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RESEARCH ARTICLE

COMPARATIVE STUDIES BETWEEN THE MOTHER PLANT AND THE MICROPROPAGATED PLANTLETS OF BLACK BERRY (*RUBUS FRUTICOSUS* L.)

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ABSTRACT

Blackberry (*Rubus fruticosus* L.) is a shrub with great importance because of its fruits and /or aerial parts have a wide use in pharmaceutal, medicinal and industrial healthcare purposes, in-addition to their nutritional value. In the present study, inter simple sequence repeats (ISSR) and sequence-related amplified polymorphism(SRAP) analyses were used to evaluate genetic stability of *Rubus fruticosus* L.' Triple Crown' micropropagated plantlets and compare or detect possibly existing genetic variation between them and their donor mother plant. Introduced shoot tips of *Rubus fruticosus* L.' Triple Crown' were micropropagated in North Sinai Research Station, Desert Research Center. Sterilized excised shoot tips of them were cultured on MS basal salt medium supplemented with benzyl adenine 0.5 mg/L. Then developed shoots successfully transferred to multiplication media for several subcultures and subsequently to rooting medium. Genomic DNA of five micropropagated plantlets (samples) that were phenotypically normalregenerates and essentially identical with their mother plant, in-addition to their donor (mother plant) was extracted using modified CTAB method. Ten ISSR primers and twenty three different SRAP primer combinations were used for this study. Comparisons of each ISSRand SRAPbanding patters average ofmean percentage of similarity which calculated by Nei & Li similarity coefficient and dendrogams constructed based on the UPGMA clustering method showed that the micropropagated plantlets (samples) exhibited somaclonal variation and not true-to-type. Also, polymorphic information content and marker index values for the two markers indicated the presence of polymorphism between the studied samples and their mother plant. In-addition, the discriminating capacity and efficiency of ISSR and SRAP markers for genetic analyses were high, but SRAP markers had better capacity for comparative study and quantifying genetic diversity between *Rubus fruticosus* L.'Triple Crown' micropropagated plantlets (samples) and their mother plant.

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INTRODUCTION

Blackberries often termed" Brambles"are diverse group of species and hybrids in the genus *Rubus*. They belong to family *Rosacea* L. *Rubus* is one of the most diverse genera of flowering plants in the world. Blackberry (*Rubus fruticosus* L.) has a great economically importance as its fruits are a concentrated source of valuable nutrients and bioactive constituents of therapeutic interest and consider a functional food. Also, its aerial parts contain vitamins, steroids, lipids, minerals, flavonoids, glycosides, terpenes, acids and tannins. In-addition, it possess diverse pharmacological activities such as anti-filamentary, anti-microbial, anti-diabetic, anti-diarrheal and antiviral (Verma *et al*., 2014 and Zia *et al*., 2014). The shrub is believed to have its origin in Armenia and is now distributed throughout Europe, Asia, and North and South America.

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Researches concerning this economically important species have been focused on applicationof different *in-vitro* culture methods for production an elite material (new cultivars) in high demand for breeding,industry production of secondary metabolites, conservation of biodiversity, efficient regeneration, rapid dissemination and large-scale micropropagation of the good cultivars release(Dziadczyk *et al*., 2013, Gajdosova *et al*., 2015 and Vescan *et al*., 2012). The application of in-vitro culture techniques has associated risk including the occurrence of somaclonal variation (Larkin and Scowcroft, 1981 and Vujovic *et al*., 2010). Though, whether somaclonal variation may imply an advantage as a source of variability for new lines, or a disadvantage for propagation of an elite cultivar, it is important to achieve rapid and easy techniques to assess the genetic stability of the propagated plants at the earliest stage of plant growth. These propagated plantlets should be true-to-type or type off. A variety of molecular techniques has been developed and could be used as DNA fingerprinting strategy to provide plant genetic stability and genetic relationship information (Khan *et al*., 2013).

Although no single technique is universally applicable (Arif *et al*., 2010; Doveri *et al*., 2008 and Robarts and Wolfe, 2014) and all molecular marker approaches have inherent strengths and weaknesses so the use of only one type of molecular marker to assess the genetic stability of an in-vitro production system may be inadequate. ISSR (inter-simple sequence repeat) markers are simple and faster; need only a little amount of DNA and no need of radioactivity tests. These markers have been used for the detection of somaclonal variation in various micropropagated plants (Carvalho *et al*., 2004; Martins *et al*., 2004; Modgil *et al*., 2005 and Ramage *et al*., 2004). ISSR is highly discriminative, reliable and cost- effective (Lakshmanan *et al.*, 2007 and Mehrotra *et al*., 2012). Meanwhile, SRAP (sequence related amplified polymorphism, a more recently developed,dominant marker PCR technique (Li and Quiros, 2001), is simple, inexpensive and effective for producing genome-wide fragments with high reproducibility and versatility. It targets open reading frames (ORFs) and allows easy isolation of bands for sequencing (Uzun *et al*., 2009). So, SRAP used for a variety of purposes in different plants, including germplasm identification, map construction, gene tagging, genomic and cDNA fingerprinting, map based cloning, evaluate genetic diversity and assess genetic stability of several micropropagated species (Al-Saleem *et al*., 2014; Amar, 2012; Deepak *et al*., 2011 and Zhu *et al*., 2014). The present study aimed to use inter simple sequence repeats (ISSR)and sequence-related amplified polymorphism(SRAP) analyses to evaluate genetic stability of*Rubus fruticosus* L. 'Triple Crown' micropropagated plantlets and compare possibly existing genetic variation between them and their donor mother plant. Also, compare between discriminating capacity and efficiency of ISSR and SRAP markers for genetic analysis.

