

Development of a multiplexed microsatellite set for fingerprinting red raspberry (*Rubus idaeus*) germplasm and its transferability to other *Rubus* species

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Received 18 October 2010; accepted 2 March 2011

Abstract. This study aimed to develop a standardised and cost effective protocol for fingerprinting red raspberry germplasm using microsatellite (SSR) markers. Twenty SSRs well distributed through the raspberry genome were screened on 16 genetically diverse cultivars to assess polymorphism, allele size range, quality of amplification, ease of scoring, and discriminating power (DF). We selected published SSR markers that were highly informative, consistently easy to score, provided good genome coverage and could be conveniently amplified into two multiplexed reactions. As a proof of concept, the fingerprinting set was used to genotype 37 red raspberry cultivars of various origins and 19 advanced selections from our breeding programme. The multiplexed set amplified consistently the same products originated by single reactions and it reliably discriminated all non-clonal accessions including full-sib genotypes. Furthermore the set was tested on 19 accessions of eight related *Rubus* species to assess its transferability. Provided control samples are included, this set will permit research groups to share data accurately and easily. The use of DNA fingerprinting to ensure trueness-to-type of clonally propagated horticultural crops is increasingly common and this multiplexed set constitutes a reliable, convenient and economic tool for raspberry genotyping.

Keywords: Genotyping, trueness-to-type, SSR, caneberry

1. Introduction

Red raspberry (*Rubus idaeus* L.) belongs, as many other temperate fruit crops, to the Rosaceae family. The *Rubus* genus comprises several hundreds of species and hybrids of which most of the cultivated types belong to one of the two largest subgenera: *Rubus* (blackberries) and *Idaeobatus* (raspberries). The raspberry subgenera are diploid ($2n = 2x = 14$) and comprise the European red raspberry (*R. idaeus* L.), the North American red raspberry (*R. strigosus* Michx.), the black raspberry (*R. occidentalis* L.) and their hybrid, aptly known as the purple raspberry (*R. ×neglectus* Peck), whilst blackberries species vary greatly in ploidy.

Worldwide production of raspberries exceeds 500,000 Mt per year with the Russian Federation, the EU, Serbia and the US as the largest producers [1]. New raspberry cultivars become available every year and the nursery business is thriving and complex. Approximately 1,600 ha are currently dedicated to raspberries in the UK with more than 80% of production currently being grown under protection. Annual domestic production is around 14,000 t with a value in the region of £95M [2].

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Raspberry cultivars are propagated clonally from their roots or *in vitro* using meristem cultures and are commercialised as small rooted plants or as dormant canes carrying the root system. Propagation stocks can go through several rounds of vegetative multiplication during which the absence of fruit makes morphological verification very difficult and errors can be extremely costly. Thus, ensuring trueness-to-type of the material during this process as well as at the point of sale is critical. Furthermore, reliable DNA fingerprinting is essential to protect breeders' rights, police illegal fruit sales and should be routinely incorporated to propagation protocols to ensure cultivar integrity.

Early attempts to characterise raspberry germplasm using molecular markers concentrated in non sequence-specific markers such as RAPDs and AFLPs. RAPDs have been used to determine the relationships within and between *Rubus* species [3, 4] and for cultivar identification [5]. Likewise, AFLPs have been employed in the assessment of genetic diversity in *Rubus* [6]. However, in recent years, due to their well documented advantages for diversity studies and linkage map construction, SSRs have become the markers of choice for genetic characterisation of rosaceous species including apple [7], pear [8], cherry [9], peach [10] and strawberry [11]. Caneberries are no exception; SSRs have been developed from a number of different *Rubus* species [12–16] and recently EST-SSRs have been developed in raspberry and blackberry [17, 18]. Additionally, some studies have tried to determine their usefulness for genetic studies in raspberry and blackberry [3, 16].

Despite their advantages over other molecular markers, reproducible and cost-effective SSR fingerprinting requires a careful choice of markers. Ideally a good fingerprinting set would consist of highly polymorphic markers that are distributed throughout the genome and that display robust amplification profiles (low tendency to A-additions, no wavering of large fragments etc.). Such sets have been proposed for a number of rosaceous crops such as strawberry [11], cherry [9] and pear [19]. Additionally, SSR markers can often be combined in multiplexed reactions, in particular when using fluorescently labeled primers, and/or different reactions can be pooled together for electrophoresis thus saving time and money [20]. Ideally the markers selected for a core fingerprinting set should be suitable for multiplexed PCR amplification allowing high-through put and economical genotyping [11, 19].

