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RESEARCH ARTICLE

Efficient, one step and cultivar independent shoot organogenesis of potato

Amanpreet Kaur[1] · M. Sudhakara Reddy[1] · Anil Kumar[1]

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Abstract An efficient, one step and genotype independent protocol of shoot organogenesis was developed from leaf and internodal explants taken from microshoots of different cultivars of potato (*Solanum tuberosum* L.). Initially, microshoots were cultured on basal Murashige and Skoog medium additionally supplemented with 10 µM $AgNO_3$ (MS1 medium) to achieve healthy shoot growth required to get the quality explants. Shoot organogenesis was induced from both types of explants (leaf and internodal) on MS1 medium variously supplemented with 6-benzyladenine (BA) and gibberellic acid (GA_3). Maximum explants were induced shoot organogenesis on MS1 medium supplemented with 10 µM BA and 15.0 µM GA_3 from both the cultivars namely 'Kufri Chipsona 1' and 'Kufri Pukhraj'. Among the types of explants used, better response was observed from internodal segments as compared to leafs. This optimized medium combination was found to be equally effective for all the eight cultivars tested namely 'Kufri Pukhraj', 'Kufri Chipsona 1', 'Kufri Chipsona 2', 'Kufri Jyoti', 'Kufri Surya', 'Kufri Chandramukhi', 'Kufri Khyati' and 'Desiree'. The clonal uniformity of the regenerated shoots was confirmed using random amplified polymorphic DNA and inter-simple sequence repeats markers.

Keywords Clonal fidelity · ISSR · RAPD · Shoot organogenesis · Silver nitrate · *Solanum tuberosum*

✉ Anil Kumar
adatta@thapar.edu

[1] Department of Biotechnology, TIFAC-Centre of Relevance and Excellence in Agro and Industrial Biotechnology (CORE), Thapar University, Patiala 147001, India

Introduction

India is the fourth largest producer of potato (*Solanum tuberosum* L., family Solanaceae), the most important *rabi* vegetable crop (Bansal and Trehan 2011; Solomon and Baker 2001). It is conventionally propagated vegetatively using tubers from previous year crop (Simmonds 1997). The propagules get infected by viruses and other pathogens during repeated cycles of conventional vegetative propagation, thus, limiting the lifespan of superior cultivars (Simmonds 1997). In vitro propagation offers the advantage to meet such challenges (JayaSree et al. 2001; Le Roux and Van Staden 1991; Lokossou et al. 2009). However, on basal MS medium, shoot cultures of potato were reported to show stunted growth with rudimentary leaves (Joyce and Cassels 2002; Turhan 2004; Sarkar et al. 1997). Thus the improvement of shoot growth with normal expanded leafs is important for obtaining aseptic explants for regeneration and other biotechnological interventions for the trait specific improvement.

Shoot organogenesis is an effective mode of in vitro regeneration, besides its utility for undertaking trait specific improvement programme. After the first report of shoot organogenesis from potato tuber discs (Lam 1975), many workers reported shoot organogenesis using different explants (Lentini et al. 1990; Ooms et al. 1987; Webb et al. 1983; Yee et al. 2001) and protoplasts (Haberlach et al. 1985). However, most of the reported protocols are cultivar-specific (Al-Hussaini et al. 2015; Mani et al. 2014; Pal et al. 2011) and there is a need to develop a plant regeneration protocol that is effective across the cultivars (Saker et al. 2012; Wheeler et al. 1985). Moreover, the majority of reported protocols are complicated by the requirement of two stage sequential culture on different media combinations with an elaborated emphasis on the requirement of

zeatin or zeatin riboside as essential cytokinin for potato shoot organogenesis (JayaSree et al. 2001; Laboney et al. 2013; Sharma and Millam 2004; Yee et al. 2001). Thus, a simple one step protocol of shoot organogenesis is likely to benefit a trait specific improvement programme and commercial micropropagation.

Therefore, the present study was taken up with an objective to develop an efficient, one step, and cultivar independent shoot organogenesis protocol and to subsequently improve shoot growth of potato. The use of BA as a sole source of cytokinin will also help reducing operational cost of in vitro propagation of potato. The regenerated plants were tested for clonal uniformity and also evaluated for the production of minitubers.

Materials and methods

Plant material, chemicals and culture conditions

Cultures of *S. tuberosum* L. cvs. 'Kufri Pukhraj', 'Kufri Chipsona 1', 'Kufri Chipsona 2', 'Kufri Jyoti', 'Kufri Surya', 'Kufri Chandramukhi', 'Kufri Khyati' and 'Desiree' were available at plant tissue culture facility at TIFAC-CORE, Thapar University, Patiala (India). All these cultivars are high yielding and recommended for different agroclimatic zones of India. The cultivars 'Kufri Chipsona 1 and 2' are processing grade cultivars used for making chips and French fries. All tissue culture grade chemicals including plant growth regulators (PGRs) were purchased from HiMedia laboratories (Mumbai, India). Cultures were maintained on Murashige and Skoog medium (Murashige and Skoog 1962) containing sucrose (3%, w/v) and agar (0.7%, w/v) (basal MS medium) in 300 ml culture bottles (Kasablanka, Mumbai) and were sub-cultured at regular interval of 21 days. All cultures were incubated at 25 ± 1 °C under the light intensity of 42 µmol m^{-2} s^{-1} provided by cool fluorescent lamps (Phillips, Mumbai, India) in a photoperiod regime of 16-h light and 8-h dark cycle.

