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Structural Genomic Resources in *Fragaria* Genus

Julio Bonet • Amparo Monfort*

IRTA, Centre de Recerca en Agrigenomica (CSIC-IRTA-UAB). Campus UAB, Edifici CRAG. Bellaterra 08193- (Barcelona), Spain

Corresponding author: * **amparo.monfort@irta.es**

ABSTRACT

Strawberry is the most widely grown of the berries, and the most economically important soft fruit. It has an interesting history. *Fragaria vesca*, known as the wood strawberry, a diploid species (2n=14) with hermaphrodite flowers, has small, aromatic fruit and is found in localised markets in Europe, northern Asia, northern Africa and North America. Additionally, many species of *Fragaria* L. (Rosaceae) occur throughout northern temperate regions with one species extending into South America. A range of ploidy levels is observed within the genus, with naturally occurring diploid, tetraploid, hexaploid and octoploid species and natural hybrids. The strawberry cultivated worldwide, *F. x ananassa*, is derived from the chance hybridisation between two octoploid (2n=56) native American species, *F. virginiana*, and *F. chiloensis*, both usually dioecious, in European botanic gardens. There is a well characterised collection of molecular markers in *Fragaria* species, used principally to develop a saturated genetic linkage map of diploid *Fragaria*. The map has over 600 transferable molecular loci from an interspecific cross between *F.* vesca and another closely-related diploid species, *F. bucharica* (FV×FB) and includes microsatellites (SSRs), T-DNA insertion site markers, gene-specific and STS markers, RFLPs and SCAR markers and single nucleotide polymorphism (SNP) markers. Comparative mapping studies have shown a high level of macrosynteny between the diploid and octoploid *Fragaria* genomes.

Keywords: diploid, maps, molecular markers, octoploid, SSRs

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INTRODUCTION

Since 1981, advances in structural genomics in *Fragaria* has been largely due to the improvement and availability of different types of molecular markers in *Fragaria* species, as well as markers developed in other Rosaceae species and transferred to *Fragaria*. Molecular markers arise from DNA mutations generated spontaneously, usually located in non-coding regions, and their pattern of inheritance is valuable for applications such as breeding, fingerprinting and genome annotation. Substitutions on a single position, larger rearrangements (insertions or deletions) or number variations on tandem-repeated DNA sequences generate marks or labels that can be easily identified and tracked by DNA visualization assays.

To select an appropriate marker, their different features should be taken into account. The stability of the mutation, as the euchromatic/heterochromatic position within the genome, critically determines the polymorphism rates. As EST-derived markers are usually well conserved in different taxa, they are a good choice for syntenic or phylogenetic studies, while genomic-derived markers offer more polymorphic loci between closely related individuals within a species. Genomic data or the *in silico* genomic resources of the species, accessing high technology, are crucial for the election of a marker, and while no sequence knowledge is necessary for arbitrarily-primed markers, it is needed for SSR, SNP, CAPS and STS markers.

ISOENZYMES

Isoenzymes are different molecular forms of a particular enzyme. They can be electrophoretically separated due to the specific migration pattern generated by the different electric charge and molecular weight. Hancock and Bringhurst (1978, 1979) published the first studies of variation in

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phosphoglucoisomerase (PGI) and peroxidase (PX) enzymes in natural populations of *F. vesca*, *F. chiloensis* and *F. virginiana*. PGI, leucine aminopeptidase (LAP) and phosphoglucomutase (PGM) variation were first employed in the characterization of diploid and cultivated strawberry cultivars (Arulsekar and Bringhurst 1981; Bringhurst *et al.* 1981). In their study on the inheritance pattern of PGI and LAP enzymes in cultivated strawberry, Arulsekar *et al.* (1981) showed for the first time that four active *Pgi* loci segregate following a disomic Mendelian pattern. Apart from some controversial studies, the diploidized nature of the octoploid *Fragaria* genome has been accepted by the scientific community (Ahmadi *et al.* 1990; Lerceteau-Köhler *et al.* 2003; Sargent *et al.* 2009b).

The association of a particular trait and an isoenzyme locus has never been observed in cultivated strawberry. Williamson *et al.* (1995) reported the inheritance of the yellow fruit colour and its linkage to the *Sdh* (shikimate dehydrogenase) locus in an intraspecific F_2 population of *F. vesca* Baron Solemancher x *F. vesca* Yellow Wonder, where SDH segregated in a 1: 2: 1 ratio, and fruit colour segregated 3 red/1 yellow. A few months later, Yu and Davis (1995) identified an isozyme locus weakly linked to the non-runnering trait using the same diploid varieties as Williamson *et al.* (1995) as parental lines for several F_2 and F_3 offspring. Both traits were mapped by Davis and Yu (1997) in the first linkage map of the diploid strawberry genome.

