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EFFICIENCY OF ORGANOGENESIS IN PEPPER (*Capsicum annuum* L.) USING DIFFERENT EXPLANTS

Sahar S.Taha
Vegetable Crops Dept., Faculty of Agric., Cairo Univ.

ABSTRACT

In vitro regeneration from leaf, cotyledon and hypocotyl explants of three pepper cultivars, namely Ouskare hybrid, California Wonder and Anahem was achieved by direct organogenesis. California wonder cv. showed the best response while Anahem cv. showed the least response. Leaf and cotyledon explants regenerated more shoots than hypocotyl, maximum callus and bud were produced on Murashige and Skoog (MS) medium containing 5 mg/l IAA plus 5 mg/l BA. Elongation of shoot buds derived from different explants was achieved on MS medium containing 2.0 mg/l BA or 0.5 mg/l IAA plus 2.0 mg/l BA. Elongated shoots were excised and rooted on MS basal medium either without plant growth regulators or with 0.5 mg/l IBA or 0.2 mg/l NAA. Plantlets acclimatized and transplanted to soil in the greenhouse showed normal development and grew to maturity bearing normal fruits with seeds.

Abbreviation: IAA indole acetic acid; BA N^6-benzyladenine; IBA indole-3-butyric acid; NAA α–naphthalene acetic acid

Keywords: Pepper, Organogenesis, Regeneration and Explant.

INTRODUCTION

Pepper (*Capsicum annuum,* L.) ranks among the most important vegetable crops world wide. It is highly regarded as a vegetable and a condiment for its sayour and perfume. In addition, to its use as a food, its powder disinfects the oral and gastric mucous membranes and destroys the pathogenic bacteria in the intestine (Bosland and Votava 1999). Pepper is susceptible to many pathogens including viruses, fungi, bacteria and nematodes and to severe climatic conditions, essentially temperature extremes that are limiting factors for its cultivation (Pochard *et al.*, 1992).

Biotechnology provides an interesting tool for accelerating the selection of pepper genotypes with desired quality traits and resistance to biotic and abiotic stress. However, in order to utilize biotechnological techniques, successful *in vitro* regeneration of complete and fertile plants at adequate capacity is required (Mezghani *et al.*, 2007).

Propagation of plants through tissue culture offers a unique advantage over conventional propagation methods such as rapid multiplication of valuable genotypes, expeditious release of improved cultivars, production of disease-free plants, non –seasonal production, germplasm conservation and facilitating their easy exchange (Sanatombi and Sharma 2008).

Relative success on shoot organogenesis was reported from foliar explants (Husain *et al.*, 1999; Venkataiah *et al.*, 2003) or cotyledon and hypocotyl explants (Gunay and Rao 1978; Phillips and Hubstenberger 1985; Agrawal *et al.*, 1989 Ochoa -Alejo and Ireta – Moreno 1990; Arroyo and Revilla 1991; Szasz *et al.*, 1995 ; Christopher and Rajam 1996; Ramirez - Malagon and Ochoa- Alejo 1996; Frank - Duchenne *et al.*, 1998; Husain *et al.*, 1999; Arous *et al.*, 2001; Pozueta – Romero *et al.*, 2001; Venkataiah *et*

al., 2001; Mathew 2002; Golegaonkar and Kanthar -ajah 2006; Venkataiah *et al.*, 2003 ; Khan *et al.*, 2006; Sanatombi and Sharma 2008) and somatic embryogenesis from immature zygotic embryos (Binzel *et al.*, 1996) or anthers and isolated microspores (Barany *et al.*, 2005; Koleva -Gudeva *et al.* 2007) . Genetic engineering in case of pepper is still restricted by the low morphogenetic potential of this species (Steinitz *et al.*, 1999; Mathew 2002). However, regeneration is frequently reported to consist of bud -like structures and rarely of well developed shoots (Ochoa -Alejo and Ireta –Moreno 1990; Arroyo and Revilla 1991; Ramirez -Malagon and Ochoa – Alejo 1996; Steinitz *et al.*, 1999). Strong influence of culture conditions and genotypes was also demonstrated (Ochoa -Alejo and Ireta -Moreno 1990; Szasz *et al.*, 1995; Christopher and Rajam 1996; Ramirez -Malagon and Ochoa -Alejo 1996; Venkataiah *et al.*, 2003; Sanatombi and Sharma 2008)

The present investigation, involved culture of leaf, cotyledon and hypocotyl explants of three pepper cultivars to determine the regeneration potential of the genotypes and to develop efficient *in vitro* plant regeneration protocols for the three economically important cultivars.