Effect of silver nitrate

The shoot cultures maintained on basal MS medium were highly stunted and possessed rudimentary leaves. To achieve normal shoot growth and leaf expansion, microshoots of cvs. 'Kufri Pukhraj', and 'Kufri Chipsona 1' were sub-cultured on MS medium fortified with different concentrations of silver nitrate ($AgNO_3$; 0–20 µM). Shoot length, the number of leaves, leaf size and so forth were recorded after 21 days of culture. Best silver nitrate concentration was also tested in remaining six cultivars ('Kufri Chipsona 2', 'Kufri Jyoti', 'Kufri Surya', 'Kufri Chandramukhi', 'Kufri Khyati' and 'Desiree') under study.

Shoot organogenesis

Internode and leaf explants were obtained from cultures growing on basal MS medium containing 10 µM $AgNO_3$ (MS1 medium). Fully expanded leafs (3rd–5th leaf) and internodes from 21 days old cultures of both the cultivars ('Kufri Pukhraj' and 'Kufri Chipsona 1') were excised and used as explants. For shoot organogenesis, leafs and internodal segments were cultured on MS1 medium variously supplemented with N^6-benzyladenine (BA; 0–15 µM) and gibberellic acid (GA_3; 0–20 µM). Cultures were maintained on the same medium for 10 weeks. The appropriate care was taken while culturing the leaves that their adaxial surface was facing the medium. Optimum medium combination was also tested for shoot organogenesis from both the explants in all other cultivars of potato used in this study ('Kufri Chipsona 2', 'Kufri Jyoti', 'Kufri Surya', 'Kufri Chandramukhi', 'Kufri Khyati' and 'Desiree').

Clonal fidelity of regenerated plants

PCR based random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers were used to study clonal fidelity of regenerated plants. The plants regenerated through shoot organogenesis were grown in pots separately. Plants were selected randomly for isolation of DNA by CTAB method (Doyle and Doyle 1990). PCR amplifications using 40 primers of RAPD and 20 primers of ISSR were carried out (Table 1) as described by Bansal et al. (2014). The PCR amplification conditions were initial denaturation for 4 min at 94 °C followed by 41 cycles of denaturation at 94 °C for 1 min, annealing at 40 °C for 45 s for RAPD and 55 °C for 45 s for ISSR, primer extension at 72 °C for 1.30 min. Final extension was carried out at 72 °C for 5 min. Amplified products were separated on 1.2% (w/v) agarose gel, stained with ethidium bromide and visualised under UV trans-illuminator (Gel Doc Mega; Biosystematica, USA).

Acclimatization and minituber production

Micropropagated plants were initially transferred to the polythene bags and kept in a polyhouse with high relative humidity (>90%) for 10 days. These were then planted in tunnels protected with wire mesh and irrigated with microsprinklers. Plants were grown for 100 days and dehaulmed to develop the skin of tubers. After 15 days of dehaulming the tubers were harvested to record the yield.

Table 1 The details of the primers used for RAPD and ISSR amplifications from regenerated plants of cvs. 'Kufri Pukhraj' (KP) and 'Kufri Chipsona 1' (KC1) and number of bands amplified with each primer

