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EMBRYO CULTURE AS AN ALTERNATIVE WAY TO COMPLETE THE DEVELOPMENT OF EMBRYOS RESULTING FROM THE INTERSPESFIC CROSS BETWEEN *A. odorum* AND *A. cepa*

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ABSTRACT

The widely spread onion belonging to *Alium cepa* L., is know to be severely affected by the soil born disease, white rot. Infected soils totally prevents the production of onion for a period up to ten years, which dramatically reduced the economic production of onion in Egypt and limited the exportation of Egyptian onion during the last decade. There were no source of resistance in the cultivated onion. As an attemptation to pick out a source for resistance, a survey was made among other relatives belonging to *Alium* sp. Resistance was found in *Alium odorum sp.* and *Alium tuberosum* sp. Interspesific crosses were made for many years to transfer resistance ability to Egyptian onion cultivars, but normal crossing was always incomplete and embryos fail to develop normally. Tissue culture techniques were used to resque the embryos resulted from the cross between *A. cepa* and *A. odorum*. Embryos were collected and an array of media with different hormone combinations were examined. In this study a very rare percent of embryos succeeded to complete its development and produced plantlets.

Keywords: Tissue culture, Embryo culture, inter-specific cross, white rot, *Alium* sp.

INTRODUCTION

White rot disease is a serious soil born disease that dramatically reduced the annual production of onion (*A. cepa* L.,) in Egypt. The infected soils can't be cultivated with onion for a period extends up to ten years. The cultivated onion belonging to *A. cepa* L., is known to be highly sensitive to the infection. Surveying the cultivated onion cultivars revealed that there are no available source of resistance. Sources for resistance were found in onion relatives i.e. *A. tuberosum* and *A. odorum*. Interspecific crosses between Alium sp. are very difficult task to achieve, embryos always fail in completing it's development. Few days after pollination the endosperm separates from the Zygot nucleus and eventually the embryo dies (personal communications), during the last decade many work has been reported in the use of tissue culture to generate new cultivars through somaclonal variation (Muller *et al.*, 1990. Limacero and Vazquez 1992 and Geum–Sook *et al.*, 1999), In vetro propagation by direct shoot praliferation (Pandy *et al* 1992) and haploid plant production through gynogensis in many species such as *A. cepa* (Compion *et al.*, 1990 and Compion *et al., 1992*), *Hordeum Valgure* (Sim Noean 1976), *Oryza Sativa* (Assilian 1980), *Nicotiana tabocum* (Zhu *et al.,* 1981) and *Zea maiz* (Troung and Demarly,1984). In *A. cepa* production of haplaid plants have been reported by culturing infertized ovules and Overies

(Guha and John 1986 and Compion and Alloni 1990 and 1999, Campion *et al.*, 1992 and Campion *et al.*, 1995).

In onion a relatively high percentage of multiple shoot structures were formed on mature flower buds or overies in two stages induction/ differentiation procedure in which no callus stage was involved (Luthar and Bohanec 1999), Such procedure would be superior to others, since regeneration through callus can cause undesired chromosomal abarations resulting in phenotypic instability as demonstrated in other Alium species such as *A. cyaneum* (Lee *et al.*, 1998); *A. fistulosum* (Lee and Oono 1999); *A. tuberosum* (Geum-Sook *et al.*, 1999) and Andrej *et al.*, (2002).

In the present study an array of media combination were tested to overcome the problem of incomplete development of the embryos resulting from the inter-specific cross between *A. copa* and *A. tubrosun* or *A. odorum*.

MATERIALS AND METHODS

Embryos of the cross between *A. Cepa* and *A. tubrosum* or *A. odorum* were kindly donated by Dr. Khadiga A.. Bebars, Onion Res. Dept, Field Crops Res. Inst., A.R.C. Different media combinaions were used including MS, B5 and BDS basal media supplemented with different growth regulators as shown in Table (1).

Table (1): Midia composition used for emberyo emergence (I_1, I_2 and I_3) and for regeneration (R_1 and R_2).

Composition	Midium				
	I_1	I_2	I_3	R_1	R_2
Macro and micro elements (gmL^{-1})					
B5 (Gamborg 1968)	+	-	-	-	-
MS (Morashige and skoog 1962)	-	-	+	+	+
BDS (Dunstan and short 1977)	-	+	-	-	-
Suppliments (mgL^{-1})					
$Na\ H_2\ PO_2 - H_2O$	-	-	250	250	250
Vitamins (mgL^{-1})					
Mio –inositol	100	100	500	500	500
Thiamin – Hcl	10	2	2	2	2
Pyrodoxine – Hcl	1	1	1	1	1
Nicotenic acid	1	1	1	1	1
Glycine	-	-	0.2	0.2	0.2
Folic acid	-	-	-	1.0	1.0
Biotin	-	-	0.01	0.01	0.01
Growth regulators (mgL^{-1})					
BA	2	2	2	-	-
IAA	-	-	-	-	1.5
NAA	-	-	-	1	-
2, 4-D	2	2	-	-	-
Suerose (mgL^{-1})	100	100	30	10	30
Agar (mgL^{-1})	6	6	6	6	6

I_1 = modified M_2 medium described by Murean (1989).
I_2 = modified F_5 medium described by Compion *et al.*, (1992)
I_3, G_1, G_2 modified A_2 (B_1 and C_1 Compion and Alloni 1990)

Flower buds were excised 7 days after cross pollination and surface sterilized in sodium hypochlorate 0.25% (W./V) for five minutes, followed by three rinses in sterile distilled water. Twenty flower buds were plated onto petri dishes containing induction media (either I_1, I_2 or I_3). Petri dishes were sealed with parafilm and incubated at 25°C with 16/8 photoperiod provided by white cool fluoresent tubes.