MATERIALS AND METHODS

This investigation was carried out in the Tissue Culture Laboratory, Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza, Egypt. during the period from 2007 to 2008. Seeds of the three cultivars of pepper were used tow of them were sweet (Ouskare hybrid, California Wonder cv.) while the third (Anahem cv.) was hot pepper. Seeds were washed with running tap water for 30 min followed by immersing in 20% commercial bleach for 30 min and then washed with sterile distilled water. They were aseptically sown on MS basal medium in a growth room at 25±2 °C for 5 days in dark followed by 16 h daily photoperiod. Hypocotyl, cotyledon and leaf explants were derived from four -five week old *in vitro* germinated seedlings.

Explants were cultured on MS medium containing MS salts with 3% (m/v) sucrose. The pH of the medium was adjusted to 5.8 with 1 m NaOH and 1 m HCl before adding growth regulators. The media were then dispensed in jars and autoclaved at 121°C for 20 min. All cultures were maintained in a growth chamber at a temperature of 25 ± 2°C and 16 h photoperiod provided by white fluorescent tubs.

Hypocotyl segment (1 cm long) and whole leaf or cotyledon were aseptically cut and cultured in jars containing bud induction medium. The explants were placed ad axial side down on bud induction media.

The culture media used in the present study were as follows:

M_1- 1.0 mg/l IAA+1.0 mg/l BA
M_2- 1.0 mg/l IAA+2.0 mg/l BA
M_3- 1.0 mg/l IAA+5.0 mg/l BA
M_4- 2.0 mg/l IAA+2.0 mg/l BA
M_5- 5.0 mg/l IAA+10.0 mg/l BA
M_6- 5.0 mg/l IAA+5.0 mg/l BA
M_7- 1.0 mg/l IAA+10.0 mg/l BA

The adventitious shoot buds induced on hypocotyl, cotyledon and leaf explants were excised from the remaining parts of the explants, cut into smaller pieces, and then cultured in jars containing bud elongation medium consisting of MS medium supplemented with different concentrations of BA and IAA.

The elongation media were as follows:

M_1- 0.0 mg/l IAA+1.0 mg/l BA
M_2- 0.0 mg/l IAA+ 2.0 mg/l BA
M_3- 0.5 mg/l IAA+1.0 mg/l BA
M_4- 0.5 mg/l IAA +2.0 mg/l BA
M_5- 1.0 mg/l IAA+1.0 mg/l BA
M_6- 1.0 mg/l IAA+2.0 mg/l BA

The percentage number of shoot obtained from a particular explant was recorded after 6 weeks. The elongated shoot buds (about 2 cm long) were excised and cultured in jars containing rooting media consisting of MS media free hormone, 0.5 mg/l IBA and 0.2 mg/l NAA. The percentage number of roots (including the main roots and their branches) were recorded after three weeks of culture.

The rooted plantlets were gently removed from the flasks and the roots were washed in tap water to remove traces of agar. The plantlets were then transplanted in perforated paper cups containing sand: soil (1:1) and kept covered with clear polythene bags having a few holes for the initial 10 days. The plantlets were watered daily with tap water to maintain high humidity. After 10 days, humidity was gradually decreased by increasing the size of holes in the polythene bags. Twenty days later, the polythene bags were completely removed. Four weeks old hardened plants were then transplanted in bigger earthen pots or to the field.

All the experiments were repeated three times and each treatment for shoot bud induction and rooting of the shoot buds consisted of ten replicates. All experiments were arranged in completely randomized design. Data were subjected to analysis of variance as described by Steel and Torrie (1960).

RESULTS AND DISCUSSION

The leaf, cotyledon and hypocotyl explants produced shoot buds directly on the shoot induction medium within four weeks of culture. Leaf and cotyledon explants produced buds mainly from the petiolar end, while hypocotyl explants produced buds initially from the cut ends and later from all over the surface. Initially, the buds appeared as small green bulges but later it developed into small leafy structures, which elongated into shoots on the elongation medium.

The cultivars California Wonder and Ouskare responded better than Anahem (Table1 and Fig.1). Differences in regeneration potential of different Capsicum genotypes observed in the present study is similar to earlier reports (Ochoa – Alejo and Ireta- Moreno 1990; Christopher and Rajam 1996; Ramirez-Malagon and Ochoa- Alejo 1996; Venkataiah *et al.*, 2003).