S. no.	Primer	Primer sequence	No. of bands		S. no.	Primer	Primer sequence	No. of bands	
	RAPD		KP	KC1				KP	KC1
1	RAPD1	AGCGCCATTG	10	9	27	RAPD32	TCGGCGATAG	3	2
2	RAPD2	CTTCCCCAAG	–	3	28	RAPD33	CAGCACCCAC	3	1
3	RAPD3	AGGGCGTAAG	5	4	29	RAPD34	TCTGTGCTGG	2	1
4	RAPD4	CTGGGGGACT	–	5	30	RAPD35	TTCCGAACCC	–	3
5	RAPD 5	ACCGCGAAGG	3	–	31	RAPD36	AGCCAGCGAA	–	4
6	RAPD 6	GGACCCAACC	4	3	32	RAPD37	GACCGCTTGT	–	3
7	RAPD 7	GTCGCCGTCA	3	–		Total number of bands		86	75
8	RAPD 8	TCTGGTGAGG	4	3		ISSR			
9	RAPD 9	TGAGCGGACA	4	2	1	ISSR-4	$(AC)_8(GC)_2$	–	4
10	RAPD11	TTGCACGGG	1	5	2	ISSR-5	$(AC)_8$	–	4
11	RAPD12	GTGTGCCCCA	4	2	3	ISSR-6	$(CA)_8TG$	4	3
12	RAPD 13	CTCTGGAGAC	1	4	4	ISSR-8	$(GA)_8TA$	5	–
13	RAPD15	GGGGTGACGA	3	–	5	ISSR-9	$(GC)_8T$	3	–
14	RAPD18	GAGAGCCAAC	4	–	6	ISSR-10	$(GC)_8A$	3	–
15	RAPD20	ACCCGGTCAC	4	–	7	ISSR-11	$(GC)_8AT$	6	–
16	RAPD21	CAGGCCCTTC	4	3	8	ISSR-13	$(CT)_8A$	–	4
17	RAPD22	TGCCGAGCTG	3	2	9	ISSR-15	$(GT)_8A$	–	1
18	RAPD23	AGTCAGCCAC	3	3	10	ISSR-16	$(GT)_8C$	–	1
19	RAPD24	AATCGGGCTG	1	3	11	ISSR-17	$(AT)_8C$	–	1
20	RAPD25	AGGGGTCTTG	1	3	12	ISSR-19	$(AT)_8GC$	3	–
21	RAPD26	GGTCCCTGAC	1	2	13	ISSR-20	$(AT)_8$	5	4
22	RAPD27	GAAACGGGTG	1	2	14	ISSR-21	$(GA)_8TG$	6	4
23	RAPD28	GTGACGTAGG	3	–	15	ISSR-22	$(GA)_8C$	6	3
24	RAPD29	GGGTAACGCC	4	1	16	ISSR-23	$(GA)_8CT$	3	3
25	RAPD30	GTGATCGCAG	4	1	17	ISSR-24	$(GA)_8CA$	3	2
26	RAPD31	CAATCGCCGT	3	1	18	ISSR-25	$(GA)_8CC$	5	5
						Total number of bands		52	39

The size of amplified fragments varied from 500 to 2500 bp

Statistical analysis

Each experiment consisted of five replicates with five explants in each replicate and experiments were repeated three times. Data were transformed into log values and analysed by analysis of variance (ANOVA) and means were compared using Duncan's multiple range test (DMRT) or by unpaired *t* test at $P \leq 0.05$ using GraphPad Prism 5 software (GraphPad, San Diego, CA, USA).

Result

The shoot cultures of all the cultivars of *S. tuberosum* maintained on basal MS medium showed stunted growth with rudimentary leaves. When microshoots of cvs. 'Kufri Pukhraj' and 'Kufri Chipsona 1' were cultured on MS medium containing different concentrations of $AgNO_3$ (0–20 μM), improved shoot growth and expansion of leafs was observed. Optimum growth and leaf expansion was observed on basal MS medium fortified with 10.0 μM $AgNO_3$ (MS1 medium; Table 2). When other cultivars namely 'Kufri Chipsona 2', 'Kufri Jyoti', 'Kufri Surya', 'Kufri Chandramukhi', 'Kufri Khyati' and 'Desiree' were subcultured on MS1 medium normal shoot growth with leaf expansion was observed (Figs. 1, 2a). Subsequently, the shoots were maintained on MS1 medium and all explants for subsequent experiments were taken from the microshoots growing on MS1 medium.

Shoot organogenesis

Initially, the medium for shoot organogenesis was optimized from fully expanded (3rd–5th) leafs and internodal segments

Table 2 The effect of silver nitrate concentrations in the basal MS medium on growth and leaf expansion of microshoots of *S. tuberosum* cvs. 'Kufri Pukhraj' (KP) and 'Kufri Chipsona 1' (KC1)

Conc. of silver nitrate (μM)	Mean number of leaves per shoot		Mean length of shoots (cm)		Size of leaves	
	KP	KC1	KP	KC1	KP	KC1
0	$0.00^{c,A}$	$0.00^{c,A}$	$3.88^{a,A}$	$4.60^{a,A}$	–	–
5	$4.22^{b,B}$	$7.77^{a,A}$	$2.49^{b,A}$	$3.27^{b,A}$	+	+
10	$4.10^{b,A}$	$5.88^{b,A}$	$2.57^{b,A}$	$2.44^{b,A}$	++	++
15	$6.77^{a,A}$	$5.77^{b,A}$	$2.77^{ab,A}$	$2.22^{b,A}$	+++	+++
20	$6.22^{a,A}$	$6.44^{ab,A}$	$1.65^{b,A}$	$2.83^{b,A}$	+++	+++

Data were recorded after 21 days of incubation and analysed by ANOVA. Mean values followed by same lowercase letters within columns are not significant at $P < 0.05$ by DMRT. Means between cultivars were compared by unpaired t-test and those which are significant at $P < 0.05$ are marked with different uppercase letters. Each '+' sign is approximately equal to 0.5 cm

