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Original article

Efficient and reproducible *in vitro* regeneration of *Solanum lycopersicum* and assessment genetic uniformity using flow cytometry and SPAR methods

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ABSTRACT

In the present study, we develop an efficient and reproducible *in vitro* regeneration system for two cultivars *viz*., Jamila and Tomaland of *Solanum lycopersicum* L., an economically important vegetable crop throughout the world. Sterilization of seeds with 2.5% (v/v) NaOCl was found to be most effective, about 97% of seeds germinated on cotton in magenta box moistened with sterile half strength (½)Murashige and Skoog (MS) medium. Regeneration efficiency of cotyledonary leaf (CL) and cotyledonary node (CN) explants derived from 08 days old aseptic seedling were assessed on MS medium supplemented with different concentrations of auxins and cytokinin. CL explants were found more responsive in comparison to CN in both the cultivars. Types of basal media were also assessed and found to have a significant effect on shoot regeneration. Highest regeneration frequency and maximum number of shoots were standardized from CL explants on MS medium supplied with 6-benzyl adenine (BA; 5.0 μM), indole-3-butyric acid (IBA; 2.5 μM) and Kinetin (Kin; 10.0 μM). *In vitro* regenerated microshoots were rooted on ½MS medium containing 0.5 μM indole-3-butyric acid (IBA). Regenerated plantlets with well-developed roots and shoot system were successfully acclimated to *ex vitro* condition. Genetic uniformity of tissue culture raised plantlets was first time evaluated using flow cytometry and single primer amplification reaction (SPAR) methods *viz*., DAMD and ISSR. No significant changes in ploidy level and nuclear DNA content profile were observed between *in vitro* propagated plants and normal plants of both the cultivars. Similarly, the SPAR analysis also revealed monomorphic banding patterns in regenerated plantlets of *S. lycopersicum* verifying their genetic uniformity and clonal fidelity. This efficient regeneration system can be used as a fast and reproducible method for genetic transformation of this important vegetable crop.

1. Introduction

Solanum lycopersicum L. (Solanaceae) commonly known as 'tomato' is a one of the most important vegetable crops in the world (Bhatia et al., 2004; Foolad, 2004). It is considered as 'poor man's orange, because of having substantial quantities of mineral and vitamins (Devi et al., 2008). It is an important nutritive crop and can be grown in short period of time with high productivity. Cultivation of this important crop is increasing day by day because of its high economic values in the vegetables market. It is a rich source of minerals (iron), vitamins (A and C), organic acid, essential amino acids, dietary fibers and can be used in preserved foodstuffs

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like ketch-up, sauce, chutney, soup, paste etc. (Block et al., 1992; Gerster, 1997; Rao and Agarwal, 2000). Tomato productivity in Saudi Arabia is below the international levels due to various constraints in cultivation. Biotic stresses are contributing significantly to this low productivity both in open field and polyhouse cultivation. Cultivation of tomato suffers from serious losses due to infestation by insects and pests and the diseases they transmit. Therefore, there is an urgent for biotechnological interventions to increase the productivity of this crop. Culture of plant cell, tissues *in vitro* is an integral part plant biotechnology, which has been be exploited for *in vitro* regeneration and genetic improvement of this crop, is the first and quintessential step towards genetic transformation of plants. Without a reliable, reproducible and efficient system to regenerate genetically identical plants from a small mass of transformed cells, it is not possible to produce a complete genetically modified plant. There are several reports on adventitious *in vitro* cultivation in tomato from various explants (Compton and Veillux, 1991; Moghaleb et al., 1999; Brichkova et al., 2002; Raziuddin et al., 2004; Mohamed et al., 2010; Liza et al., 2013). However, improvement and standardization of *in vitro* regeneration and shoot multiplication protocols is still imperative due to its diverse morphogenic potential of different explants and genotypes (Tomsone et al., 2004).

