Analysis of genetic stability of in vitro propagated potato microtubers using DNA markers

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Abstract The genetic stability of in vitro propagated potato microtubers was assessed using random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers. Microtubers were developed through in vitro from potato microplants using standardized protocols. The microtubers were conserved for 1 year under three different culture media and consequently microplants were regenerated for the DNA analyses. During the study, a total of 38 (10 RAPD, 11 ISSR, 12 SSR and 5 AFLP) primers produced a total of 407 (58 RAPD, 56 ISSR, 96 SSR and 197 AFLP) clear, distinct and reproducible amplicons. Cluster analysis revealed 100 % genetic similarity among the mother plant and its derivatives within the clusters by SSR, ISSR and RAPD analyses, whereas AFLP analysis revealed from 85 to 100 % genetic similarity. Dendrogram analysis based on the Jaccard's coefficient classified the genotypes into five clusters (I–V), each cluster consisting of mother plant and its derivatives. Principal component analysis (PCA) also plotted mother plant and its genotypes of each cluster together. Based on our results, it is concluded that AFLP is the best method followed by SSR, ISSR and RAPD to detect genetic stability of in vitro conserved potato microtubers. The in vitro conservation medium (T2) is a safe method for conservation of potato microtubers to produce true-to-type plans.

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Introduction

Plant tissue culture is recognized as one of the valuable components of biotechnology methods because of its potential to rapid multiplication of “true-to-type” genotypes and to conserve valuable germplasm. Tissue culture technologies have been proven very valuable in potato for maintenance of healthy stocks and rapid propagation of planting materials (Smulders and de Klerk 2011). Most potato seed propagation systems around the world maintain elite clones by tissue culture. In vitro clonal propagation is used to produce micro or mini tubers for healthy seed stocks identical to mother plant after repeated sub-culturing. In general, clonal propagation through tissue culture should generate individuals identical to the mother plant from which they were sub-cultured. However, the past studies have shown that in vitro cultures pose a problem of genetic stability caused by genetic and epigenetic variations (somaclonal variation) in regenerants. This may lead to phenotypic changes in the propagated clones and alternation of in the agronomic performance (Vazquez and Linacero 2010). So in the clonal regeneration, one of the most crucial concerns of curators is to retain genetic stability of in vitro propagating material. Hence a quality check up for true to type planting material at an early stage of development is considered to be very useful in plant tissue culture (Zilberman and Henikoff 2007). Although molecular basis of genetic variations are now becoming apparent that involves transcription, transposable element, chromosomal rearrangement, gene amplification, gene mutation (Alvarez et al. 2010). Molecular markers serve as an important tool to check the genetic uniformity and true to type nature of micropropagated plants.

Methods to detect genetic changes have become more streamlined and exhaustive with next-generation sequencing

technologies. Among the various DNA-based markers, random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) are still a reliable alternative. RAPD is simple, dominant, quick and easy assay marker which requires low quantities (5–50 ng) of template DNA, and randomly distributed throughout the genome. ISSR technique is also simple, fast, cost effective and a highly reliable technique in which a single sequence repeat motif is used as a primer for amplification of regions between microsatellites. ISSRs use longer primers (15–30 mers) as compared to RAPD primers (10 mers), which permit the subsequent use of high annealing temperature leading to higher stringency. In the past RAPD and ISSR markers have been proven to be quite efficient in detecting genetic fidelity in several plant species such as *Prunus dulcis* (Martin et al. 2004), *Solanum* species (Aversano et al. 2009), *Cymbopogon martinii* (Bhattacharya et al. 2010), *Gynura bicolor* (Liu et al. 2011), *Malus domestica* (Pathak and Dhawan 2012), *Simmondsia chinensis* (Kumar et al. 2011), *Gerbera jamesonii* (Bhatia et al. 2011) and *Lilium orientalis* (Liu and Yang 2012).

