INT. J. BIOL. BIOTECH., 3 (4): 669-672, 2006.

EFFECT OF LIGHT AND DARK CULTURE CONDITIONS ON CALLUS INDUCTION AND GROWTH IN CITRUS (*CITRUS RETICULATA* BLANCO.)

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ABSTRACT

Explants such as hypocotyls, epicotyls and cotyledons obtained from germinated seedlings, and juice vesicles and albedo tissues from immature citrus (*C. reticulate*) fruits were cultured under light and dark culture conditions to record any effect on callus initiation and growth. The seedling emergence was observed to be higher in Early Feutrall than Kinnow; andit was found to be lower under light incubation. Callus initiation was maximum in cotyledon and epicotyl explants of both cultivars and culture conditions. Juice vesicle explants of Kinnow yielded maximum callus under dark incubation. For callus development the same pattern was followed. Maximum callus growth and embryogenic callus was noticed in cotyledon, juice vesicle, albedo and epicotyl explants.

Key words: Cotyledon, albedo, epicotyl, mandarins, *in vitro*,

INTRODUCTION

Citrus (*Citrus reticulate* Blanco) is one of the most important fruit crops of the world. In Pakistan, citrus keeps its own importance due to the largest growing fruit of country with annual production of 1.6 MMT (MINFAL, 2005). Despite its excessive cultivation, its plantation has some problems such as diseases, insect pests, alternate bearing, and pre- and post-harvest losses.

The genetic improvement of perennial woody plants often takes many years using traditional plant breeding method (Cameron and Frost, 1968). Moreover, the breeding of citrus cultivars by conventional methods is hampered by the complexity of their genetic systems. This is serious obstruction to introducing important characteristics like disease resistance and stress tolerance to salt, herbicides, cold and drought from wild species of citrus to commercial cultivars. The incorporation of new technologies such as *in vitro* selection, protoplast fusion and genetic transformation methods into citrus improvement programs offers new opportunities to fully utilize this variability for both scion and rootstock (Grosser and Gmitter, 1990; Fleming *et al*., 2000). In citrus the utilization of *in vitro* selection and protoplast technology relies on the availability of embryogenic callus cultures from the desired species and cultivars. Embryogenic callus cultures are also a good source for *in vitro* cryopreservation of large plants (Kobayashi *et al.*, 1990). There are several reports on nucellar callus induction, embryogenesis and plant regeneration in different citrus species and cultivars (Grosser and Gmitter, 1990; Koc *et al.*, 1992; Pérez *et al.*, 1998; Tomaz *et al.*, 2001). In citrus, somatic embryogenesis is induced either by the lack of growth regulators or reduction of sucrose concentration in the culture medium in contrast to other plant species (Kochba *et al.*, 1978). *In vitro* growth of explant cultures from citron, grapefruit, sweet orange and mandarin fruit was stimulated by addition of orange juice to a basal MS medium containing, 2,4-D (2 mg/l) and Kinetin (0.5 mg/l) (Einset, 1978). Starrantino and Caponnetto (1990) cultured immature embryos from ripe fruits of 5 citrus cultivars on MS and MT medium with or without benzyl adenine (BA) and Kinetin. The highest frequency of embryogenic callus was obtained with ovules of cultivars Torocco, Moro and Bellandona and medium with either 10 mg BA or 5-10 mg each of BA and Kinetin per litre. Somatic embryos and plantlets were obtained upon transfer of calli to MT medium supplemented with 50 g lactose or 20 ml glycerol per litre. Callus induction and shoot regeneration has also been reported from citrus rootstocks by using different explants on MS media with NAA (10 mg/l) and BAP (1 mg/l) (Grewal *et al*., 2000). But, in this study the effects of light and dark incubation conditions on callus initiation and growth from different explants of two mandarin cultivars were examined.

