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DETERMINAREA STABILITĂȚII GENETICE A MATERIALULUI SĂDITOR DE AFIN ȘI MUR OBȚINUT PRIN MICROPROPAGARE CU AJUTORUL MARKERILOR MOLECULARI RAPD ȘI SRAP

MOLECULAR ANALYSIS OF GENETIC STABILITY OF MICROPROPAGATED BLACKBERRY AND BLUEBERRY PLANTS USING RAPD AND SRAP MARKERS

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Abstract

Micropropagation of blackberry (*Rubus fruticosus* L.) and blueberry (*Vaccinium corymbosum* L.) plants is a successful alternative for plant material production. Among the micropropagation methods developed axillary bud stimulation is the most commonly used method for mass propagation of different plants and considered the most reliable method to guarantee genetic stability of the regenerated plants obtained by micropropagation. Although, a major problem of this type of propagation method is the somaclonal variation which can occur among the subcultured plants of the parental line, especially after a high number of subcultures. Therefore, the aim of this research was to evaluate the genetic stability of the micropropagated plants of two blackberry cvs. 'Loch Ness' and 'Chester Thornless' and three blueberry cultivars ('Aurora', 'Draper' and 'Liberty') using RAPD and SRAP markers. Our results reveal that, no genetic variations were shown amongst the mother plants and micropropagated plants of the investigated species providing thus further evidence for the genetic stability.

Cuvinte cheie: micropropagare, markeri moleculari, uniformitate genetică.
Key words: micropropagation, molecular markers, genetic stability.

1. Introduction

The interest of berry fruits has increased recently not only among small farmers but also on a larger scale to their great economic importance and health-promoting properties. Therefore, in order to satisfy the increasing demand for planting material, the commercial use of tissue culture technology could be a major source of a profitable planting material production. Previous research results suggest that, proliferation of axillary shoots in tissue culture is a very simple and reliable in vitro propagation method which provides genetically identical regeneration of the plants compared to the mother plant (Debnath, 2017, Goyal et al., 2012). The occurrence of somaclonal variation is a possible drawback of both in vitro cloning and germplasm preservation. Continuous maintenance of cultures may often result in chromosomal rearrangements and mutations. As in vitro culture promotes genetic disturbances due to many factors, the confirmation of genetic stability is of immense significance to assure the genetic uniformity of in vitro raised plants at an early stage (Alizadeh et al. 2015). Therefore the aim of this research was to evaluate the genetic stability of the micropropagated blackberry and blueberry plants to reveal the applicability of two molecular marker systems, namely RAPD and SRAP.

2. Material and methods

2.1. Plant material and culture conditions

The experiments have been carried out using plant material from blackberry and blueberry orchards, both established using the planting material obtained by in vitro micropropagation at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Standard plants of blackberry and blueberry were produced from shoot tips. Tissue cultures of blackberry cvs. (*Rubus frutticosus* L., 'Loch Ness' and 'Chester Thornless') were obtained on Murashige and Skoog (1962) stock solutions (MS) according to the protocol described by Fira et al. (2014) containing micro and macro elements such as Myo-inositol 100 mg/l, Thiamine 1 mg/l, Pyridoxine 0.5 mg/l, nicotinic acid 0.5 mg/l, 30 g/l sugar, 50 g/l wheat starch and 0.5 mg/l 6-Benzyladenine (BA). Both cultivars were propagated in vitro for 10 subcultures, after which they were rooted and acclimatized ex vitro.

