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Direct Organogenesis and Genetic Characterization of *Solanum pseudocapsicum* L. *in vitro* Regenerated Plants

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Abstract

Solanum pseudocapsicum L. is an unexplored plant of Solanaceae having horticultural and medicinal importance. Earlier the *in vitro* morphogenic potential of this plant species has not been explored. Induction of direct organogenesis was obtained from internodes, leaf bases and root explants from *in vitro* grown seedlings and plants in MS supplemented with different concentrations and combinations of BAP (0.44 - 8.88 μM/l) and IAA (0.29 - 2.85 μM/l). Direct organogenesis was achieved in 90% of internodes, 65% of leaf bases and 75% of root explants. The optimum regeneration medium was MS supplemented with 4.44 μM/l BAP and 1.43 μM/l IAA. The regeneration rate observed six weeks after culture was approximately 20, 9 and 12 shoot buds/explants from internodes, leaf bases and root explants, respectively. cDNA RAPD analysis from organogenic stages was carried out and revealed distinct gene expression pattern. *In vitro* rooting in 100% shoots was achieved on MS within ten days of culture. Successfully hardened rooted plants (R_0) on transfer to the field produced flowers, fruits and viable seeds (R_1). Genetic characterization of R_0 and R_1 plants revealed stability and uniformity at the chromosomal and genomic DNA level.

Introduction

Differentiation and re-differentiation of plant cells occur *in vivo* with ease while its replication *in vitro* still faces difficulties. Totipotency, a prerequisite for biotechnological improvement of crops is still either not attempted or poorly understood in many plant species. Some plant species are highly amenable under *in vitro* condition, while some others are moderate and many important

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crops are still considered recalcitrant. Influence of genotypes on *in vitro* growth of cultured tissues and their regeneration have been reported to vary from cultivar to cultivar and effects of genotype pose one of the greatest constraints to the tissue culture and micropropagation of plants (George and Deberg 2008, Trujillo-Moya et al. 2011). The ability to control the organogenesis *in vitro* is very important for different biotechnological applications (Phillips 2004). The family Solanaceae contains many important crops such as potato, tomato, brinjal, capsicum, as well as many well-known species with horticultural and medicinal values (Hawkes 1999). However, at the species and cultivar level all members of the Solanaceae are not *in vitro* friendly. Influence of genotypes is well-known and the genus *Capsicum* is still considered recalcitrant due to its unpredictable behaviour in *in vitro* condition.

Solanum pseudocapsicum L., popularly known as the winter cherry with horticultural and medicinal importance remains not so far investigated biotechnologically. Morphologically, the species shares some important characteristics with the genus *Capsicum.* The species grows in different parts of the world but it has no known cultivars. However, the species is important for its antimicrobial, antitumoral, antiviral, antispasmodic, antihypertensive, antioxidant and hepato-protective properties (Dhar et al. 1973, Vijayan et al. 2002, 2003, 2004, Badami et al. 2005, Aliero et al. 2006). Its major bioactives are solacasine, β-caryophylle, and o-methylsolanocapsine (Aliero et al. 2006).

Considering the above facts, the targeted objectives were to explore the *in vitro* organogenic competence of the species and subsequent studies at the molecular level. Our results have demonstrated its high *in vitro* organogenic competence, differential gene expression during organogenic development and genomic stability of the R_0 and R_1 plants.

Materials and Methods

Mature fruits of *Solanum pseudocapsicum* L. were collected from the Mungpoo hills of Darjeeling District (4500 ft above sea level), West Bengal, India during the month of October. After cleaning and following standard surface sterilization protocol**,** the isolated seeds (20 in number) were placed on MS for germination. Internode segments from 2 weeks old *in vitro* seedlings as well as leaf bases and root segments excised from *in vitro* grown plants were used as primary explants in our experiments. Voucher specimen of this species was identified from the Botanical Society of India (BSI), Howrah, West Bengal, India. MS was used for germination of seeds, while MS supplemented with different combinations and concentration of BAP (0.44 - 8.88 μM/l) and IAA (0.29 - 2.85 μM/l) with 3% (w/v) sucrose and 0.80% agar-agar were used to study its effect on *in vitro*

morphogenesis on different explants, elongation of shoots and regeneration of whole plants. The pH of culture media was adjusted between 5.6 and 5.8 before autoclaving at 17 lb pressure and 121°C for 17 min. All the aseptic cultures were incubated at 22 ± 2°C under a photoperiod of 16 hrs light/8 hrs dark cycles with a light intensity of 45 μE/m^2/s. Eight experimental sets were prepared and each set contained minimum 5 explants. All the experiments were repeated thrice. Elongated shoots 2 - 4 cm long were placed only on MS for induction of roots. Data regarding the frequency and number of roots induced per shoots were noted after every two weeks of culture. Hardening and transfer of healthy plantlets to the field were performed following the protocol of Dafadar et al. (2012).