MATERIALS AND METHODS

Cotyledon, hypocotyl, epicotyl, juice vesicle, albedo, shoot tip and nodal segments of *C. reticulate*, Kinnow and Early Feutrall were used as explants. Seed, shoot tip and node were first washed in tap water, sterilized in 95 % ethanol for 1 minute having 1 to 2 drop Tween 20 and then rinsed three times with autoclaved distilled water. The

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explants were then immersed in sodium hypochlorite solution (10 %) for 5 minutes followed by 3-4 rinses with autoclaved distilled water. Seeds were cultured on MS media after removing seed coat under aseptic conditions. For juice vesicles and albedo explants, surface sterilization of fruits was made by dipping fruits in 70% ethyl alcohol for 2 to 3 minutes and then flamed.

Media used for juice vesicle, albedo, shoot tip and nodal segments was MS media with 2,4-D (2 mg/l) + Kinetin (0.5 mg/l). Seeds of two cultivars were cultured on MS media. Cotyledons, epicotyls and hypocotyls from germinated seedlings were later cultured on MS + 2, 4-D (2 mg/l) + Kinetin (0.5 mg/l) for callus induction. After inoculation of seeds on MS media, cultures were incubated under light and dark conditions at 25°C for germination. Experiment was laid out in Completely Randomized Design (CRD) with three replications and data was analyzed according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

The seed germination of Kinnow and Early Feutrall was investigated under dark and light conditions. Seeds showed higher germination potential under dark culture conditions (70.2 %) than under light (54.9 %). Early Feutrall showed higher seedling emergence (68.1 %) as compared to kinnow (56.9 %) (Table1).

Table 1. Effect of light and dark culture conditions on seed germination (%) in citrus.

Treatment	Cultivars		Means
	Kinnow	Early Feutrall	
Light incubation	45.8	63.9	54.9 b
Dark incubation	68.0	72.2	70.2 a
Means	56.9 b	68.1 a	

Comparison of means of cultivar (Table 2) showed variable results for callus initiation. Kinnow yielded higher (32.1 %) callus than Feutral's early (25.1%). The interaction of treatments and cultivars resulted in higher callus initiation under dark culture conditions both in Kinnow (36.2%) and Feutral's early (27.8%). The explants individually also revealed different response for callus initiation under light and dark culture conditions. Juice vesicles and albedo explants of Kinnow showed maximum callus initiation (70.4%) under dark. Epicotyl of Kinnow initiated the highest callus (64.9%) under light culture conditions (Table 2). Early Feutrall explant cotyledon showed maximum callus under light (68.1%) and dark (65.9%). Shoot tips and nodes showed poor callus initiation in both culture conditions. **Nito and Iwamasa (1990)** induced callus from juice vesicles of Satsuma mandarin on MS medium supplemented with NAA, kinetin and gibberellins but they did not explored the effect of dark or light incubation on callus growth.

Table 2. Effect of light and dark culture conditions on callus induction (%) in different explants of citrus.

Explant	Kinnow			Early Feutrall		
	Light	Dark	Explant x Treatment	Light	Dark	Explant x Treatment
Cotyledon	36.7 e	41.6 d	39.2 cd	68.1 ab	65.9 b	67.0 a
Hypocotyl	31.1 f	25.6 g	28.3 e	10.3 i	18.0 h	14.2 f
Epicotyl	64.9 b	41.3 d	53.1 d	32.4 f	49.2 c	40.8 c
Vesicles and albedo	4.5 jk	70.4 a	37.4 d	0.4 l	5.6 j	3.0 g
Shoot tips and node	2.7 jkl	2.1 kl	2.5 g	0.0 l	0.3 l	0.2 h
Incubation x Variety	28.0 b	36.2 a		22.2 c	27.8 b	
Means of Fruit	32.1			25.0		

Comparison of means of cultivars for callus development (Table 3) showed overall variable results. Kinnow yielded higher (31.6%) callus than Early Feutrall (26.7%). The explants individually also depicted different response for callus development under both culture conditions. Juice vesicles and albedo explants of Kinnow showed maximum callus development (71.1%) under dark (Fig.1). Epicotyl of Kinnow showed the highest callus development (59.3%) under light culture conditions (Table 3). Cotyledon (51.2%) and epicotyl (55.0%) explants of Kinnow also showed high callus development percentage. Cotyledon explants of Feutral's early showed maximum callus development under light (63.2%) and dark (62.6%). Response of explants for callus initiation in both cultivars was also observed. Shoot tips and nodes showed poor callus development under both culture conditions. Erner and Reuveni (1981) also induced callus cultures derived from albedo of "Shamouti" orange and citron of commerce.