The purpose this investigation was to use available SSR markers for the development of a robust and cost-effective multiplexed genotyping set for the accurate fingerprinting and thus reliable identification of red raspberry cultivars and breeding lines.

2. Materials and methods

2.1. Plant material and DNA extraction

Sixteen red raspberry cultivars from a range of different origins and including both primocane and florican fruiting types were chosen to optimise the SSR set, namely 'Autumn Bliss', 'Autumn Treasure', 'Cascade Delight', 'Cowichan', 'Grushovka Leningradskaya', 'Glen Ample', 'Glen Rosa', 'Haida', 'Heritage', 'Himbotop', 'Latham', 'Malling Jewel', 'Malling Leo', 'Octavia', 'Rubaca' and 'Tulameen'. An additional set of 21 cultivars and 19 advanced selections from EMR's breeding programme (Table 1) were then analysed with the optimised set. Finally, four black raspberry cultivars ('Cumberland', 'Haut', 'Jewel' and 'MacBlack'); six blackberry cultivars ('Arapaho', 'Aurora', 'Black Satin', 'Darrow', 'Kotata' and 'Loch Ness'); four hybrid berries ('Logan Thornless', 'Marionberry', 'Sunberry' and 'Tayberry') and five accessions of related species, namely *R. coreanus*, *R. crataegifolius*, *R. odoratus*, *R. spectabilis* and *R. strigosus*, were also analysed with the optimised set to assess its transferability. All genotypes are maintained as part of the field or pot *Rubus* germplasm collection at East Malling Research (EMR). To determine both the range of alleles and the ability of the fingerprinting set to distinguish closely related material, we included primocane and florican-fruiting cultivars from diverse origins as well as interrelated genotypes. DNA was extracted using the DNeasy Plant minikit (Qiagen GmbH, Germany) according to the manufacturer's protocol. DNA was re-suspended in 200 µl AE buffer (Qiagen GmbH, Germany) and was diluted 1 : 200 for use in PCR.

2.2. Marker selections, PCR conditions and product detection and scoring

Based on previous experience and reported marker information, a total of 20 SSR markers previously mapped in raspberry [16, 17] were tested in 16 genetically-diverse red raspberry cultivars to assess polymorphism, quality of

Table 1

Parentage and/or origin of the *Rubus* genotypes (by species) analysed in this study as well as their bearing type – where PF = primocane fruiting and SF = floricanes fruiting – and cultivar year of release. All accessions are diploid ($2n = 2x = 14$) unless stated

Genotype	Parentage or accession	Type (date)
<u>Red raspberry selections</u>		
EM6564/87 ¹	EM3655/47 × Kitsilano	SF
EM6631/64 ¹	EM6429/90 × Tulameen	SF
EM6685/2 ¹	EM6429/6 × Malahat	SF
EM6686/25 ¹	Glen Ample × EM6305/12	SF
EM6686/26 ¹	Glen Ample × EM6305/12	SF
EM6710/132 ¹	EM6481/17 × EM6535/1	PF
EM6727/26 ¹	EM6482/112 × EM6442/155	PF
EM6727/63 ¹	EM6482/112 × EM6442/155	PF
EM6732/96 ¹	EM6413/59 × EM6488/58	SF
EM6739/75 ¹	Glen Ample × EM6399/84	SF
EM6741/89 ¹	SCRI9059C1 × (Himboqueen × Julia)	SF
EM6761/55 ¹	(Himboqueen × Julia) × (EM5928/22 × Glen Ample)	SF
EM6769/75 ¹	Polana × EM6531/62	PF
EM6780/81 ¹	Joan Squire × EM6592/11	PF
EM6803/185 ¹	Octavia × (EM3655/47 × Kitsilano)	SF
EM6804/122 ¹	Tulameen × Octavia	SF
EM6804/94 ¹	Tulameen × Octavia	SF
EM6881/102 ¹	EM6727/35 × Polka	PF
EM6886/91 ¹	Maravilla open pollination	PF
<u>Red raspberry cultivars</u>		
Autumn Bliss ²	Derivative of Landmark, M. Promise and <i>R. articus</i>	PF (1983)
Autumn Treasure ²	EM6304/36 × EM6330/96	PF (2005)
Brice ³	J. Squire × (93119/4 × (Terri-Louise × J.Squire))	PF (2005)
Cascade Delight ⁴	Chilliwack × WSU994 (= deriv. of Meeker and Haida)	SF (2003)
Cowichan ⁵	Newburg × Qualicum	SF (2001)
Erika ⁶	Tulameen × Autumn Bliss	PF (2007)
Esquimalt ⁵	Comox × Glen Ample	SF (2003)
Grushovka Leningradskaya ⁷	Unknown	SF (?)
Glen Ample ⁸	Derivative of G. Prosen, Meeker, Preussen, M. Promise	SF (1996)
Glen Doll ⁸	Glen Rosa × SCRI8605C2	SF (2006)
Glen Fyne ⁸	SCRI8631D1 × SCRI8605C2	SF (1992)
Glen Lyon ⁸	(SCRI6820/35 × Haida) × (SCRI703/36 × G. Prosen)	SF (1994)
Glen Rosa ⁸	Derivative of G. Prosen, Meeker, Preussen, M. Promise	SF (1994)
Haida ⁵	Malling Promise × Creston	SF (1972)
Heritage ⁹	(Milton × Cuthbert) × Durham	PF (1969)
Himbotop ¹⁰	Autumn Bliss × Himboqueen	PF (2008)
Ivars ⁷	Unknown	SF (?)
Joan Squire ³	(J. Squire × J. Irene) × (A. Bliss × <i>R. spectabilis</i>)	PF (1994)
Kiwigold	Yellow sport of Heritage	PF (1997)
Latham ¹¹	King × Loudon	SF (1920)
Malahat ⁵	Meeker × BC7853/116	SF (1996)
Malling Hestia ²	EM3689/5 × EM3655/48	SF (2006)
Malling Jewel ²	Derivative of Preussen, Lloyd George and Pyne's Royal	SF (1980)
Malling Juno ²	EM6166/79 × EM6304/36	SF (2005)
Malling Leo ²	EM791/45 × EM765/30	SF (1975)

