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Genetic analysis of somaclonal variation among callus-derived plants of tomato

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Genetic stability in tissue-cultured tomato plants was examined by randomly amplified polymorphic DNA (RAPD) analysis. Picloram was used for the first time as alternative auxin, along with benzyladenine (BA) for callus induction in tomato. Calli were induced from leaf explants on Murashige and Skoog's (MS) medium supplemented with 8.88 µM BA and 4.13 µM picloram. Regeneration was obtained after culturing freshly-induced calli on MS medium containing 17.7 µM BA alone. Microshoots were rooted in the presence of 10 µM indole-butyric acid (IBA) on MS medium. DNA samples from the mother plant and 11 randomly selected regenerated plants, obtained from a single callus, were subjected to RAPD analysis for the detection of putative somaclones. Six arbitrary decamer primers produced polymorphic amplification products. In this set of experiments, fifteen non-parental bands were observed, of which three were shared and twelve unique. The estimation of genetic similarity coefficient based on RAPD band-sharing data indicated that ten regenerated plants were more than 95% similar to the mother plant, except one, LS5, which was found to be distinctly different. This report demonstrates the feasibility of easy induction of regenerative calli by using combination of picloram and BA and the possibility of detecting genetic variation through RAPD analysis among callus-regenerated plants in tomato at an early stage of growth.

IDENTIFICATION of possible somaclonal variants at an early stage of development is considered to be very useful for quality control in plant tissue culture, transgenic plant production and in the introduction of variants. Somaclonal variability often arises in tissue culture as a manifestation of epigenetic influence or changes in the genome of differentiating vegetative cells induced by tissue culture conditions[1,2]. Any genetic change induced by *in vitro* conditions of tissue culture is expected to generate stable plants carrying interesting heritable traits. However, such random changes are not desirable in plant transformation experiments. Therefore, their early detection is considered to be very useful in plant tissue culture and transformation studies. Randomly amplified polymorphic DNA (RAPD) based detection of genetic polymorphism[3,4] has found successful application in describing somaclonal variability in regenerated individuals of several plant species[5–7]. In the present paper, we report successful induction of regenerative calli from tomato leaf explants, cultured on Murashige and Skoog's (MS) medium supplemented with picloram (4-amino-3-5-6-trichloropicolinic acid, a common herbicide) (as an auxin) and benzyladenine (BA; as cytokinin), and the extent of genetic variability in the plants regenerated from one of these calli as examined through RAPD analyses.

Lycopersicon esculentum Mill cv. Sakthi procured from Agricultural College, Thiruvananthapuram was used in this study. Leaf explants, from a field-grown plant (mother plant), were sequentially washed under running water and with 0.1% labolene (Qualigens, India) for 10 min each. Their surface was disinfected with 70% ethanol for 2 min and then treated with 0.1% w/v mercuric chloride solution for 3 min. Finally, they were washed 3–4 times with sterile distilled water and inoculated aseptically on MS basal medium[8] containing combinations of BA (4.2–17.7 µM) and picloram (4.13–8.26 µM). Regeneration of calli was attempted on MS medium containing BA alone, picloram alone and combinations of both. The pH of all media was adjusted to 5.8 and 0.8% (weight/volume) agar was added prior to autoclaving at 103 kpa for 20 min. Cultures were incubated under a 12 h photo-period with light intensity of 3000 lux at 26 ± 1°C.

DNA was extracted from fresh *in vitro* leaves of regenerated tomato plants and the mother plant by cetyltrimethyl ammonium bromide (CTAB)-based procedure[9]. RAPD was performed as described by Williams *et al.*[4] using random decamer primers (M/s Operon Inc, USA). Polymerase chain reaction (PCR) was carried out in presence of 1X Taq DNA polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM Mg Cl_2, 0.001% gelatin), 100 µM dNTPs, 5 picomole single random primer, 25 ng template DNA, 0.5 unit of Taq DNA polymerase (Bangalore Genei, India) in a total volume of 20 µl overlayed with mineral oil (Sigma Chemical Co). PCR amplification was performed in automated thermal cycler (PE480) programmed for 45 cycles of 1.0 min denaturation at 94°C, 1.0 min annealing at 37°C and 2 min polymerization at 72°C, followed by a final extension step at 72°C for 10 min. The amplification products were resolved by electrophoresis in 1.2% agarose (USB) gels in 0.5X TBE buffer at 5 V/cm for 3 h and documented on Gel Doc 1000 (Bio-Rad).