MATERIAL AND METHODS

Elite *Rubus fruticosus* L. 'Triple Crown' rooted plants were acclimatized in green house at North Sinai Research Station, Desert Research Center through years 2014-2015 were used. This cultivar was introduced jointly by USDA- Beltsville, Maryland and Pacific West Agric. Research Service-Germany in 1996. Then seedlings of this cultivar were bought to North Sinai Research Station (DRC) and Fac. of Agric., Assiut Univ. Egypt from Germany. *Blackberry, Rubus fruticosus* L.'Triple Crown' was micropropagated in Tissue Culture Unit- North Sinai Research Station as follows: - plants shoot tips were excised, disinfected using sodium hypochlorite solution, washed several times with sterilizedwaterand were cultured on Morashige and Skoog (MS) basal medium (Morashige and Skoog, 1986) which were supplemented with benzyl adenine 0.5mg /L. These shoot tips were developed to shootlets which were successfully transferred to multiplication media for several sub-cultures and developed shootlets subsequently transferred to rooting medium. Five (5) of phenotypically normal micropropagated plantlets (samples) were chosen randomly for usage in this genetic analysis, in-addition to their mother plant (donor).

DNA Extraction

Genomic DNA was isolated from the studied blackberry samples and donor plant by a modified cetyl-trimethyl ammonium bromide (CTAB) method (Tewary and Suryanarayana, 2007 and Saghai-Maroof *et al*., 1984). The quality and quantity of extracted DNA was tested by spectrophotometer and 0.8% agarose gel electrophoresis.

ISSR-PCR Amplification

A set of ten (10) ISSR primers was used to amplify the genome of the studied materials (Table1). PCR amplification were performed in 20µl reaction volume containing, 2µl of genomic DNA, 1.2µl of primers, 0.4µl dNTPs Mix, 1.5µl $MgCl_2$,0.3µl*Taq* DNA polymerase (5 Unit/µl), 2.0 µl PCR bufferand 12.6µl double distilled water. PCR amplification was carried out with 94ºC for initial denaturing,followed by 35 cycles of denaturing at 94ºC for 30s,annealing (considering Tm of primers)for 45s and extension at 72ºC for 2 min. This wasfollowed by a final extension stage for 7 min. at 72ºC. Amplification reaction products were separated on 1.5% agarose gels 1xTAE running buffer. The run was performed at 80V for 180 min. After electrophoresis, staining performed by ethidium bromide. A marker of 1Kb plus DNA Ladder 1µg/µl (Gene Ruler^{TM}that contains a total of fifteen bands ranging from 20.000 to 75 bp was used. Bands were detected on UV-transilluminator and photographed by gel documentation system Biometra Bio-Doc Analyze 2000.

Table 1. List of ISSR primers and their sequences

Primer	Primer Sequence	Primer	Primer Sequence
HB15	(GTG)3 GC	844B	(CT)8 GC
HB04	(GACA)4	HB10	(GA)6 CC
17898A	(CA)6 AC	844A	(CT)8 AC
17898B	(CA)6 GG	807	(AG)8 T
17899A	(CA)6 AG	814	(CT) 8TG

SRAP-PCR Amplification

Thirty-eight SRAP primers consisted of 19 forward and 19 reverse primers (Table2) (were synthesized by Sigma-Aldrich) were screened initially for ease of amplification, overlap between samples and lack of multiple bands. Each sample was then amplified using each of primers. Twenty-three different SRAP primers combinations that produced clear, amplified bands were used for studying genetic stability analysis. Each 20µl PCR reaction mixtures consisted of 1.5 mM $MgCl_2$, 20ng genomic DNA, 0.2 mM dNTPs, 0.5 µM primers (forward and reverse), and 1υ*Taq* polymerase (5u/µl). PCR amplification was performed under the following conditions: 5 min of denaturing at 94ºC, 5 cycles of 1 min of denaturing at 94ºC, 1 min of annealing at 35.0ºC, and 1 min of extension at 72.0ºC. This was followed by 35 cycles of 1 min of denaturing at 94.0ºC, 1 min of annealing (temperature raised to 50.0ºC), 1 min of extension at 72.0ºC and final extension of 10 min at 72.0ºC (Wen *et al*., 2011). All amplification products were visualized on a 1.5% agarose gel. A 100 bp DNA ladder 1µg/µL (GeneRuler^{TM}) was used as molecular standard. The SRAP bands were stained using ethidium bromide. Bands were detected on UV. Transilluminator photographed by gel documentation system Biometra Bio Doc Analyze 2000. SRAP analysis was repeated twice for each DNA sample.