Moreover, SSR and AFLP are the most reliable and reproducible techniques to assess genetic stability of the in vitro propagated clones. Microsatellites consist of randomly repeated units of short nucleotide motifs (2–8 bp). SSRs have advantages of high genomic abundance throughout the genome, codominant, locus-specific, able to detect heterozygotes and homozygotes, high level of polymorphism, informative, high reproducibility and more robust. AFLP marker combines the RFLP and PCR-based techniques, which was developed by Vos et al. (1995). AFLP is highly efficient, reproducible, high genomic abundance and dominant marker. AFLP can be generated for any organism without initial investment in primer/probe development and sequence analysis. Like RAPD and ISSR, in the past SSR and AFLP markers have also been used to detect genetic fidelity in many plants such as *Elaeis guineensis* (Matthes et al. 2001), *Hordeum brevisubulatum* (Li et al. 2007), *Solanum tuberosum* (Zarghami et al. 2008; Dann and Wilson 2011), *Pinus pinaster* (Marum et al. 2009) and *Ocotea catharinensis* (Hanai et al. 2010). The aim of the present study was to assess the genetic stability of in vitro conserved potato microtubers by RAPD, ISSR, SSR and AFLP markers and also confirmed in vitro conservation protocol.

Materials and methods

Plant materials

Five in vitro propagated potato genotypes/cultivars were used to assess genetic stability by RAPD, ISSR, SSR and AFLP markers (Table 1). In vitro plants were maintained at

Table 1 Potato genotypes used in genetic stability analysis through molecular markers

SN	Sample	Code ^a
1.	IC in vitro	IC in vitro
2.	IC T1	IC T1
3.	IC T2	IC T2
4.	IC T3	IC T3
5.	Kufri Sindhuri in vitro	KS in vitro
6.	Kufri Sindhuri T1	KS T1
7.	Kufri Sindhuri T2	KS T2
8.	Kufri Sindhuri T3	KS T3
9.	IWA-I in vitro	IWA-I in vitro
10.	IWA-I T1	IWA-I T1
11.	IWA-I T2	IWA-I T2
12.	IWA-I T3	IWA-I T3
13.	KCM in vitro	KCM in vitro
14.	KCM T1	KCM T1
15.	KCM T2	KCM T2
16.	KCM T3	KCM T3
17.	IWA-III in vitro	IWA-III in vitro
18.	IWA-III T1	IWA-III T1
19.	IWA-III T2	IWA-III T2
20.	IWA-III T3	IWA-III T3

^a Code & SN of the corresponding genotypes were used in result/discussion, SN were used to depict gel pictures of the genotype

Central Potato Research Institute (CPRI), Shimla. Plantlets were multiplied using leafy nodes of the plantlets in the propagation medium (MS medium + 30 g l⁻¹ sucrose + 7 g l⁻¹ agar) at 20 °C under environment controlled chamber.

In vitro conservation

Twenty-days old in vitro plants were induced for microtubers on the tuber inducing medium (MS medium + 80 g l⁻¹ sucrose + 10 ml l⁻¹ BAP) without agar. Microtubers (1–2 nos.) were conserved at 6 °C on the conservation media [T1: MS medium + 80 g l⁻¹ sucrose + 10 ml l⁻¹ BAP + 7 g l⁻¹ agar; T2: MS medium + 40 g l⁻¹ sorbitol + 7 g l⁻¹ agar; and T3: MS medium + 20 g l⁻¹ sucrose + 40 g l⁻¹ sorbitol + 7 g l⁻¹ agar]. After 1 year of in vitro conservation, plants raised through micropropagation from original, T1, T2 and T3 microtubers and used for genetic stability analysis. At CPRI, the most commonly used conservation medium is T2, and in addition to original and T2, experiments were also conducted in T1 and T3 media to assess genetic variation.

DNA analysis

Plant DNA was isolated from 100 mg leaves collected from fresh in vitro sub-cultures using the GenElute Plant

Genomic DNA MiniPrep Kit (Sigma-Aldrich, St. Louis, USA). DNA quality and quantity were determined with NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA), and quality was also assessed on 0.8 % (w/v) agarose gel. The isolated genomic DNA was used for RAPD, ISSR, SSR and AFLP analyses.

