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EFFECT OF GROWTH REGULATORS ON *IN VITRO* REGENERATION OF TOMATO PLANTS

Sharaf, A. N.*; Sawsan S. Yousuf*; H.I. Ahemaidan and A.G. Mabrouk***

*** Genetics Department, Faculty of Agriculture, Cairo University.**

****Desert Research Center**

ABSTRACT

An investigation on *Lycopersicon esculentus* cvs. Castle Rock (CR), Strain B and Super-Marmand (Tomato) was conducted to standardize the method of high frequency regeneration. Cotyledon and hypocotyl from 12 days old seedlings were used. Comparison was made on cotyledon and hypocotyl explants cultivated on 4 different regeneration media of three cultivars. The best results for shoot primordial numbers per explants were observed of hypo-cotyledon explants with MS medium containing 1mg/L BA of cultivars Castle Rock and Super-Marmand, was reached (91-87% respectively), while the best results for shoot primordial numbers per explants reached the highest value (83%) for strain B by using MS medium supplemented with 0.5 mg/L BA compared to the other treatments. While, the best results for shoot primordial numbers per explants were observed of cotyledon explants with MS media containing 2.0 mg/L BA of cultivars Castle Rock, strain B and Super-Marmand compared to the other treatments, was reached (79,72 and 80%, respectively). The percentage of shoots forming roots was reached the highest value 95 and 90 % of Castle Rock (CR) and Super-Marmand, respectively by using MS medium supplemented with 1.0 mg/ L IBA, while the best results of shoots forming roots was reached the highest value (92%) for strain B by using MS medium supplemented with 0.5 mg/L IBA and 0.5 mg/L NAA. Tomato plants are able to regenerate within 2 months and the young plantlets can be transferred to pots for hardening. Hardening procedure for tissue culture raised plantlets was standardized using sterile mixture of sand and soil in plastic pots.

INTRODUCTION

Tomatoes have great economic importance in greenhouse and field production in Egypt. Fresh and processed tomatoes are an essential component of the human diet contributing to a large intake of the vitamins, minerals and fiber. The ripening of tomato fruits is sensitive to heat stress and causes primary deleterious effects in producing desirable phtynutrients such as carotenoids. There is an increasing demand for enhancing quality and extending shelf-life of fruits. Economically significant attributes for the fresh market tomatoes include size and shape, color, taste and flavor, gloss, presence of external and internal defects, texture and softening (Grierson and Kader, 1986). Fruit ripening is composed of a complicated array of biochemical and physiological processes involving evolution of ethylene, breakdown of chloroplasts, development of chromoplasts leading to accumulation of pigments such as carotene and lycopene, development of aroma and flavor, softening of fruit tissues and increased susceptibility to pathogens (Giovannoni, 2001). Ripening in many types of fruits including tomato is impaired at elevated temperatures (Biggs *et al*., 1988; Paull and Chen, 2000). Tomato fruit stored at a temperature of 30^{o}C and above show abnormal ripening including lack of lycopene accumulation, a slow down in

chlorophyll degradation, tissue softening, and decreased ethylene production (Biggs *et al*., 1988; Kagan Zur *et al*., 1995; Picton and Grierson, 1988). Ramakrishna, *et al*. (2003) have recently identified a novel ripening regulated small heat shock gene, *vis1*, which modifies pectin depolymerization and juice thickness in tomato fruit. The molecular function of *vis1* has been studied in transgenic tomato lines expressing sense and antisense transcripts of *vis1* under the control of CaMV 35S promoter. The ectopic expression of *vis1* significantly enhanced juice viscosity. On the other hand, impaired expression of *vis1* resulted in significantly lowered juice viscosity in several independent transgenic lines.

MATERIAL AND METHODS

Tomato seeds of cvs. Castle Rock (CR), Strain B and Super-Marmand were obtained from the Horticulture Research Institute, ARC, Giza, Egypt and used in all regeneration experiments.