Table 1: Effect of explant type and culture medium on the weight of callus, buds and shoots in pepper

Cultivar	Explant	M_1	M_2	M_3	M_4	M_5	M_6	M_7	Mean
Ouskare	Leaf	0.80	073	4.13	1.66	3.90	2.80	1.80	2.56
	Cotyledon	0.39	0.34	0.30	0.29	1.88	1.90	1.63	0.96
	Hypocotyl	0.39	0.32	0.93	0.44	1.97	2.33	1.63	1.15
Mean		0.53	0.46	1.79	0.80	2.58	2.34	1.96	1.46
California Wonder	Leaf	0.54	0.57	4.07	0.90	2.70	3.37	2.07	2.03
	Cotyledon	0.46	0.57	2.73	0.60	2.23	3.30	1.40	1.61
	Hypocotyl	0.36	0.41	1.56	0.50	1.80	2.20	1.50	1.91
Mean		0.45	0.52	2.79	0.67	2.24	2.96	1.66	1.61
Anahem	Leaf	0.70	0.60	2.3	1.07	2.20	2.80	1.80	1.64
	Cotyledon	0.41	0.42	1.17	1.30	1.77	1.87	1.27	1.17
	Hypocotyl	0.39	0.51	1.07	0.65	0.86	1.10	0.67	0.74
Mean		0.50	0.51	1.51	1.00	1.61	1.92	1.25	1.19
Mean	Leaf	0.68	0.64	3.50	1.21	2.93	2.98	1.89	1.98
	Cotyledon	0.42	0.44	1.40	0.73	1.96	2.36	1.43	1.25
	Hypocotyl	0.38	0.41	1.19	0.53	1.54	1.88	1.27	1.03
Mean		0.49	0.50	2.03	0.82	2.14	2.41	1.53	1.42

(M_1, 1mg/ l IAA + 1mg/ lBA ; M_2 , 1mg / l IAA + 2mg/ l BA; M_3, 1mg / l IAA +5mg / l BA; M_4, 2 mg/l IAA + 2 mg / l BA; M_5, 5mg / l IAA + 10mg /l BA ; M_6,5 mg/ l IAA +5 mg/l BA and M_7,1 mg / l IAA +10 mg/ l BA)

L.S. D at 0.05

Cultivars (Cv)	0.17	Cv X E	0.21		
Culture medium (Cm)	0.18	Cm X E	0.31		
Explants (E)	0.12	Cv X E X Cm	0.53	Cv X Cm	0.31

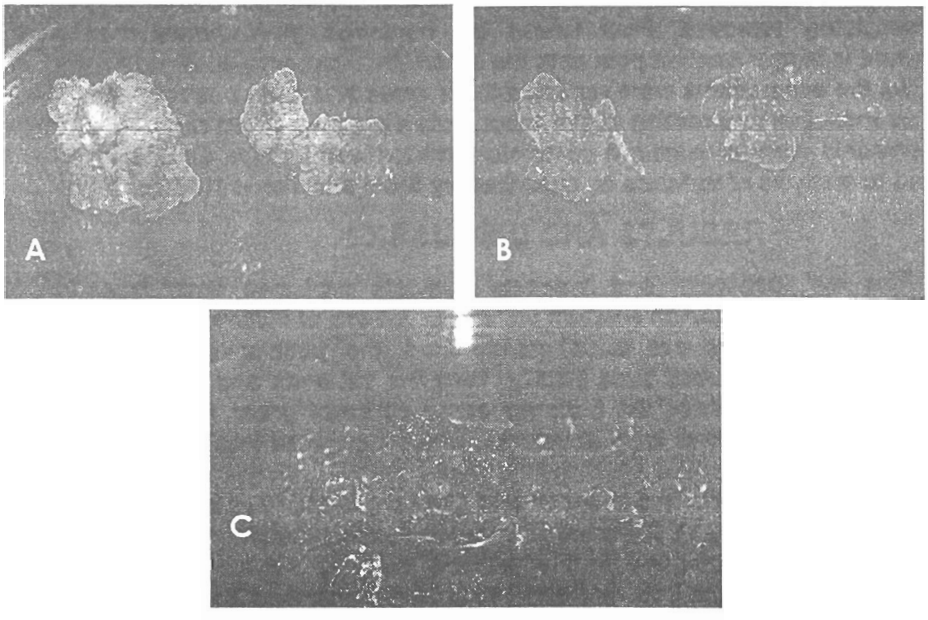

Fig 1: Different morphogenetic responses of pepper. (A) Production of callus; (B) Mixed bud – like structures and callus and (C) Short shoots.