Data Analysis

Only bands that could be unambiguously scored across all samples were used in this study. Amplified ISSR and SRAP fragments were scored for band presence (1) or absence (0). The resulting data matrix was used to calculate the genetic similarity (GS) index, by Neiand Li similarity coefficient (Nei and Li, 1979). Genetic relationships among samples were estimated using an Unweighted Pair-Group Method with Arithmetic mean (UPGMA) cluster analysis of GS matrix,

implemented NTSYS-Pc v2.1a software (Rohlf, 2000). The polymorphism information content (PIC) for each primer was calculated to estimate its allelic variation according to the formula (Smith *et al*., 1997).

$$PIC = 1- \sum^{n}_{j-1} P_{ig}^{2}$$

Where P_{ij} is the frequency of the ith allele for marker j and the summation extends over n alleles, calculated for each ISSR and SRAP marker. Comparisons of discriminating capacity, level of polymorphism and informativeness were calculated according to Anderson *et al.* (1993), Demey *et al.* (2004) and Powell *et al*. (1996).

RESULTS

Genetic stability of micropropagated plantlets (samples) using ISSRtechnique

The ten ISSR primers used generated 144 clear and scorable bands across the studied five micropropagated *Rubus fruticosus* L.'Triple Crown' micropropagated plantlets (samples) and their mother plant (donor) (Figure 1 and Table 3).

The highest and the lowest number of polymorphic bands per assay were 6.00 and 0.00 bands, respectively (Table 3). The mean value of polymorphism was 23.60%, with the highest value for the primer 814 (50.00%) and the lowest value for the 844A (0.00%).

The results showed genetic variation among different samples (micopropagated plantlets) as well as between samples and their mother plant. Since this experiment was performed starting from a single individual mother plant, the polymorphism in banding patter reveal somaclonal variation in samples. The average of PIC index was 0.93 that showedefficiency of ISSR primers to separate studied samples, but some primers were more efficient in differentiation between samples than others. Data revealed that the maximum PIC (0.95) was observed for primers HB15 and 17899B and the minimumPIC (0.91) was obtained with primers 814 and 844B. Marker index (MI) was calculated for all primers, with an average of 3.185. The MI values for ISSR primers ranged between 0.00 and 5.7. This feature has been used to evaluate the discriminatory power of molecular marker systems in some plant species (Kumar *et al*., 2011). These results coincide with previous results of Esmaeili *et al*. (2014), Khan *et al*. (2013) and Mehrotra *et al*. (2012).

Table 2. The forward and reverse SRAP primers and their sequences used in the assessment of genetic stability of the blackberry micropropagated plants

Forward Primer	Forward primer sequence (3'- 5')	Reverse Primer	Reverse primer sequence (5' - 3')
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me2	TGAGTCCAAACCGGAGC	Em2	GACTGCGTACGAATTTGC
Me3	TGAGTCCAAACCGGAAT	Em3	GACTGCGTACGAATTGAC
Me4	TGAGTCCAAACCGGACC	Em4	GACTGCGTACGAATTTGA
Me5	TGAGTCCAAACCGGAAG	Em5	GACTGCGTACGAATTAAC
Me6	TGAGTCCAAACCGGACA	Em6	GACTGCGTACGAATTGCA
Me7	TGAGTCCAAACCGGACG	Em7	GACTGCGTACGAATTCAA
Me8	TGAGTCCAAACCGGACT	Em8	GACTGCGTACGAATTCTG
Me9	TGAGTCCAAACCGGAGG	Em9	GACTGCGTACGAATTCAG
Me10	TGAGTCCAAACCGGAAA	Em10	GACTGCGTACGAATTCAT
Me11	TGAGTCCAAACCGGAAC	Em11	GACTGCGTACGAATTCTA
Me12	TGAGTCCAAACCGGAGA	Em12	GACTGCGTACGAATTCTC
DN06	TGAGTCCAAACCGGTAA	Em13	GACTGCGTACGAATTCTT
DN07	TGAGTCCAAACCGGTCC	Em14	GACTGCGTACGAATTGAT
DN08	TGAGTCCAAACCGGTGC	Em15	GACTGCGTACGAATTGTC
DN09	TGAGTCCAAACCGGTCA	Em16	GACTGCGTACGAATTCGA
DN10	TGAGTCCAAACCGGGCT	Em17	GACTGCGTACGAATTAGC
DN11	TGAGTCCAAACCGGTAG	Em18	GACTGCGTACGAATTGAG
DN12	TGAGTCCAAACCGGTGT	Em19	GACTGCGTACGAATTGCC