The experiments were set up in a randomized design. Data were examined by ANOVA to detect significances between means. Means differing significantly were compared using DMRT at a 5% probability level. Variability of data has also been expressed as the mean ± standard error (SE).

Healthy root tips (1 cm long) from germinated seedlings of donor and regenerated (R_0) plants were pre-treated with PDB for over 5 hrs and fixed in methanol-acetic acid (3 : 1) and stored at –20°C. Enzymatic maceration and air-drying (EMA) and Giemsa staining of fixed roots were carried out basically following Fukui (1996) with required modifications (Jha and Yamamoto 2012). Chromosomes were re-stained with 0.1 mg/ml chromomycin A_3 (CMA). The slides were observed under a fluorescence microscope (Zeiss Axioscope 2) with normal and BV filter cassette and photographed using ProgRes® CapturePro 2.8.8 software. Gametic chromosomal analysis was carried out from flower buds of *in vitro* grown plants following the aceto-carmine staining method. Photographs were taken under Ziess photomicroscope using ProgRes Capture^R C_3 software.

Genomic DNA isolation was carried out from leaf tissue samples of donor and *in vitro* regenerated plants (R_0)**.** DNA isolation from 1 g tissues of each samples was performed following the protocol of Dafadar et al. (2012). The quality of DNA samples was estimated by spectrophotometer and by agarose gel (0.8%) electrophoresis.

DNA fingerprint profiles of donor and R_0 regenerants were compared to evaluate clonal fidelity and their genetic stability. DNA samples isolated from donor and three randomly selected R_0 regenerants were subjected to PCR to generate fingerprint profiles using a total of 30 random decamer primers obtained from OPERON Technologies, USA. PCR amplifications reactions were carried out in a total volume of 25 μl. Reaction mixture contained 1 × PCR buffer, 2 mM $MgCl_2$, 100 μM dNTP, 1U of Taq DNA polymerase (Fermentas, LIFE

SCIENCES), 1 μM decamer random primers (GeNei, Bengaluru, India) and 100 ng of template DNA. RAPD reactions were performed in a thermal cycler (BioRad) consisting of an initial hot start step for 2 min at 94°C followed by 45 cycles of denaturation for 1 min at 94ºC, annealing for 1 min at 35ºC and extension for 1 min at 72ºC. The final extension step was done at 72ºC of 10 min. The amplified products were resolved in 1.5% agarose gel and stained with ethidium bromide, using tris-borate EDTA (TBE) as electrophoresis buffer. The size of amplicons were calculated by EcoRI/HindIII double digested lambda DNA ladder and 100 bp DNA ladder (GeNei, Bengaluru, India) and were visualized under UV light and documented using the Gel Doc equipment (BioRad). RAPD analysis using each primer was repeated at least thrice, in order to establish the reproducibility of banding pattern of the DNA samples studied. Total RNA was isolated from different developmental stages (0 - 28 days) of internodal segments of *S. pseudocapsicum* during organogenesis following the protocol of Bandyopadhyay et al. (2013). The amplified DNA products were separated on 2% agarose gel.

Results and Discussion

Eighty per cent seed germination was recorded within two weeks of culture and seedlings attained a length of 1 - 4 cm on MS (Fig. 1A). Aseptic internodal segments of the germinated seedlings (1 cm long) were placed on basal medium as well as media supplemented with different combinations and concentrations of BAP (0.44 - 8.88 μM/l) and IAA (0.29 - 2.85 μM/l) to study the responses (Table 1). Observations were recorded from ten days onward up to 42 days (Table 1). No response was noted on MS. However, on all hormones containing media combinations the cut ends of 20 - 90% internodal segments responded and green shoot apical meristems were visible within 14 days of culture (Fig. 1B). Differentiations of variable numbers (2 - 15) of tiny shoot buds from the internode explants (Fig. 1B), root explants (Fig. 1C) and leaf base explants (Fig. 1D) were found almost in all the combinations of BAP and IAA (Table 1) within four weeks of culture. Maximum number of shoot buds (more than 20) per internodal segments were noted in explants placed on MS supplemented with 4.44 μM/l BAP and 1.43 μM/l IAA within 6 weeks of culture (Fig. 1E). The same experimental sets (Table 1) were extended for leaf base and root explants. Direct organogenesis was obtained in 65% leaf base and 75% root explants. Detailed results are presented in Table 1. Maximum number of shoot bud induction 9 and 12 explants was recorded from leaf bases and root explants on the same medium which induced highest number of shoot buds from internodal explants, i.e. MS 4.44 μM/l BAP and 1.43 μM/l IAA. Direct induction of shoot buds was observed