Leaf explants were also found to be more responsive than cotyledon and hypocotyl of capsicum in earlier studies (Agrawal *et al.*, 1989, Christopher and Rajam 1996, Venkataiah *et al.*, 2003). Similarly, better responsiveness of leaf explants than other explants in all genotypes was detected in the present study.

The shoot buds induced in the bud induction media were rosettes of small leaf – Like structures that failed to elongate into normal shoots on the same medium.

Steinitz *et al.*, (1999) and several methods were employed for elongating the rosettes like the use of low doses of BA and IAA (Phillips and Hubstenberger 1985, Venkataiah *et al.*, 2003). Phenyl acetic acid (Husain *et al.*, 1999), gibberellic acid (Szasz *et al.*, 1995), silver nitrate (Hyde and Phillips 1996) and achieving *ex vitro* elongation after transplantation (Arroyo and Revilla 1991, Ebida and Hu 1993, Hyde and Phillips 1996). In the Present study, elongation of shoots was achieved on MS medium containing 0.5 mg/ l IAA with 2 mg/ l BA within 2 weeks of culture, the buds derived from hypocotyl explants failed to grow to shoots in all media, and the growth of the shoot bud derived from leaf and cotyledon explants was very slow and elongated after about 5 weeks in cv. Anahem (Table2). MS medium containing 0.3 uM BAB (0.07 mg/l) was found to be more effective for shoot elongation from buds induced in pepper tissue cultures (Phillips and Hubstenberger 1985, Venkataiah *et al.*, 2003). The buds derived from culturing the Leaf explants produced a high percentage of elongated shoots compared with the buds derived from culturing the cotyledon and hypocotyl explants.

The excised elongated shoots (about 3 cm long) rooted in the rooting medium. Rooting of the shoot buds derived from all the three explants of the three cultivars was achieved on a medium containing 0.5 mg/l IBA. Roots induced on these medium was long, sometimes with branches accompanied by further elongation of the shoots (Table 3).

The effectiveness of IBA on rooting of *in vitro* regenerated plantlets has been reported earlier (Agrawal *et al.*, 1989; Christopher and Rajam 1994; 1996; Szasz *et al.*, 1995). Husain *et al.*, (1999) reported higher effectiveness of NAA in inducing rhizogenesis of the regenerated shoots in capsicum. However, in the present study, the roots induced on medium containing NAA were short and thick.

The plantlets rooted on medium containing NAA or IBA were used for transplantation. Four- week old rooted plantlets were transplanted to paper cups. These were hardened within 10-15 days. New apical leaves appeared and the plantlets remained well when transferred to bigger earthen pots or in the field. The plantlets showed 70% survival during acclimatization and transplantation.

This study thus shows the importance of choosing the suitable type of explants, the genotype and the culture medium for *in vitro* regeneration of capsicum cvs. The Leaf and the cotyledon explants are found to be more responsive than the hypocotyl and MS medium containing 5 mg/l IAA with 5 mg/l BA was the best medium for shoot bud induction.

Table 2: Effect of explant type and culture medium on the percentage of elongated shoots in pepper.

Cultivar	Explant	M_1	M_2	M_3	M_4	M_5	M_6	Mean
Ouskare	Leaf	41.33	91.33	31.33	80.00	21.00	66.67	56.28
	Cotyledon	22.33	51.33	18.00	43.33	11.67	35.67	30.39
	Hypocotyl	0.00	0.00	16.03	22.00	0.00	12.00	8.34
Mean		21.22	47.56	21.79	50.44	10.89	38.11	31.67
California Wonder	Leaf	47.33	86.67	28.33	70.33	39.67	54.33	54.44
	Cotyledon	41.67	60.00	23.00	55.67	18.33	40.67	39.89
	Hypocotyl	0.00	0.00	12.00	6.33	3.33	3.67	4.22
Mean		29.67	48.89	21.11	44.11	20.44	32.89	32.85
Anahem	Leaf	35.00	38.00	15.00	44.67	13.67	36.67	30.50
	Cotyledon	13.67	21.00	6.00	17.33	4.00	10.67	12.11
	Hypocotyl	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean		16.22	19.67	7.00	20.67	5.89	15.78	14.20
Mean	Leaf	41.22	72.00	24.89	67.00	24.73	52.56	47.07
	Cotyledon	25.89	44.11	15.67	38.78	11.33	29.00	27.46
	Hypocotyl	0.00	0.00	9.35	9.46	1.11	5.22	4.19
Mean		22.37	38.70	16.63	38.41	12.41	28.93	26.24

Due to presence of zero value, 0.01 was added to all values, thereafter statistical analyses were done.