After three weeks flower buds were transferred to fresh media and incubated at the same conditions until embryos emerged. Enlarged emerged embryos were transferred to propagation media in 200ml glass jars and incubated at 22°C with 16-8 h photoperiod until shoots developed, Basal swelling of the shoot lead to the formation of a small bulls on R_1 medium.

Shoots with small bulbs were transferred to rooting media composed of $^1/_2$ Ms media free of growth regulators and roots were developed on the resulting plantlets that reached 5cm long plantlets were potted on 1:1:1 vermiculite: soil: sand.

RESULTS AND DISCUSSION

A total number of 201 buds of *A. odorum* x *A. cepa* and 218 buds of *A. Tuberosum* x *A. cepa* were cultured on the three initiation media (I_1,I_2 and I_3) based on either B5, MS or BDS major and minor salts ,respectively. The availability of cross pollinated buds determined the number of buds tested. Figure 1 represents the cultured flower buds on initiation media ten days after pollination. Buds started to swellen and grow for three weeks where some of which started to emergeo, there were no bud emergence after six weeks of incubation.

From 419 cultured buds of the two crosses (Figure1-A), nine buds were able to emerge with a percentage ranged from 3.92 to 5.81% (Table2). Emerged embryos were excised and sub-cultured on the same media for further three weeks.

Table (2) : Number of embryos percentages for both emergence and regeneration for the two crosses in different midia.

Medium	No. cultured Embryos		% emerged embryos		% regeueration	
	H_1	H_2	H_1	H_2	H_1	H_2
I_1	52	73	-	-	-	-
I_2	86	94	5.81	4.26	-	-
I_3	63	51	3.17	3.92	-	-
Total	201	218	-	-	-	-
R_2	-	-	-	-	0.28	-
R_3	-	-	-	-	-	-

H_1 = Cross *Alium odorum* x *Alium cepa* L.
H_2 = Cross *Alium tuberosum* x *Alium cepa* L.

I_2 medium based on BDS major and minor salts and supplemented with some amino acids and phyto-hormones (as shown in table1), proved to be most suitable for flower bud emergence. Four embryos of the total 86

buds of H_1 cultured on I_2 medium were able to emerge (5.81%), while, 4.26% was able to emerge from H_2 on the same medium followed by I_3 medium (3.17 and 3.92%%) for H_1 and H_2, resp. Whereas, not a single bud was able to emerge when cultured on I_1 initiation medium (Table2).

Three weeks after subculture on the same induction medium, the emerged embryos were transferred to regeneration media (either R_1 or R_2), only two embryos started to regenerate and evolved green leaves on R_1 medium. The two embryos that produced green leaves belonged to the cross *A. odorum* x *A. cepa* (Figure1-B) no sign of regeneration were observed on the cultured flower buds of the cross *A. Tuberosum* x *A. cepa*.

Embryos emerged 3-6 weeks after initiation in I_2 medium and 5-6 weeks on I_3 medium. Embryos emerged directly from the ovules. Following the transfer to regeneration media, developing embryos formed individual plantlets (Figure1-B) and in some plantlets ($^1/_7$), the basal portion of the shoot swelled to make small bulbs. These plants (10 plantlets after desiccation) were transferred to rooting media (MS-basal medium free of growth regulators) as shown in figure 1-C. plantlets developed well roots in the jars. Only one plantlet survived when potted in the greenhouse but unfortunately, it failed to complete it's life cycle to give seeds. In genus Alium, many workers have reported that plant production through gynogensis is a difficult task to achieve and that regeneration rates were very rare (Guha and John 1986, Compion and Alloni 1990 and 1999, Compion *et al.*, 1992, Compion *et al.*, 1995, Lee *et al.*,1998 and Luther and Bohanec 1999).

Our results although being incomplete and failed to produce a whole hybrid plant in the greenhouse that didn't gave us the chance to achieve the planned cytological work, it gives a great hope that by the next trials the frequency of embryos that regenerate will be more successful and that this work have identified the most suitable medium for embryo emergence and regeneration, this will help us in the future time to re-try the same procedure to get an onion hybrid that could be tested for resistance to white rot disease the aim of our work.

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استخدام طرق زراعة الأجنة كوسيلة بديلة لإستكمال تطور الآجنة الناتجة من التهجين بين *Alium odorum & Alium cepa*

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يعتبر البصل التابع لجنس *Alium cepa* هو أكثر الأنواع المنزرعة انتشارا والذى يتميز بشدة القابلية للإصابة بمرض العفن الأبيض، مما يؤدى إلى عدم صلاحية التربة المصابة بالفطر لاستخدامها فى إنتاج البصل لفترات قد تصل إلى عشرة أعوام مما يحد من إنتاج البصل المصرى وتصديره وخاصة إلى الاسواق الأوروبية ولا يوجد طرز وراثية منزرعة مقاومة لهذا المرض الا أنه بدراسة الأنواع القريبة التابعة لجنس Alium أمكن الحصول على مصدر للمقاومة فى كل من *A. odorum & A. tuberosum* .

التهجين بالطرق التقليدية بين البصل المنزرع *A. cepa* والاجناس القريبة لم تنجح للعديد من السنوات حيث أن الأجنة لم تتم تطورها بصورة طبيعية، لذلك استخدمت طرق زراعة الانسجة النباتية كمحاولة لانقاذ الأجنة المتكونة بالتهجين ما بين *A. cepa & A. odorum* وبالفعل امكن الحصول على نسبة اسنيلاء ضئيلة من خلال استزراع الأجنة عند عمر عشرة أيام من تاريخ التهجين على بيئات متعددة والتى امكن من خلالها تحديد أنسب بيئة لاستزراع الأجنة والحصول على بادرات من الهجين النوعى *A. dorum X A. cepa*.