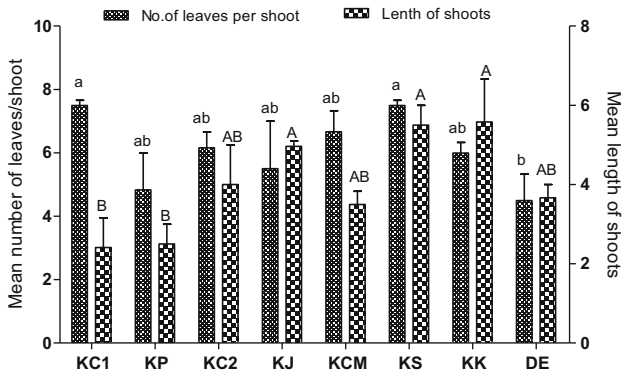

Fig. 1 The response of eight important cultivars of potato ['Kufri Pukhraj' (KP), 'Kufri Chipsona 1' (KC1), 'Kufri Chipsona 2'(KC2), 'Kufri Jyoti' (KJ), 'Kufri Chandramukhi' (KCM), 'Kufri Surya' (KS), 'Kufri Khyati' (KK) and 'Desiree' (DE)] on MS1 medium (basal MS medium containing 10 μM $AgNO_3$). Data were recorded after 30 days of culture. Means denoted by *same lower case letter* (number of leaves per shoot) and *upper case letter* (length of shoots) are not significant at $P = 0.05$ by DMRT

taken from cultivars namely 'Kufri Pukhraj' and 'Kufri Chipsona 1' and best shoot organogenesis was observed on MS1 medium variously supplemented with BA (0–1.5 μM) and GA_3 (0–20 μM). Depending on the medium composition, nodular structures were observed from both types of explant (Fig. 2b, c). Shoot organogenesis was observed only on MS1 medium supplemented with both BA and GA_3 (Table 3; Fig. 2d, e). In both the cultivars, maximum number of explants (both leaf and inter node) differentiated shoots on medium supplemented with 10 μM BA and 15 μM GA_3 (Table 3). Response from leaf explants was higher in case of 'Kufri Pukhraj', whereas from nodal segments better response was recorded in case of cultivar 'Kufri Chipsona 1'. The frequency of shoot organogenesis was significantly higher in internodal explants as compared to leaf explants. Internodal explants also showed early shoot organogenesis (within 3 weeks of culture), whereas leaf explants took longer to differentiate shoots (about 5 weeks to initiate shoot organogenesis). It was interesting to note that none of the leaf explant of cv. 'Kufri Pukhraj' showed shoot organogenesis on medium lacking GA_3. When optimized medium was tested for all the eight cultivars using both the types of explants, a similar response pattern was observed with higher response from internodal explants as compared to leaf explants. Maximum response among all the cultivars from internodal segments was observed in 'Kufri Chipsona 1' and in case of leaf explants maximum response was observed in cultivar 'Kufri Khyati' (Fig. 3). The shoots rooted on MS1 medium were successfully acclimatized (Fig. 2f) with more than 90% survival and plants were transferred to field (Fig. 2g) for the production of minitubers. Minituber yield from the plants is depicted in Table 4. The average yield of tubers was 26.6 g per plant in case of 'Kufri Pukhraj' and 24.1 g per plant in case of 'Kufri Chipsona 1'. The number of tubers of >10 g was more in case of 'Kufri Pukhraj' than 'Kufri Chipsona 1' (Table 4).

Clonal fidelity

In the present study shoot organogenesis was achieved from leaf and internode explants of eight cultivars of potato. Therefore, it was important to establish the clonal fidelity of the regenerated shoots vis-à-vis mother cultures. Out of total 60 primers tested (40 RAPD and 20 ISSR); amplification of DNA fragments was observed in 26 RAPD and 13 ISSR primers (Table 1). All the markers (both RAPD and ISSR) scored (independently for two cultivars namely 'Kufri Pukhraj' and 'Kufri Chipsona 1') were monomorphic (Fig. 4a–d). Maximum of 10 bands (for RAPD1 primer) and minimum of 1 band (for many primers) were scored (Table 1) and size of amplified fragments ranged from 200 bp to 2000 bp. In the case of cultivar 'Kufri Pukhraj', a total of 138 markers were scored (86 for RAPD and 52 for ISSR), whereas in the case of