(M_1, 1.0 mg / l BA ; M_2, 2.0mg/l BA; M_3, 0.5 mg/ l IAA + 1.0mg / l BA; M_4, 0.5 mg / l IAA + 2.0 mg / l BA; M_5, 1.0 mg / l IAA + 1.0 mg / l BA and M_6, 1.0 mg / l IAA + 2.0 mg / l BA)

L.S. D at 0.05

Cultivars (Cv)	**1.86**	**Cv X E**	**4.54**		
Culture medium (Cm)	**3.21**	**Cm X E**	**4.54**		
Explants (E)	**1.86**	**Cv X E X Cm**	**7.80**	**Cv X Cm**	**2.62**

Table 3: Effect of explant type and culture media on the percentage of rooting in pepper.

Cultivar	Explant	M_1	M_2	M_3	Mean
Ouskare	Leaf	70.00	86.67	83.33	80.00
	Cotyledon	70.00	83.33	100.0	84.44
	Hypocotyl	60.00	66.67	53.33	60.00
Mean		66.67	78.89	78.89	74.81
California Wonder	Leaf	63.33	100.0	90.00	84.44
	Cotyledon	63.33	100.0	86.67	83.33
	Hypocotyl	56.67	90.00	40.00	62.22
Mean		61.11	96.67	72.22	76.67
Anahem	Leaf	90.00	100.0	83.00	91.11
	Cotyledon	90.00	90.00	86.67	88.89
	Hypocotyl	0.00	0.00	0.00	0.00
Mean		60.0	63.33	56.56	59.96
Mean	Leaf	74.44	95.56	85.56	85.19
	Cotyledon	74.44	91.11	91.11	85.56
	Hypocotyl	55.56	76.67	51.11	61.11
Mean		62.59	79.63	69.22	70.48

Due to presence of zero value, 0.01 was added to all values, thereafter statistical analyses were done.

M_1, MS free hormone; M_2 0.5 mg / l IBA and M_3, 0.2 mg / l NAA.

L.S. D at 0.05

Cultivars (Cv)	**5.24**	**Cv X E**	**9.07**		
Culture medium (Cm)	**5.24**	**Cm X E**	**9.07**		
Explants (E)	**5.24**	**Cv X E X Cm**	**15.01**	**Cv X Cm**	**9.07**

MS medium containing 2mg/l BA or 1.0 mg/l IAA and 2.0 mg/l BA were found to be the best medium for elongation of the shoot buds while medium containing 0.5 mg / l BA induced efficient rooting of the shoot buds.

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كفاءة تكوين الاعضاء فى الفلفل عن طريق زراعة اجزاء نباتية مختلفة

سحر سميح طه

قسم الخضر كلية الزراعة ، جامعة القاهرة

تم الحصول على نباتات كاملة عن طريق الانتاج المباشر للاعضاء بزراعة الاوراق الحقيقية والاوراق الفلقية والسويقه الجنينية السفلى . واعطى الصنف كاليفورنيا وندر افضل استجابة بالنسبة لتكوين البراعم الخضرية يليه الهجين أوسكار ثم الصنف أناهيم وأدى زراعة الاوراق الحقيقيه والاوراق الفلقية الى انتاج عدد لكبر من البراعم الخضرية بالمقارنة بزراعة السويقة الجنينية السفلى . وافضل نسبة من الكالس تم الحصول عليها بزراعة الاجزاء النباتية على بيئة موراشيجي وسكوج المضاف اليها ٥ ملجم / لتر اندول استيك اسيد + ٥ ملجم /لتر بنزيل امينو بيروين وعند زراعة البراعم الخضرية على بيئة الاستطالة لوحظ استطالة البراعم الخضرية عند استخدام بيئة موراشيجي وسكوج المضاف اليها ٢ ملجم /لتر بنزيل أمينو بيورين أو استخدام ٥. ملجم / لتر أندول استيك اسيد + ٢ ملجم / لتر بنزيل امينو بيورين وزرعت الافرع الخضرية المتكونة على بيئة موراشيجي وسكوج بدون منظمات نمو أو مضافا اليها ٥. ملجم / لتر اندول بيوتريك أسيد أو ٢. ملجم / لتر نفتالين حمض الخليك نقلت النباتات المتكونة الى التربة بعد عملية الاقلمة تحت الصوب البلاستيكية وكانت طبيعية من حيث النمو و شكل الثمار والبذور.