However, there are some limitations of *in vitro* technique because of the occurrence of spontaneous genetic or epigenetic changes leading to cytological abnormalities, phenotypic mutations, sequence changes (Kaeppler et al., 2000), and DNA methylation in *in vitro* regenerated plants. These variations may affect the quality and quantity of plants as well as genetic transformation through various approaches. The present study was aimed to formulate an efficient and reproducible regeneration system of this economically important crop by optimization of various parameters *viz.*, genotypes, sterilizing agents, types of explants, medium and auxin and cytokinin balance for *in vitro* multiplication and plant regeneration. Furthermore, the genetic stability of the *in vitro* developed plants was also assessed for the first time by flow cytometry and SPAR methods (DAMD; directed amplification of minisatellite DNA and ISSR; intersimple sequence repeat polymorphic DNA).

2. Materials and methods

2.1. Experimental materials and surface sterilization

Certified mature seeds of tomato (*Solanum lycopersicum*) cvs. Jamila and Tomaland were purchase from local seed market were used as starting experimental materials. The seeds were thoroughly washed under tap water in the laboratory for 30 min, followed by soaking in 5% (v/v) liquid detergent solution for 5 min. After several washes with sterile ultrapure water, the seeds of both the cultivars were surface sterilized with different sterilizing agents *viz.*, mercuric chloride (Merck, Germany), sodium hypochlorite (Clorox Co. Saudi Arabia) at different concentration and time of exposure (Table 1). The sterilized seeds were immediately washed 4–5 times with sterile ultrapure water.

2.2. Seed germination and growth conditions

The sterilized seeds were aseptically transferred on cotton in magenta vessels moistened with half strength (½) Murashige and Skoog [1962; (MS)] medium. The pH of the medium was adjusted to 5.8 using 1 N NaOH or 1 N HCl before being autoclaved at 121 °C for 20 min. The cultured seeds were incubated in dark for 48 h and thereafter maintained under 50 μmol m^{-2} s^{-1} light provided by cool white fluorescent lamp for a photoperiod of 16 h at 24 ± 2 °C in a growth chamber. Data on seed germination were recorded after 08 days of inoculation.

2.3. In vitro shoot initiation and multiplication

For *in vitro* shoot induction, cotyledonary leaf (CL) and cotyledonary node (CN) explants of both the cultivars were excised form 8 days old aseptic seedlings and transferred to MS medium supplemented with BA (5.0 μM) and IBA (0.5 μM) and kept in dark for two days at 24 ± 2 °C. After two days, the explants were cultured on MS medium supplemented different with concentration and combination plant growth regulators [6-benzyladenine (BA; 0.5, 2.5, 5.0, 10 μM) Kinetin (Kin; 0.5, 2.5, 5.0, 10 μM) and indole-3 butyric acid (IBA; 0.5, 2.5 and 5.0 μM). All of the cultures were transferred onto the fresh medium after every two weeks. The frequency with which explants produced shoots and the number of shoots per explant was recorded after eight weeks of culture.

After standardizing the most suitable growth regulator combination, different plant growth media was also evaluated and compared to perceive the best suitable media for regeneration and multiplication. Gamborg medium [Gamborg et al., 1968; (B5)], Nitsch medium [Nitsch and Nitsch, 1969; (NN)] and White medium [White, 1943; (W)] were used for *in vitro* shoot regeneration from cotyledonary leaf and cotyledonary node.

2.4. Rooting of regenerated shoots

In vitro regenerated shoots measuring about 4–5 cm in length were excised individually under aseptic condition and transferred to half-strength (½)MS medium supplemented with NAA, IAA or IBA (0, 0.1, 0.5, 1.0 and 2.0 μM) for *in vitro* rhizogenesis. Data were recorded on the percentage of shoots forming roots and the mean

Table 1
Effect of $HgCl_2$ and NaOCl on sterilization of seeds of *Solanum lycopersicum* L. cvs. Jamila and Tomaland on ½MS medium.

$HgCl_2$ (% w/v)	NaOCl (% v/v)	Treatment duration (min)	Germination (%)		Remarks
			Jamila	Tomaland	
0.01	–	3	92	90	Contaminated
0.1	–	3	48	47	Delayed germination
0.2	–	3	0	0	No germination
0.5	–	3	0	0	No germination
1.0	–	3	0	0	No germination
	0.5	10	92	90	Contaminated
	2.5	10	97	96	No contamination
	5.0	10	76	76	Delayed germination
	10.0	10	44	42	Delayed germination

Evaluation were made from 8 days old culture.

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