Shoot fragments of blueberry cvs. (*Vaccinium corymbosum* L., 'Aurora', 'Draper' and 'Liberty') were propagated on McCown's Woody Plant medium (WPM) stock solutions (Lloyd and McCown, 1981) containing micro and macro elements, 100 mg/l of Sequestrene 138, 100 mg/l of Myo-inozitol, 2 mg/l of vitamin B1, 1 mg/l of vitamin B6, 1 $mg \cdot L^{-1}$ of nicotinic acid, 1 $mg \cdot L^{-1}$ zeatin, 30 g/l of sugar and 5 g/l Plant agar. All the three blueberry cultivars were micropropagated in vitro for 5 subcultures, and then rooted and acclimatized in ex vitro conditions. For culture media preparation, tap water was used from the public administration of Cluj-Napoca (https://www.casomes.ro/wp-content/uploads/2019/10/Buletin-calitate-apa-Cluj.pdf). After all the ingredients were added, the pH of the medium was adjusted to 5.8 for blackberry and 5 for blueberry cvs. For blackberry cultures, the medium was solidified with starch, and 100 ml medium was dispensed in each 720 ml glass jar with air ventilation provided by the filters made up of autoclavable plastic sponge. After media dispersion, the culture vessels were autoclaved at 121°C for 30 minutes. In the multiplication stage, five mini-shoots were introduced into the medium/vessel until they had 2/3-3/4 parts in contact with the culture medium. The blueberry culture was carried out in 720 ml glass jars with polypropylene lids without filters. Plant agar was used as gelling agent to solidify the media. After media dispersion (100 ml/jar), the culture vessels have been autoclaved at 121°C for 20 minutes. The multiplication stage consisted of introducing twelve mini-shoots/vessel into the medium until they had 3/4 parts in contact with the culture medium. Both blackberry and blueberry cultures were maintained at 24±1°C under fluorescent white light (33.6 µmol m^{-2} s^{-1}) during a photoperiod of 16:8 h light and dark cycles. The duration of one cycle was 10 weeks. The new blackberry plantlets were rooted and hardened through hydroculture as described by Clapa et al. (2013). Blueberry plantlets were rooted and hardened ex vitro using a potting mix of acid peat and perlite (1:1) in mini greenhouses (Clapa et al., 2018).

2.2. DNA extraction and PCR amplification

For DNA extraction and molecular analyses young blackberry plant leaves were used from the mother pants and from the 10th subcultures from both *R. fructicosus* 'Loch Ness' and 'Chester Thornless' cvs. For blueberry DNA extraction, young leaves were harvested from mother plants (*V. corymbosum* 'Aurora', 'Draper', 'Liberty') and from ex vitro hardened plants odtained from the 5th subculture.

DNA isolation was carried out according to CTAB-based method as published by Lodhi et al. (1994) and improved by Pop et al. (2003). DNA purity and concentration were determined with a NanoDrop 1000 spectrophotometer. The DNA samples were diluted to 50 ng/µl by using sterile distilled water.

Genetic stability of the in vitro-grown plantlets was tested by using RAPD markers for both *V. corymbosum* cvs. and *R. fruticosus* cvs. and SRAP markers for *R. fruticosus* cvs.

For RAPD analysis, PCR amplification reactions were carried out according to the protocols provided by Williams et al. (1990) and Pop et al. (2011). Reaction mixtures (25 µl total volume) were prepared of 5 µl of 50 ng/µl DNA, 9.3 µl distilled H_2O for PCR reactions, 2 µl PVP (Polyvinylpyrrolidone), 5 µl GoTaq Flexi green buffer (Promega Corp., Madison, WI, USA), 2.5 µL MgCl2 (Promega Corp., Madison, WI, USA), 0.5 µL dNTP mix (Promega Corp., Madison, WI, USA), 0.5 µL RAPD primer (Microsynth, Balgach, Switzerland), and 0.2 µl GoTaq polymerase (Promega Corp., Madison, WI, USA).

DNA amplification was performed in a 96 Well Gradient Palm-Cycler CG1-96 (Corbett Research, Sydney, Australia) with 20 decamer primers (Table 1) with 1 cycle of 3 min at 95°C, followed by 45 cycles of 1 min at 93°C, 1 min at 34°C and 1 min at 72°C. After a final incubation of 10 min at 72°C the samples were stored at 4°C prior to analysis.