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Green morphogenetic calli were induced on leaf explants within three weeks of culture on MS medium supplemented with BA (8.9 μM) and picloram (4.13 μM). Regeneration of multiple shoots (8–12/callus) was noticed within two weeks following transfer to MS medium containing 17.7 μM BA alone (Figure 1). Regenerated shoots were rooted on MS medium supplemented with 1 mg/l indole 3-butyric acid (IBA). After gradual acclimatization they were transferred to pots.

In earlier reports, use of α-naphthaleneacetic acid (NAA) or indole-3-acetic acid (IAA) has been described for callus induction and regeneration in tomato[10]. However, there are many reports of successful usage of picloram as an auxin-like agent, along with BA or kinetin (as cytokinins) for effective induction of regenerative calli in a number of plant species[11–16]. To the best of our knowledge, we did not find any previous information about the use of picloram as callogenic agent in tomato tissue culture.

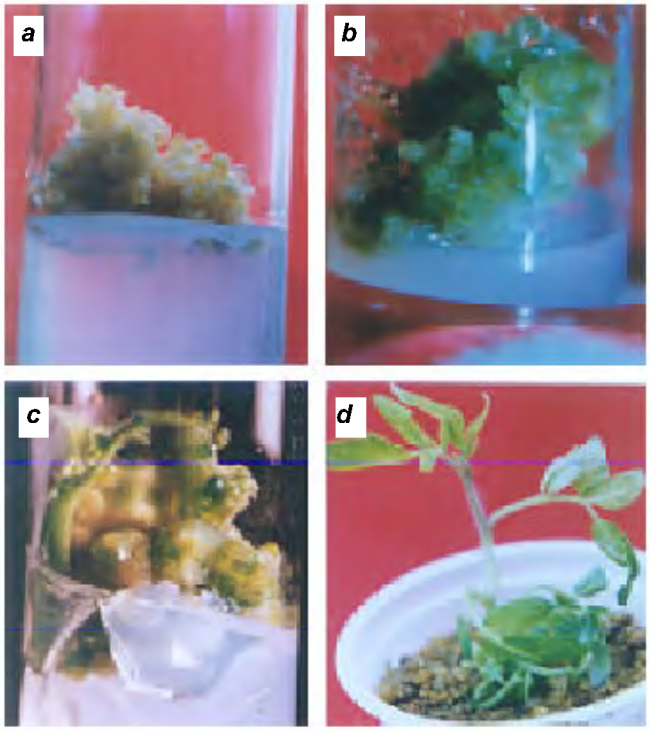

Figure 1. Different stages of indirect organogenesis in tomato. ***a***, Morphogenetic callus; ***b***, Green callus showing initiation of shoot buds; ***c***, Elongated shoot buds; and ***d***, Plant transferred to soil.

Further, we examined somaclonal variation amongst 11 plants regenerated from a picloram-induced callus by RAPD using 20 random primers. Out of 20 random decamer primers tested, only 10 primers successfully produced scoreable RAPD bands (200 bp to 2.5 kb) for all the plants. Six primers (A15, AC7, AC8, AC9, AC10, C15) produced polymorphic RAPD profile for 11 regenerated plants (Table 1). For example, A15 primer produced two polymorphic non-parental bands, A15–10 and A15–12 (Figure 2). Genetic similarity between the regenerated plants and the mother plant was scored by comparing their RAPD profile for each of the above primers and calculating the coefficient of genetic similarity as described previously[17] (Table 2). A total of 84 RAPD band positions were recorded, out of which 35 were polymorphic. Fifteen polymorphic bands were absent in the RAPD profile of the mother plant out of which three were shared, while twelve others were unique. Since, even single base change at the primer annealing site is manifested as appearance or disappearance of RAPD bands, it could be suggested that tissue culture conditions have induced varied amount of genetic changes in different regenerated plants. Some of these changes appeared identical in different plants as represented by appearance of non-parental bands A15–10 (in LS7, 10 and 11), A15–12 (in LS1, 2, 3, 5 and 9) and AC10–4 (in LS5 and 6). Such commonness of genetic variation in these plants could be because they were all derived from the same callus.

Table 1. Presence of non-maternal polymorphic RAPD bands in tomato regenerants

Plants	P	LS1	LS2	LS3	LS4	LS5	LS6	LS7	LS8	LS9	LS10	LS11
Polymorphic bands												
A15–10	–	–	–	–	–	–	–	+	–	–	+	+
A15–12	–	+	+	+	–	+	–	–	–	+	–	–
AC7–6	–	–	–	–	–	–	+	–	–	–	–	–
AC7–9	–	–	–	–	+	–	–	–	–	–	–	–
AC8–10	–	–	–	+	–	–	–	–	–	–	–	–
AC9–3	–	–	+	–	–	–	–	–	–	–	–	–
AC9–4	–	–	+	–	–	–	–	–	–	–	–	–
AC9–7	–	–	+	–	–	–	–	–	–	–	–	–
AC9–8	–	–	+	–	–	–	–	–	–	–	–	–
AC10–4	–	–	–	–	–	+	+	–	–	–	–	–
AC10–6	–	–	–	–	–	+	–	–	–	–	–	–
AC10–9	–	–	–	–	–	+	–	–	–	–	–	–
AC10–15	–	–	–	–	–	+	–	–	–	–	–	–
AC10–16	–	–	–	–	–	+	–	–	–	–	–	–
AC10–21	–	–	–	–	–	+	–	–	–	–	–	–
C15–8	–	+	–	–	–	–	–	–	–	–	–	–