Table 3. Levels of genetic information generated from DNA of *Rubus fruticosus* L .' Triple Crown' mother plant and its micropropagated plantlets by ten ISSR primers

Primer	Total bands	No.ofmonom-orphic bands	% Mono-morphism	No.ofpolym-orphic bands	% Poly morphism	PIC	MI	D
HB04	13	8	61.5	5	38.5	0.93	4.65	0.933
HB10	16	10	62.5	6	38.0	0.94	5.70	0.95
HB15	16	11	68.8	5	31.3	0.95	4.65	0.96
807	17	14	82.4	3	17.7	0.94	2.84	0.95
814	8	4	50.0	4	50.0	0.91	3.65	0.93
844A	11	11	00.0	0	0.00	0.91	0.00	0.92
844B	14	12	85.7	2	14.3	0.93	1.86	0.94
17898A	15	12	80.0	3	20.0	0.94	2.82	0.95
17899A	16	13	81.3	3	18.75	0.94	2.82	0.954
17899B	18	15	83.3	3	16.67	0.95	2.85	0.96
Total	144	110	76.4	34	23.6	9.34	31.85	9.44
Average	14.4	11.0	76.4	3.4	23.6	0.93	3.185	0.944

Also, the number of amplified DNA fragments by each primer ranged from 18 and 8 produced by primers 17899B and 814, respectively. Analysis of banding patterns revealed total 34 polymorphic bands with an average of 3.40 bands per primer.

To assess the genetic variation or relationships between thestudied R. *fruticosus* L. mother plant and its micropropagated samples the UPGMA dendrogram was constructed based on Neiand Li genetic distance matrix (Figure 2).

Genetic stability of the micropropagated plantlets (samples) using SRAP

Twenty–three SRAP primer combinations used generated clear bands across the studied *Rubus fruticosus* L.'Triple Crown' micropropagated plantlets (samples) and their mother plant (donor) (Figure 3 and Table 4).

Total polymorphic bands were 135.00 with an average of 5.87 bands per primers combination. The highest and the lowest number of polymorphic bands per assay were 9.00 and 3.00 bands, respectively (Table 4). The mean value of polymorphism was 5.87%, with the highest value for primers combination Em16R-DN10F (75.0%) and the lowest value for primers combination Em9R-DN6F and Em20R-Me9F (43.0).

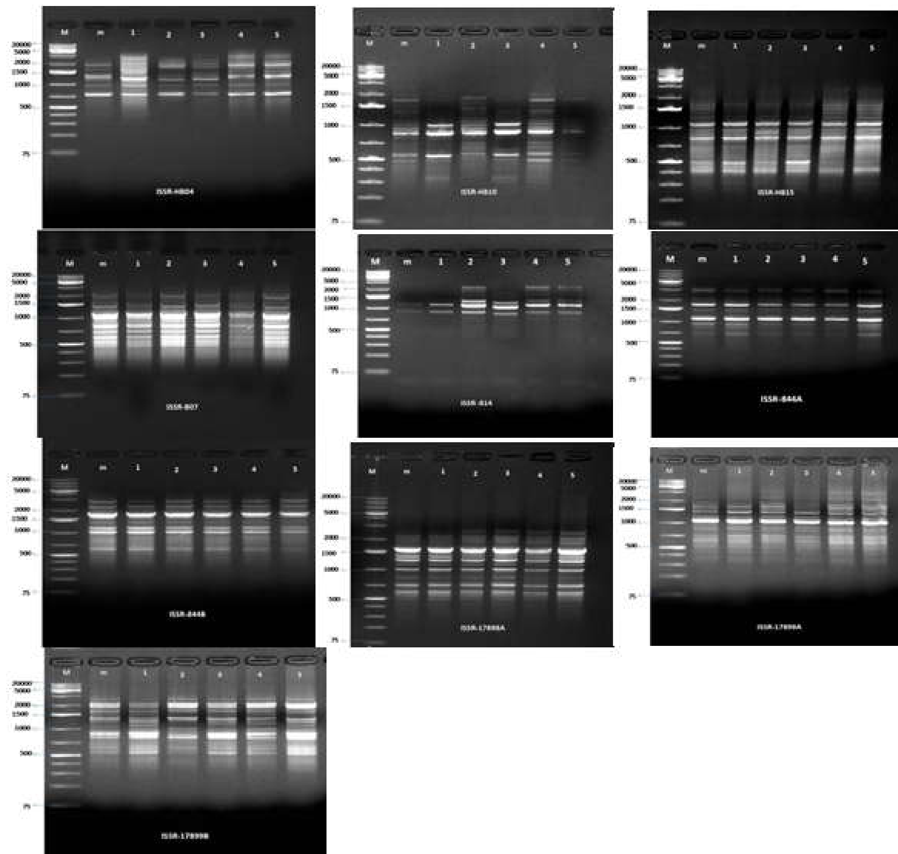


Figure 1. Inter simple sequence repeat (ISSR) amplification pattern obtained from DNA of *Rubus fruticosus* L.'Triple Crown' mother plant (Lane1) and its micropropagated plantlets (Lanes 2-6) generated by 10 primers. M: GeneRuler^{TM} 1 Kp DNA Ladder Plus