To perform SRAP analysis, PCR reactions were carried out according to the Li and Quiros (2001) method, but the reaction volumes were adjusted to 15 µl. The reaction mixtures contained 50 ng genomic DNA, 0.3 µM of each primer (forward and reverse) (Kaneka-Eurogentec, Belgium), 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 5X Green PCR buffer, 1 U Go Taq DNA polymerase (Promega, USA) and nuclease-free water (Sigma-Aldrich GmbH, Germany). DNA amplification was carried out in a 96 Well Gradient Palm-Cycler CG1-96 (Corbett Research, Sydney, Australia) with 24 primer combinations (Table 2) with the following cycles: 5 min of denaturation at 94°C, five cycles of 1 min of denaturation at 94°C, 1 min of annealing at 35°C and 1 min of elongation at 72°C and then 35 cycles (94°C for 1 min; 50°C for 1 min and 72°C for 1 min) with a final elongation step of 10 min at 72°C.

PCR amplifications were repeated twice for each RAPD and SRAP primer combination to ensure the reproducibility of results. The amplified products from both SRAP and RAPD techniques were subjected to electrophoresis on a 2% agarose gel using TAE buffer. The gels were stained with ethidium bromide and the electrophoretic profiles were visualized under UV light. Gel images were analysed using TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK) to determine the range of molecular weight (bp) of both RAPD and SRAP amplified bands.

3. Results and discussions

3.1. Genetic stability of micropropagated plantlets using RAPD markers

Blackberry. The 20 RAPD primers used in this study, out of which six generated clear and reproducible bands across the investigated nine micropropagated *Rubus fruticosus* 'Loch Ness' and 'Chester Thornless' plantlets and their mother plants (Table 1). The number of amplified DNA bands by each RAPD primer ranged from 4 to 12 in *Rubus fructicosus* 'Loch Ness' and from 3-11 in *Rubus fruticosus* 'Chester Thornless'. The highest number of monomorphic bands in *R. fructicosus* 'Loch Ness' cultivar was obtained with OPB-10 primer, while the lowest number of bands was generated by OPAB 11 primer. In 'Chester Thornless' cultivar the highest number of amplified fragments was 11 generated by OPD 19 primer, while the lowest number of monomorphic bands (3) was obtained by OPAB 11 primer. Each RAPD primer generated amplification products ranging in size from 350 bp (OPD-19) to 2600 bp (OPA 04) (Table 3). Thus, the results of the RAPD analyses revealed that all the bands were monomorphic across all the samples and no polymorphism was detected (Fig. 1) indicating the true to type nature of the micropropagated plants to the mother plants. According to previous reports the micropropagated plants derived from shoot tips and axillary buds maintain clonal stability (Ostray et al. 1994; Samantaray and Maiti, 2010). Besides, the composition of the culture media might determine genetic variations, especially morphological variations due to the excessive use of auxins and/or cytokinins in the culture media. Genetic stability can be maintained in in vitro plantlets if lower concentrations of growth regulators are used in the culture media (Debnath, 2017). Our previous findings also confirm that the micropropagated *Rubus* plantlets were genetically identical when compared to the mother plant even after a large number of subcultures when 0.5 mg/l BA was used (Borsai et al., 2019). Our results revealed that RAPD marker system can provide reliable results regarding genetic stability of plants. Similar results have been reported by Gajdošová et al. (2006) who tested the same method with other woody plant species including *Rubus* cultivars as well.

Blueberry. Out of the 20 RAPD markers used in this study, eight RAPD primers resulted clear and scorable DNA bands. The number of scorable bands for each RAPD primer varied from 3 (OPB -08) to 13 (OPB-10) in 'Aurora' cultivar; from 4 (OPB10) to 9 (OPB 8, OPA10,OPA 01) in 'Draper'. In case of the 'Liberty' cv. the highest number of amplified bands recorded was 9 generated by OPA 01 and OPA 10 RAPD primer, while the lowest number of bands recorded 6 provided by OPB10 and OPB 8 primers. The amplification products' size generated by the RAPD markers in blueberry cultivars were ranging from 250 bp (OPB-11, OPB 08) to 2300 bp (0PA 10) (Table 4). Our results show that the in vitro regenerated plants of highbush blueberry cvs. 'Aurora', 'Draper' and 'Liberty' did not show any genetic variation between the mother and micropropagated plants. The most representative profiles obtained by two RAPD markers (OPB 10 and OPB 11) are shown in Figure 2. As the electrophoretic profiles show, the 2 RAPD primers exhibited similar banding patterns for both the mother and micropropagated plants. These patterns reveal the genetic uniformity of the micropropagated plants as compared to the mother plants. Our findings are consistent with previous results regarding RAPD marker system declared as a reliable molecular technique that had been used to identify cultivars and clones to determine inter-relatedness in many woody species, including *Vaccinium* sp. (Gajdošová et al. 2006; Persson and Gustavsson, 2001; Arce-Johnson et al., 2002: Божидай et al. 2016)