Shoot regeneration:

Cotyledon and hypo-cotyledon sections were used as explants from seedlings grown for 12 days, nods were taken leaves and preexisting buds were removed. Explants were cut into two pieces longitudinally and cultured on MS salts without growth regulators. All cultures of regeneration and transformation treatments were carried out using the MS salts sucrose 30g/L, myoinsitol 100mg/L, Nitsch vitamins 1000x 1ml/L supplemented with different concentrations of growth regulators and the pH of the medium was adjusted to 5.6 by KOH befor adding the phytagel, In addition, the MS salts without growth regulators were used as a standard tomato regeneration medium. All *in vitro* cultured plant materials were incubated in a controlled growth chamber at 25°C±2 and 8/16 hr (dark/light) photoperiod. Two different kinds of explants were applied to MS media which contain different concentrations; 0.5, 1.0, 2.0, 3.0 mg/L of benzyl adenine (BA). After 3 weeks, shoot primordial numbers per explants were scored, and shoot formation is subcultured at regular 4 weeks intervals.

Rooting efficiency:

For this step, the root morphogenesis efficiency of shoots- derived from the tissue culture of the three cultivars. Shoots, 3-5 cm in length, were transferred and implanted in jars containing MS medium supplemented with 1.0, and 2.0 mg/L IBA or 0.5 mg/L IBA and 0.5 mg/L NAA and were incubated at 25 $\pm$1°C under 16 h photoperiod. The efficiency of root formation was calculated as average of total number of shoots forming roots.

Acclimatization stage (Reestablishment of plantlets in soil).

Plantlets produced from rooting stage were transferred from the test tubes under tap water to minimize injury and to free the roots from phytagel. The plantlets were transferred to pots containing a mixture media of peat moss and sand (1:1); plastic pots enveloped in polyethylene bags were incubated under 3000 Lux light intensity derived from cool white fluorescent lamps for 16 hours photo period at 25 $\pm$1°C in growth cabinets. After three weeks polyethylene bags were completely opened and after 4 weeks more

polyethylene bags were removed and plantlets were maintained under greenhouse conditions.

RESULTS AND DISCUSSION

In this study, shoot regeneration system of tomato cvs. Castle Rock (CR), Strain B and Super-Marmand *via* direct shoot organogenesis using the proximal zone of the hypocotyledon and cotyledon explants were established.

Tomato regeneration:

The shoot regeneration ability for the cultivars Castle Rock (CR), Strain B and Super-Marmand were examined using the medium supplemented with BA. The best results for shoot primordial numbers per explants were observed of hypo-cotyledon explants with MS medium containing 1mg/L BA of cultivars Castle Rock and Super-Marmand, was reached (91-87% respectively), while the best results for shoot primordial numbers per explants reached the highest value (83%) for strain B by using MS medium supplemented with 0.5 mg/L BA compared to the other treatments. While, the best results for shoot primordial numbers per explants were observed of cotyledon explants with MS media containing 2.0 mg/L BA of cultivars Castle Rock, strain B and Super-Marmand compared to the other treatments, was reached (79,72 and 80%, respectively) as shown in table (1) and figures (1).

Table (1): Effect of growth regulator (BA) on establishment of shoot formation from cotyledon and hypo-cotyledon sections of tomato cvs. Castle Rock (CR), Strain B and Super-Marmand.

Conc. Of BA (mg/L)	hypo-cotyledon explants			cotyledon explants		
	% of explants forming shoots			% of explants forming shoots		
	C.R	S.B	S.M	C.R	S.B	S. M
0.5	80	**83**	82	50	58	60
1.0	**91**	75	**87**	69	61	71
2.0	60	70	74	**79**	**72**	**80**
3.0	73	64	65	71	65	70

C.R. = Castle Rock S.B. = Strain B S.M. = Super-Marmand

Rooting:

In the present study, *in vitro* elongated shoots for at least 3 cm and shoots of tomato cvs. Castle Rock (CR), Strain B and Super-Marmand were cultured on MS medium supplemented with 1.0, and 2.0 mg/L IBA or 0.5 mg/L IBA and 0.5 mg/L NAA for 4 - week's culture period.

Results in table (2) show that the percentage of shoots forming roots was reached the highest value 95 and 90 % of Castle Rock (CR) and Super-Marmand, respectively by using MS phytagel gelled nutrient medium supplemented with 1.0 mg/ L IBA under 28 days of light comparing with the other treatments; (85 and 89%) for Castle Rock and (75 and 79%) for Super-Marmand as shown in figure (2), while the best results of shoots forming roots was reached the highest value (92%) for strain B by using MS medium supplemented with 0.5 mg/L IBA and 0.5 mg/L NAA compared to the other treatments (78 and 86%).