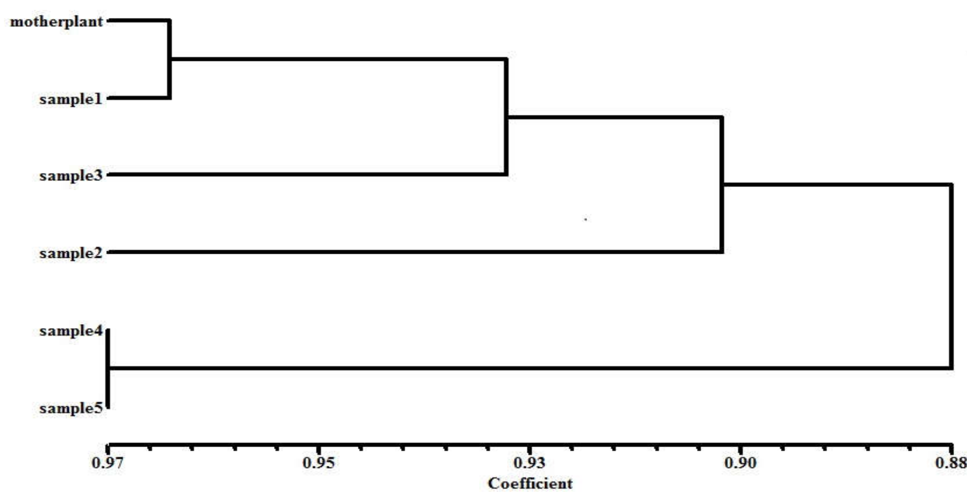


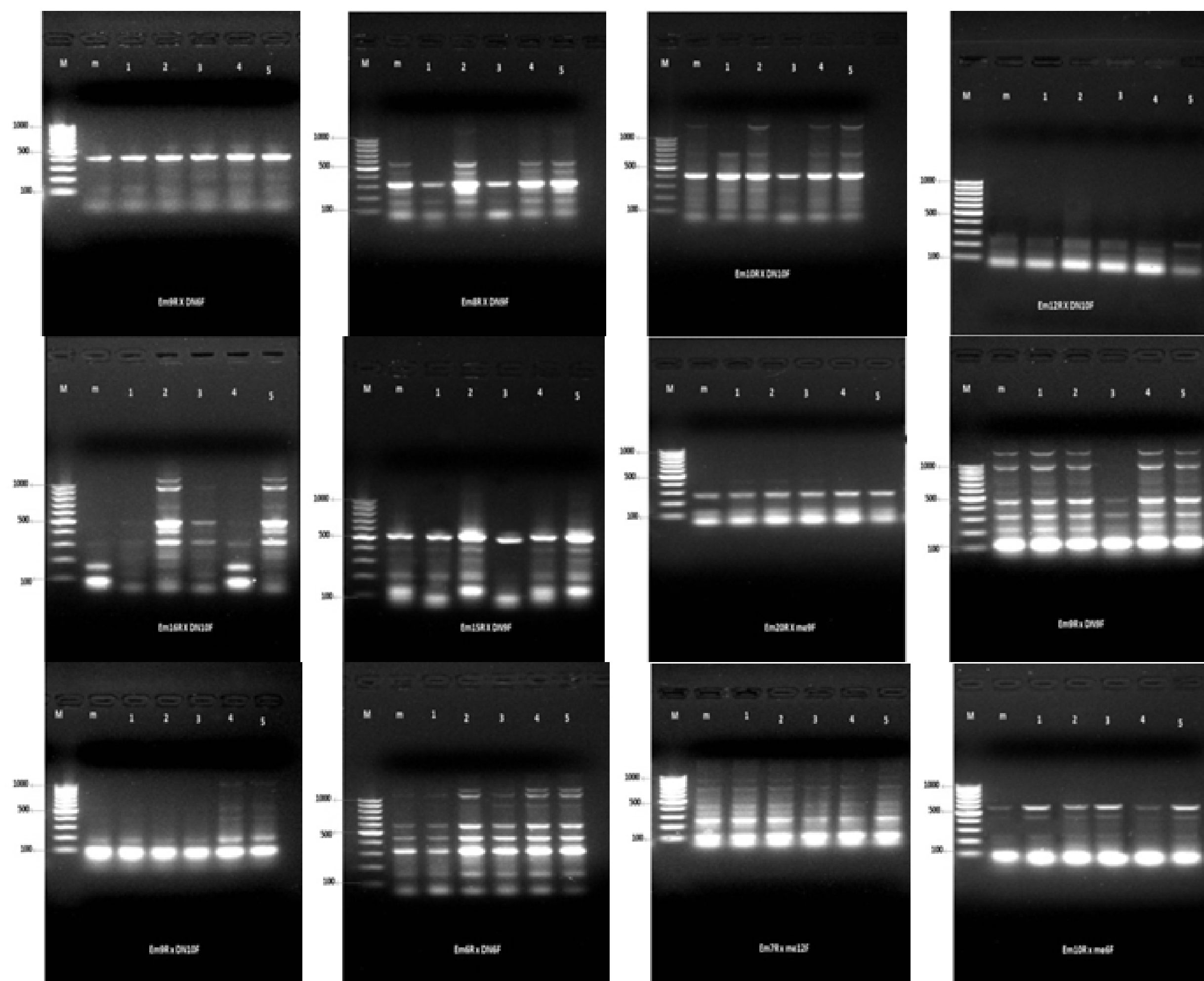
Figure 2. Dendrograms showing genetic similarities among the studied *Rubus fruticosus* L.'Triple Crown' micropropagated plantlets and their mother plant constructed using Nei& Li similarity coefficients based on ISSR markers (UPGMA)