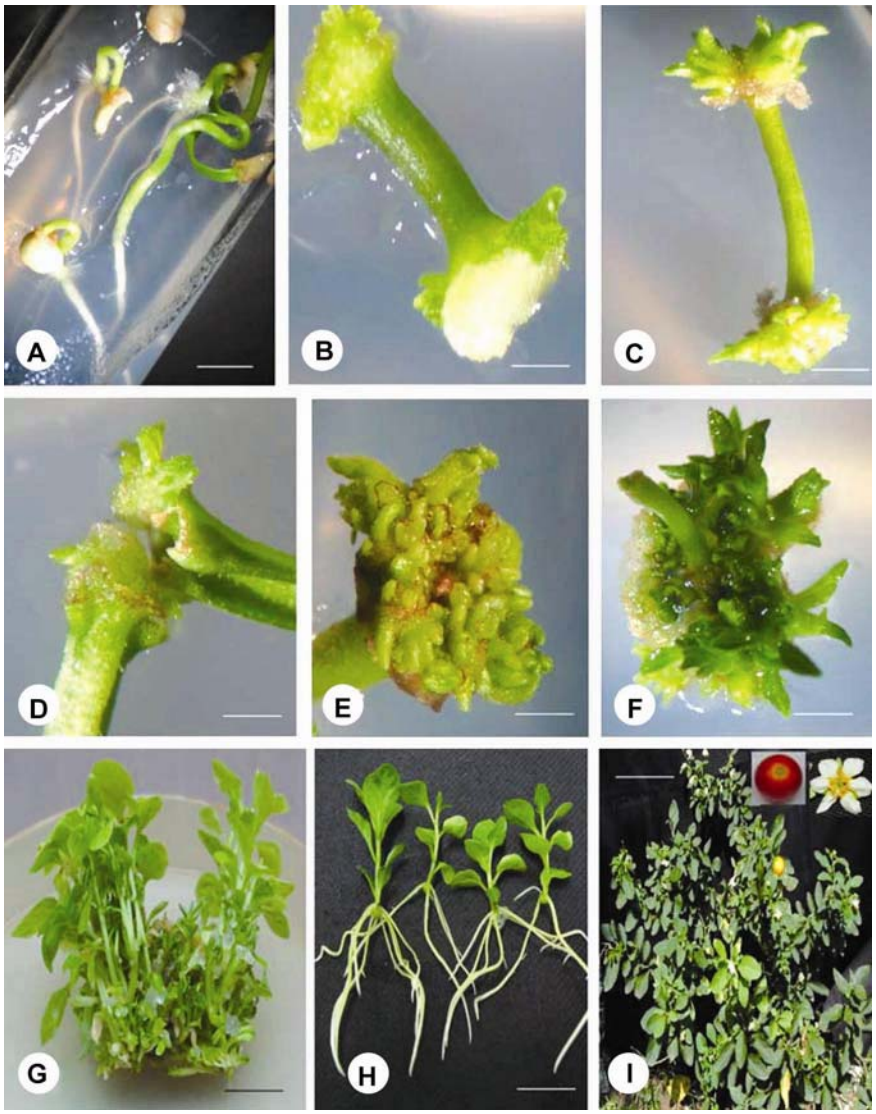

Fig. 1. A. *In vitro* germination of *Solanum pseudocapsicum* seeds. Scale bar = 0.5 cm. B. Direct induction of shoot buds on the internodal segment within two weeks of culture. Scale bar = 0.2 cm. C. Direct induction of shoot buds on the *in vitro* grown root explants within two weeks of culture. Scale bar = 0.2 cm. D. Direct induction of shoot buds on the *in vitro* grown leaf bases within two weeks of culture. Scale bar = 0.2 cm. E. Showing maximum (more than 20) shoot bud induction from a single internodal segment on MS with 4.44 μM BAP and 1.29 μM of IAA within six weeks of culture. Scale bar = 0.2 cm. F. Development of shoot buds on MS with 4.44 μM BAP and 1.29 μM of IAA. Scale bar = 0.2 cm. G. 10 - 12 week's old elongated shoots. Scale bar = 1 cm. H. Induction of healthy roots on MS. Scale bar = 1.2 cm. I. Hardened plant growing in the field with large number of flowers. Magnified view of a single flower and ripe fruit (Inset). Scale bar = 10 cm.

