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Short communication

Assessment of genetic integrity of plants regenerated by organogenesis from callus culture of strawberry

Anuradha, S.K. Sehrawat and D.S. Dahiya

Department of Horticulture, CCS Haryana Agricultural University, Hisar 125004

ABSTRACT

The nodal segments of strawberry cultivar Ofra were cultured on MS medium supplemented with different concentrations of auxins and cytokinins for indirect regeneration *via* callus formation. MS medium supplemented with 4.0 mg/l NAA and 2.0 BAP yielded the highest percentage of callus. The highest response of shoot regeneration was obtained in MS medium fortified with 2.0 mg/l kinetin and 0.5 mg/l IAA for regeneration of shoots from callus. The regenerated shootlets were rooted on half-strength MS medium. The plantlets thus developed were hardened successfully. Some morphological variations were found in plants generated by callus. On the basis of variations, *in vitro* raised plantlets were subjected to evaluation of genetic uniformity using RAPD markers. Out of 21 primer screened, six primers showed amplification and the RAPD profiles obtained were found to be reproducible and monomorphic. Results revelaed that tissue culture raised plants of strawberry were genetically identical and clonally uniform.

Key words: *In vitro* propagation, strawberry, auxins, cytokinins, genetic fidelity.

In order to obtain true to the type plantlets with the aid of tissue culture, it is quite necessary to examine the genetic uniformity of *in vitro* raised plants from time to time. During *in vitro* cultures, however it comes to surveillance that many of regenerated plants are not genetically identical to their parents and these plants are known as somaclonal variants. Somaclonal variation although thought to be beneficial for generating better performing clones, can sometimes be considered a serious problem as it may lead to creation of non true-to-the-type plants which differ in genetic makeup from that of their parent. Clonal fidelity is one of the major concerns in commercial propagation. Strawberries are too amenable to *in vitro* somaclonal variation (Kaushal *et al*., 4). Production of true-to-the-type propagules and their genetic stability are pre-requisites for the application of *in vitro* propagation of strawberry. Ascertaining clonal fidelity using molecular marker techniques is the most reliable method. RAPD (random amplified polymorphic DNA) is a reliable technique for identification of genetic variation. Strawberries have been extensively analyzed for clone identification, mapping and diversity studies using RAPD marker (Gantait *et al.*, 2). Objective of the present study was to determine the appropriate growth regulator concentration and combination to establish a mass production system of callus raised plants of strawberry cultivar Ofra and to assess the genetic fidelity of the *in vitro* raised plants using RAPD markers. This can be a useful for establishing a reliable micropropagation system for the production of genetically uniform plants.

The experiment was carried out at the Centre of Plant Biotechnology and Department of Horticulture, CCS Haryana Agricultural University, Hisar during the 2014-15. Explants were collected from the healthy plants in July maintained at Experimental Polyhouse of the Department. Young tender vegetative nodal segments of 5-10 cm length were excised. The MS medium was supplemented with BAP, kinetin, IAA and NAA alone or in combinations for direct regeneration and for callus induction. Calli were cultured on MS medium supplemented with different concentration of BAP and kinetin at lower concentration in combination with auxins (NAA, IAA). The cultures were maintained in thermal insulated tissue culture room with temperature of around 25 ± 2°C and 4000 lux illumination uniformly. Half strength MS medium was used for induction of roots in all cultures. The rooted plantlets were transferred to 3:1:1 mixture of cocopeat, perlite and sand in plastic pots and acclimatized under greenhouse conditions. The data of all the experiment recorded during the present investigation were subjected to statistical analysis using Completely Randomized Design using software OP STAT.

