J. Hortl. Sci.
Vol. 6(2):130-132, 2011

Influence of growth regulators and explant on plant regeneration in tomato

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ABSTRACT

Influence of different growth regulators was studied on *in vitro* growth and regeneration of tomato (*Solanum esculentum*) explants derived from hypocotyls and cotyledons of aseptically grown seedlings. On the basis of regeneration frequency, number of shoot primordia and shoots produced per explants, it is concluded that the best regeneration is achieved on Murashige and Skoog (MS) medium supplemented with 0.5 mg L^{-1} of indole-3-acetic acid and 1.0 mg L^{-1} zeatin. In all the genotypes studied, a good percentage of regeneration frequency was observed in hypocotyl explants used.

Key words: Cotyledon, explants, genotype, hypocotyl, shoot primordia

INTRODUCTION

Tomato (*Solanum esculentum*) is the second most popular vegetable next to potato in the world (Bhatia *et al*, 2006). It is considered an important vegetable crop, and a model species for introduction of agronomically important genes into dicotyledonous crop plants. The most often used pathway of regeneration in tomato is via shoot organogenesis from callus, leaf or cotyledon explants, or, directly from thin cell layers of inûorescence (Bhatia *et al*, 2004). *In vitro* regeneration through organogenesis and somatic embryogenesis can be used for multiplication of genetically identical clones and is an integral part of genetic transformation procedures. *In vitro* morphogenetic responses of cultured plants are affected by different components of culture media and it is important to evaluate their effect on plant regeneration. Although advances have been made towards better understanding of metabolic processes related to regeneration (Cairney *et al*, 2000), determining conditions for *in vitro* plant regeneration is still largely at an empirical stage. Thus, *in vitro* regeneration can be difficult to achieve in some plant species or in particular genotypes within the species. Among *Lycopersicon* species, *L. peruvianum* is considered to be highly organ-specific in its response, and regeneration of shoots from roots has already been documented (Koornneeff *et al*, 1993). Other genotypes have also been described for their ability to form shoots on calli derived from hypocotyls (*L. pimpinellifolium* WV700; Faria and Illg, 1996), cotyledons (*L. esculentum* cv. UC82; Hamza and Chupeau, 1993) and suspension cells (*L. esculentum* cv. VFNT; Meredith, 1979). In the present study, an attempt has been made to compare effects of various growth regulators on shoot induction and plant regeneration in tomato.

MATERIAL AND METHODS

Tomato (*Solanum esculentum*) cultivars, *Pusa Ruby*, *Ailsa Craig* and *Arka Vikas*, were used in the present investigation. Seeds were purchased from the local market of Lucknow city. Seeds of cv. *Ailsa Craig* were procured from Research Institute of Crop Production (France).

Surface-sterilization of seeds was done for 15 min with 1% sodium hypochlorite solution and seed were rinsed three to four times with sterile (autoclaved) water. Thereafter, the seeds were allowed to germinate in glass containers with half-strength MS medium comprising MS salts (Murashige and Skoog, 1962), 2.5 mg l^{-1} thiamine, 1.0 mg l^{-1} pyridoxine, 1.0 mg l^{-1} nicotinic acid and 1.5% (w/v) sucrose. The generation medium was solidified with 0.8% (w/v) agar. Cultures were incubated initially for two days in dark at 26±1°C temperature. These were maintained under a photoperiod of 16h illumination, with a light intensity of 50µmol m^{-2} s^{-1}.

In vitro grown seedlings were used as the source for

Table 1 Adventitious shoot regeneration in tomato explants and cultivars on MS medium supplemented with various growth regulators. The data were recorded at 5 weeks from culture

	Number of shoots and shoot primordia per explant (± SE)					
	Pusa Ruby		Arka Vikas		Ailsa Craig	
Explant	Hypocotyl	Cotyledon	Hypocotyl	Cotyledon	Hypocotyl	Cotyledon
Medium MS1	3.10±0.31	0	4.98±0.89	0	4.95±0.12	0
Medium MS2	6.31±1.12	5.90±1.22	6.69±1.14	5.45±0.86	6.98±1.03	5.78±0.32
Medium MS3	2.43±0.40	4.12±1.91	3.51±0.24	5.40±0.90	2.97±0.11	5.24±1.02
Medium MS4	3.49±1.01	4.39±1.85	3.48±0.11	4.67±0.12	4.26±1.01	5.31±1.05
Medium MS5	3.18±1.20	3.12±0.44	5.61±0.92	4.62±0.73	4.02±0.71	5.09±0.89

± SE = Standard Error; Each treatment is an average of 25 replicates

two types of explant: hypocotyl and cotyledon segment. Each cotyledonary leaf was cut transversely into two segments (proximal and distal), which were placed with their adaxial surface in contact with the medium whereas, hypocotyls were cut into three segments (lower, middle and upper), and were placed horizontally on the surface of regeneration medium. For induction of regeneration, the following media were used:

(a) MS1 {MS medium without growth regulators (control)}

(b) MS2 {MS medium + 0.5 mg l^{-1} IAA+ 1.0 mg l^{-1} zeatin}

(c) MS3 {MS medium +1.2 mg l^{-1} BAP + 0.5 mg l^{-1} NAA}

(d) MS4 {MS medium + 2.5 mg l^{-1} BAP+ 0.4mg l^{-1} NAA}

(e) MS5 {MS medium + 3.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA}

Culture conditions and statistical analysis

Culture media were adjusted to pH 5.8 before autoclaving at 121ºC and 15lb/in^2 for 20 min. Cultures were incubated in a growth chamber at 26±2ºC under 16h light (2000 Lux) and 8h dark. Experiments were designed in Completely Randomized Design (CRD) and data are presented with standard error (SE) (Snedecor and Cochran, 1967). Regeneration of explants was assessed at five weeks from culture. The following parameters were evaluated:

(1) Frequency of regeneration (Number of regenerating explants /Number of plated explants) x 100

(2) Number of shoots and shoot primordial per explant plated

RESULTS AND DISCUSSION

In vitro morphogenic response of cultures was seen to be affected by different components of culture media, especially by concentration of growth hormones. It is, therefore, important to evaluate hormonal effects on plant regeneration. Tomato is one of the most studied plants owing to its importance as a crop species, and its several advantages in genetic, molecular and physiological studies

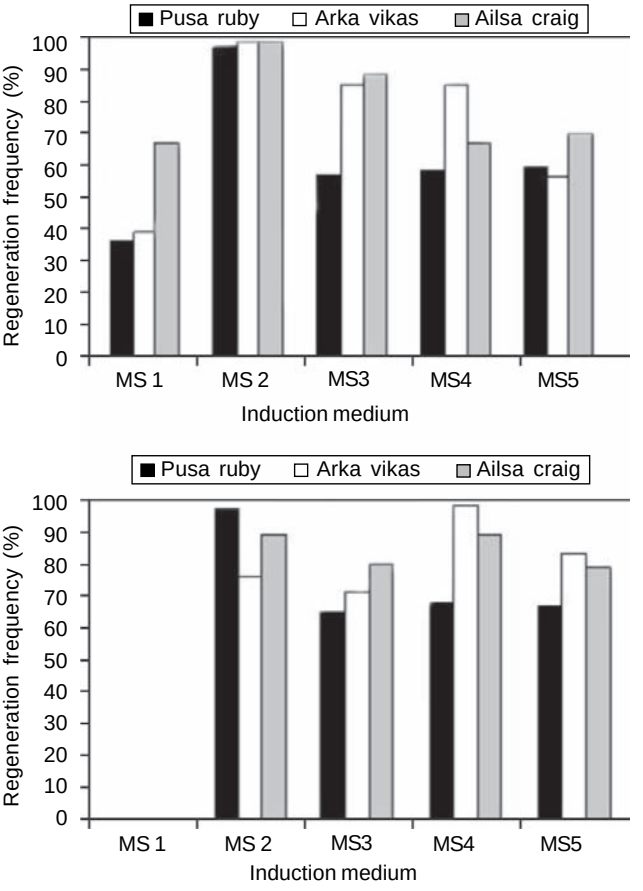

Fig 1. Regeneration frequency of cotyledons across cultivars

(McCormick *et al*, 1986). Two types of explants, derived from cotyledons and hypocotyls, were isolated from seedlings of three tomato cultivars. Forty to forty five segments from each type of explant were cultured on MS Medium variously supplemented with different growth regulators. Frequency of adventitious shoot regeneration was seen to differ with the type of explants, and, type and concentrations of growth regulators added to the medium. Medium supplemented with 0.5 mg l^{-1} IAA +1.0 mg l^{-1} zeatin (MS2 medium) was the most effective (100%) at inducting adventitious shoots from hypocotyl explants in all cultivars

studied (Fig.1). Regeneration frequency of cotyledons was cultivar-dependent and renged from 67% to 100% in media supplemented with various concentrations of BAP and NAA, and from 75% to 100% in medium supplemented with zeatin.

Results of our experiment confirm the positive influence of growth regulators on number of shoots regenerating from tomato cotyledons and hypocotyls. In all the cultivars, zeatin-supplemented media gave higher number of shoots and shoot-primordia per explant (Table 1). We observed weaker effect of BAP on adventitious shoot regeneration in tomato compared to zeatin, and this corresponded with frequency of regeneration for a particular cultivar. Results of our experiments are in very close proximity to findings of other workers (Nogueira *et al*, 2001). The most efficient medium for *in vitro* regeneration of tomato is induction medium supplemented with the cytokinin zeatin.

ACKNOWLEDGEMENT

The authors are thankful to Director, National Botanical Research Institute, Lucknow, for his kind help during the course of the above study.

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(MS Received 12 July 2011, Revised 16 August 2011)