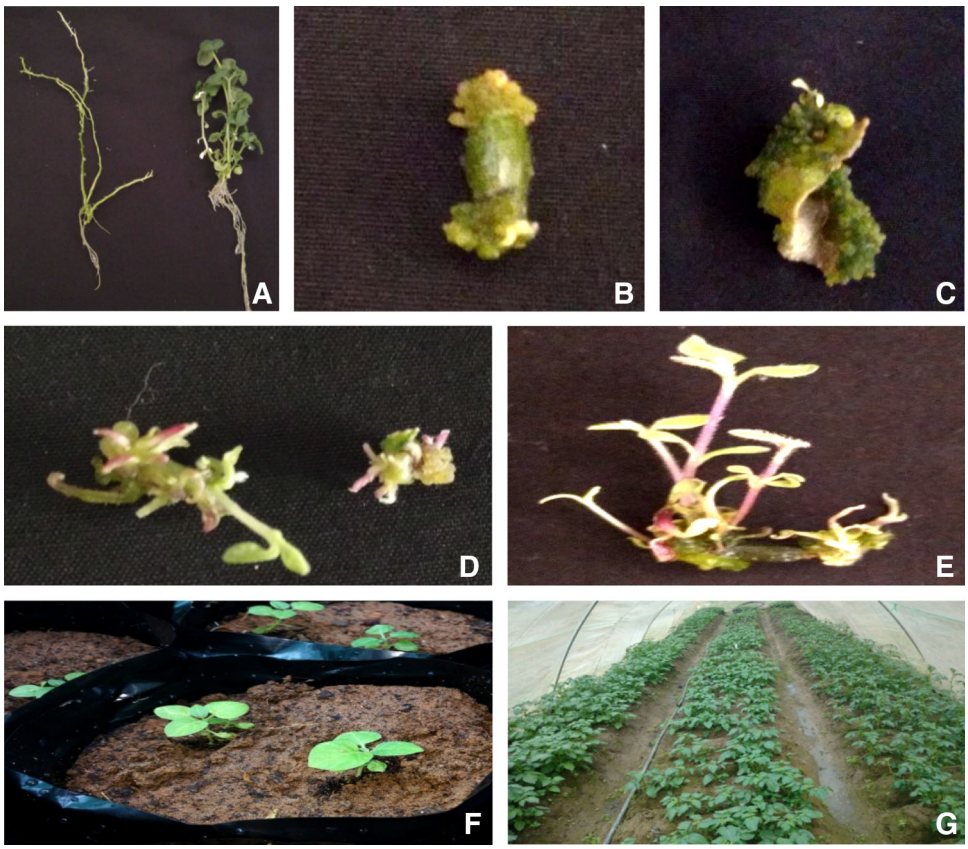

Fig. 2 Shoot organogenesis in *S. tuberosum* **a** shoot from cultures growing on basal MS medium (*left*) and on MS containing 10 μM $AgNO_3$ (MS1) medium (*right*); **b**, **c** explants showing nodular callus on MS1 medium supplemented with 10 μM BA and 15 μM GA_3, **b** internodal segments and **c** leaf; **d**, **e** actively growing shoots from the explants (**d** cultivar 'Desiree'; **e** cultivar 'Kufri Chipsona1'); **f** plants transferred to pots for acclimatization; **g** actively growing plants in field

cultivar 'Kufri Chipsona 1' 114 markers were scored (75 for RAPD and 39 for ISSR). All the regenerated plants were found to be true-to-type to that of mother cultures.

Discussion

In this study, one step shoot organogenesis protocol have been developed for two important Indian cultivars of *S. tuberosum* namely, 'Kufri Pukhraj' and 'Kufri Chipsona1' and tested for six more cultivars (total eight) to validate the efficacy of the protocol across the cultivars. In this protocol, BA was used as sole source of cytokinin for shoot organogenesis from nodal and leaf explants. Earlier, majority of workers reported the requirement of zeatin or zeatin riboside for shoot organogenesis in potato (Cearley and Bolyard 1997; Molla et al. 2011; Zel and Medved 1999). Since shoots were regenerated through organogenesis, the regenerated plants were also tested for the clonal fidelity. An efficient regeneration protocol (through shoot organogenesis and/or somatic embryogenesis) is also a prerequisite to undertake trait specific improvement programme (Aggarwal et al. 2010; Tavazza et al. 1988). Earlier, there are reports on shoot organogenesis in potato (Cearley and Bolyard 1997; Hansen et al. 1999; Yee et al. 2001) and all these studies reported a complicated two step protocol, which is a bottle neck in undertaking an efficient trait specific improvement programme of potato. Moreover, genotype specific response of potato cultivars to plant growth regulators (PGRs) has also been reported by many researchers (Khokan et al. 2009; Pal et al. 2011; Wheeler et al. 1985). Therefore, one step and cultivar independent shoot organogenesis protocol using BA as sole cytokinin reported in this study is likely to be useful in potato improvement and also commercial propagation.