Modified CTAB (Cetyl Trimethyl Ammonium Bromide) method of Murray and Thompson (5), modified by Saghai-Maroof *et al.* (6) and Xu *et al.* (9) was used for extraction of total DNA from both tissue cultured plants and mother plants. Twenty one RAPD primer were used for assaying genetic uniformity. PCR

*Corresponding author's E-mail: anuradha2917@gmail.com

was performed in a volume of 10 µl. The variations were made in genomic DNA concentration (25, 50 and 100 ng), *Taq* DNA polymerase concentration (1.0 and 2.0 units), primer concentration (0.5 and 1 µl) and annealing temperature (36, 37 and 40°C). All possible combinations were tried in 10 µl of reaction mixture for PCR amplification. Amplification was carried out in PTC-100 programmable thermal cycler. PCR conditions for RAPD analysis included an initial pre-denaturation step of 3 min. at 94°C and following 45 cycles of amplification of denaturation 94ºC at 1 min., annealing for 36°C and an extension at 72°C for 1 min. and final extension was carried out at 72°C for 7 min. The amplified products were resolved on a 2% agarose gel in TBE (Tris-borate EDTA) buffer stained with ethidium bromide using 100 bp ladder as reference. PCR amplified products were visualized under UV light and photographed using VSD Image Master of Pharmacia, Biotech.

Among the used hormonal supplements, explants of strawberry showed high response to callus formation on MS medium supplemented with BAP. The specific concentration of growth regulators, nutrients and incubation condition modify the normal physiology of explants and induce dedifferentiation and redifferentiation of tissue. Depending on the auxins and cytokinin concentration of culture media calli were initiated from cut surfaces. The application of hormones disturbs the internal system and leads to dedifferentiation and differentiation of tissue. Among the used hormonal supplements, auxins in combination with cytokinins (NAA & BAP) was found to be the most effective for callus induction (Table 1), which is concomitant with previous reports of Ara *et al*. (1).

Table 1. Effect of growth regulators on callus induction and No. of days taken for callus initiation in strawberry cultivar Ofra.

Growth regulator (mg/l)	No. of days taken for callus initiation	Callus induction (%)
2.0 NAA	15.00 ± 0.58	81.67 ± 0.67
4.0 NAA	15.00 ± 0.00	73.33 ± 1.67
5.0 BAP	16.33 ± 0.33	70.67 ± 2.08
5.0 BAP + 0.1 NAA	13.00 ± 0.58	83.33 ± 0.58
5.0 BAP + 0.1 IAA	16.00 ± 0.58	80.33 ± 0.33
4.0 NAA + 2.0 BAP	11.00 ± 0.58	87.33 ± 1.67
4.0 NAA + 2.0 Kin.	17.67 ± 0.33	71.67 ± 1.67
$CD_{0.05}$	1.44	4.63

*Basal medium was MS

The results showed that (MS media + 2.5 mg/l Kin. + 0.5 mg/l IAA) was best for shoot regeneration followed by (MS medium + 1.0 mg/l BAP + 0.1 mg/l NAA) from nodal segment derived callus. It was observed that higher concentration of cytokinins with lower concentration of auxins promoted shoot regeneration from callus. The calli which were placed on MS medium without growth regulators failed in regenerating shoots (Table 2). Karim *et al*. (3) reported that addition of kinetin in combination of BA and NAA resulted in efficient shoot induction and multiplication from calli, while Ara *et al.* (1) reported that combination of BA and NAA produced maximum number of shoots. Synergistic effect of BA and Kin. has been reported to be the best for shoot regeneration in strawberry (Sood *et al*., 7).

Table 2. Effect growth regulators on shoot multiplication (21 days) from nodal segment derived callus of strawberry cv. Ofra.

Growth regulator (mg/l)	No. of shoot	No. of leaves	Shoot length
Control	0.0	0.0	0.0
0.5 BAP	3.07 ± 0.07	2.90 ± 0.20	3.67 ± 0.00
0.5 BAP + 0.1 NAA	5.33 ± 0.19	4.84 ± 0.16	3.14 ± 0.23
1.0 BAP + 0.1 NAA	6.11 ± 0.11	5.50 ± 0.28	5.11 ± 0.22
1.0 BAP + 0.5 NAA	3.44 ± 0.29	3.64 ± 0.21	3.07 ± 0.13
2.0 Kin. + 0.1 IAA	4.33 ± 0.28	3.83 ± 0.16	5.67 ± 0.33
2.5 Kin. + 0.1 IAA	4.66 ± 0.19	4.68 ± 0.22	4.52 ± 0.26
3.0 Kin. + 0.1 IAA	6.00 ± 0.00	5.19 ± 0.09	4.07 ± 0.07
2.0 Kin. + 0.5 IAA	4.62 ± 0.03	4.54 ± 0.02	5.41 ± 0.07
2.5 Kin. + 0.5 IAA	6.96 ± 0.04	5.89 ± 0.11	5.33 ± 0.19
3.0 Kin. + 0.5 IAA	4.26 ± 0.13	3.63 ± 0.32	4.78 ± 0.22
$C.D._{0.05}$	0.49	0.59	0.61

*Basal medium was MS, control = No growth regulator.