Table 1. Effect of BAP and IAA on direct induction of shoots from various explants of *S. pseudocapsicum*. MS with or without additives was used for all the sets.

Expt. sets	BAP (µM)	IAA (µM)	No. of explants showing shoot induction (%)			No. of shoots/ explants (6 weeks)±S.E			% of shoots elongated (up to 6 weeks)		
			Internodes	Leaf bases	Roots	Inter-nodes	Leaf bases	Roots	Internodes	Leaf bases	Roots
1	-	-	0	0	0	0	0	0	0	0	0
2	0.44	0.29	20.00 ± 0.58^{a}	11.50 ± 0.16^{a}	14.00 ± 0.81^{a}	2 ± 0.33^{a}	1 ± 0.56^{a}	1 ± 0.87^{b}	0.00	0.00	0.00
3	2.22	0.29	80.00 ± 2.31^{b}	50.01 ± 1.11^{b}	59.00 ± 1.22^{b}	6 ± 0.57^{b}	3 ± 0.85^{a}	4 ± 1.31^{b}	15.33 ± 0.33^{b}	2.20 ± 0.13^{a}	5.00 ± 1.21^{a}
4	3.33	0.29	81.00 ± 1.73^{b}	53.22 ± 1.02^{b}	63.50 ± 0.95^{b}	7 ± 0.33^{c}	4 ± 1.20^{b}	6 ± 1.33^{c}	16.00 ± 0.58^{b}	3.50 ± 1.22^{b}	8.32 ± 0.99^{b}
5	4.44	0.29	84.33 ± 1.20^{bc}	57.03 ± 0.85^{a}	65.00 ± 1.07^{a}	12 ± 0.33^{d}	4 ± 1.32^{c}	7 ± 0.68^{a}	17.67 ± 0.33^{c}	3.50 ± 1.27^{c}	8.85 ± 1.02^{a}
6	4.44	1.43	**90.60 ± 1.75^{b}**	**65.00 ± 0.75^{a}**	**75.00 ± 0.69^{b}**	**20 ± 0.33^{e}**	**9 ± 0.85^{b}**	**12 ± 0.59^{a}**	**21.00 ± 0.58^{c}**	**8.22 ± 1.21^{b}**	**16.00 ± 0.68^{c}**
7	4.44	2.85	89.33 ± 1.20^{cd}	62.55 ± 1.03^{c}	70.58 ± 1.23^{d}	16 ± 0.33^{e}	6 ± 1.21^{e}	8 ± 1.33^{a}	19.23 ± 0.33^{c}	4.00 ± 1.11^{d}	11.19 ± 0.54^{d}
8	8.88	1.43	85.00 ± 2.08^{d}	58.66 ± 1.66^{b}	68.44 ± 2.05^{d}	14 ± 0.33^{e}	5 ± 0.98^{c}	7 ± 1.65^{b}	15.67 ± 0.58^{c}	5.00 ± 0.65^{a}	13.55 ± 1.22^{d}

Each value represents the mean ± S.E. Means having different letters in superscript are significantly different from each other ($p \leq 0.05$), according to DMRT (Wang et al. 2009).

on all type of explants within two weeks of culture. However, after completion of six weeks it was noted that organogenic competence of roots and leaf bases was lesser than internodal segments (Fig. 1C, D, E; Table 1). Repeated experiments with the reproducible results led us to conclude that high organogenic competence exists in juvenile cells and tissues of *S. pseudocapsicum. In vitro* organogenesis in many important members Solanaceae were either influenced by their genotypes or not consistent and required high amounts of auxins and cytokinins (Sundari et al. 2010, Kothari et al. 2010). Whereas, the present report for the first time reveals that *in vitro* organogenesis in *S. pseudocapsicum* required relatively low amount of cytokinin and auxin not only during induction phase (Fig. 1 B - E), but also for their multiplication and elongation (Fig. 1 F,G). Even, we have achieved 100% root induction on MS (Fig. 1 H), within ten days of culture, and large number of field ready hardened plants were ready within 12 - 15 weeks time. R_0 plants showed strong adaptability and survival rate in the 40ºC of Kolkata. Small sized (1 ft) field grown plants started to produce flowers within 10 - 12 weeks after transferring them to the field and continued to flower (Fig. 1I) over a longer period of time (April - December).