Analysis of banding patterns revealed total number of amplified bands was 225 bands and the number of amplified DNA fragments by each primers combination ranged from 6.00 and 16.00 bands.

Genetic variation among the studied samples and their mother has been obviously shown. The average of PIC index was 0.94 that showed efficiency of SRAP primers to separate studied samples, and some primers combinations were more efficient

Table 4. Levels of genetic information generated from DNA of *Rubus fruticosus* L.'Triple Crown' mother plant and its micropropagated plantlets by 23 SRAP primer combinations

Primer combination	Total bands	No.ofmon omorp-hic bands	% Poly morphism	No.ofpolymo rp-hic bands	% Poly morphism	PIC	MI	D
Em19R-DN10F	6	2	43	4	57	0.92	3.68	0.93
Em19R-DN9F	7	3	33	4	67	0.91	3.64	0.94
Em19R-DN6F	16	8	50	8	50	0.96	7.7	0.97
Em14R-DN10F	12	5	42	7	58	0.95	6.62	0.96
Em13R-Me7F	15	9	60	6	40	0.95	5.71	0.97
Em12R-Me5F	9	2	22	7	78	0.94	6.57	0.95
Em12R-Me2F	12	4	33	8	67	0.98	7.86	0.99
Em12R-Me6F	9	2	22	7	78	0.96	6.73	0.97
EM12R-Me1F	10	5	50	5	50	0.93	4.65	0.95
Em12R-DN10F	5	2	40	3	60	0.85	2.55	0.91
Em10R-DN10F	12	4	33	8	67	0.95	7.59	0.96
Em9R- DN6F	7	4	57	3	43	0.91	2.73	0.92
Em8R-DN9F	9	3	33.3	6	66.7	0.94	5.64	0.95
Em20-Me9F	7	4	57	3	43	0.91	2.72	0.92
Em16R-DN10F	12	3	25	9	75	0.97	8.75	0.98
Em15R-DN9F	11	4	36.0	7	64	0.92	6.48	0.94
Em10R- Me6F	7	2	28.6	5	71.4	0.93	4.67	0.94
Em9R-DN10F	6	3	50	3	50	0.96	3.88	0.90
Em9R-DN9F	11	4	36	7	64	0.94	6.56	0.98
Em7R-Me12F	11	6	54.5	5	45.5	0.94	4.7	0.95
Em6R-Me8F	8	2	25	6	75	0.93	5.57	0.94
Em6R-DN6F	13	6	46.2	7	54	0.95	6.67	0.99
Em2R-DN6F	10	3	30	7	70	0.95	6.65	0.96
Total	255	90	40	135	60	21.6	128	2.19
Average	9.78	3.91	40	5.87	60	0.94	5.58	0.95