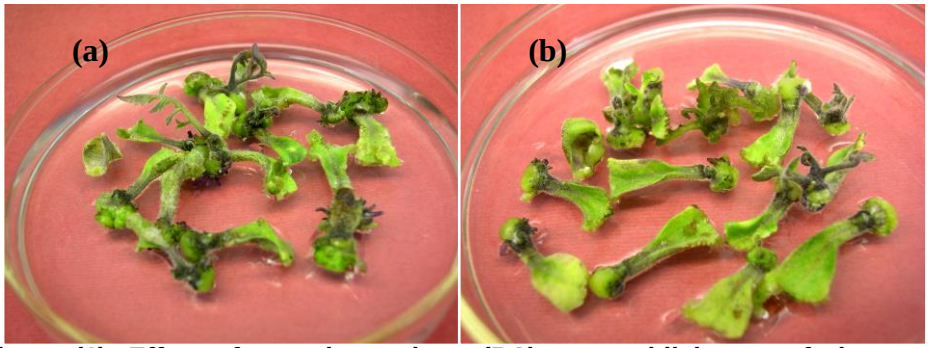

Figure (1): Effect of growth regulator (BA) on establishment of shoot formation from tomatoes hypo-cotyledon sections, (a) 0.5 mg/L BA, (b) 1.0 mg/L BA.

Table (2): The efficiency of shoots forming roots for tomato cvs. Castle Rock (CR), Strain B and Super-Marmand after growing on MS nutrient medium supplemented with different auxin concentration.

Auxin concen. (mg/L)		% of shoot forming roots		
IBA	NAA	**C.R**	**S.B**	**S. M**
1.0	0.0	**95**	86	**90**
2.0	0.0	85	78	75
0.5	0.5	89	**92**	79

Acclimatization:

Plants produced from rooting stage which were containing 4 cm, then rinsed once with water and then transfer into pots containing equal parts of peat and sand, then incubated under transparent plastic bags on 16h photo period at 25°C for 2 weeks before transfer to a green house, after 4 weeks from transfer into green house, they were repotted into sterile soil consists equal parts of peat and sand (v/v) as shown in figure (2).

Many available methodologies for in vitro regeneration of commercial tomato varieties promote not only the production of normal shoots but also individual leaves, shoots without apical meristems and vitrified structures. All these abnormal formations influence and diminish the regeneration efficiency. At the basis of this phenomenon lies callus development (Plana *et al.,* 2006). Therefore, the major goal of this study was conducted to standardize the method of high frequency regeneration of some tomato strains.

In this study, the best results for shoot primordial numbers per explants were observed with MS medium containing 1 mg/L BA, while the best results for shoot primordial numbers per explants reached the highest value by using MS medium supplemented with 0.5 mg/L BA compared to the other treatments. While, the best results for shoot primordial numbers per explants were observed of cotyledon explants with MS media containing 2.0 mg/L BA. The percentage of shoots forming roots was reached the highest value by using MS medium supplemented with 1.0 mg/ L IBA, while the best results of

shoots forming roots was reached the highest value by using MS medium supplemented with 0.5 mg/L IBA and 0.5 mg/L NAA. Tomato plants are able to regenerate within 2 months and the young plantlets can be transferred to pots for hardening.

Figure (2): Rooting and Acclimatization of tomato plants cv. Castle Rock.

Consistent with these results, The portion including the proximal part of hypocotyls and the radicle was cultured on medium consisting of MS salts, 4 mg/L thiamine, 100 mg/L mio-inositol and 3% sucrose. After two-three weeks, 60% explants showed adventitious shoot formation. No changes in the morphological characteristics of regenerated plants and fruits were observed as compared with parents. Karyotypic analysis of regenerated plants showed no variations in chromosome number. The optimized procedure offers the advantage of tomato plant regeneration avoiding callus formation, which enables normal plant recovery with an efficiency ranging from 1.45 to 2.57 shoots per explant (Plana *et al.,* 2006).

