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Technical note

EFFECT OF BAP AND IAA ON SHOOT REGENERATION IN COTYLEDONARY EXPLANTS OF COSTA RICAN MELON GENOTYPES

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Palabras clave: Melón "criollo", *Cucumis melo* L., regeneración de plantas, cultivo in vitro, morfogénesis.
Keywords: "Criollo" melon, *Cucumis melo* L., plant regeneration, in vitro culture, morphogenesis.

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RESUMEN

Efecto del BAP y el AIA en la regeneración de brotes a partir de explantes cotiledonares de genotipos de melón costarricense. Para establecer una metodología para la regeneración del melón criollo (C*ucumis melo* L), se investigó la influencia del genotipo (OSO-1, OSO-2, OSO-3, PQRG-1, PQRG-2, PQRG-3, y EM-1) y la interacción de N^6-bencilaminopurina (BAP) (0,1, 0,5 y 1,0 mg.l^{-1}) con ácido indolacético (AIA) (0, 0,05 y 0,5 mg.l^{-1}) en la inducción de brotes y regeneración de plantas. Independientemente de la concentración de BAP y AIA, el mayor porcentaje de formación de brotes se obtuvo en EM-1>OSO-1>PQRG-3>OSO-2>PQRG-2>PQRG-1>OSO-3. Por otra parte, independientemente del genotipo, el mayor porcentaje de formación de brotes se obtuvo con 0,5 mg.l^{-1} BAP y 0,05 mg.l^{-1} AIA o 1 mg.l^{-1} BAP y 0 mg.l^{-1} AIA. El protocolo de cultivo in vitro establecido puede ser utilizado para la micropropagación de genotipos "criollos" de melón.

ABSTRACT

Cultured cotyledon explants of OSO-1, OSO-2, OSO-3, PQRG-1, PQRG-2, PQRG-3, and EM-1 "criollo" melon (C*ucumis melo* L) genotypes were evaluated with regard to their morphogenic response to combinations of N^6-benzylaminopurine (BAP) (0.1, 0.5 and 1.0 mg.l^{-1}) with indolacetic acid (IAA) (0, 0.05 and 0.5 mg.l^{-1}). Regardless of BAP and IAA concentration in the shoot induction medium, the highest shoot formation percentages were obtained using EM-1>OSO-1>PQRG-3>OSO-2>PQRG-2>PQRG-1>OSO-3. On the other hand, independently of the genotype, the shoot induction medium supplemented with 0.5 mg.l^{-1} BAP and 0.05 mg.l^{-1} IAA or 1 mg.l^{-1} BAP and 0 IAA mg.l^{-1} resulted in the highest average of shoots. Culture of cotyledons of the genotypes evaluated on induction medium supplemented with different BAP and IAA resulted in a different response. The in vitro culture protocol developed in this study will be useful in micropropagation of "criollo" melon genotypes.

INTRODUCTION

Melon (*Cucumis melo* L.) is one of the economically important crops of the Cucurbitaceae family and it is grown extensively in tropical, subtropical and temperate countries. The world production of melons in 2005 was about 28 million tons (Pech et al. 2007). In Costa Rica, "criollo" melons are widely cultivated by small farmers in Carrillo, Liberia, Puntarenas, Orotina, Aguirre and Cañas. Althought "criollo" melons are not exported they are very appreciated by local consumers due to their aroma and flavor. Currently, "criollo" melons are not characterized but represent a pool

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of germplasm extremely variable in shape, size and color, aroma and flavor.

This crop is susceptible to viral, fungal and bacterial pathogens, and insects. The main production problems encountered for melon in Costa Rica are virus infections, caused by Cucumber Mosaic Virus (CMV), Watermelon Mosaic Virus (WMV), Zucchini Yellow Mosaic Virus (ZYMV) and Papaya Ringspot Virus (PRSV); and the melon aphid (*Aphis gossypii*); which is considered as the most important vector in the spread of cucurbit viruses in Costa Rica (Rivera et al. 1993). Hord et al. (2001), demonstrated the widespread occurrence of CMV in a wide range of climatic zones and crops in Costa Rica. The incidence of CMV infection can approach 100% causing significant losses in export melon in Costa Rica (García 1998).

The findings mentioned above emphasize the importance of appropriate control measures, such as the use of resistant cultivars (Hord et al. 2001). Moreover, this species has not been subject to enough genetic or biotechnological investigations. Therefore, plant biotechnology techniques and genetic transformation represent alternatives to incorporate genes for virus resistance into "criollo" and commercial varieties of melon. Nevertheless, a reliable and efficient in vitro culture system is essential for improvement of melons through genetic transformation.

