GENETIC IDENTITY OF RASPBERRY 'POLANA' PLANTLINGS EXAMINED USING MICROSATELLITE MARKERS

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Abstract

Raspberry cultivars are clonally propagated and therefore all plants belonging to a single cultivar represent the same genotype. Cultivar integrity of raspberry plantlings placed on the market in Bosnia and Herzegovina (B&H) is based on examining of morphological traits, which is not a reliable tool for genetic identification. In this study plantlings declared as cultivar 'Polana' were genotyped using seven microsatellites, in order to gain preliminary insight into the genetic integrity of raspberry plantlings marketed in B&H. Plant tissue (leaves) from 10 raspberry plants were randomly sampled from a batch of plantlings sold by major fruit nursery in Bosnia and Herzegovina. Along with these samples, four reference cultivars with confirmed identity ('Polka', 'Autumn Bliss', 'Heritage' and 'Polana') were also included in the study. Seven primer pairs amplified 31 alleles, or on average 4.4 alleles per locus. UPGMA cluster analysis, based on the Jaccard similarity coefficient, revealed that among the ten samples declared as 'Polana' plantlings only five were genetically identical to any of the other samples. The cluster analyses also exposed that none of the ten samples declared as 'Polana' seedlings were in fact identical or even closely related to the 'Polana' reference cultivar or any of the other reference cultivars. These findings clearly show that the genetic identity of primocane raspberry plantlings , currently sold in Bosnia and Herzegovina, needs to be tested using objective and reliable methods rather than simple morphologic observation.

Key words: *cultivar integrity, morphological traits, DNA*

Introduction

Red raspberry (*Rubus idaeus*), along with strawberry, represents the most commercially important berry fruit currently cultivated in Bosnia and Herzegovina (B&H). Raspberry cultivars are clonally propagated and therefore all plants belonging to a single cultivar possess an identical genetic makeup (same genotype). In order for raspberry plantlings to be placed on the market in B&H, they must be supervised during the nursery production in order to ensure the cultivar integrity. However, this supervision is based on examining morphological traits,

which are oftentimes not a reliable tool for the verification of genetic identity. Unlike morphological traits, microsatellite DNA markers have proven to be an excellent tool for examining the cultivar integrity among raspberry genotypes (Fernandez-Fernandez et al., 2011). A number of different microsatellites or SSRs (Simple Sequence Repeats) have been developed for R. idaeus (Graham et al., 2002; Castillo et al., 2010) and are available for this purpose. Microsatellite markers have previously been used in genetic studies of several fruit crops in Bosnia: apple (Gasi et al., 2010; Gasi et al., 2013a, Gasi et al., 2013d), pear (Gasi et al., 2013b), plum (Halapija-Kazija et al., 2014) and cherry (Gasi et al., 2013c). However, until now SSRs have not been used for genetic analyses of berry fruits in this country.

In this study, genetic identity of plantlings declared as cultivar 'Polana' were genotyped using microsatellites, in order to gain preliminary insight into the genetic integrity of raspberry plantlings marketed in Bosnia and Herzegovina.

Table 1. Seven SSR markers developed by Castillo et al. (2010), used in this study

SSR marker	forward primer	reverse primer
RhM001	GGTTCGGATAGTTAATCCTCCC	CCAACTGTTGTAAATGCAGGAA
RhM003	CCATCTCCAATTCAGTTCTTCC	AGCAGAATCGGTTCTTACAAGC
RiM019	ATTCAAGAGCTTAACTGTGGGC	CAATATGCCATCCACAGAGAAA
RhM023	CGACAACGACAATTCTCACATT	GTTATCAAGCGATCCTGCAGTT
RhM011	AAAGACAAGGCGTCCACAAC	GGTTATGCTTTGATTAGGCTGG
RhM043	GGACACGGTTCTAACTATGGCT	ATTGTCGCTCCAACGAAGATT
RiM015	CGACACCGATCAGAGCTAATTC	ATAGTTGCATTGGCAGGCTTAT

Materials and methods

Plant tissue (leaves) from 10 raspberry plants, randomly sampled from a batch of plantlings sold by a major fruit nursery in Bosnia and Herzegovina, were used for the isolation of genomic DNA applying CTAB method (Doyle and Doyle, 1987). All of the sampled plants were declared by the nursery to be the plantlings derived from the primocane raspberry cultivar 'Polana'. Along with these samples, four reference cultivars with confirmed identity ('Polka', 'Autumn Bliss', 'Heritage' and 'Polana') were also included in the study. Seven primer pairs (Table 1) used for SSR amplifications have previously been published by Castillo et al. (2010). Polymerase chain reaction (PCR) amplification of SSR sequences was performed in a Veriti TM Thermal Cycler (Applied Biosystems, Foster City, CA) using fluorescently labelled primers. All PCR amplifications were performed as described in Castillo et al. (2010). The detection of SSR products was conducted on ABI 310 automated sequencer (Applied Biosystems). PCR product (1 µL) was added to a master mix containing 9 µL of deionized formamide and 0.5 µL GeneScan-350 Rox size-standard (Applied Biosystems). Samples were heated at 95 ˚C for 5 min and immediately cooled down on ice. SSR profiles were scored using GeneMapper Software ID v3.2 (Applied Biosystems). UPGMA cluster analysis, based on Jaccard similarity coefficient, was calculated using the obtained SSR data, in NTSYS software (Rohlf, 1993) and visualized by MEGA 5 software (Molecular Evolutionary Genetics Analysis), (Tamura et al., 2011).