Random amplified polymorphic DNA (RAPD) analysis of the potato genotypes was performed using random decamer primers (Operon Biotechnologies GmbH, Cologne, Germany). RAPD primers used in the analysis are summarized in Supplementary Table 1. The polymerase chain reaction (PCR) amplification and analysis was performed as described by Sarkar et al. (2011) and Tiwari et al. (2010). Inter simple sequence repeat (ISSR) analysis was performed using genomic DNA by ISSR primers summarized in Supplementary Table 1. The polymerase chain reaction (PCR) was performed in a Mastercycler Gradient (Eppendorf, Hamburg, Germany) in a total volume of 10 μ l and consisted of 50 ng DNA templates in 1 $\times$ PCR buffer, 2.5 mM MgCl₂, 200 μ M dNTP, 0.5 μ M of primer, 1 Unit Taq Polymerase (Qiagen). The PCR procedure included: 5 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C, with a final extension of 8 min at 72 °C. Gel documentation and analysis was performed like RAPD analysis. Simple sequence repeat (SSR) analysis was carried out by SSR primers summarized in Supplementary Table 1. The PCR amplification was followed as original procedures of Ghislain et al. (2009) described by Tiwari et al. (2013) followed by analysis of PCR products as per the manufacturers' instruction in '3500 Genetic Analyzer' (Applied Biosystems, California, USA). Amplified fragment length polymorphism (AFLP) analysis was carried out according to the original protocol of Vos et al. (1995) using primer sequences listed in Supplementary Table 2 as described in the AFLP® Plant Mapping Protocol in '3500 Genetic Analyzer' (Applied Biosystems).

Scoring and data analysis

A data matrix was constructed on the basis of presence (1) or absence (0) of bands of the amplified DNA fragments. Missing data were scored as '9'. All reactions were repeated at least twice, and only distinct, reproducible, polymorphic and well-resolved bands across all runs were considered for analysis. In the AFLP, peak intensity (>100) and band size (>90 bp) were considered for analysis. DNA fragments of low visual intensity, which could not be readily distinguished as present or absent, were considered to be ambiguous markers and were not scored. Genetic diversity analysis was performed with the program NTSYS-PC 2.21 (Rohlf 2006). A similarity matrix was calculated by Jaccard's coefficient and the dendrogram was generated using unweighted pair-group method (UPGMA) clustering

method. To assess the genetic association of the potato genotypes, a principal component analysis (PCA) was conducted using NTSYS-PC 2.21.

Results

The protocols used in this study involved use of microtubers for in vitro conservation followed by micropropagation of tissue culture plants. To validate efficiency of in vitro conservation, it was essential to ascertain the genetic status of resultant progeny. RAPD, ISSR, SSR and AFLP markers were employed to characterize the genetic similarity of the mother plant and its derivatives. Out of the 10 RAPD primers, all resulted in two to seven scorable bands per primer. These 10 RAPD primers generated 58 amplicons in total, ranging from 150 to 3,000 bp in size. The number of bands in the selected primers varied from 3 (OPB05) to 8 (OPB04 and OPB08), with an average of 5.8 bands per RAPD primer (Table 2). Genetic stability was determined based on the bands appearance and they showed identical to mother plant. RAPD profiles of OPB08 and OPB10 markers (Fig. 1) and dendrogram analysis based on the Jaccard's similarity coefficient revealed 100 % genetic similarity among the mother plants and its derivatives (Fig. 5). In the dendrogram, micropropagated potato genotypes were characterized into five clusters namely cluster I [IC in vitro (1), IC T1 (2), IC T2 (3) and IC T3 (4)], cluster II [KS in vitro (5), KS T1 (6), KS T2 (7) and KS T3 (8)], cluster III [IWA-I in vitro (9), IWA-I T1 (10), IWA-I T2 (11) and IWA-I T3 (12)], cluster IV [KCM in vitro (13), KCM T1 (14), KCM T2 (15), and KCM T3 (16)], and cluster V [IWA-III in vitro (17), IWA-III T1 (18), IWA-III T2 (19), and IWA-III T3 (20)]. RAPD analysis revealed true-to-type plants within each cluster viz., cluster I: mother plant 1 to regenerants 2, 3 and 4; cluster II: mother plant 5 to regenerants 6, 7 and 8; cluster III: mother plant 9 to regenerants 10, 11 and 12; cluster IV: mother plant 13 to regenerants 14, 15 and 16; and cluster V: mother plant 17 to regenerants 18, 19, and 20. In principal component analysis (PCA), variation among the first three principal components accounted for 46.95, 18.22 and 15.79 % variation, respectively of the total variation summing up to 80.97 %. Four clusters (I, II, III and IV) established by the cluster analysis are clearly separated by the PCA (data not shown).