Efficient regeneration of melon have been reported via organogenesis (Moreno et al. 1985, Kathal et al. 1986, 1988, Orts et al. 1987, Dirks and van Buggenum 1989, Niedz et al. 1989, Chee 1991, Tabei et al. 1991, Molina and Nuez 1995, Yadav et al. 1996, Abrie and van Staden 2001, Liborio et al. 2001, Curuk et al. 2002); and somatic embryogenesis (Moreno et al. 1985, Oridate and Oosawa 1986, Tabei et al 1991, Debeaujon and Branchard 1992, Oridate et al. 1992, Gray et al. 1993, Guis et al. 1997, Abrie and van Staden 2001, Liborio et al. 2001, Kintzios et al. 2002, Rhimi et al. 2006). Despite all these studies, melon is still considered a difficult plant to regenerate, besides successful regeneration is highly related to the genotype (Pech et al. 2007). The purpose of this study was to establish a simple shoot regeneration method for 7 genotypes of Costa Rican "criollo" melon.

MATERIALS AND METHODS

Plant material and explant preparation

Seven "criollo" melon (*Cucumis melo* L.) genotypes denominated OSO-1, OSO-2, OSO-3, PQRG-1, PQRG-2, PQRG-3 and EM-1 were collected in "Finca Instituto de Desarrollo Agrario" (Paquera, Puntarenas, Costa Rica) and used as a source of explants. The genotypes have not been genetically or morphologically characterized.

After removal of the seed coat, the decoated seeds were washed in 70% (v/v) ethanol for 10 min, disinfected in 4.5% (v/v) sodium hypochlorite (NaOCl) supplemented with 8 drops of Tween 20 (Sigma, St. Louis, MO, USA) for 20 min followed by an immersion in the fungicide Benomyl (Piscis, Costa Rica) at a concentration of 100 $mg.l^{-1}$ for 5 min. Finally, seeds were washed 3 times with sterile distilled water.

Disinfected seeds were cultured in tubes (100x20 mm) containing 20 ml of ¼ strength Murashige and Skoog (1962) medium (MS) with 30 $g.l^{-1}$ sucrose and 8 $g.l^{-1}$ agar. The pH was adjusted to 5.6 with NaOH before autoclaving for 21 min at 121°C and 1.05 $kg.cm^{-2}$. Explants were cultured with 16 h light photoperiod (30 $\mu mol.m^{-2}.s^{-1}$) at 26±2ºC.

Shoot regeneration

Cotyledons (1 cm^2) of "criollo" melon genotypes were excised from in vitro grown seedlings after 3 days of germination. These explants were cultured on tubes (100x20 mm) containing 20 ml of shoot induction medium, which consisted of MS mineral salts and vitamins, BAP (0.1, 0.5 and 1 $mg.l^{-1}$) and IAA (0, 0.05 and 0.5 $mg.l^{-1}$), 30 $g.l^{-1}$ sucrose and 8 $g.l^{-1}$ agar (Table 1) to comprise 9 treatments. The pH was adjusted to 5.6 with NaOH before autoclaving for 21 min at 121°C and 1.05 $kg.cm^{-2}$. Cultures

Table 1. Effect of BAP and IAA concentration on shoot regeneration from cotyledons of EM-1, OSO-1, OSO-2, OSO-3, PQRG-1, PQRG-2 and PQRG-3 "Criollo" melon genotypes (*Cucumis melo* L.) after 3 weeks of culture on shoot induction medium.

Treatment (mg.l^{-1})		Variable	"Criollo" melon genotype						
IAA	BAP		EM-1	OSO-1	OSO-2	OSO-3	PQRG-1	PQRG-2	PQRG-3
0	0.1	Shoot regeneration (%)	0±0[a]	41±12	44±11	27±14	21±8	25±11	38±10
	0.5		92±6	30±10	83±8	82±12	54±10	26±10	58±10
	1		87±7	75±10	100±0	47±13	58±10	68±10	58±10
0.05	0.1		17±8	46±11	54±11	40±16	42±10	41±12	42±10
	0.5		79±9	68±10	75±11	55±16	58±10	50±11	79±8
	1		62±10	73±12	71±10	40±16	50±10	56±13	70±10
0.5	0.1		29±9	47±11	0±0	11±11	12±7	56±12	38±10
	0.5		79±9	73±10	40±16	10±10	70±9	42±11	67±10
	1		58±10	59±12	44±18	35±13	20±8	47±12	46±10
P values			<0.0001	0.0159	<0.0001	0.0249	<0.0001	0.1557	0.02

a Mean±se

were maintained with 16 h light photoperiod (30 μmol.m^{-2}.s^{-1}) at 26±2ºC. Twenty-four explants were cultured per treatment.