Microshoots cultured on basal MS medium showed stunted growth and have rudimentary leaves, which posed problems with the availability of quality explants for the development of regeneration protocols. Such problems have earlier been attributed to the accumulation of ethylene in the cultures of potato (Saltveit 2005) and incorporation of $AgNO_3$ (an inhibitor of ethylene action) into the medium resulted in normal shoot growth and leaf expansion. Earlier, silver ions have been reported to influence the growth of potato microshoots (Sandra and Maira 2013; Turhan 2004). Further, shoot organogenesis was achieved only on MS medium containing 10.0 μM $AgNO_3$ (MS1 medium) and on basal MS medium, shoot organogenesis was not observed in any of the media combinations tested (data not shown) emphasizing the requirement of $AgNO_3$ for normal shoot growth and shoot organogenesis in a kind of sealed culture vessels used in plant tissue culture studies.

Table 3 The effect of plant growth regulators in MS1 medium on shoot organogenesis from leaf and internodal explants of *S. tuberosum* L. cv. 'Kufri Chipsona 1' and 'Kufri Pukhraj'

PGR conc		Percent explants showing shoot organogenesis		Mean number of shoots regenerated per explant		Percent explants showing shoot organogenesis		Mean number of shoots regenerated per explant	
BA	GA_3	'Kufri Pukhraj'				'Kufri Chipsona 1'			
		Leaf	Internode	Leaf	Internode	Leaf	Internode	Leaf	Internode
0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0	$0.00^{d,B}$	$33.33^{cd,B}$	$0.00^{d,B}$	$4.00^{ef,A}$	$26.66^{ab,A}$	$46.55^{bc,A}$	$2.83^{abc,A*}$	$3.33^{cde,A*}$
5	5	$13.33^{b,A}$	$66.66^{a,A}$	$17.22^{a,A*}$	$25.33^{a,A*}$	$23.33^{ab,A}$	$66.77^{ab,A}$	$1.00^{de,B*}$	$2.78^{de,B*}$
5	10	$0.00^{d,B}$	$29.99^{d,B}$	$0.00^{d,B}$	$29.22^{a,A}$	$20.22^{ab,A}$	$49.88^{abc,A}$	$2.83^{abc,A}$	$6.88^{ab,B}$
5	15	$19.99^{b,A}$	$39.99^{bc,A}$	$7.16^{b,A*}$	$13.22^{bc,A*}$	$4.44^{c,B}$	$51.46^{abc,A}$	$2.50^{abcd,B*}$	$3.77^{cde,B*}$
5	20	$13.33^{b,A}$	$46.66^{b,A}$	$3.33^{c,A}$	$12.88^{bc,A}$	$20.22^{ab,A}$	$33.33^{cd,B}$	$3.50^{abc,A*}$	$5.50a^{bcd,B*}$
10	0	$0.00^{d,B}$	$0.00^{g,B}$	$0.00^{d,B*}$	$0.00^{f,B*}$	$13.33^{b,A*}$	$4.44^{g,A*}$	$3.50^{abc,A*}$	$3.00^{de,A*}$
10	5	$6.66^{c,A}$	$39.99^{bc,A}$	$3.33^{c,A}$	$13.33^{bc,A}$	$6.66^{c,A}$	$16.66e^{f,B}$	$4.00^{a,A*}$	$5.33^{abcd,B*}$
10	10	$13.33^{b,A}$	$59.99^{a,A}$	$5.00^{bc,A}$	$13.44^{bc,A}$	$13.44^{b,A}$	$45.00^{bc,B}$	$2.33^{bcd,A*}$	$3.88^{bcde,B*}$
10	15	$49.99^{a,A}$	$73.33^{a,A}$	$6.88^{b,A*}$	$8.10^{cde,A*}$	$35.22^{a,A}$	$83.66^{a,A}$	$1.00^{de,B}$	$4.25^{bcde,A}$
10	20	$33.33^{a,A*}$	$29.99^{d,B*}$	$3.33^{c,A*}$	$7.33^{de,A*}$	$14.44^{b,B}$	$64.99^{ab,A}$	$2.00^{cd,A}$	$5.66^{abcd,A}$
15	0	$0.00^{d,A}$	$13.33^{f,A}$	$0.00^{d,A*}$	$3.00^{ef,A*}$	$0.00^{d,A}$	$20.22^{def,A}$	$0.00^{e,A}$	$2.16^{de,A}$
15	5	$16.66^{b,A}$	$39.99^{bc,A}$	$6.88^{b,A*}$	$6.66^{de,A*}$	$6.66^{c,A}$	$29.99^{cd,A}$	$1.00^{de,B}$	$6.33^{abc,A}$
15	10	$6.66^{c,B}$	$33.33^{cd,A}$	$2.22^{cd,A}$	$11.55^{bcd,A}$	$20.22^{ab,A*}$	$13.33^{f,B*}$	$1.33^{de,A}$	$8.16^{a,A}$
15	15	$0.00^{d,B}$	$16.66^{e,B}$	$0.00^{d,B}$	$15.33^{b,A}$	$20.22^{ab,A*}$	$30.00^{cde,A*}$	$3.16^{abc,A}$	$5.77^{abcd,B}$
15	20	$19.99^{b,B}$	$0.00^{g,B}$	$4.33^{bc,A*}$	$0.00^{f,B*}$	$39.77^{a,A*}$	$35.55^{cd,A*}$	$3.83^{ab,A*}$	$5.33^{abcd,A*}$