(Continued)

Table 1
(Continued)

Genotype	Parentage or accession	Type (date)
Malling Minerva ²	EM5030/3 × SCRI7269/67	SF (2005)
Marcela ³	Joan Squire × Autumn Bliss	PF (2005)
Nagrada ⁷	Kolkhoznitsa × Lloyd George	SF (?)
Octavia ²	Malling Hestia × Glen Ample	SF (2002)
Polana ¹²	Heritage × Zeva Herbsternte	PF (1991)
Polka ¹²	P89141(=Autumn Bliss derivative) open pollination	PF (2001)
Rannaya Sladkaya ⁷	Red Antwerp derivative	SF (?)
Rubaca ¹³	Rucanta × Latham	SF (1993)
Rutrago ¹³	Klon4a × Tragilo	SF (1980)
Sugana ⁶	Tulameen × Autumn Bliss	PF (2008)
Tulameen ⁵	Nootka × Glen Prosen	SF (1989)
Valentina ²	EM6225/11 × EM5588/81	SF (2005)
<u>Black raspberry cultivars</u>		
Cumberland ¹⁴	Gregg selfed	SF (1896)
Haut ¹⁵	(Manteo selfed) × (Bristol selfed)	SF (1980)
Jewel ⁹	NY.29773 × Dundee	SF (1973)
MacBlack ¹⁶	unknown	
<u>Blackberry cultivars</u>		
Arapaho ¹⁷ (4x)	(Ark.550 × Cherokee) × Ark.883	SF (1993)
Aurora ¹⁸ (8x)	ORUS616 × ORUS73	SF (1961)
Black Satin ¹⁹ (4x)	(US 1482 × Darrow) × Thornfree	SF (1974)
Darrow ⁹ (4x)	(Eldorado × Brewer) × Hedrick	SF (1958)
<u>Hybrid-berry cultivars</u>		
Logan Thornless ²⁰ (6x)	Thornless mutation of Loganberry on unknown origin	SF (1933)
Marionberry ¹⁸ (6x)	Chehalem (6x) × Olallie (6x)	SF (ca.1950)
Sunberry ² (6x)	<i>R.ursinus</i> (8x) × (M. Jewel (4x) selfed)	SF (1967)
Tayberry ⁸ (6x)	Aurora (8x) × SCRI626/67 (4x)	SF (1979)
<u>Accessions from other <i>Rubus</i> spp</u>		
<i>R. coreanus</i>	L646	SF
<i>R. crataegifolius</i>	L1032	SF
<i>R. odoratus</i>	L992	SF
<i>R. spectabilis</i>	L544	SF
<i>R. strigosus</i>	L518	SF