The number of leaves per explants was maximum with (MS medium + 2.5 mg/l Kin. + 0.5 mg/l IAA). This increase in number of leaves may be attributed to the synergistic effects of kinetin and IAA. Length of shoots was recorded maximum (5.67 cm.) on MS medium supplemented with 2.0 mg/ l Kin. and 0.1 mg/l IAA. It was observed that as concentrations of kinetin increased, length of shoots decreased. Whereas, BAP at higher concentrations exhibited synergistic effect with lower concentrations of NAA (0.1 mg/l). Cytokinins are responsible for division of cells, which are elongated by auxins due to their ability to increase cell wall plasticity and lowering in cell wall resistance to the penetration of solutes.

In the present work, the genetic fidelity of tissue cultured clones was also determined so that true-to-the-type character of calli derived plants could be established. Twenty one primers were used, out of which six primers showed amplification (Table 3). The results revealed that the tissue culture raised plants were genetically identical and uniform. Although, minor morphological variation were recorded in some plants regenerated via callus. The RAPD profiles of calli derived plants were identical to the mother plant irrespective of the minor phenotypic variations (Fig. 1). The phenotypic variations in calli derived plants might be due to higher concentrations of growth regulators. Similar results were reported by who reported epigenetic changes in tissue culture raised plants due to applications of plant growth regulators, but such trait is not passed to their offspring through sexual cycle or might entirely disappear during plant maturation. Sutan *et al*. (8) also found that epigentic variation due to higher concentration of growth regulators but at molecular basis tissue cultured plants were true to the type.

It can be concluded from the present study that MS medium + 4.0 mg/l 0 NAA + 2.0 mg/l BAP was best for maximum callus induction in minimum number of days. MS medium supplemented with 2.5 mg/l Kin. and 0.5 mg/l IAA was most effective for multiple shoot formation and number of leaves for both direct regenerated shoots and for callus derived shoots.

Table 3. Random primers showing amplification.

Primer	Sequence
OPB 1	'GTTTCGCTCC'
OPB 2	TGATCCCTGG'
OPB 3	'CATCCCCCTG'
OPB 4	'GGACTGGAGT'
A-05	'GGGATATCGG'
OPA 10	'GACCGCTTGT'

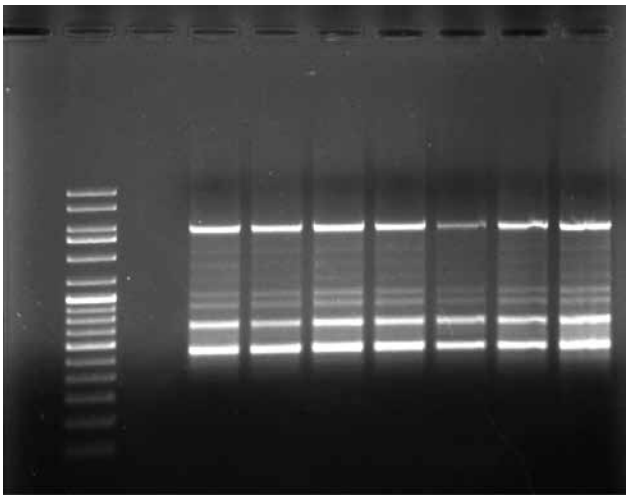

Fig. 1. RAPD Profile of indirect regenerated plants showing monomorphic bands.

Whereas, shoot length was recorded maximum on MS medium + 2.0 mg/l Kin. + 0.1 mg/l IAA. RAPD profile of *in vitro* raised plants were monomorphic and identical to the field grown mother plants.

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