Out of the 11 ISSR markers, all primers resulted in two to eight scorable bands, which were selected for further study. These 11 ISSR primers generated 56 amplicons, ranging from 180 to 1,200 bp in size. The number of bands for each primer varied from 2 (ISSR1, ISSR5 and ISSR2105) to 11 (ISSRP93), with an average of approximately 5 bands per ISSR primer (Table 2). ISSR profiles by ISSRP93 and ISSRAm2 markers (Fig. 2) and dendrogram analysis based

Table 2 RAPD, ISSR, SSR and AFLP amplicons profiles of in vitro propagated potato genotypes

SN	Primers	Band size range (bp)	No. of total bands	Size of each band (bp)
RAPD				
1.	OPB01	480–1000	5	480, 530, 800, 900, 1000
2.	OPB02	500–920	4	500, 700, 850, 920
3.	OPB03	300–1000	6	300, 420, 510, 700, 770, 1000
4.	OPB04	250–2000	8	250, 400, 500, 600, 730, 1000, 1500, 2000
5.	OPB05	600–1000	3	600, 750, 1000
6.	OPB06	200–700	6	200, 250, 315, 400, 500, 700
7.	OPB07	150–1000	7	150, 450, 520, 610, 725, 900, 1000
8.	OPB08	330–1600	8	330, 450, 560, 670, 710, 900, 1200, 1600
9.	OPB09	380–3000	6	380, 660, 900, 1500, 2000, 3000
10.	OPB10	325–700	5	325, 460, 500, 600, 700
ISSR				
1.	ISSR1	400–700	2	400, 700
2.	ISSR5	400–600	2	400, 600
3.	ISSR19	440–650	4	440, 500, 550, 650
4.	ISSR1425	300–700	3	300, 500, 700
5.	ISSR2103	320–900	7	320, 400, 600, 650, 700, 800, 900
6.	ISSR2105	300–400	2	300, 400
7.	ISSRMC1	180–1000	7	180, 230, 330, 400, 700, 800, 1000
8.	ISSRMC2	320–1200	5	320, 380, 400, 700, 1200
9.	ISSRP92	300–1500	8	300, 385, 460, 500, 580, 700, 1000, 1500
10.	ISSRP93	300–1180	11	300, 380, 400, 415, 500, 520, 580, 680, 800, 820, 1180
11.	ISSRAm2	280–1000	5	280, 500, 600, 700, 1000
SSR				
1.	STIKA	175–230	6	175, 190, 200, 213, 222, 230
2.	STU6	176–200	6	175, 180, 189, 193, 198, 200
3.	STM5127	230–272	9	230, 237, 240, 243, 247, 264, 269, 271, 272
4.	STM5114	232–304	9	232, 257, 264, 279, 282, 287, 291, 295, 304
5.	STM1053	126–170	7	126, 130, 135, 138, 142, 164, 170
6.	STI0012	147–185	7	147, 153, 163, 166, 170, 180, 185
7.	STPoAc58	106–240	11	106, 112, 143, 160, 168, 174, 180, 185, 225, 230, 240
8.	STM0019	108–209	11	108, 115, 120, 128, 151, 162, 170, 178, 186, 200, 209
9.	STM0031	161–174	4	161, 166, 170, 174
10.	STM1104	161–175	4	161, 167, 171, 175
11.	STM1052	114–218	12	114, 128, 135, 141, 151, 160, 166, 180, 186, 198, 208, 218
12.	STM1106	110–158	10	110, 117, 122, 130, 135, 139, 145, 149, 153, 158
AFLP				
1.	E11+MseCAC	92–216	28	92, 95, 98, 100, 103, 105, 107, 111, 113, 116, 120, 123, 127, 131, 134, 136, 140, 142, 144, 153, 156, 160, 164, 172, 174, 188, 190, 216
2.	E13+MseCTG	93–238	39	93, 95, 98, 100, 103, 105, 108, 109, 111, 115, 117, 120, 123, 125, 126, 128, 132, 138, 140, 141, 142, 147, 152, 154, 157, 164, 168, 175, 179, 180, 181, 187, 188, 191, 199, 200, 211, 234, 238
3.	E32+MseCAC	91–236	43	91, 93, 96, 98, 100, 101, 103, 104, 108, 109, 110, 113, 115, 117, 120, 122, 127, 128, 131, 133, 136, 138, 141, 144, 147, 149, 153, 156, 158, 160, 163, 167, 168, 169, 172, 175, 190, 216, 217, 221, 226, 231, 236
4.	E35+MseCTG	91–312	44	91, 96, 98, 100, 104, 108, 112, 113, 115, 119, 120, 123, 125, 126, 131, 134, 137, 140, 142, 154, 155, 156, 161, 163, 169, 170, 174, 175, 180, 188, 191, 199, 203, 206, 211, 217, 231, 233, 236, 240, 264, 267, 294, 312
5.	E36+MseCAC	90–232	43	90, 94, 96, 99, 101, 103, 105, 107, 109, 111, 113, 115, 116, 118, 119, 121, 124, 126, 129, 131, 134, 136, 137, 138, 141, 143, 144, 145, 147, 149, 151, 153, 154, 157, 161, 169, 176, 178, 184, 190, 200, 227, 232