¹ East Malling Research breeding line (UK); ² Bred at East Malling Research (UK); ³ Bred by Medway Fruits / ReDeva (UK); ⁴ Bred at Washington State University (USA); ⁵ Bred at Agriculture and Agri-Food Canada (BC – Canada); ⁶ Bred by Agricultural Research Council (CRA) (Italy); ⁷ Bred or selected in research institutes of the former USSR; ⁸ Bred at Scottish Crop Research Institute (UK); ⁹ Bred in Geneva, New York (USA); ¹⁰ Bred in Switzerland; ¹¹ Bred in Minnesota (USA); ¹² Bred at the Research Institute of Pomology and Floriculture (Poland); ¹³ Bred at the Max Planck Institute (Germany); ¹⁴ Bred or selected in Pennsylvania (USA); ¹⁵ Bred or selected in Maryland (USA); ¹⁶ Probably bred or selected by E. Lowden in Ontario (Canada); ¹⁷ Bred in Arkansas (USA); ¹⁸ Bred in Oregon (USA); ¹⁹ Bred or selected by J.N. Hull in Illinois (USA); ²⁰ Bred or selected by G. Bauer in California (USA).

amplification and ease of scoring. This group included two to four markers from each of the seven raspberry linkage groups (Table 2) to ensure good genome coverage.

Initial PCRs were performed individually for each primer pair in 12.5 μ l reactions containing 0.2 μ M forward primer, 0.2 μ M reverse primer labelled with fluorescent dyes 6-FAM, VIC, NED or PET (Applied Biosystems), 0.2 mM of each dNTP, 1.5 mM MgCl₂ and 0.25 U of Amplitaq[®] polymerase in GeneAmp[®] buffer of 10 mM Tris-HCl (pH 8.3) and 50 mM KCl (all reagents from Applied Biosystems). The PCR cycling regime consisted of an initial 3 min denaturation step at 94°C, ten touch-down cycles comprising a 30 s denaturation at 94°C followed by 90 s of annealing starting at 60°C and decreasing, 1°C per cycle, down to 50°C and 60 s of extension at 72°C. Subsequently, 25 more identical cycles were conducted with an annealing temperature of 50°C followed by a final 30 min extension step at 60°C. PCR products were loaded undiluted to be separated by electrophoresis on an ABI 3100 prism genetic analyser (Applied Biosystems). Data generated were then collected and analysed using the GENESCAN and GENOTYPER (Applied Biosystems) software and checked visually by at least two researchers. Failed reactions were repeated taking care to include samples that worked in the original screen to ensure consistency of scoring.

2.3. Multiplexed PCR reactions

Eight SSRs were chosen as the core fingerprinting set for red raspberry taking into account polymorphism, linkage group and ease of scoring in 16 genotypes. These eight markers were grouped into two separate multiplexed reactions, MP_A and MP_B (Table 2), and used to screen all genotypes. Each multiplex included equimolar amounts of four markers each one labelled with a different fluorescent molecule (6-FAM, VIC, NED or PET). Multiplexed reactions were carried out in 13- μ l reactions using Type-it[™] Microsatellite PCR kit (Qiagen) according to the manufacturer instructions and thermal cycling as above. Products from multiplexed reactions were diluted 1 : 50, 1 : 100 and 1 : 200 prior to analysis and separated by electrophoresis on an ABI 3100 prism genetic analyser (Applied Biosystems). Data generated were then collected and analysed using the GENESCAN and GENOTYPER (Applied Biosystems) software and checked visually by at least two researchers. Amplicons were compared to those produced by the same markers on single PCR and failed reactions were repeated alongside previously scored genotypes to ensure consistency of scoring.

2.4. Data analysis

Usefulness of the markers was evaluated based on amplification of the 16 test cultivars. Number of alleles and number of unique genotypes per marker were noted and discrimination power was calculated as $DP = 1 - \sum P_i^2$ where P_i is the frequency of the i^{th} genotype (Table 2).

A data matrix was created from the scored peaks for all 56 *Rubus* accessions amplified with the eight selected primer pairs. Data were analysed following the methods described by Marchese et al. [21]. The peaks were scored as present (1) or absent (0) for each accession and then all data were combined into a single matrix for clustering analysis with unweighted pair group method with arithmetic mean (UPGMA) using PAUP (Phylogenetic Analysis Using Parsimony) software [22], from which a phenogram showing phenetic similarity was then constructed with TREEVIEW [23]. Phylogenetic bootstrapping was performed using 10,000 replicates to establish support for relationships inferred from the UPGMA analysis.

3. Results

3.1. Single PCR performance and marker selection

All 20 primers amplified one or two alleles in each of the 16 test genotypes. However Rub120a, which was seemingly homozygous in many genotypes, could not achieve amplification for three samples even after various attempts, thus suggesting the presence of 'null' alleles (data not shown).