Moreover, many authors reported the best method of high frequency regeneration of some tomato strains using methods different from the one that is mentioned in this study. Rao *et al.* (2005) reported multiple shoots were induced from leaf explants of *Lycopersicon esculentum* cultivar MicroTom, within 20-25d, on MS medium supplemented with 8.9 mM benzylaminopurine (BAP) plus 1.14 mM indole-3-acetic acid (IAA). For rooting, elongated microshoots were excised and transferred onto MS medium supplemented with 4.9 mM indole-3-butyric acid (IBA). Well-developed roots and flower raceme were obtained on d 7 and 13, respectively, upon transfer of the microshoots onto rooting medium

Also, Brasileiro *et al.* (1999) reported that different growth regulators combinations were tested. Calli were induced on media supplemented with 1.0 mg/L gibberellic acid (GA3), 0.05 mg/L NAA plus 0.1 mg/L 6-benzylaminopurine (BAP), or with 1.0 mg/L BAP plus 1.0 mg/L NAA. The medium containing 1.0 mg/L BAP and 1.0 mg/L NAA produced the highest calli frequency, and promoted plant regeneration by indirect organogenesis, when calli were transferred to 0.01 mg/L BAP and 0.001 mg/L NAA. Plants

regenerated presented tetraploid cells and rare diploid cells. These tetraploid plants could be used as source for further obtainment of trisomic lines, for the purpose of genic localization studies and protein compounds analysis.

Additionally, the effect of different growth regulators on in vitro growth and plant regeneration of tomato (*Lycopersicon esculentum* Mill.) explants, derived from hypocotyls and cotyledons of aseptically grown seedlings, was studied. With regard to the regeneration frequency, number of shoot primordia and shoots per explant, the best regeneration medium was the MS medium supplemented with 1 mg/L of zeatin and 0.1 mg/L of indole- 3-acetic acid. In all genotypes studied, 100% frequency of regeneration was observed when hypocotyl explants were used (Gubi *et al.,* 2004).

Moreover, Correa-Aragunde et al. (2004) found that lateral root formation induced by the synthetic auxin NAA was prevented by 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (CPTIO) in a dose-dependent manner.

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تأثير منظمات النمو على أعادة التكشف لنباتات الطماطم معمليا

أحمد نجيب شرف*، سوسن سامى يوسف*، حميد ابراهيم أحميدنو أحمد جمعه مبروك***

*** قسم الوراثه –كلية الزراعة – جامعة القاهرة**

**** مركز بحوث الصحراء**

تهدف الدراسة البحثية الى تحديد الطرق القياسيه للحصول على أعلى نسب نمو لأصناف الطماطم Castle Rock (CR), Strain B, Super- Marmand حيث تم استخدام منطقة Cotyledon and hypocotyl بعد تنمية البذور لمدة ١٢يوم وتمت المقارنة بين Cotyledon وhypocotyl على أربعة بيئات نمو مختلفة للاصناف السابقه. وكانت أفضل النتلئج بالنسبة لعدد النموات الخضرية لكل منفصل نباتي عند استخدام hypo-cotyledon كانت عند التنمية على بيئة M.S (تحتوي على ١ مجم/لتر بنزايل أدينين) لصنفي Castle Rock (CR) و Super- Marmand بنسبة(٩١-٨٧%). بينما كانت أفضل النتائج بالنسبة لعدد النموات الخضرية عند التنمية على بيئة M.S (تحتوي على ٠,٥ مجم/لتر بنزايل أدينين) للمنفصل النباتي لصنف Strain B هى (٨٣%) مقارنة بالمعاملات الاخرى. وتشير النتائج بالنسبة لعدد النموات الخضرية لكل منفصل نباتي عند استخدام Cotyledon كانت عند التنمية على بيئة M.S (تحتوي على ٢ مجم/لتر بنزايل أدينين) وذلك للاصناف الثلاثة(Castle Rock (CR وSuper- Marmand وStrain B بمقارنةهذه النتائج بنتائج المعاملات الاخرى وصلت النسبة الى(٧٩,٧٢- ٨٠ %). وأثبتت النتائج أن النسبة المئوية للنموات الخضرية في مرحله تكويت الجذور لصنفي Castle Rock (CR)وSuper- Marmand وصلت الى(٩٥ – ٩٠ %) على التوالي باستخدام بيئة M.S مزودة ب ١ مجم/لتر اندول بيوترك اسيد. بينما النسبة المئوية للنموات الخضرية في مرحله تكويت الجذور لصنفStrain B وصلت الى (٩٢%) باستخدام بيئة M.S مزودة ب ٠,٥ مجم/لتر اندول بيوترك اسيد و ٠,٥ مجم/لتر نفثالين اسيتك اسيد. وتم اقلمه نباتات الطماطم داخل الصوبة.