Fig. 1 RAPD profiles of potato genotypes generated by **a** OPB08 and **b** OPB10 markers. $M=100$ bp ladder (Fermentas)

on the Jaccard's similarity coefficient revealed 100 % genetic similarity among the mother plants and its derivatives (Fig. 5). In the dendrogram, micropropagated true-to-type potato genotypes were characterized into five clusters (I–V) like RAPD. In principal component analysis (PCA), variation among the first three principal components accounted for 54.71, 21.58 and 11.32 % variation, respectively of the total variation summing up to 87.62 %. Five clusters (I, II, III, IV and V) established by the cluster analysis are clearly separated by the PCA and plotted separately onto PCA plot (data not shown).

Out of the 12 SSR primers, all resulted in four to twelve scorable bands per primer. These 12 SSR primers generated 96 amplicons in total, ranging from 106 to 304 bp in size. The number of bands in the selected primers varied from 4 (STM0031 and STM1104) to 12 (STM1052), with an average of 8 bands per SSR primer (Table 2). To illustrate, Fig. 3 shows SSR profiles generated by the marker STU6 of mother plant KS in vitro (5) identical to KS T1 (6), KS T2 (7), and KS T3 (8). Moreover, dendrogram analysis based on the Jaccard's similarity coefficient revealed 100 % genetic

similarity among the mother plants and its derivatives (Fig. 5). In the dendrogram, micropropagated true-to-type potato genotypes were characterized into five clusters (I–V) like RAPD and ISSR. In principal component analysis (PCA), variation among the first three principal components accounted for 41.32, 20.31 and 15.32 % variation, respectively of the total variation summing up to 76.96 %. Five clusters (I, II, III, IV and V) established by the cluster analysis are clearly separated by the PCA (data not shown).

Out of the 5 AFLP primers, all resulted in 7 to 13 scorable bands per primer. These 5 AFLP primers generated 197 amplicons in total, ranging from 90 to 312 bp in size. The number of bands in the selected primers varied from 28 (E11+MseCAC) to 44 (E35+MseCTG), with an average of approximately 40 bands per primer (Table 2). To illustrate, Fig. 4 shows AFLP profiles generated by E13+MseCTG primer combination in mother plant KS in vitro (5) identical to KS T1 (6), KS T2 (7), and KS T3 (8). Genetic stability was determined based on the bands appearance and they showed almost identical to mother plant, except very minor fragments. However, unlike above markers, dendrogram

Fig. 2 ISSR profiles of potato genotypes generated by **a** ISSRP93 and **b** ISSRAm2 markers. $M=100$ bp ladder (Fermentas)

Fig. 3 SSR profiles generated by marker STU6 of potato genotypes **a** KS in vitro, **b** KS T1, **c** KS T2, and **d** KS T3

analysis based on the Jaccard's similarity coefficient revealed 85–100 % genetic similarity among the mother plants and its derivatives (Fig. 5). In the dendrogram, micropropagated true-to-type potato genotypes were characterized into five clusters (I–V) like RAPD, ISSR and SSR. In principal component analysis (PCA), variation among the first three principal components accounted for 23.73, 20.90 and 19.48 % variation, respectively of the total variation summing up to 64.11 %. Five clusters (I, II, III, IV and V) established by the cluster analysis are clearly separated by the PCA (data not shown).