Shoots regenerated were excised from the original explant and transferred to baby food jars, containing 20 ml of MS medium and closed with polyethylene food wrap (Glad, Costa Rica), to promote elongation and root formation

Percentage of shoot regeneration, callus formation and abnormal shoot development (shoots with deformed cotyledons) were evaluated after 3 weeks of culture.

Statistical analysis

Data were analyzed by one-way ANOVA and mean values separated with Tukey Unequal N HSD at $p<0.05$ using STATISTICA 6.0 (StatSoft, Tulsa, OK, USA).

RESULTS

Shoot regeneration

A protocol for shoot organogenesis and the regeneration into plants was developed (Figure 1A). On media with different combinations of plant growth regulators (BAP and IAA), yellowish callus and small multiple shoots areas become visible at both ends of the explant after 3 weeks of culture (Figure 1B, 1D and 1E). These callus cultures were not embryogenic but exhibited shoot formation (Figure 1C), suggesting an indirect regeneration pathway. Transfer of multiple shoots to elongation medium produced more shoots (Figure 1F) and plants with leaves (Figure 1G) and roots (not in the picture). Plants were transferred to soil and appeared to be morphologically normal and fertile (data not shown).

Culture of cotyledons on shoot induction medium supplemented with different BAP and IAA resulted in a differential response. Table 1 shows the percentage of shoot regeneration. With the EM-1 genotype, the highest average of shoots was obtained using 0.5 and 1 mg.l^{-1}BAP combined with 0, 0.05 and 0.5 mg.l^{-1} IAA, respectively. For OSO-1, OSO-2 and OSO-3 genotypes best results were obtained using 0.5 or 1 mg.l^{-1} BAP with 0 mg.l^{-1} IAA. In the PQRG-1 genotype, the highest shoot average was obtained using 0.5 mg.l^{-1} BAP combined with 0.5 mg.l^{-1} IAA. For