P, Mother plant; LS1–LS11, Regenerated plants; +, Presence of a non-maternal band; –, Absence of a non-maternal band.

Table 2. Matrix of similarity coefficient* based on RAPD profiles for 6 selected primers

	P	LS1	LS2	LS3	LS4	LS5	LS6	LS7	LS8	LS9	LS10	LS11
P	1	0.97	0.95	0.96	0.97	0.90	0.95	0.99	0.96	0.99	0.98	0.99
LS1		1.00	0.92	0.93	0.95	0.90	0.92	0.96	0.93	0.98	0.95	0.96
LS2			1.00	0.94	0.90	0.85	0.89	0.93	0.89	0.93	0.92	0.93
LS3				1.00	0.99	0.88	0.92	0.95	0.92	0.95	0.95	0.95
LS4					1.00	0.95	0.94	0.98	0.91	0.96	0.97	0.98
LS5						1.00	0.90	0.90	0.86	0.92	0.90	0.90
LS6							1.00	0.95	0.92	0.93	0.94	0.93
LS7								1.00	0.97	0.97	0.99	1.0
LS8									1.00	0.92	0.96	0.97
LS9										1.00	0.96	0.98
LS10											1.00	0.99
LS11												1.00

*Similarity coefficient ($S = 2 \times n_{ab}/n_a + n_b$) was calculated from band sharing data as described by Nei and Li[17]. n_{ab} is number of bands shared by two lanes, while n_a and n_b represent number of bands present in lane a and lane b, respectively. P represents mother plant and LS1 to LS11 represent regenerants from different experiments.

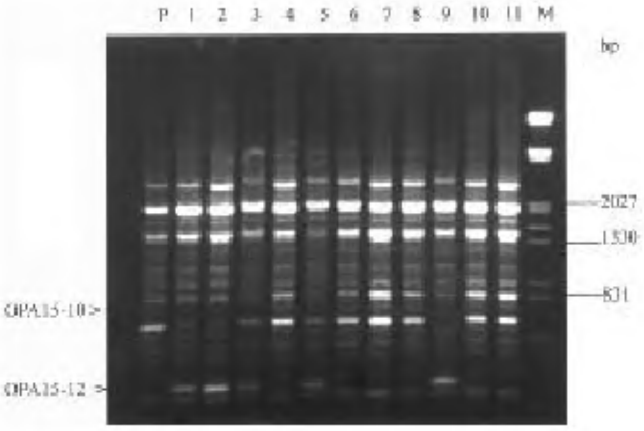

Figure 2. RAPD pattern generated by OPA-15 primer from genomic DNA of the mother plant (lane P) and regenerated plants LS1 to LS11 (lane, 1–12). Lane M contains lambda DNA digested with *Eco*RI and *Hind*III as molecular weight markers. Arrowheads indicate polymorphic bands A15–10 and A15–12.

Analysis of the coefficient of genetic similarity among the different plants indicated that all the regenerated plants had varied degree of genetic difference from the mother plant as well as among themselves, except for LS7 and LS11, which reported identical RAPD profiles. The genetic similarity between the mother and the regenerated plant was high (average of 96%) and ranged between 99% (for LS7 and LS11) and 90% (for LS5). Evidently, LS5 had accumulated maximum genetic changes (as represented by presence of 7 non-maternal and absence of 5 maternal RAPD bands in this plant) and had lowest average coefficient of genetic similarity (0.89%) to other regenerated plants. The *in vitro* regeneration process was shown to be mainly responsible for the RAPD banding difference among the phenotypically different somaclones in earlier studies on peach and beet[6,7].

Results obtained here indicated induction of random changes in the genomic organization during differentiation of tomato plants from callus culture under given *in vitro* conditions, (viz. explant type, growth hormones, temperature and light conditions). The findings here are in line with the earlier reports on application of RAPD in describing genetic polymorphisms among regenerated plants in several other plants, viz. *Apium* species, and *Prunus* species[5,18]. RAPD analysis could also be useful for studying the genetic influence of different hormonal combinations during morphogenesis.

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