Data were recorded after 6 weeks of incubation and analysed by ANOVA. Mean were compared by DMRT within columns and values followed by same lowercase letter are not significant within at $P < 0.05$. Means between cultivars and explants were compared by unpaired t-test and those which were insignificant at $P < 0.05$ were donated with same uppercase letters in case of cultivars and with '*' signs in case of explants

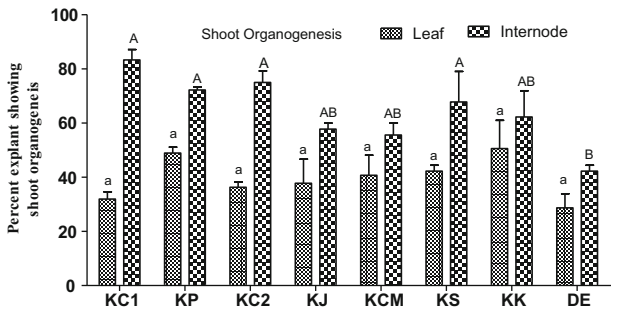

Fig. 3 Shoot organogenesis response from leaf and internodal explants taken from eight cultivars of potato ['Kufri Pukhraj' (KP), 'Kufri Chipsona 1' (KC1), 'Kufri Chipsona 2'(KC2), 'Kufri Jyoti' (KJ), 'Kufri Chandramukhi' (KCM), 'Kufri Surya' (KS), 'Kufri Khyati' (KK) and 'Desiree' (DE)] on MS1 medium supplemented with 10 μM BA and 15 μM GA_3. Data were recorded after 40 days of inoculation. Means denoted by *same lower case letter* (leaf explants) and *upper case letter* (internodal explant) are not significant at $P = 0.05$ by DMRT

Earlier, most of the studies emphasized the requirement of zeatin or zeatin riboside for shoot organogenesis (Cearley and Bolyard 1997; Molla et al. 2011; Zel and Medved 1999), yet in the present study shoot organogenesis was achieved using BA as sole source of cytokinin. Further, one-step shoot organogenesis protocol reported in this study is simple and cost effective for propagation and trait specific improvement. Moreover, it was observed that in case of leaf explants of cultivar 'Kufri Pukhraj', incorporation of GA_3 was also essential for shoot organogenesis as no shoot organogenesis was observed in any of the combination lacking GA_3. The requirement of GA_3 along with zeatin for shoot induction in potato has also been reported earlier (Hansen et al. 1999). Although, in this study clonal and explant specific response was observed, yet the medium formulation worked out for the optimum response was effective for both the explants (leafs and internodes) and also for all the other cultivars tested, thus ascertaining the efficacy of this protocol across the explants and cultivars tested.

Amongst two explants used, shoot organogenesis was higher from internodal segments in all the cultivars. The effect of explant on shoot organogenesis of potato has been reported earlier by some workers (Sabeti et al. 2013; Seabrook and Douglass 2001; Yee et al. 2001). The present findings are in line with the earlier reports of Sabeti et al. (2013), who also reported better response from internodal explants over other explants such as leaf and minitubers for regeneration of plants. However, there are also reports

Table 4 The performance of in vitro propagated plants in the field with respect to number ans size of minitubers produced

Cultivar	Number of tubers per plant (mean ± SE)	Number of tubers in different weight ranges (mean ± SE)		
		0–5 g	5–10 g	≥10 g
Kufri Pukhraj	26.6 ± 2.66	18.6 ± 2.05	1.8 ± 0.51	6.1 ± 1.29
Kufri Chipsona 1	24.10 ± 2.74	18.4 ± 2.41	3.1 ± 0.50	2.6 ± 0.40

The plants were acclimatized in poly house and transferred to tunnels (for protected cultivation) and grown for 100 days. 100 plants of each cultivars were planted and data were recorded by randomly selecting 15 plants of each cultivar