Marker polymorphism and discrimination power (DP) varied considerably between primer pairs. RhM001 was found to be the least useful having revealed only two different alleles and two unique genotypes (DF = 0.49) whereas

Table 2

SSR markers tested detailing source for primer sequence, raspberry linkage group (LG), number of alleles amplified (N(16)), number of unique genotypes (UG) and discrimination power (DP) in the 16 cultivar used for preliminary evaluation as well as multiplex reaction and dye; total number of alleles and size range allele in 16 and 56 red raspberry genotypes (multiplexed SSR markers, only)

Marker	LG	N (16)	UG	DP ¹	Multiplex (Dye)	N	Range	
							16	56
Rubus285a ²	1	9	14	0.92	MP.B (NED)	11	169–193	163–193
Rubus124a ²	1	4	6	0.69	n/a	n/a	143–152	n/a
Rubus270a ²	2	8	10	0.84	MP.A (NED)	11	156–209	156–209
RhM003 ³	2	6	9	0.83	MP.B (VIC)	9	196–214	182–214
RubruitC1 ²	2	3	4	0.73	n/a	n/a	151–161	n/a
RhM001 ³	2	2	2	0.49	n/a	n/a	235–237	n/a
Rubus223a ⁴	3	7	10	0.86	MP.A (VIC)	10	135–172	135–172
Rub120a ⁵	3	4	5	0.66	n/a	n/a	180–188	n/a
Rubus16a ²	3	6	5	0.61	n/a	n/a	132–167	n/a
Rubus110a ²	4	9	12	0.88	n/a	n/a	143–188	n/a
Rubus57a ²	4	7	9	0.86	n/a	n/a	163–194	n/a
Rubus275a ²	5	12	14	0.92	MP.A (PET)	16	116–184	114–186
Rubus105b ²	5	7	10	0.88	n/a	n/a	160–195	n/a
RiM019 ³	5	9	9	0.86	MP.B (PET)	12	167–220	167–220
Rub5a ⁵	5	4	5	0.76	n/a	n/a	154–159	n/a
Rubus123a ²	6	10	11	0.85	MP.A (6–FAM)	15	142–183	135–204
RiM036 ³	6	3	5	0.70	n/a	n/a	297–314	n/a
Rubleaf102 ²	6	3	4	0.68	n/a	n/a	221–234	n/a
RhM011 ³	7	11	12	0.88	MP.B (6–FAM)	13	266–316	266–316
Rub265a ⁵	7	6	8	0.80	n/a	n/a	132–154	n/a

¹ $DP = 1 - \sum P_i^2$ where P_i is the frequency of the i^{th} genotype; ²Published by Graham *et al.* 2004; ³Published by Castillo *et al.* 2010; ⁴Published by Graham *et al.* 2002; ⁵Published by Graham *et al.* 2006.

Rubus275a and Rubus285a were the most informative amplifying 12 and 9 different alleles respectively; both revealed 14 unique genotypes each (DF = 0.92). Similarly, the quality and consistency of amplification and the ease of scoring also varied greatly among the markers. Markers Rubus57a and Rub5a presented series of three or more consecutive alleles in 1-bp steps that made consistent scoring difficult. Rubus110a and Rub265a amplified products with pronounced stuttering and/or inconsistent non-templated nucleotide addition, making allele size assignment complicated. These were discarded from the core fingerprinting set regardless of their polymorphism. The best overall primer from each linkage group was chosen with the exception of LG4 for which neither of the SSRs tested was satisfactory. Other markers from this linkage group had previously been evaluated for fingerprinting (data not shown) and found to be equally unsuitable. Therefore two additional markers from linkage groups 2 and 5 were chosen to make up the set of eight.

3.2. Multiplexed PCRs and genotype analysis

The amplification from multiplexed reactions with the SSR Type-it™ Microsatellite PCR kit (Qiagen) was consistent with previous scores for single reactions but the product yield was very high causing requiring dilution prior to electrophoresis to allow accurate scoring; a 1 : 100 dilution was the most suitable.

Although the number of alleles detected for each of the eight selected SSR markers increased when we analysed all 56 individuals, the allele range did not varied substantially (Table 2), suggesting that the 16 genotypes chosen for the preliminary test were satisfactorily representative.

The similarity of the fingerprints revealed by the multiplexed set for each accession is shown in the phenogram (Fig. 1) and two clusters of genotypes related by known pedigree information have been highlighted. However, the level of bootstrap support for these clusters was below the 75% cut-off value to be considered statistically significant. From known pedigree information, supported by their position on the phenogram, six genetically-diverse and commonly-available raspberry cultivars were proposed for the harmonizing of scoring across different future studies; allele sizes revealed by MP_A and MP_B on these genotypes are provided (Table 3).