To assess clonal fidelity we have studied somatic and gametic chromosomes of donor and R_0 plants for the first time in this species. Somatic chromosomal analysis was conducted by enzyme maceration and Giemsa staining not only to assess numerical stability but also to identify the heterochromatic regions within the individual chromosomes. Approximately 50 metaphase plates from the donor and R_0 plants were studied. Our results revealed 2n = 24 small chromosomes in donor and regenerated plants (Fig. 2A). Karyotype formula was determined as 9 m + 2 Sm + 1 Sm. Fluorescent banding with chromomycin A_3 (CMA), conducted in our studies for the first time revealed distinct CMA signals associated with the GC rich heterochromatic regions in 9 out of 12 chromosome pairs (Fig. 2B). Gametic chromosome analysis from R_0 plants revealed n = 12 bivalents without any irregularities (Fig. 2 C, D). Giemsa and CMA karyotypes are presented in Fig. 2E, F) and it shows similarity with a South American population of *S. pseudocapsicum* (Acosta et al. 2012). Our chromosomal analysis at the somatic and gametic level thus confirms numerical stability of the *in vitro* regenerated R_0 plants at the chromosome level.

RAPD profiles of the donor and R_0 regenerants were generated using 30 random primers (OPA-01 to OPA-20 and OPB-01 to OPB-10) of which 15 generated distinct, reproducible amplified products (Table 2). A total number of 100 amplification products were detected with an average of 6.67 bands per primer. The amplicons ranged in size from 0.31 kb (OPA-10 primer) to 1.75 kb