Discussion

Considering the importance of ensuring genetic stability of micropropagated plants in any conservation program, it is imperative to choose a procedure that does not induce variation. Occurrence of genetic variations is a serious problem in micropropagation of crop species because of their unpredictable nature (Rahman and Rajora 2001). The variations generated during tissue culture processes are generally caused by chromosomal rearrangements and single gene mutations. Detection and analysis of genetic variation can help in understanding the molecular basis of various biological phenomena in plants. Variations induced in

tissue cultured plants are most likely to be reflected in the banding profiles developed by different marker systems (Phillips et al. 1994). To uncover genetic changes, in this study we used RAPD, ISSR, SSR and AFLP profiles with mother plant and its derivatives. Molecular markers analyses demonstrated that the mother plant and its clonal derivatives could be grouped together in a single cluster. DNA analyses revealed 100 % similarity among mother plant and its derivatives by SSR, ISSR and RAPD markers whereas, 85–100 % similarity by AFLP markers and indicated a true-to-type progeny. PCA analysis demonstrated that the mother plant and its derivatives of the corresponding clusters were plotted together. The present data are similar to reports of others. There are a number of findings in the literature which report similar results for detection of genetic fidelity using molecular markers in different crops such as *Solanum aculeatissimum* (Ghimire et al. 2012), *Olea europaea* (Leva and Petruccioli 2012), *Saccharum officinarum* (Tawar et al. 2008), *Dendrocalamus hamiltonii* (Agnihotri et al. 2009), *Capparis deciduas* (Tyagi et al. 2010), *Vanilla* spp. (Sreedhar et al. 2007), *Dioscorea bulbifera* (Narula et al. 2007) and *Musa* spp. (Lakshmanan et al. 2007). For example, Kumar et al. (2011) assessed genetic fidelity with similarity level of 100 % of micropropagated plants and mother plants of jojoba using RAPD and ISSR markers and indicated axillary bud multiplication can also be used

Fig. 4 AFLP profiles generated by marker E13+MseCTG primer combination of potato genotypes **a** KS in vitro, **b** KS T1, **c** KS T2, and **d** KS T3

Fig. 5 Dendrogram of the potato genotypes based on the Jaccard's coefficient of **a** RAPD, **b** ISSR, **c** SSR and **d** AFLP markers

as one of the safest modes for the production of true-to-type plants. Bhatia et al. (2011) evaluated genetic fidelity with 100 % similarity of in vitro propagated plants and mother plant of gerbera using RAPD and ISSR marker. Zarghami et al. (2008) evaluated genetic stability up to a level of 97–100 % similarity in cryopreserved and non-cryopreserved potato using AFLP markers. However, Liu and Yang (2012) analysed micropropagated progenies and mother plant of lily could be grouped together in one major cluster with similarity level of 92 % and indicated that direct shoot formation from explant regeneration is a safe method for multiplication of true-to-type plants. Dann and Wilson (2011) observed genetic (8.75–15.63 % polymorphisms) and epigenetic (12.56–26.13 % polymorphisms) variations among regenerants of potato derived from long-term nodal tissue culture and cell selection. Aversano et al. (2009) demonstrated that under in vitro culture conditions *Solanum* genotype affects the integrity of the genome and absence of polymorphism at plastid level confirms the greater genetic stability of cytoplasmic DNA. Li et al. (2007) demonstrated genetic and epigenetic instabilities induced by tissue culture in wild barley using AFLP, S-SAP and MSAP markers. Based on our results, it is concluded that AFLP is the best method followed by SSR, ISSR and RAPD for detection of genetic stability of in vitro conserved potato microtubers. Similarly, previously McGregor et al. (2000) analysed 39 potato cultivars using RAPD, ISSR, AFLP and SSR markers and concluded AFLP is the best followed by a multi-locus SSR, RAPD, ISSRs and single locus SSR. Later, Ghislain et al. (2009) developed robust and highly informative SSR markers for potato characterization and they were demonstrated in the present study to assess genetic stability. Moreover, in our study ISSR and RAPD markers produced similar findings, however, due to high annealing temperature ($T_a=55\text{ }^{\circ}\text{C}$) and more specific PCR products of ISSR was given merit over RAPD ($T_a=36\text{ }^{\circ}\text{C}$).

Extensive research has been conducted on genetic and epigenetic changes that may be attributed to the somaclonal variations during tissue culture. It was observed that presence or absence of variations during tissue culture depends upon the source of explant and the mode of regeneration (Goto et al. 1998), including levels of growth substances that are used (Martin et al. 2006). Plants regenerated from axillary buds or from other meristematic tissue showed the lowest tendency for genetic variation (Joshi and Dhawan 2007). Application of axillary branches and somatic embryogenesis are better methods for generation of genetically stable clones. However, organogenic differentiation is very prone to somatic variation (Pontaroli and Camadro 2005). Our results demonstrated that since, there were no variations observed among the in vitro conservation media T1, T2 and T3, the most commonly used T2 was considered ideal for in

vitro conservation of potato microtubers to generate true-to-type plants.

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