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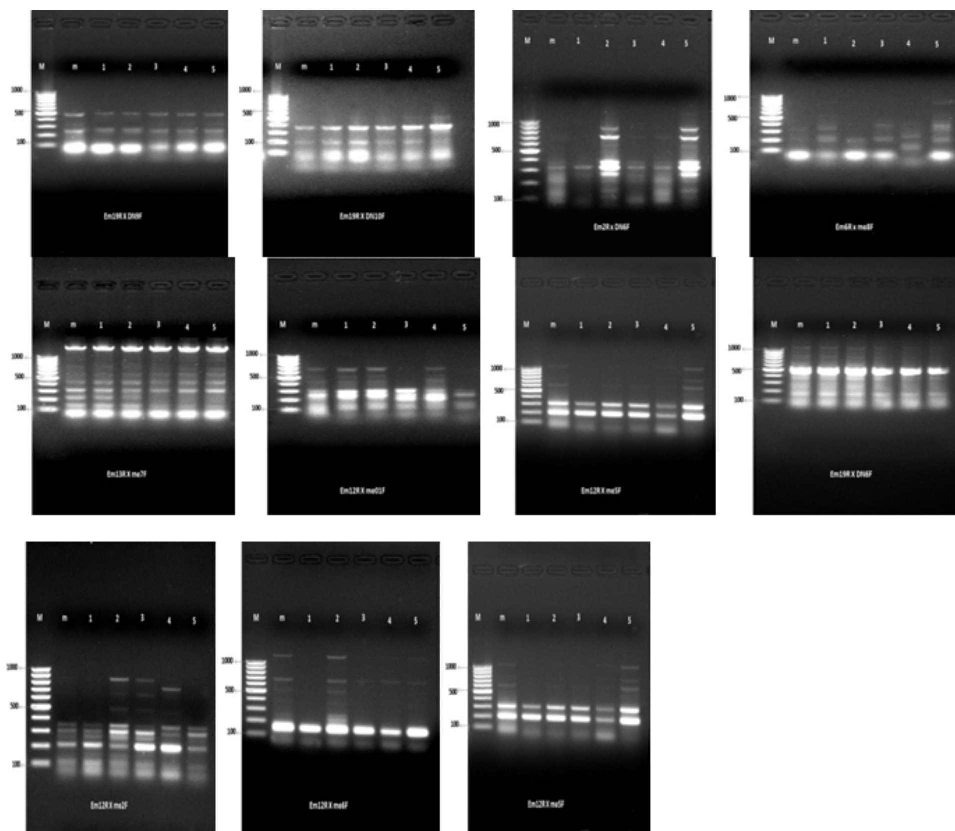

Figure 3. Sequence related amplified polymorphism (SRAP) amplification pattern obtained from DNA of *Rubus fruticosus* L.'Triple Crown' mother plant (Lane1) and its micropropagated plantlets (Lanes 2-6) generated by 23 primer combinations. M: GeneRuler^{TM} 1 Kp DNA Ladder Plus

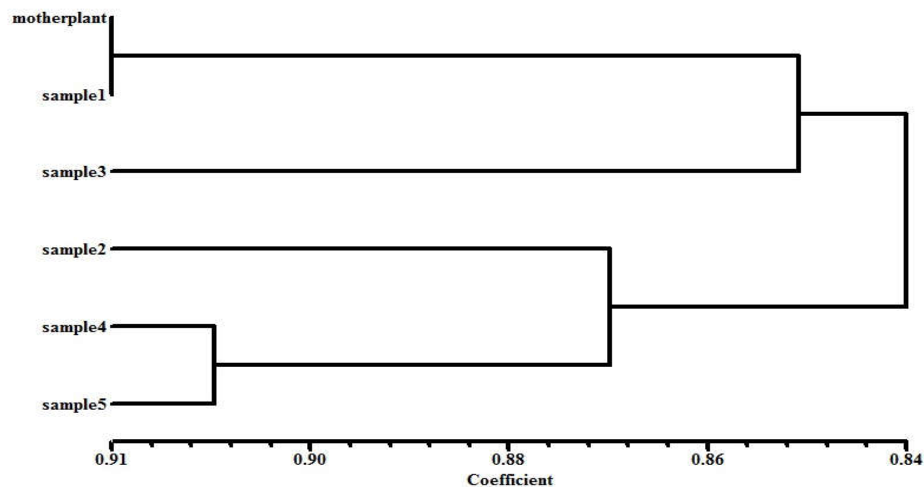

Figure 4. Dendrograms showing genetic similarities among the studied *Rubus fruticosus* L.'Triple Crown' micropropagated plantlets and their mother plant constructed using Nei& Li similarity coefficients based on SRAP markers (UPGMA).

than others. Data revealed that the maximum PIC (0.98) was observed for primers combination Em12R-Me2F and the minimum PIC (0, 91) was obtained with primers combinations Em19R-DN9F, Em9R-DN6F and Em20R-Me9F.Marker index was calculated for all primers combinations with an average of 5.58. The MI values for SRAP primers combinations ranged between 2.55 and 8.75. Similar results were reported by Li *et al*.(2014), Sun *et al.* (2015) and andMuniswamy (2017). To assess the genetic variation among the studied *R.fruticosus* L. mother plant and its micropropagated plantlets the UPGMA dendrogram was constructed based onNei&Li genetic distance matrix. Dendrogram based on clustering (Figure4) revealed genetic instability.

Comparisons of the level of polymorphism and discriminating capacity of ISSR and SRAP molecular markers are shown in Tables 3 &4. Slightly higher PIC values were obtained with SRAP markers. However, the level of polymorphism and discriminating capacity of all primers were highly effective in discriminating the analyzed samples. Tables 3&4 shows information obtained with both markers, where the mean number of polymorphic bands was 5.87 loci for SRAP and 3.40 loci for ISSR. So, SRAP produced more polymorphic bands for assay unit. Similar results were reported byMishra *et al*. (2011).