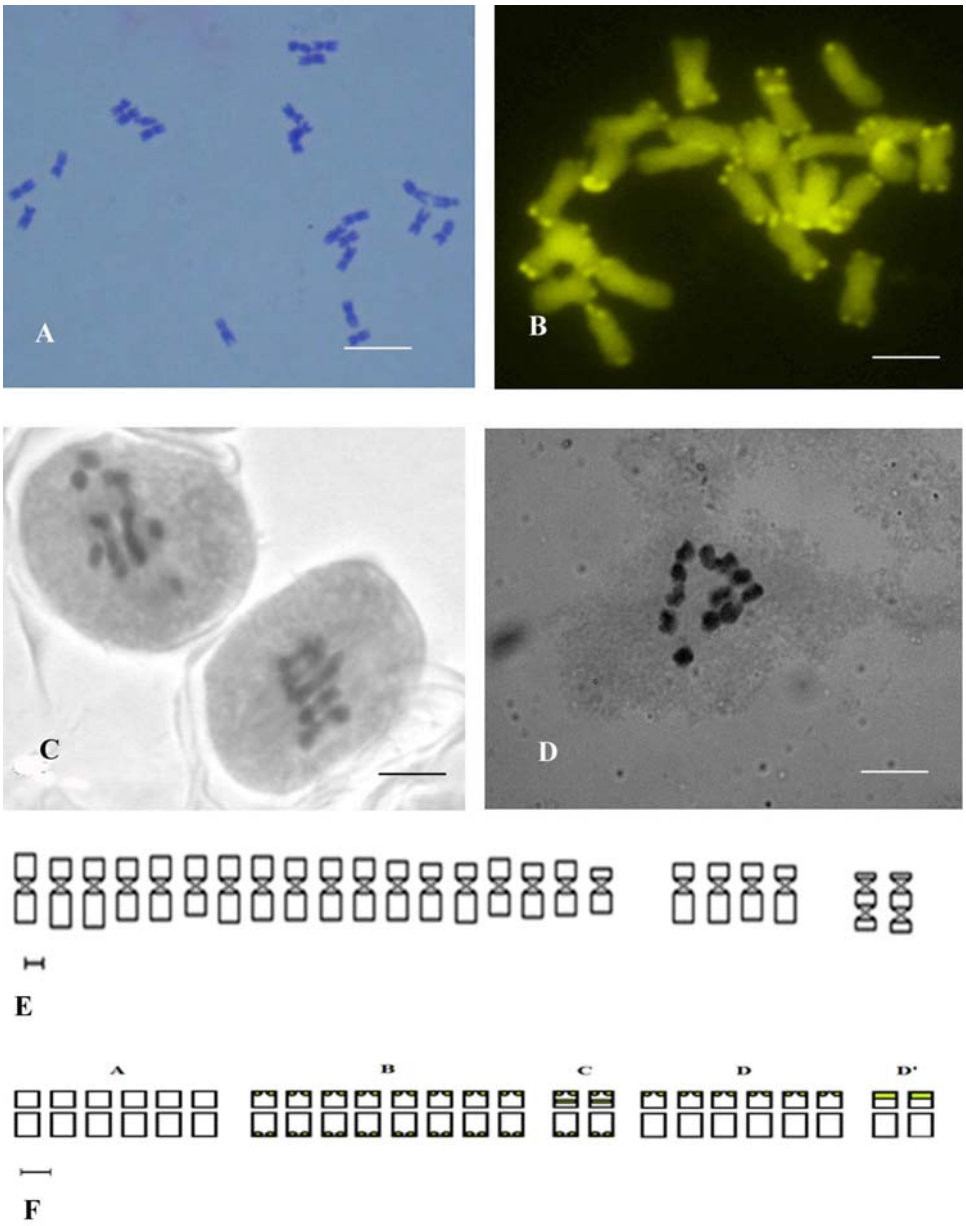

Fig. 2. A. Giemsa stained somatic chromosomes of *Solanum pseudocapsicum* showing 2n = 24 small chromosomes. Scale bar = 6 μm. B. Chromomycin A_3 (CMA) positive fluorescent banding with telomeric and interstial bands. Scale bar = 5 μm. C and D. Meiotic analysis showing normal n = 12 bivalents. Scale bar = 2.5 μm. E and F. Karyotype showing normal chromosomes morphology (E). Scale bar = 6 μm and CMA banding pattern (F). Scale bar = 5 μm.

(OPA-20 primer). The number of scoreable bands for each RAPD primer varied from 4 to 11. Fingerprint profiles of the culture regenerants (Fig. 3A, B) were monomorphic with respect to the mother plant. Monomorphic RAPD profiles

across 100 amplification products indicate homogeneity among the culture regenerants and genetic uniformity with that of the donor plant.