3.3. Transferability of the fingerprinting set to other *Rubus* species

The set produced good amplification in blackberry and black raspberry accessions (Table 3). As expected on account of their ploidy levels, all markers amplified more than two alleles from the majority of blackberry and hybrid berry genotypes. Only two markers failed to amplify every accession of these crops (*Rubus*223a did not generate any products in three blackberries and nor did RiM019 that also failed to amplify two black raspberries), nonetheless the set fully discriminated all the cultivars tested. Hybrid berries proved less polymorphic and, despite excellent amplification, we could not distinguish between ‘Tayberry’ and ‘Sunberry’. Most markers in the set also amplified successfully the majority of other *Rubus* accessions (Table 3).

4. Discussion

SSR markers are abundant, co-dominant and easily transferable, allowing for the same locus to be analysed reliably and compared across a wide range of germplasm. After the initial investment in their development, SSRs are inexpensive and straight-forward to use and several markers can be multiplexed in the same reaction to further decrease genotyping costs [20].

Sets of multiplexed SSRs have been proposed for high-throughput genotyping in other fruit crops such as strawberry [11] and pear [19]. Previous studies in *Rubus* have concentrated in determining the usefulness of particular sets of markers for mapping [3, 13, 14]. Castillo et al. presented a set of SSR markers derived from blackberry and red raspberry genomic DNA and evaluated their usefulness for fingerprinting a range of cultivars of both crops [16]. However they presented no multiplexed set and no set of harmonizing genotypes was suggested. We are not aware of any convenient and/or widely accepted set of SSRs for raspberry fingerprinting.

In this investigation, we report a fingerprinting set for red raspberry comprising eight SSR markers multiplexed into two PCR reactions that we have shown to be highly discriminating in a wide range of red raspberry genotypes. Although no individual SSR could differentiate all non-clonal genotypes, each of the two multiplexes could discriminate them with two exceptions (MP_A could not distinguish between ‘Joan Squire’ and ‘Brice’ and MP_B could not distinguish between ‘Malling Minerva’ and ‘EM6727/63’). When both multiplexes were combined, the resulting fingerprinting set satisfactorily discriminated between even closely related genotypes; parents and their off-spring as in the case of ‘Tulameen’ × ‘Autumn Bliss’ seedlings ‘Erika’ and ‘Sugana’ as well as between siblings (EM6727/26 and EM6727/63; EM6686/25 and EM6686/26; EM6804/94 and EM6804/122; ‘Glen Ample’ and ‘Glen Rosa’; ‘Erika’ and ‘Sugana’) whilst producing consistent amplification for clonal variants (‘Heritage’ and its sport, ‘Kiwigold’) and for the same genotypes across different runs. The markers selected in this study remained reliable and produced reproducible results in multiplexed reactions allowing substantial savings in labour and consumables alike.

Red raspberry cultivars are, for the most part, complex hybrids derived from a relatively small genetic pool; a few founding clones of *R. idaeus* (European red raspberry) and *R. strigosus* (North-American red raspberry) variously inter-crossed through many generations form the background of most modern cultivars. Closely related species such as *R. occidentalis*, *R. spectabilis* and *R. crataegifolius* were introduced as donors of particular traits (e.g. pest resistance or fruit quality attributes). As a result, and not surprisingly, genetic distances between the genotypes included in this study are small. Nonetheless some groupings related to pedigree can be observed in the phenogram (Fig. 1). Group ‘A’ is almost entirely composed of cultivars from the Scottish raspberry breeding programme at SCRI (‘Glen’ series) or cultivars and selections partly derived from their germplasm (e.g. ‘Tulameen’, and ‘EM6732/96’). On the other hand Octavia (‘Glen Ample’ derivative), appears to group more closely to its other progenitor (‘Malling