3.2. Genetic stability of micropropagated plantlets using SRAP markers

Our results show that out of the 24 SRAP marker combinations used (Table 2), 12 generated clear and scorable bands across the investigated *Rubus* species obtained by micropropagation and their mother plants. The total number of monomorphic bands amplified were 69 for 'Loch Ness' and 79 for 'Chester Thornless' cultivar. The number of amplified DNA fragments by each primer ranged from 3 to 7 in 'Loch Ness' and from 4-8 in 'Chester Thornless' (Table 5). The size of the amplification products ranged from 200 generated by Me4-Em5, Me4-Em6 and Me6-Em7 primer combinations to 1900 generated by Me8-Em2 primers (Table 5). Our results are consistent with previous results reported by Borsai et al., 2019, where 10 of SRAP primers have been already tested to asess the genetic fidelity between *Rubus* mother plants and micropropagated plantlets obtained from the 9th subculture. In both cases, the primer combination and SRAP marker system used proved to be an easy and powerful tool for genetic stability assessments with higher precision and less effort than phenotypic and karyologic determinations (Cloutier and Landry,1994). Similar studies have been carried out to check possible genetic variations between *R. fruticosus* L.' Triple Crown' micropropagated plants and mother plant using SRAP and ISSR marker systems (AbdAlla et al, 2017). Other studies show that among ISSR and SRAP markers, SRAP markers are considered more efficient method for genetic variation analyses than ISSR markers due to multiloci and multi allelic features (Zaefizadeh and Goliev, 2009).

4. Conclusions

This study investigated the genetic stability between mother plant and micropropagated plants of *R. fruticosus*, *V. corymbosum* and revealed that no genetic variations were detected between micropropagated plantlets and their donor plants. RAPD and SRAP markers proved to be a simple, realiable and powerful tools for genetic stability confirmation.