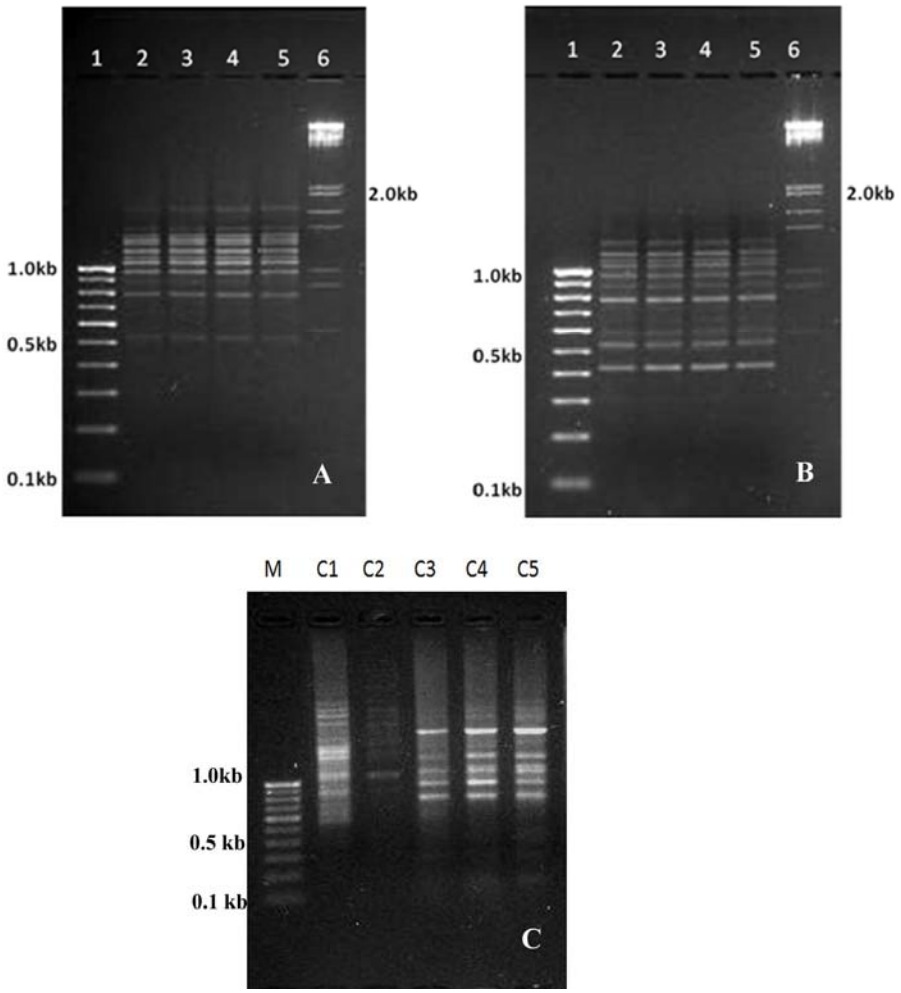

Fig. 3. A. RAPD profile of *S. pseudocapsicum* regenerated by the primer OPA 13 (5′-CAGCACCCAC-3′): Lane 1 - 100 bp DNA marker, lane 2- donor plant, lane 3 - 5 R_0 regenerants, lane 6- 1 kb DNA marker. B. RAPD profile regenerated by the primer OPA 18 (5′-TCTGTGCTGG-3′): Lane 1- 100 bp DNA marker, lane-2 donor plant, lane 3 - 5 R_0 regenerants, lane 6- 1 kb DNA marker. C. cDNA RAPD profile of samples (0–28 days) generated with primer OPA 2. Lanes correspond to M- 100 bp ladder, C1- and C2- day 0, C3- day 14, C4- day 21, C5- day 28 aged organogenic tissues.

RAPD technique applied to cDNA based templates can be useful to study patterns of gene expression unraveling the genetic basis of biological responses (Agarwal et al. 2008). To detect differentially expressed genes during

organogenesis, cDNA RAPD was performed using internodal explants aged 0, 14, 21 and 28 days of culture i.e., before and after induction of organogenesis. cDNA RAPD profiles were regenerated using 30 primers of which 9 primers (Table 3) generated distinct, scoreable amplicons. cDNA RAPD profile showed two distinct banding patterns in the sample tissues. Molecular expression of genes was almost similar in organogenic samples aged 14, 21 and 28 days**,** whereas in day 0 un-organogenic samples at the expression level was different (Fig**.** 3C). Five distinct scoreable bands were found in 14, 21 and 28 days old organogenic tissues, while 8 bands were found in day 0 internodal tissue samples. One monomorphic band (1 kb) was present in all samples, whereas one 800 bp band was present only in 14, 21 and 28 days old organogenic samples (Fig**.** 3C). Further genetic analysis may throw some light in the identification of organogenic genes in this species.

Table 2. Sequence of 15 RAPD primers used in the study.

Primer	Primer sequence	No. of scorable bands	Size range of amplified products (kb)
OPA-02	5′-TGCCGAGCTG-3′	8	0.58 - 0.91
OPA-03	5′-AGTCAGCCAC-3′	8	0.41 - 1.02
OPA-05	5′-AGGGGTCTTG-3′	8	0.58 - 1.30
OPA-07	5′-GAAACGGGTG-3′	5	0.56 - 1.10
OPA-08	5′-GTGACGTAGG-3′	6	0.46 - 1.42
OPA-09	5′-GGGTAACGCC-3′	5	0.33 - 1.15
OPA-10	5′- GTGATCGCAG-3′	4	0.31 - 1.15
OPA-13	5′-CAGCACCCAC-3′	11	0.52 - 1.65
OPA-14	5′-TCTGTGCTGG-3′	4	0.48 - 1.07
OPA-18	5′-TCTGTGCTGG-3′	11	0.48 - 1.07
OPA-20	5′-GTTGCGATCC-3′	10	0.54 - 1.75
OPB-01	5′-GTTTCGCTCC-3′	7	0.32 - 1.12
OPB-03	5′-CATCCCCCTG-3′	5	0.88 - 1.30
OPB-04	5′-GGACTGGAGT-3′	4	0.47 - 0.95
OPB-05	5′-TGCGCCCTTC-3′	4	0.53 - 0.92
Total	**15**	**100**	

Biotechnological investigation of *S. pseudocapsicum* in the present study has demonstrated high organogenic competence, stability of the genome in producing a large number of genetically uniform and fertile plants within 12 - 15 weeks, GC rich regions within the chromosomes and differential gene expression

during direct organogenic events. The results may be useful to understand molecular regulations of gene expression as an aid to future genetic improvement of crops.

Table 3. List of primers and their sequences used for cDNA RAPD analysis.