DISCUSSION

Somaclonal variation was coined to refer to the genetic variation among regenerate plants from *in-vitro* cultures of plant cells, calli and organs (Larkin and Scowcroft, 1981 and Skirivin *et al*., 1994). Typical genetic alterations are: changes in chromosome numbers (polyploidy and aneuploidy), chromosome structure (translocations, deletions, insertions and duplications) and DNA sequence (base mutations and gene activation/ inactivation including transposons (Kaeppler *et al*., 2000 and Largia *et al*., 2015). In spite of somaclonal variation may imply disadvantage for the propagation of an elite cultivar, it may be an advantage as a source of variability for novel variations for crop breed improvement. Induction of somaclonal variability in *in-vitro* culture is one of the methods for widening the genetic bases of any crop (Amzad *et al*., 2003, Devi *et al*., 2014, Hrahsel *et al*., 2014 and Li *et al*., 2005). Two types of markers were used to be beneficial for analysis of genetic variation as a different markers target different regions in the genome (Hrahsel *et al*., 2014 and Phulwaria *et al*., 2014). Hence, in the present study, ISSR and SRAP two PCR based DNA markers were adopted for evaluation of genetic variability among and between*Rubus fruticosus* L.'Triple Crown' micropropagated plantlets and their donor mother plant. Based on results of this study we are able to characterized genetic variability among the studied genotypes.

Where, ISSR analysis revealed polymorphic and monomorphic bands with an average of 23.6 % and 76.4 %, respectively. Also, monomorphic and polymorphic bands ratio were 40 % and 60 %, alternatively as revealed by SRAP analysis. Also, PIC (polymorphic information content and D (Discriminating power) of SRAPs were slightly higher than of ISSRs. In comparisons performed based on genetic stability, ISSR and SRAP markers, many reports have indicated the occurrence of genetic variation among the micropropagated --plantlets of different plant species by using ISSRs (Esmaeili *et al*., 2014; Khan *et al*., 2013; Lakshmanan *et al*., 2007; Mehrotra *et al*., 2012 and pourjabar *et al*., 2009). However, few studies were conducted in this purpose such as Devi *et al*., 2014 and Peng *et al*., 2015).The weaking or disappearance of some shared bands might often be because of segregation of parental heterozygosity (Guo *et al*., 2006) or because that some gene loci readily mutate, while other are more conservative. A few new bands emerge might be because of *De novo* genomic changes (Guo *et al*., 2006) or gene amplification in long- term culture in-vitro. The frequency of these variations varies with culture conditions, culture time, propagation patter, number of subcultures, choice and concentration of growth regulators, culture variability, explants source and age of culture (Gaafar and Saker, 2006;Gajdosova *et al*., 2006; Kilinc *et al*., 2014; Jin *et al*., 2008 and Vujovic *et al*., 2010). Moreover, the loci detected by ISSR were scattered throughout the genome which targeted non-functional regions, which were susceptible to surrounding environment. So, Khan *et al*. (2013), Mehrotra *et al*. (2012) and Peng *et al*. (2015) reported, ISSRtechnique is highly discriminative and has been successfully used for the detection of somaclonal variation among phenotypically normal regenerants and essentially identical with theirmother plant. On other hand, SRAPs, polymorphism in SRAP assay may result from small insertions and deletions or changes in nucleotide sequence (Li and Quiros, 2001). Therefore, differences in banding pattern from parentto offspring may be the result of mutation (Darnell *et al*., 1990 and Huchett & Botha 1995).

Zaefizadeh and Goliev (2009) reported that SRAP markers possess multiloci and multi in allelic features which make them potentially more efficient for genetic variation analysis. In-addition, only amplified target region of open reading frame (ORF), the functional and relatively conservative regions (Li andQuiros, 2001; Peng *et al*., 2015 and Zietkiewicz *et al*., 1994). In-spite of, SRAPs may be slightly effective and discriminativethan ISSRs with the present study, usage of ISSRs and SRAPs may be useful in generating "all- sided" information. Because, ISSR markers target the region within the microsatellite repeats whereas, SRAP markers preferentially detect polymorphism in coding sequences which are usually conserved among closely related cultivars and species with low mutation rate (Chen *et al*., 2013 and Mishra *et al*., 2011).

Conclusion

In this comparative study, genetic variation was detected between *Rubus fruticosus* L.'Triple Crown' micropropagated plantlets and their donor mother plant. ISSR and SRAPmarkers were powerful tool for Characterized this genetic variability but, SRAPs slightly more efficient than ISSRs. And, PIC (polymorphic information content) and D (Discriminating power) of SRAPs were slightly higher than ISSR. Also, simultaneous use of differenttypes of molecular markers may be useful in generating "all- sided" information. Further understanding of the level of genetic variation between these economically importantplantlets would provide an important input in designing appropriate breeding exercises for production an elite material (new cultivars) in high demand for breeding, industry production of secondary metabolites.

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