RFLP (RESTRICTION FRAGMENT LENGTH POLYMORPHISM)

With the advent of restriction enzyme technology (Smith 1970), nucleotide sequence specific systems were developed worldwide. The first report on genetic linkage of restriction fragments in plants was done by Polans *et al*. in 1985. A robust, reliable and transferable technique, restriction fragment length polymorphism (RFLP) provides mostly co-dominant polymorphisms. It is not commonly used today, as it is time consuming, involves expensive, radioactive or toxic reagents and requires large quantity of high quality genomic DNA (Agarwal *et al.* 2008). Hybridization-based markers such as RFLP, have the advantage of being highly transferable between distantly related species.

No RFLP probes have been developed in the *Fragaria* nuclear genome so far, but *Prunus* derived probes have been shown to be a valuable tool for mapping in *Fragaria* (Viruel *et al.* 2002). Vilanova *et al.* (2008) mapped 40 polymorphic *Prunus* EST-derived RFLP markers in the diploid *Fragaria* reference population when comparing strawberry and peach genomic structure. This comparison comprised 71 common markers and revealed that only 24% of the probes gave poor or no hybridization in the *Fragaria* genome, suggesting that *Prunus* coding sequences are essentially conserved in the strawberry genome.

ARBITRARILY-PRIMED MARKERS

With the establishment of PCR procedures in the late 1980s, PCR-derived techniques dramatically increased the availability of molecular markers, largely due to the simplicity of the procedures; nanogram (rather than microgram) quantities of DNA are required. Arbitrarily-primed marker systems such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphisms (AFLP) normally generate a large number of dominant markers. They are based on different amplification patterns of small PCR fragments generated by short primer pair combinations. No knowledge of the DNA sequence for the targeted gene or genomic region is required, as the primers bind somewhere in the sequence, and therefore markers can be quickly amplified and detected in any organism. However, reproducibility is sometimes a problem with these markers, in particular RAPDs (Jones *et al.* 1997), and they are extremely difficult to transfer between different populations. DNA amplification fingerprinting (DAF) (Bentley and Bassam 1996) and randomly amplified DNA fingerprinting (RAF) (Waldron *et al*. 2002) techniques, with higher annealing temperatures used in the PCR, seem to be more reproducible and transferable than RAPDs.

Despite these difficulties, RAPD markers were the method of choice for the identification of strawberry varieties (Congiu *et al.* 2000), germplasm characterization (Kuras *et al.* 2004) and construction of linkage maps in diploid and octoploid species of *Fragaria* (Davis and Yu 1997; Sugimoto *et al.* 2005; Sargent *et al.* 2009b). Linkage between RAPD markers and a trait in the cultivated strawberry was first reported using bulked-segregant analysis (BSA) of individuals resistant and susceptible to *Phytophthora fragariae* (Haymes *et al.* 1997). The authors identified seven RAPD markers linked to the *Rpf1* locus, which is one of the major sources of genetic resistance to red stele root rot, caused by the soil-born fungus *P. fragariae*. Two RAPD markers were also found to be linked to the everbearing locus in octoploid strawberry in the first map-based association study in *F* x *ananassa* (Sugimoto *et al.* 2005).

The major advantage of AFLP over the earlier arbitrarily-primed PCR protocols is that they generate a large number of segregating markers (Vos *et al.* 1995) mostly corresponding to unique positions on the genome so they can be exploited as landmarks in genetic and physical mapping. AFLP analysis is usually preferred for increasing marker density or to identify additional markers linked to specific chromosomal regions by BSA (Capomaccio *et al.* 2009; Kamei *et al.* 2010). This technique was applied for the first time in *Fragaria* by Lerceteau *et al.* in 2003 during the construction of the first public linkage map on the cultivated strawberry *Fragaria* x *ananassa*, and subsequently in related studies (Rousseau-Gueutin *et al.* 2008; Weebadde *et al.* 2008; Sargent *et al.* 2009b).

ISSR (inter-simple sequence repeat) is a general term for a genome region between two adjacent microsatellite loci. These regions are often polymorphic between different species or varieties, and have been used to generate arbitrary primers. These primers are designed containing different SSR motifs, and PCR products result from the variable amplified region. The result is a mix of a variety of amplified DNA strands which are generally short but variable in length. The small genome size of the diploid *Fragaria* has led to the identification of variable ISSR regions (Korbin *et al.* 2002; Albani *et al.* 2004).