Growth of subcultures was found to be dependent on the activity of sweet lime juice but was less effective than orange juice.

Table 3. Effect of light and dark culture conditions on callus development (%) in citrus mandarins.

Explant	Kinnow			Early Feutrall		
	Light	Dark	Explant x Treatment	Light	Dark	Explant x Treatment
Cotyledon	45.3 e	51.2 d	47.2 c	63.2 b	62.6 b	62.9 a
Hypocotyl	14.3 fg	17.5 f	15.9 e	8.4 h	12.4 gh	10.4 f
Epicotyl	59.3 bc	55.1 cd	57.2 b	58.7 c	57.5 c	58.1 b
Vesicles and albedo	3.1 i	71.1 a	37.1 d	1.1 i	2.9 i	2.0 g
Shoot tips and node	0.0 i	0.8 i	0.4 g	0.0 i	0.0 i	0.0 g
Incubation x Variety	24.0 c	39.1 a		26.3 b	27.1 b	
Means of Fruit	31.6			26.7		

The cultivars showed variable response for callus growth under both culture conditions. Kinnow explants showed more callus growth under light incubation as compared to dark (Table 4). In contrast, Early Feutrall yielded more callus growth under dark condition. The comparison of explants represented maximum callus growth by cotyledons in both cultivars. In case of Kinnow mandarin, there was the same callus growth rate under light and dark culture conditions. In Early Feutrall cotyledon explants responded well under light incubation condition rather dark. Hypocotyl explants in Early Feutrall had similar callus growth rate under both culture conditions. Juice vesicles and albedo explants in Kinnow showed higher callus growth under light than dark. In Early Feutrall, juice vesicles and albedo failed to show any response for callus growth.

Table 4. Effect of light and dark culture conditions on growth and appearance of citrus callus.

Explants	Light culture condition				Dark culture condition			
	Kinnow		Early Feutrall		Kinnow		Early Feutrall	
	Growth	Color	growth	Color	growth	Color	growth	Color
Cotyledon	++	Brownish	++	Brownish	++	Brownish	+	Brownish
Hypocotyls	- -	Brownish	-	Light brown	-	Light brown with some creamy white	-	Brown
Epicotyls	++	Brownish	++	Same	-	Brownish	+	Brown
Juice vesicles and albedo	++	Light green	-	Light green	+	Light green with creamy white growth	-	Light green
Shoot tips and nodes	-	Same	-	Same	-	Same	-	Same

In conclusion, genotypic variation existed regarding callus initiation and growth under light and dark incubation. The explants responded differently under both incubation conditions. Callus initiation had significant differences except cotyledon explant of Early Feutrall but callus development was mostly irresponsive of light and dark culture conditions.

REFERENCES

Cameron, J.W. and H.B. Frost (1968). Genetics, breeding and nucellar embryony. In: *The Citrus Industry II*. (W. Reuther, L.D. Batchelor and H.J. Webber eds.). Univ. Calif. Div. Agric. Sci., Univ. of California Press, Berkeley, pp. 325-370.