Results and discussion

Seven primer pairs amplified 31 alleles (Table 2), or on average 4.4 alleles per locus, among all the 14 analysed raspberry samples. Higher values for the average alleles per locus (7.9), among the same SSR loci, have been published by Castillo et al. (2010). However, in that study 48 different raspberry genotypes were examined. Overall, the seven primer pairs used in our research provided sufficient variation for clear distinction among four reference cultivars. The same cluster analyses revealed that among the ten samples declared as 'Polana' plantlings, only five were genetically identical to any of the other samples. Considering that raspberry plantlings are clonally propagated, all true offspring should share the same SSR profile. The cluster analyses also revealed that none of the ten samples declared as 'Polana' plantlings were in fact identical or even closely related to 'Polana' reference cultivar or any of the other

Table 2. SSR profiles (allele sizes expressed in base pairs) of 10 raspberry plants, declared as plantlings of the cultivar 'Polana', and four reference cultivars, obtained through the analyses of seven SSR loci.

Genotypes	**RhM001**		**RhM003**		**RiM019**		**RhM023**		**RhM011**		**RhM043**		**RiM015**	
'Polka'	239	241	202	202	180	182	195	195	289	291	371	373	351	351
'Autumn Bliss'	227	229	191	193	182	217	187	195	279	279	373	373	349	351
'Heritage'	227	236	193	202	180	182	187	195	281	291	371	371	349	351
'Polana' ref.	236	236	200	202	182	195	187	195	281	281	371	373	351	351
Sample 1	227	236	200	202	174	182	187	195	279	279	373	373	349	351
Sample 2	227	236	195	202	170	178	187	195	285	285	373	373	349	359
Sample 3	227	236	200	202	174	182	187	195	279	279	373	373	349	351
Sample 4	227	236	200	202	174	182	187	195	279	279	373	373	349	349
Sample 5	227	236	200	202	182	182	187	195	279	279	373	373	349	351
Sample 6	227	236	200	204	180	182	187	195	285	285	373	373	349	349
Sample 7	227	236	191	193	174	182	187	195	279	279	373	373	349	351
Sample 8	227	236	191	193	174	182	187	195	279	279	373	373	349	351
Sample 9	227	236	191	193	174	182	187	195	279	279	373	373	349	351
Sample 10	227	236	200	202	182	182	187	195	279	279	373	373	335	339

UPGMA cluster analysis, based on Jaccard similarity coefficient, revealed that among the reference cultivars, 'Heritage' and the reference 'Polana' samples clustered the closest (Figure 1). Similar findings have already been reported by Bassil et al. (2012). Also, considering that 'Heritage' is a parent of the cultivar 'Polana' this result was entirely expected.

reference cultivars. The obtained results indicate that investigated samples are at least in partgenerative offspring, which probably arose through spontaneous hybridization between unknown raspberry genotypes. These findings clearly show that the supervision of nursery production, based on morphological traits, does not guaranty cultivar integrity.

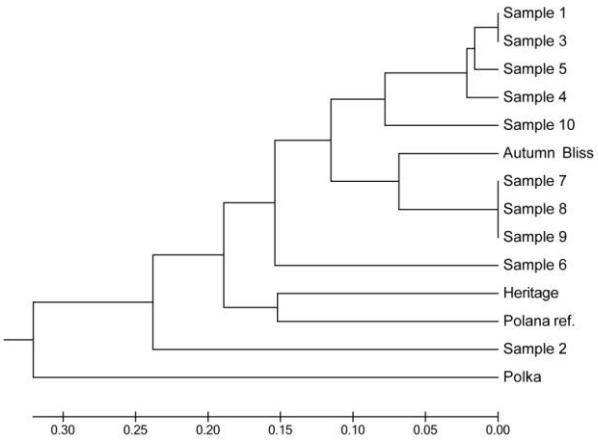

Figure 1. UPGMA cluster analyses of 10 raspberry plants, declared as plantlings of the cultivar 'Polana', and four reference cultivars

Conclusions

The genetic identity of primocane raspberry seedlings, currently sold in Bosnia and Herzegovina, needs to be tested using a more objective and reliable method than simple morphologic observation. The results of this study indicate that DNA markers are a necessary tool for this task, among which microsatellites can be considered as highly suitable.

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