SCAR

Arbitrary markers closely linked to agronomically interesting traits can be converted to sequence characterised amplified regions (SCAR) for large scale use (Paran and Michaelmore 1993). To date, only three economically important traits have been successfully linked to a SCAR marker in *Fragaria*; SCAR markers are difficult to develop. However, published functional SCAR markers designed on gene sequences coding for different enzymes in other species are being transferred to *Fragaria* (Palmieri *et al.* 2008). Haymes *et al.* (2000) successfully converted a RAPD marker into two SCARs, both linked in coupling phase to the *Rpf1* gene. This association was shown to be highly conserved among 133 *Fragaria* European and North American cultivars. Lerceteau-Köhller *et al.* (2005) studied the inheritance of resistance to one of the infectious agents causing anthracnose, *Colletotrichum acutatum* in 43 strawberry cultivars. They obtained two AFLP-derived SCAR markers, closely linked to the *Rca2* gene, which correctly predicted most of the resistant/susceptible genotypes (81.4% of the cultivars for STS-Rca2_417). In diploid *Fragaria*, three SCARs linked to the *SFL* locus (seasonal flowering locus) have been reported in *F. vesca* by Albani *et al.* (2004) using a BC1 progeny of 1,049 individuals. This locus is considered to be a floral repressor that is inactivated by short days and cool temperatures in the autumn but reactivated by winter cold (Battey *et al.* 1998).

Table 1 Microsatellite markers developed in *Fragaria* species.

Species	Name	No. SSRs	Reference
F. vesca	EMFv	31	James *et al*. 2003; Hadonou *et al*. 2004
F. vesca	UDF	68	Cipriani and Testolin 2004; Cipriani *et al*. 2006
F. vesca	CFVCT	35	Monfort *et al.* 2006
F. vesca	ChFvM	22	Zorrilla-Fontanesi *et al.* 2011
F. vesca	fvp	6	Rousseau-Gueutin *et al*. 2011
F. viridis	EMFvi	22	Sargent *et al.* 2003
F. bucharica (*F. nubicola*)	EMFn	31	Sargent *et al.* 2006
F. virginiana	Fvi	7	Ashley *et al.* 2003
F. x ananassa	ARSFL, FAC	14.4	Lewers *et al.* 2005
F. x ananassa	UFFxa	14	Sargent *et al.* 2006
F. x ananassa	CFACT, CFA, EMFxa	24,5,8	Sargent *et al.* 2008
F. x ananassa	UAFv	14	Bassil *et al.* 2006
F. x ananassa	ChFaM	108	Gil-Ariza *et al.* 2006; Zorrilla-Fontanesi *et al.* 2011
F. x ananassa	Fa	4	Shimomura and Hirashima 2006
F. x ananassa	PBCESSRFXA	14	Keniry *et al.* 2006
F. x ananassa	fap	5	Rousseau-Gueutin *et al*. 2011

CAPS

Cleaved amplified polymorphic sequences (CAPS) are usually amplified from known gene sequences and digested with an appropriate restriction enzyme (RE). CAPS reveal the restriction fragment length polymorphisms caused by single base changes of SNPs, insertions/deletions, which modify restriction endonuclease recognition sites in PCR amplicons (Konieczny and Ausubel 1993). The technique is limited by mutations, which create or disrupt a restriction enzyme recognition site. To overcome this limitation, Michaels and Amasino (1998) proposed a variant of the CAPS method called dCAPS (derived cleaved amplified polymorphic sequence). In dCAPS analysis, a restriction enzyme recognition site which includes the SNP is introduced into the PCR product by a primer containing one or more mismatches to template DNA (Neff *et al.* 1998). The modified PCR product is then digested with a restriction enzyme, with the resulting restriction pattern showing the presence or absence of the mutation.

CAPS and dCAPS analyses are fast and simple for SNP genotyping compared to more expensive techniques such as resequencing or high resolution melting analysis (HRM). HRM analysis discriminates two homozygous PCR amplicons containing a nucleotide variation, as well as their heterozygote, using fluorescence dyes which can bind to the double stranded DNA without inhibiting PCR (Herrmann *et al.* 2006). This approach has been used in other Rosaceae crops as almond (Wu *et al.* 2008, 2009) and apple (Chagné *et al.* 2008), but no studies have been reported in strawberry.

Kunihisa *et al*. (2005, 2009) used cluster-specific amplification with a set of 34 CAPS markers in *F.* x *ananassa*, corresponding to alleles of single diploid loci, and correctly identified 63 different cultivars using a minimum of nine CAPS markers. These markers are especially valuable, as markers detecting only a single locus are rare in *F.* x *ananassa*. These CAPS are cluster-specific markers, as they were developed by allele-specific amplification based on the nucleotide sequence specificity, allowing the amplification of only one of the four clusters or subgenomes in the whole *F.* x *ananassa* genome.