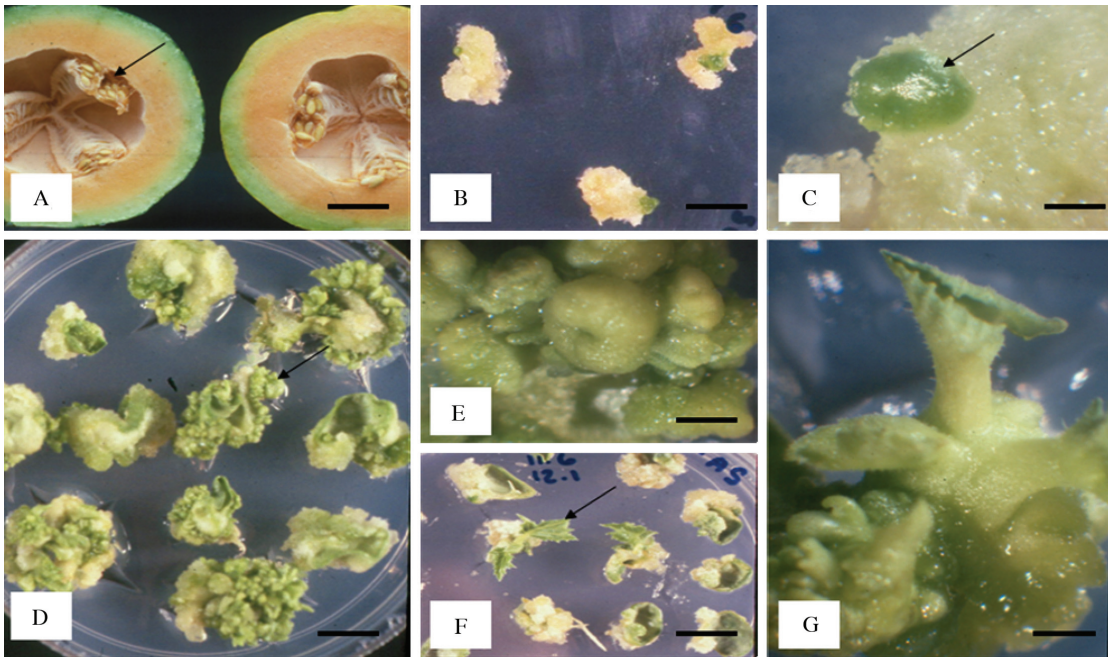

Fig. 1. Shoot induction in 7 "criollo" melon genotypes (*C. melo* L.). A) Fruit and seeds (arrow), B) Callus development on the cut edge of the cotyledon explants after 3 weeks of culture, C) Enlarge view of a shoot (arrow) formed on the surface of the callus, D) Shoot development on the cut edge of the cotyledon explants E) Enlarge view of shoot development, F) Shoots (arrow), G) Enlarged view of a regenerated shoots.

PQRG-2 and PQRG-3 genotypes, no significant differences were observed among treatments; nevertheless, the best results were obtained using 0.5 and 1 mg.l^{-1}BAP combined with 0 or 0.5 mg.l^{-1} IAA.

Regardless of BAP and IAA concentration on the shoot induction medium, shoot regeneration, callus formation, and the abnormal shoot regeneration differed significantly among the 7 genotypes evaluated. The highest shoot formation percentage was obtained with EM-1>OSO-1>PQRG-3>OSO-2>PQRG-2>PQRG-1>OSO-3; while the highest percentage of callus formation was obtained on OSO-3, OSO-1, EM-1, PQRG-2, PQRG-3, OSO-2, and PQRG-1. Abnormal shoot regeneration highest percentages were obtained with PQRG-2 and OSO-1 (Figure 2).

On the other hand, independently of the genotype, shoot induction medium supplemented with 0.5 mg.l^{-1} BAP and 0.05 mg.l^{-1} IAA or 1 mg.l^{-1} BAP and 0 IAA mg.l^{-1} resulted in the highest shoots average (Figure 3). The highest average of callus formation was obtained using 0.5 mg.l^{-1} BAP combined with 0.5 mg.l^{-1} IAA, 1 mg.l^{-1} BAP with 0 mg.l^{-1} IAA or 1 mg.l^{-1} BAP with 0.5 mg.l^{-1} IAA (Figure 3). The highest abnormal regeneration percentage was obtained when 1 mg.l^{-1}BAP was used in combination with 0.05 or 0.5 mg.l^{-1} IAA (Figure 3).

DISCUSSION

In the present study a method for regeneration of 7 Costa Rican "criollo" melon genotypes using BAP and IAA was established. Plant regeneration via organogenesis was achieved in all evaluated genotypes. Nevertheless, a genotype influence was observed since shoot regeneration

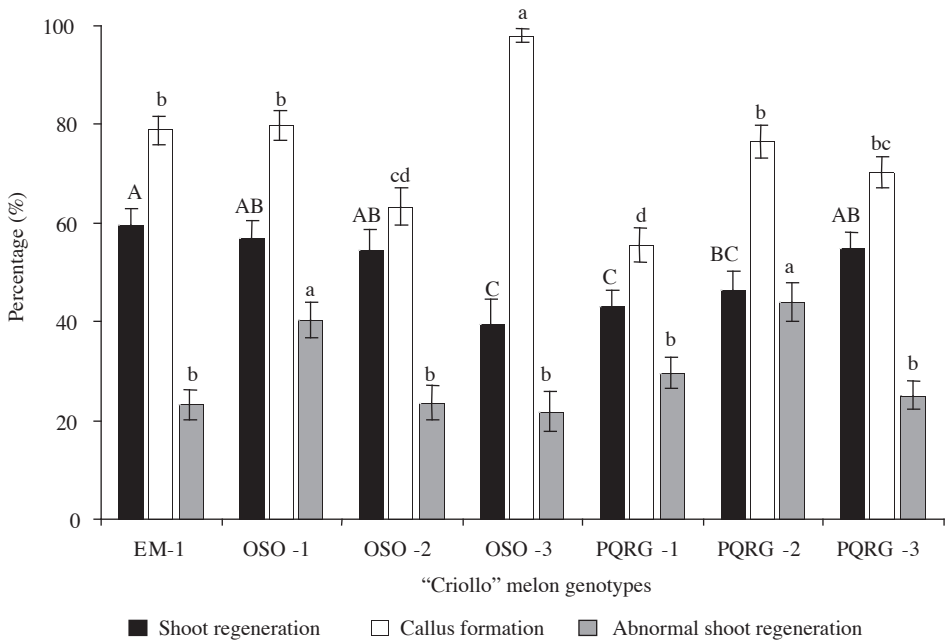

Fig. 2. Effect of the genotype on shoot regeneration, callus formation and abnormal shoot regeneration from cotyledons of "criollo" melon (*C. melo* L.) regardless the concentration of BAP and IAA on the shoot induction medium. Mean±SE. Values within columns followed by the same letter are not significantly different with the Tukey Unequal N HSD test ($p<0.05$).