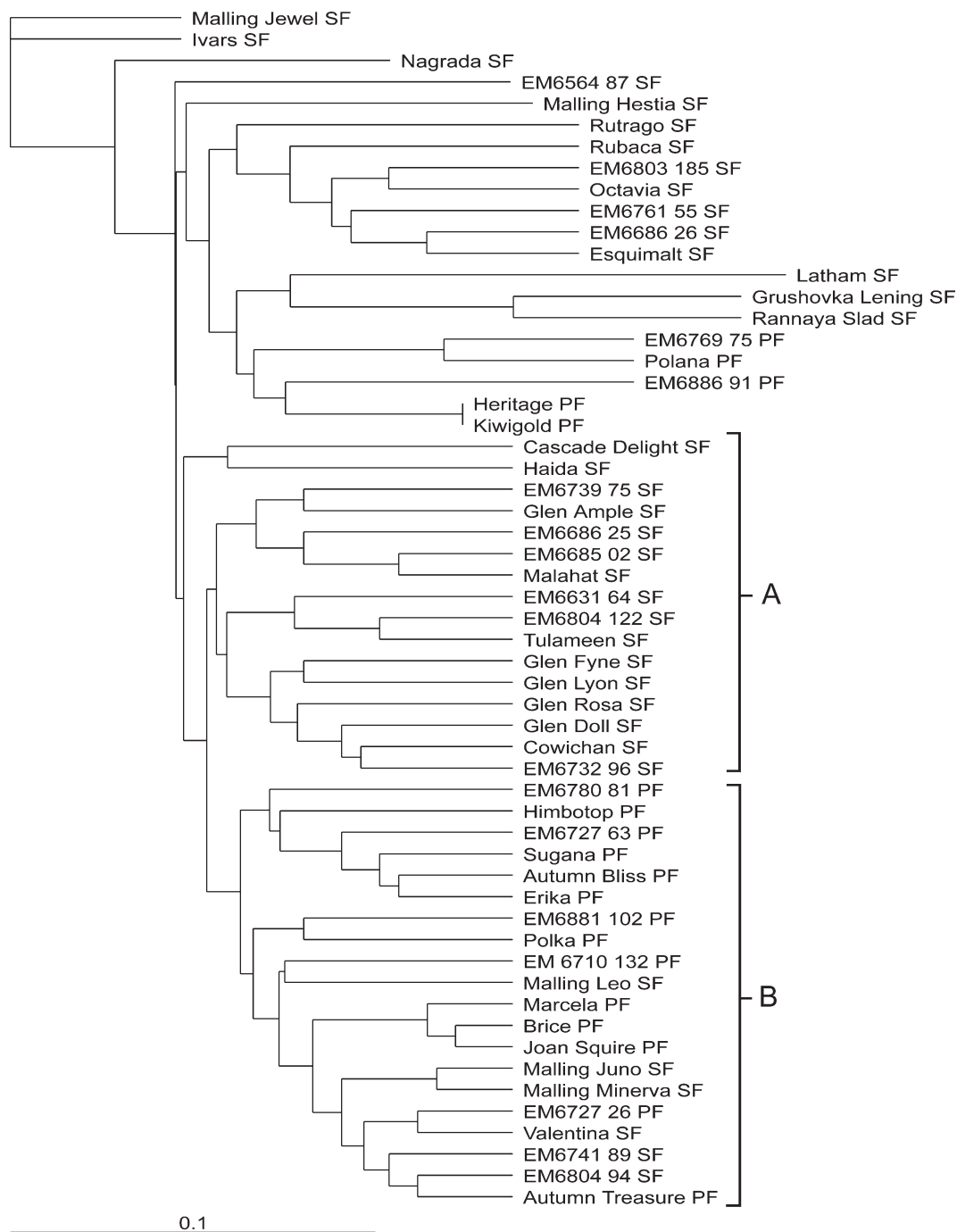


Fig. 1. A UPGMA phenogram showing similarities amongst 56 red raspberry genotypes (37 cultivars and 19 numbered selections) based on genotype data produced by eight SSR markers amplified in two multiplexes.

Table 3

Standardised allele sizes of a set of six red raspberry cultivars for all eight SSR markers as multiplexed in the fingerprinting set (MP_A and MP_B) and their transferability to other *Rubus* species

MP	SSR Marker	Proposed Red raspberry control cultivars						Transferability to other <i>Rubus</i> species*								
		Autumn Bliss	Glen Ample	Heritage	Latham	Malling Jewel	Tulameen	Size range	BR ¹	BB ²	HB ³	COR ⁴	CRA ⁵	ODR ⁶	SPE ⁷	STR ⁸
A	Rubus123a	142/183	142/146	146	150/169	148	142/148	132–252	3 (4)	9 (6)	7 (4)	2 (1)	2 (1)	1 (1)	–	2 (1)
A	Rubus223a	148/152	150	143/150	134/143	148	148	125–170	2 (4)	6 (3)	4 (4)	2 (1)	–	–	–	2 (1)
A	Rubus270a	156	186	181/207	181/209	156/188	156	136–188	4 (4)	10 (6)	7 (4)	2 (1)	2 (1)	1 (1)	2 (1)	2 (1)
A	Rubus275a	116/126	131/184	116/146	129/131	142/178	150/184	106–192	4 (4)	12 (6)	10 (4)	2 (1)	2 (1)	2 (1)	2 (1)	2 (1)
B	RhM003	196/198	196/200	198/204	196/214	196/214	196/214	188–216	3 (4)	8 (6)	7 (4)	2 (1)	2 (1)	2 (1)	2 (1)	1 (1)
B	RhM011	277/316	282/286	280/288	282/284	282/284	278/286	248–314	4 (4)	14 (6)	10 (4)	2 (1)	2 (1)	2 (1)	1 (1)	2 (1)
B	RiM019	185/220	167/181	183/185	183/192	181/185	169/185	167–220	3 (2)	6 (3)	5 (4)	2 (1)	–	–	2 (1)	2 (1)
B	Rubus285a	175/183	169/171	169/177	177/181	177/193	175/193	160–243	3 (4)	10 (6)	8 (4)	2 (1)	2 (1)	1 (1)	1 (1)	2 (1)