A set of new CAPS and dCAPS have recently been reported in the diploid strawberry *F. vesca* Hawaii 4 (Ruiz-Rojas *et al.* 2010). Seventy four SNPs were identified and mapped on the *Fragaria* reference map, in order to locate the T-DNA insertions generated by *Agrobacterium*-mediated mutagenesis. When a single dose transgenic plant was identified, thermal asymmetric interlaced PCR (hiTAIL-PCR) was used to obtain the insertion flanking regions. These regions were used to design primers for the identification of polymorphic SNPs in the parents of the *Fragaria* reference population and subsequently mapped in the population using a CAPS-dCAPS approach.

MICROSATELLITES

Simple sequence repeats (SSR) or microsatellites are tandemly arranged short sequence motifs, which occur as interspersed repetitive elements in all eukaryotic genomes (Tautz and Renz 1984). Microsatellites are among the most variable types of DNA sequence in the genome (Weber 1990), as the polymorphisms derive mainly from variability in length rather than in the primary sequence. Given their extensive polymorphisms, transferability between populations and species and their technical simplicity, they became the marker of choice in *Fragaria* mapping during the last decade and subsequently in population genetics studies and cultivar identification.

A set of more than 200 primer pairs amplifying polymorphic SSRs have been developed for *Fragaria* species (**Table 1**). Most of them have been reported in the diploid species such as *F. vesca*, *F. viridis* and *F. bucharica* (formerly *F. nubicola*; Sargent *et al.* 2006) and also in the octoploid species *F. virginiana*, the cultivated strawberry *F.* x *ananassa*, and in other rosaceous species. Whilst SSRs are excellent markers for providing a linkage framework, they are primarily developed from non-coding regions of the genome and are generally not tightly linked to genes of known function or to traits of economic importance.

In contrast to genomic SSRs, EST-derived microsatellites potentially represent functional markers (Varshney *et al.* 2005; Zorrilla-Fontanesi *et al.* 2011) as they are present in expressed regions of the genome. Around 30% of the reported SSRs in *Fragaria* belongs to expressed regions, being especially valuable for germplasm characterization (Varshney *et al.* 2005) taking into account that EST-SSRs are not as efficient as genomic SSRs for distinguishing closely related genotypes (Gupta and Varshney 2000). Besides genetic mapping and diversity studies, *Fragaria* microsatellites have been used for transferability and comparative mapping in the Rosaceae (Lewers *et al*. 2005), characterization of a high molecular weight DNA BAC library (Bonet *et al.* 2009) and the ongoing scaffold anchoring for the *Fragaria* genome sequence annotation project (Shulaev *et al.* pers. comm.).

GENE-SPECIFIC MARKERS

In spite of the availability of expressed sequences in public databases, no gene-specific markers have yet been mapped in cultivated *Fragaria*. This is probably due to the octoploid nature of the genome, as the gene dose can interfere during the characterization of an expressed, and subsequently, highly conserved locus. For this reason, the intron-exon border structure, based on sequence similarity with the most highly conserved homolog gene in a model species, can be taken into account. Coding regions flanked by introns are a good choice for primer design of polymorphic sequence-

Table 2 Locus, gene annotation and map position of mapped genes in the *Fragaria* genome.