percentage varied in each genotype. Our results confirm earlier observations, which indicate that melon regeneration via organogenesis is genotype dependent (Orts et al. 1987, Dirks and van Buggenum 1989, Debeaujon and Branchard 1992, Molina and Nuez 1995).

In this study, BAP and IAA played an important role in shoot induction in the Costa Rican "criollo" melon genotypes evaluated. It is well known that cytokinins stimulate plant cell division and participate in the release of lateral bud dormancy, induction of adventitious bud formation, growth of lateral buds, and in the cell cycle control. Whereas, auxins exert a strong influence in initiation of cell division, meristem organization giving rise to un-organized tissue (callus) or defined organs (shoots), cell expansion, cell wall acidification, apical dominance, promotion of vascular differentiation, and root formation (Gaspar et al. 1996, 2003). In this sense, manipulation of exogenous cytokinin:auxin balance could favor a developmental pattern or orient an organogenic program (Gaspar et al. 2003). Beneficial effects of BAP or kinetin in combination with IAA on shoot induction have been observed in melon by Moreno et al. (1985), Kathal et al. (1986), and Niedz et al. (1989). Liborio et al. (2001), obtained adventitious buds from cotyledon segments and leaf discs of *C. melo* using 1 $mg.l^{-1}$ BAP, as we observed in the present study. Tabei et al. (1991) indicated that IAA gave a more efficient shoot formation than naphthaleneacetic acid (NAA) and 2, 4-dichlorophenoxyacetic acid (2, 4-D) using cotyledon explants of *C. melo*.

The plant regeneration protocol developed in the present study could be used for propagation of Costa Rican "criollo" melon genotypes.

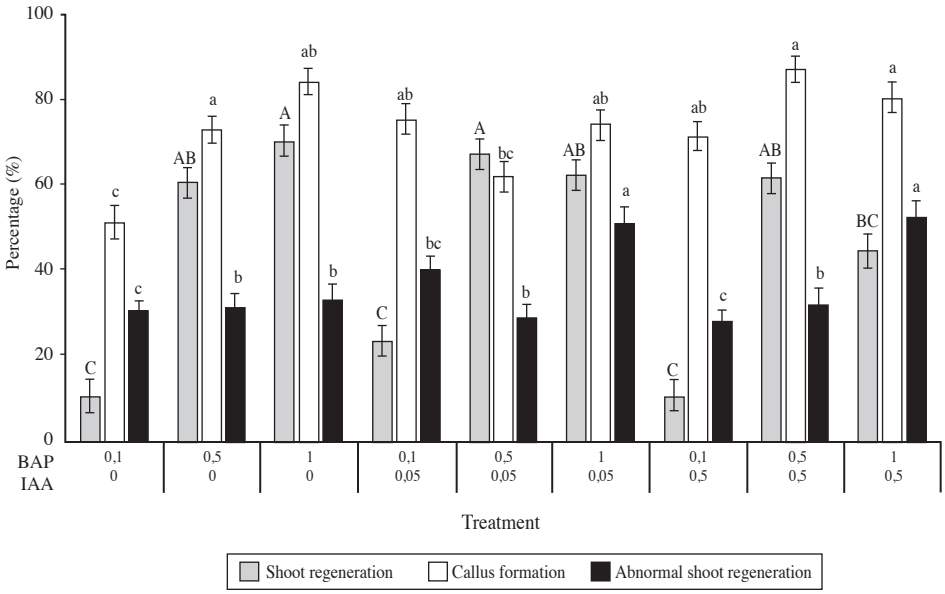

Fig. 3. Effect of BAP and IAA concentration on shoot regeneration, callus formation and abnormal shoot regeneration from cotyledons of "criollo" melon (*C. melo* L.) regardless the genotype. Mean±SE. Values within columns followed by the same letter are not significantly different with the Tukey Unequal N HSD test ($p<0.05$).

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