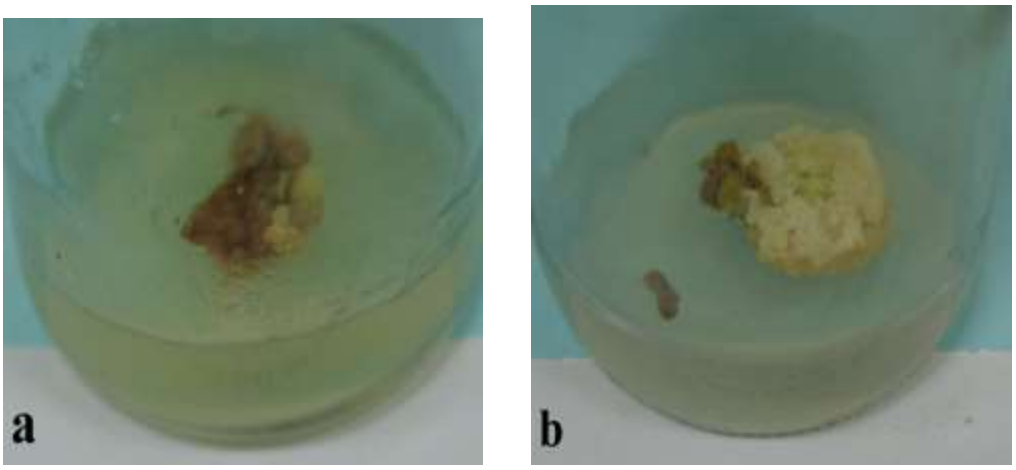

Fig. 1. a) Callus growth from albedo tissue of Kinnow mandarin under dark culture conditions; b) Callus growth from juice vesicles of Kinnow mandarin under dark culture conditions.

Einset, J.W. (1978). Stimulation of fruit explants cultures with orange juice. *Plant Physiol.*, 62: 885-888.

Erner, Y. and O. Reuveni (1981). Effect of organic acids, phenylaline and tyrosine on growth of citrus tissue cultures. *Proc. Int. Soc.Citriculture*, 1: 155-158.

Fleming, G.H., O. Oliveres-Fuster, S. Fatta Del-Bosco and J.W. Grosser (2000). An alternative method for the genetic transformation of sweet orange. *In Vitro Cell Dev. Biol. Plant*, 36: 450-455.

Grewal, H.S., A.S. Dhatt and S.S. Gosal (2000). Plantlet regeneration from callus cultures. *Plant Cell Tissue and Organ Culture*, 4: 9-16.

Grosser, J.W. and F.G. Gmitter (1990). Protoplast fusion and citrus improvement. *Plant Breed. Rev.*, 8: 339-374.

Kobayashi, A., A. Sakai and I. Oiyama (1990). Crop preservation in liquid nitrogen of cultured orange (*Citrus sinensis* Osb.) nucellar cells and subsequent plant regeneration. *Plant Cell Tissue Cult.*, 23: 15-20.

Koc, N.K., C. Can and A. Cinar (1992). Effects of some culture media on somatic embryogenesis and rooting in ovular callus of "Shamouti" orange (*Citrus sinensis* Osb.). *Tree J. Agric. Forests*, 16:140-147.

Kochba, J., P. Spiegel-Roy, H. Neumann and S. Saad (1978). Stimulation of embryogenesis in citrus tissue culture by galactose. *Naturwissenschaften*, 65: 261-262.

Minfil (2004). *Agriculture statistics of Pakistan*. Ministry of Food, Agriculture and livestock, Islamabad, Pakistan.

Pérez, R.M., A.M. Galiana, L. Navarro and N. Duran-Vila (1998). Embryogenesis *in vitro* of several *Citrus* species and cultivars. *J. Hort. Sci. Biotechnol.*, 73: 796-802.

Starrantino, A. and P. Caponnetto (1990). Effect of cytokinins in embryogenic callus formation from undeveloped ovules of orange. *Acta Horticulture*, 280: 191-194.

Steel, R.G.D. and J.H. Torrie (1980). *Principles and procedures of statistics, a biometrical approach*. McGraw Hill Book Company, New York.

Tomaz, M.L., B.M.J. Mendes, F.D.A.A. Mourão Filho, C.G.B. Demétrio, N. Jansakul and A.P.M. Rodriguez (2001). Somatic embryogenesis in *Citrus* spp., carbohydrate stimulation and histodifferentiation. *İn Vitro Cell. Dev. Biol. Plant*, 37: 446-452.

(*Accepted for publication August 2006*)