnut.

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References

Amaregouda, A., M.B. Chetti, P.M. Salimath and S.S. Kulkarni, 1994. Effect of antitranspirants on stomatal resistance and frequency, relative water content and pod yield in summer groundnut (*Arachis hypogaea* L.). *Ann. Plant Physiol.*, 8:18-23.

Barrs, H.D. and P.E. Weatherly, 1962. A re-examination of relative humidity for estimating water deficits in leaves. *Aust. J. Biol. Sci.*, 15:413-448.

Debergh, P.C. and L.J. Maene, 1981. A scheme for commercial propagation of ornamental plants by tissue culture. *Scientia Hortic.*, 14:335-345.

Dhawan, V. and S.S. Bhojwani, 1987. Hardening *in vitro* and morpho-physiological changes in the leaves during acclimatization of micropropogated plants of *Leucaena luecocephala* (Lam) De wit. *Plant Sci.*, 53:65-72.

Fuchigami, L.H., T.Y. Cheng and A. Soeldner, 1981. Abaxial transpiration and water loss in aseptically culture plum. *J. Amer. Soc. Hort. Sci.*, 106:519-522.

Grout, B.W.W. and M.J. Aston, 1977. Transplanting of cauliflower plants regenerated from maristem culture. I. Water loss and water transfer related to changes in leaf wax and to xylem regeneration. *Hort. Res.*, 17:1-7.

Ritchie, G.A., K.C. Short and M.R. Davey, 1991. *In vitro* acclimatization of Chrysanthemum and Sugar beet plantlets by treatment with paclobutrazol and exposure to reduced humidity. *J. Exp. Bot.*, 42:1557-1563.

Sutter, E.G. and M. Hutzell, 1984. Use of humidity tents and antitranspirants in the acclimatization of tissue cultured plants to the greenhouse. *Scientia Hortic.*, 23:303-312.

Steinberg, S.L., M.J. McFarland and J.W. Worthington, 1990. Antitranspirants reduces water use by peach tree following harvest. *J. Amer. Soc. Hort. Sci.*, 115:20-24.

Voyiatzis, D.G. and G.H. Me Granahan, 1994. An improved method for acclimatizing tissue cultured walnut plantlets using an antitranspirant. *HortScience*, 29:42.

Wardle, K., E.B. Dobbs and K.C. Short, 1983. *In vitro* acclimatization of aseptically cultured plantlets to humidity. *J. Amer. Soc. Hort. Sci.*, 108:386-389.