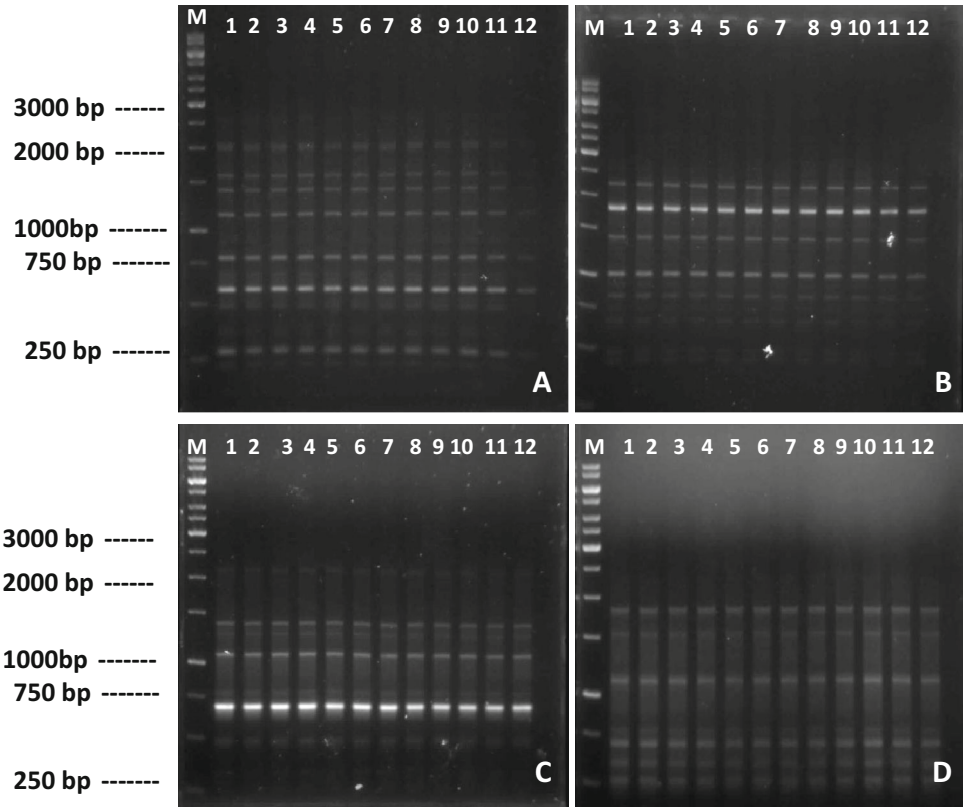

Fig. 4 Amplification profiles of RAPD (**a**, **b**) and ISSR (**c**, **d**) markers of regenerated plants from differentiated shoots of cultivars 'Kufri Pukhraj' (**a**, **c**) and 'Kufri Chipsona 1' (**b**, **d**). RAPD amplifications (**a**, **b**) were carried out using primer RAPD-1 and ISSR amplifications (**c**, **d**) were carried out using primer ISSR-25. Lanes *1* mother culture; and lane *2–12* plant grown from regenerated shoots; *M* 1-kb molecular-weight markers

highlighting the better response from leaf explants as compared to internodal explants (Ali et al. 2007; Shirin et al. 2007). The explant specific differences could be due to the variations in the endogenous levels of plant growth regulators in these explants, which could have marked effect on morphogenesis (Krikorian 2000; Pal et al. 2011).

The optimized medium was tested for all the eight cultivars under study and was found to be efficient for shoot organogenesis in all these cultivars and from both the explants. This establish the advantage of the present protocol for shoot organogenesis. Earlier there are reports highlighting the cultivar specific response of morphogenesis (Anjum and Ali 2004; Wheeler et al. 1985) and stressing the need for cultivar specific protocol of potato regeneration (Al-Sulaiman 2011). Therefore, the present one-step and cultivar independent shoot organogenesis protocol will ease the work on genetic manipulations of potato cultivars.

Since in this study regeneration of plants through shoot organogenesis was achieved. Therefore, the clonal fidelity of regenerated plants of 'Kufri Pukhraj' and 'Kufri Chipsona1' was studied using PCR based RAPD and ISSR markers. The utility of these markers in studying clonal fidelity of in vitro propagated plants is well documented (Khatab and El-Banna 2011; Kumar et al. 2010; Rani and Raina 2000). All the scored markers (both by RAPD and ISSR) were monomorphic indicating the genetic uniformity of regenerated plants (Fig. 4a–d; Table 1). Probably this could be due to short exposures time in the culture medium. Earlier, the length of culture (Bairu et al. 2006; Rodrigues et al. 1998) and the presence of a disorganized growth phase in tissue culture were considered as major

factor that may cause somaclonal variation (Rani and Raina 2000). Thus, both RAPD and ISSR markers did not reveal somaclonal variations in regenerated plants of both the cultivars.

To conclude, in this study a one-step shoot organogenesis protocol was developed from the leaf and internodal explants of potato cultivars 'Kufri Pukhraj', 'Kufri Chipsona 1', which was found to be equally effective for all the eight cultivars of potato tested. In contrast to most of earlier studies reporting the use of zeatin and zeatin riboside on shoot organogenesis of potato, the present study reports the effectiveness of BA in the presence of $AgNO_3$ on shoot organogenesis. Best shoot organogenesis was achieved on MS1 medium supplemented with 10.0 μM BA and 15.0 μM GA_3. The plants regenerated were found to be true-to-type to the mother cultures. The plants were successfully acclimatized and grown under protected cultivation to yield mini-tubers. The one step shoot organogenesis will also find application in trait specific modification of potato.

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