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References

1. Alizadeh M., Krishna H., Eftekhari M., Modareskia M., Modareskia M., 2015. Assessment of clonal fidelity in micropropagated horticultural plants. J. chem. pharm. res. 7(12), 977-990.
2. Arce-Johnson P., Rios M., Zuniga M., Vergara E., 2002. Identification of blueberry varieties using random amplified polymorphic DNA markers. Acta Hort. 574: 221-224.
3. Божидай Т. Н., Волосевич Н. Н., Кухарчик Н. В., 2016. Анализ генетической стабильности растений голубики сорта Duke, полученных в культуре in vitro. Известия Национальной академии наук Беларуси. Серия аграрных наук 1.2: 60-63.
4. Borsai O., Hârța M., Szabo K., Keleme C. D., Andrecan, F. A., Codrea M. M., Clapa, D., 2019. Evaluation of genetic fidelity of in vitro-propagated blackberry plants using RAPD and SRAP molecular markers. Horticultural Science (in press).
5. Clapa D., Fira A., Joshee, N., 2013. An efficient ex vitro rooting and acclimatization method for horticultural plants using float hydroculture. Hortscience, 48(9):1159-1167.
6. Clapa D., Bunea C., Borsai O., Pintea A., Hârța M., Ştefan R., Fira A., 2018. The Role of Sequestrene 138 in Highbush Blueberry (*Vaccinium corymbosum* L.) Micropropagation. HortScience, 53(10): 1487-1493.
7. Cloutier S., Landry B.S., 1994. Molecular markers applied to plant tissue culture. In Vitro Cell. Dev. Biol. Plant 30 (1): 32–39.
8. Debnath S. C., 2017. Molecular markers, clonal fidelity and antioxidant properties of micropropagated horticultural crops. Acta Hortic. 1155:261-268.
9. Fira A., Clapa D., Simu M., 2014. Studies regarding the micropropagation of some blackberry cultivars. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture, 71(1): 29-37.
10. Goyal P., Kachhwaha S., Kothari S. L., 2012. Micropropagation of *Pithecellobium dulce* (Roxb.) Benth-a multipurpose leguminous tree and assessment of genetic fidelity of micropropagated plants using molecular markers. Physiology and Molecular Biology of Plants, 18(2): 169-176.
11. Gajdošová A., Ostrolucká M. G., Libiaková G., Ondrušková E., Šimala, D., 2006. Microclonal propagation of *Vaccinium* sp. and *Rubus* sp. and detection of genetic variability in culture *in vitro*. Journal of Fruit and Ornamental Plant Research, 14,1; 103-119.
12. Mohamed M. AbdAlla, Nahed A. K. Rashed and Mohamed Abd Elsalam Yosef, 2017. Comparative studies udies between the mother plant and the micropropagated plantlets of black berry (*Rubus fruticosus* L.). International Journal of Current Research, 9(12): 62001-62009.
13. Murashige T., Skoog A.,1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum 15: 473-497.
14. Lloyd G., McCown B., 1981. Commercially feasible micropropagation of *Mountain laurel*, *Kalmia latifolia*, by use of shoot tip culture. Intl. Plant Prop. Soc. Proc. 30:421– 427.
15. Li G., Quiros C. F., 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theoretical and Applied Genetics, 103: 455–461.
16. Lodhi M.A., Guang-Ning Z., Weeden F.N.F., Reisch B.I.,1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Molecular Biology Reporter, 12: 6–13.
17. Ostray M.E., Hackett W., Michler C., Serres R., McCown B., 1994. Influence of regeneration method and tissue culture on the frequency of somatic variation in *Populus* to infection by *Septoria musiva*. Plant Science, 97: 209-215.
18. Persson H.A., Gustavsson B.A., 2001. The extent of clonality and genetic diversity in lingonberry (*Vaccinium vitis-idaea* L.) revealed by RAPDs and leaf-shape analysis. Molecular Ecology 10: 1385-1397.

19. Pop R., Ardelean M., Pamfil D., Gaboreanu I.M., 2003. The efficiency of different DNA isolation and purification in ten cultivars of *Vitis vinifera*. Bulletin UASMVCN. Horticulture 59: 259–261.
20. Pop R., Has I., Pop I.F., Harta M., Pamfil D., 2011. Genetic Diversity Analysis of Maize Inbred Lines from SCDA Turda-Romania Revealed by RAPD Molecular Markers. Bulletin UASMVCN. Agriculture 68(1): 277-283.
21. Samantaray S., Maiti S., 2010. An assessment of genetic fidelity of micropropagated plants of *Chlorophytum borivilianum* using RAPD markers. Biologia plantarum, 54(2): 334-338.
22. Shenoy V.B., Vasil I.K., 1992. Biochemical and molecular analysis of plants derived from embryogenic tissue cultures of napier grass (*Pannisetum purpureum* K. Schum). Theoretical and Applied Genetics. 83: 947-955.
23. Williams J.G., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic acids research 18(22): 6531-6535.
24. Zaefizadeh M, Goleiv R., 2009. Diversity and relationships among durum wheat landraces (subconvars) by SRAP andphenotypic marker polymorphism. Res J Biol Sci., 4(8):960–966.

Tables and Figures

Table 1. RAPD decamer primers and their sequences used for blueberry and blackberry genetic fidelity evaluation

Primer name	Sequences 3'-5'	Primer name	Sequences 5'-3'
OPA 01	CAGGCCCTTC	OPA 09	GGGTAACGCC
OPA 03	AGTCAGCCAC	OPA 11	CAATCGCCGT
OPA 04	AATCGCGCTG	OPB 08	GTCCACACGG
OPB 11	GTAGACCCGT	OPB 10	CTGCTGGGAC
OPAB 11	GTGCGCAATG	OPB 18	CCACAGCAGT
OPAL 20	GAACCTGCGG	OPC 04	GTGAGGCGTC
OPE 03	CCAGATGCAC	OPC 14	TGCCTGCTTG
OPH 02	TCGGACGTGA	OPD 20	ACCCGGTCAC
OPG 07	GAACCTGCGG	OPF 02	GAGGATCCCT
OPC 14	TGCCTGCTTG	OPF 13	GGCTGCAGAA