* Number of alleles amplified (number of accessions successfully amplified); ¹Black raspberry (*Rubus occidentalis*); four accessions tested; ²Blackberries (*Rubus spp.*); six accessions tested;

³Hybrid berries (raspberry x blackberry); four accessions tested; ⁴*R. coreanus*; one accession tested; ⁵*R. crataegifolius*; one accession tested; ⁶*R. odoratus*; one accession tested; ⁷*R. spectabilis*; one accession tested; ⁸*R. strigosus*; one accession tested.

Hestia') and some of its descendants. Group 'B' comprises the majority of primocane-fruiting material from various programmes, as well as some very early ripening florican-fruiting genotypes from the EMR programme ('Malling Juno', 'Malling Minerva' and 'Valentina') as well as some other EMR genotypes e.g. 'Malling Leo'. On the other hand, 'Heritage', its yellow sport 'Kiwigold' and its seedling 'Polana' seem to be further removed from other primocane-fruiting germplasm. The relationships identified using SSRs in this investigation, although coherent with pedigree information, did not carry statistically significant bootstrap support, a phenomenon previously reported in studies using SSRs in other species [21]. However, it is important to note that the purpose of the study was cultivar discrimination rather than an inference of genetic relatedness, and in this respect, SSRs remain a powerful tool.

This genotyping set will prove useful for verifying trueness-to-type of clonally propagated material as well as for aiding germplasm collection management (e.g. detection of re-propagation errors, detection of synonyms). For the former use, direct comparison with reference DNA from the original clone is sufficient; plant breeders and propagators would do well to store reference samples when embarking on large scale propagation. For the later purpose, the inclusion of a range of DNA controls covering a wide spread of allele sizes is advisable to avoid errors due to small shifts in allele sizing between runs. When comparing data generated in different laboratories following diverse methodologies (e.g. coordinated germplasm-collection management or consortium genotyping efforts) much larger differences can be found in allele assignment and thus, reference samples must be included in each analysis to allow normalization of allele sizes. Control genotypes for fingerprinting have been designated for strawberry, apple, pear and cherry [11, 24]. A reference set for red raspberry should include widely available cultivars such as 'Autumn Bliss', 'Glen Ample' and 'Tulameen'; cultivars extensively used in the breeding of modern cultivars (e.g. 'Heritage', 'Latham' and 'Malling Jewel') and perhaps, as in the case of the sets proposed for tree fruit crops, genotypes from closely related species that have contributed to modern cultivars (e.g. *R. occidentalis* and *R. spectabilis*). As identification errors can occur even in fully curated collections, control genotypes for a reference set should be centrally provided by a single reliable source such as the USDA National Clonal Germplasm Repository (Corvallis, Oregon, USA).

This study has optimised a reliable and cost-effective protocol for genotyping in *R. idaeus* that allows accurate fingerprinting of red raspberry cultivars and breeding lines. The SSR markers chosen are robust, spread throughout the raspberry genome, highly polymorphic and work consistently when multiplexed providing high quality data that can be used confidently by breeders, propagators and growers alike to document and/or verify genotype identity. Furthermore, provided common reference samples are agreed upon, it will enable comparisons between different germplasm collections facilitating the identification of possible 'rogues' and unwanted duplications.

Fresh raspberry production is a high-input business heavily reliant on high quality plant material of specific cultivars. Enormous financial losses can be incurred by breeders, propagators and growers alike when trueness-to-type of germplasm is lost. By routinely deploying DNA fingerprinting technology at critical stages in the cultivar development and propagation processes, costly errors can be avoided; therefore we expect this reliable and cost-effective fingerprinting tool to be valuable to the raspberry industry and researchers alike.

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