Sl.No.	Primer	Primer sequence
1	OPA1	5′-CAGGCCCTTC-3′
2	OPA2	5′-TGCCGAGCTG-3′
3	OPA4	5′-AATCGGGCTG-3′
4	OPA5	5′-AGGGGTCTTG-3′
5	OPA9	5′-GTGATCGCAG-3′
6	OPB2	5′-TGATCCCTGG-3′
7	OPB3	5′-TGATCCCTGC-3′
8	OPB4	5′-GGACTGGAGT-3′
9	OPB5	5′-TGCGCCCTTC-3′

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References

Acosta MC, Guerra M and **Moscone EA** (2012) Karyological relationships among some South American species of *Solanum* (Solanaceae) based on fluorochrome banding and nuclear DNA amount. Plant Syst. Evol. **298**: 1547-1556.

Agarwal M, Shrivastava N and **Padh H** (2008) Advances in molecular marker techniques and their application in plant sciences. Plant Cell Rep. **27**: 617-631.

Aliero AA, Grierson DS and **Afolyan AJ** (2006) Antifungal activity of *Solanum pseudocapsicum.* Research J**.** Bot. **1**(3): 129-133.

Bandyopadhyay S, Nandagopal K and **Jha TB** (2013) Characterization of RAM to SAM transitions in *Selaginella microphylla* grown *in vitro.* Biologia Plantarum **57** (3): 597-600.

Badami S, Prakash O, Dongre SH and **Suresh B** (2005) *In vitro* antioxidant properties of *Solanum pseudocapsicum* leaf extracts. Ind J. Pharmacol. **37**(4): 251-252

Dafadar A, Das A, Bandyopadhyay S and **Jha TB** (2012) *In vitro* propagation and molecular evaluation of a *Capsicum annuum* L. cultivar with high chromosome number (2n= 48). Sci Hortic. **140**: 119-124.

Dhar ML, Dhar MM, Dhawan BN, Srimal RC and **Tandon JC** (1973) Screening of Indian plants for biological activity. Ind. J. Exp. Biol. **11**: 43-54

Fukui K (1996) Plant Chromosome at mitosis. *In*: Plant Chromosome: Laboratory methods. (Fukui, K., and Nakayama, S. Eds.) Publication CRC Press, Florida. 1-17.

George EF and **Deberg PC** (2008) *In:* Plant propagation by tissue culture - The Background. 3rd edition (George, E, F., Hall, M., and Klerk, G.D. Eds.) Springer**,** The Netherlands. pp. 29-64.

Hawkes JG (1999) The economic importance of the family Solanaceae. *In:* Advances in Biology and Utilization, Volume IV. (Nee M, Symon DE, Lester RN, Jessop JP (Eds.) Publications Royal Botanic Gardens Kew, pp. 1-8.

Jha TB and **Yamamoto M** (2012) Application of EMA, fluorescent staining and FISH of rDNA in the analysis of *Aloe vera* (L.) Burm. f. chromosomes. Bull Fac. Agric Kagoshima Univ. **62**: 83-89.

Kothari SL, Joshi A, Kachhwaha S and **Ochoa-Alejo N** (2010) Chilli peppers - A review on tissue culture and transgenesis. Biotechnol. Adv. **28**: 35-48.

Phillips GC (2004) *In vitro* morphogenesis in plants - Recent advances. In Vitro Cell Dev Biol. Plant **40**: 342.345.

Sundari MS, Benniamin A and **Manickam VS** (2010) Micropropagation and *in vitro* flowering in *Solanum nigrum* L. A medicinal plant. Int. J. Biotechnol. **1**(1): 29-32.

Trujillo-Moya C, Gisbert C, Vilanova S and **Nuez F** (2011) Localization of QTLs for *in vitro* plant regeneration in tomato. BMC Plant Biol. **11**: 1-12.

Vijayan P, Kumar VS, Preethi V, Dhanaraj SA, Shrishailappa B and **Suresh B** (2002) *In vitro* cytotoxicity and anti-tumor properties of the total alkaloid fraction of unripe fruits of *Solanum pseudocapsicum.* Pharm. Biol. **40**(6): 456-460.

Vijayan P, Prashanth HC, Preethi V, Dhanaraj SA, Badami S and **Suresh B** (2003) Hepatoprotective effect of the total alkaloid fraction of *Solanum pseudocapsicum* leaves. Pharm. Biol. **41**: 443-448.

Vijayan P, Vijayaraj P, Setty PH, Hariharpura RC, Godavarthi A, Badami S, Arumugam DD and **Bhojraj S** (2004) The cytotoxic activity of the total alkaloids isolated from different parts of *Solanum pseudocapsicum*. Pharm. Bull. **7**(4): 528-530.