Table 2. SRAP primers used in the present study

Primer Forward	Forward primer sequence (3'-5')	Primer Reverse	Reverse primer sequence (5'-3')
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me4	TGAGTCCAAACCGGACC	Em2	GACTGCGTACGAATTTGC
Me6	TGAGTCCAAACCGGTAA	Em3	GACTGCGTACGAATTGAC
Me8	TGAGTCCAAACCGGTGC	Em4	GACTGCGTACGAATTTGA
		Em5	GACTGCGTACGAATTAAC
		Em6	GACTGCGTACGAATTGCA

Table 3. The number and range size (bp) of RAPD amplified bands in *R. fruticosus* cvs.

Primer name	Range of molecular weight of amplified bands (bp)	Number of monomorphic bands 'Loch Ness'/ 'Chester Thornless'
OPA 04	450-2600	9/6
OPB 10	650-1800	12/9
OPC 04	650-1400	6/7
OPD 19	350-2000	9/11
OPB 18	400-1500	5/5
OPAB 11	600-1800	4/3
Total		34/39

Table 4. The number and range size (bp) of RAPD amplified band in *V. corymbosum* cvs.

Primer name	Range of molecular weight of amplified bands (bp)	Number of monomorphic bands 'Aurora'/'Draper'/'Liberty'
OPA 01	500-1750	8/9/9
OPA 10	500-2300	8/9/9
OPB 08	250-1600	3/9/6
OPB 10	300-2100	13/4/6
OPB 11	250-1700	5/7/7
OPC 14	350-1700	4/5/8
OPF 02	500-1800	4/5/7
OPE 03	450-1400	6/5/8
Total		51/53/60

Table 5. Total number of bands, monomorphic bands and the range size (bp) of SRAP amplified bands in *R. fruticosus* cvs.

Primer combination	Size range of bands (bp)	Number of monomorphic bands 'Loch Ness '/'Chester Thornless'
Me1-Em3	250-1000	3/4
Me1-Em4	400-1250	4/4
Me1-Em2	300-1500	6/7
Me1-Em8	350-1300	5/5
Me4-Em4	300-1500	6/7
Me4-Em5	200-1450	7/8
Me4-Em6	200-1200	6/6
Me6-Em6	300-1300	6/5
Me6-Em7	200-1700	5/7
Me8-Em1	350-1000	4/4
Me8/Em7	250-800	3/4
Me8-Em8	300-300	4/4
Me8-Em2	**800-1900**	**5/7**
Me8-Em6	**300-1500**	**5/7**
Total		69/79

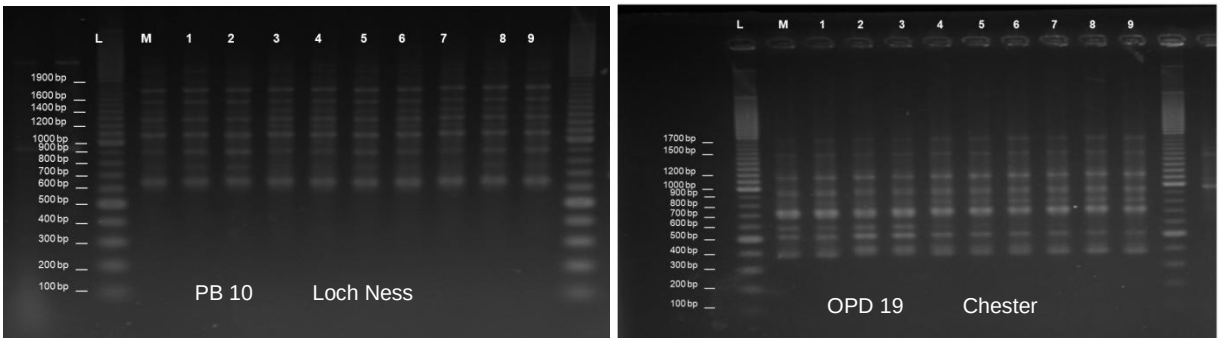

Fig 1. Electrophoretic profiles of mother plants and micropropagated plants of 'Loch Ness' cultivar generated by OPB 10 and 'Chester Thornless' cultivar generated by OPD 19 RAPD marker. L is DNA ladder of 100 bp

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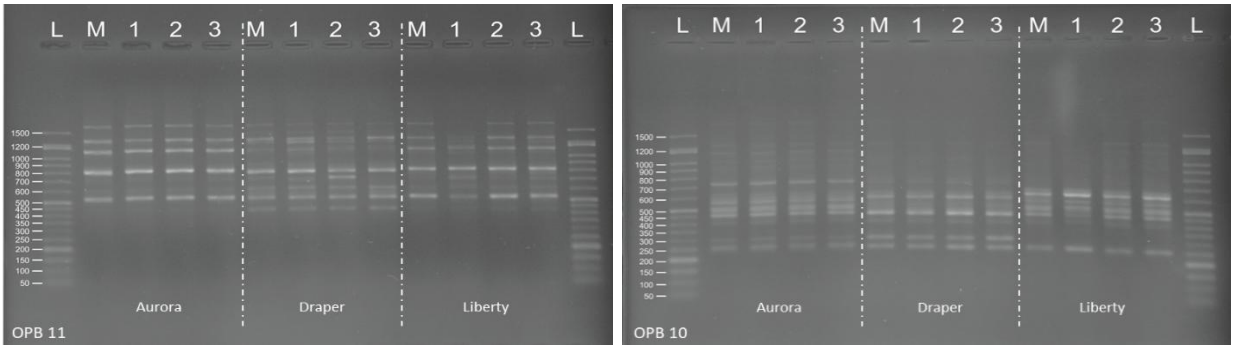

Fig 2. DNA finger printing pattern of mother plant (lane M) and micropropagated plants of blueberry cultivars 'Aurora', 'Draper' and 'Liberty' (lanes 1-3) obtained by RAPD markers OPB 11, OPB 10. Lane L is DNA ladder of 50 bp

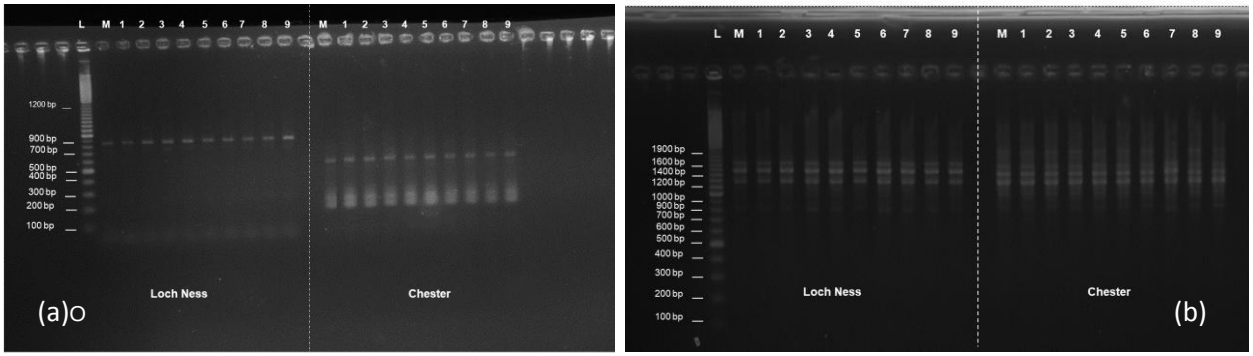

Fig. 3. Monomorphic SRAP profiles of mother plants and micropropagated clones of 'Loch Ness' and 'Chester Thornless' cultivars generated by primer combination Me8-Em2 (a), Me1-Em3 (b). Lane M shows SRAP bands from mother plant and lanes 1-9 indicate the micropropagated plants. L is DNA ladder of 100 bp