Map	Locus	Gene annotation	Position
FV x FB (Ruiz-Rojas *et al*. 2010)	ABP[1]	Auxin-binding protein	LG2 21,5
	ACO[1]	1-Aminocyclopropane-1-Carboxylate oxidase	LG6 58,2
	ADH[2]	alcohol dehydrogenase	LG2 16,9
	AKR[1]	Aldo-keto reductase superfamily member	LG4 61
	ANS[1,3]	Anthocyanidin synthase	LG5 9,2
	APX[1]	Cytosolic ascorbate peroxidase	LG3 42,3
	ARP[1]	Auxin-repressed mRNA	LG3 28
	BG-1[1]	Beta-1,3-glucanase-1	LG4 60,4
	BG-2[1]	Beta-1,3-glucanase-2	LG3 39,8
	CAD-1[1]	Cinnamyl alcohol dehydrogenase-1	LG7 17
	CAD-2[1]	Cinnamyl alcohol dehydrogenase-2	LG1 31,1
	CAD-3[1]	Cinnamyl alcohol dehydrogenase-3	LG1 54,2
	CEL-1[1]	Cellulase-1	LG4 75,3
	CEL-2[1]	Cellulase-2	LG5 20,3
	CHI[3]	chalcone isomerase	LG7 25,7
	CHS[3]	chalcone synthase	LG7 17,3
	DFR[1,3]	Dihydroflavonol 4-reductase	LG2 59,1
	DHAR[5]	DeHydro Ascorbate Reductase	LG7 0*
	EKO[1]	Ent-kaurene oxidase	LG2 14,8
	EXP-1[1]	Expansin-1	LG4 19,0*
	EXP-2[1]	Expansin-2	LG3 54,5*
	EXP-3[1]	Expansin-3	LG7 33,3*
	F3H[3]	flavanone-3-hydroxylase	LG1 43,1
	FvNES1[4]	Nerolidol Synthase 1	LG3 12,5
	GAST[1]	GAST-like gene product	LG2 52,5*
	LEAFY[1]	Leafy protein	LG3 16,1*
	LOX[1]	Lipoxygenase	LG4 27,8
	MET[1]	Metallothionein-like protein	LG6 42
	MSR[1]	Methionine sulfoxide reductase	LG5 27,5*
	MYB[1]	Transcription factor	LG5 38
	PDC[1]	Pyruvate decarboxylase	LG6 43,8
	PES[1]	Pectinesterase	LG1 37,3*
	PGLM[1]	Phosphoglyceromutase	LG6 28,4
	QR[1]	Quinone oxidoreductase	LG6 76,1
	RAN[3]	Del-like regulatory gene	LG5 14,8
	S Locus[6]	S-RNase	LG1 36
	T Locus[6]	T-Rnase	LG6 (7,3-15,2)
	ZIP[1]	Zinc transporter protein	LG6 52,4
Ever Berry x Toyonoka (Sugimoto *et al*. 2005)	EV[7]	Everbearing	(OPE07-1-OPB05-1)
Md683 x Senga Sengana (Haymes *et al*. 1997)	Rpf1[8]	*Phytophthora fragariae* resistance	(OPO-08A-OPO-16A)
Redgauntlet x Hapil (Sargent *et al*. 2009)	ACO[9]	1-Aminocyclopropane-1-Carboxylate oxidase	RG6-D 22, HA6-D
	ACP[9]	Acyl carrier protein	HA6-D
	DFR[9]	Dihydroflavonol 4-reductase	RG2-B
	bEXP-204[9]	Expansin	HA3-A
	EXP-185[9]	Expansin	RG4-D
	aEXP-177[9]	Expansin	RG5-A, HA5-A
	b-EXP-235[9]	Expansin	HA7-C
	EXP-248[9]	Expansin	HA7-D, RG7-D
	aEXP-245[9]	Expansin	RG7-C
	aLOX-374/385[9]	Lipoxygenase	RG4-B
	bLOX-376/379[9]	Lipoxygenase	HA4-B

Gene locations are given in cM with respect to the origin of the linkage group for FV x FB genes, and linkage group or flanking markers are reported for octoploid *Fragaria* genes

* The position is from a previous version of the map.

[1] Sargent *et al*. 2007. [2] Davis and Yu 1997. [3] Deng and Davis 2001. [4] Sargent *et al*. 2004. [5] Rousseau-Gueutin *et al*. 2009. [6] Boškovic *et al*. 2009. [7] Sugimoto *et al*. 2005. [8] Haymes *et al*. 1997. [9] Sargent *et al*. 2009.

specific tags (STS), as the mutation rates are higher in the introns than in the exons. Because cultivated strawberries are octoploid, the fragments amplified by one primer pair can contain various sequences derived from up to eight chromosomes (Kim *et al*. 2001). Therefore, it is necessary to consider several sequences from each target gene to correctly predict polymorphisms from the sequence data (Kunihisa *et al*. 2005).

Most studies on gene discovery have used the diploid species *Fragaria vesca*. The first gene specific marker in *Fragaria*, *ADH*, was developed by Davis and Yu (1997) from the alcohol dehydrogenase gene. They used an intron length polymorphism segregating in their mapping population. Deng and Davis (2001) developed a candidate-gene approach to determine the molecular identity of the *F. vesca c* (yellow fruit) locus. They mapped intron-length polymorphic markers for 5 candidate genes and a transcription factor involved in anthocyanin biosynthesis pathways, using two diploid *Fragaria* populations. Sargent *et al*. (2007) located 29 loci for 24 genes in the diploid *Fragaria* genome using public EST databases, predominantly from *Fragaria* mRNA sequences, as a source for primer design. Many of their primer pairs were shown to be highly transferable, amplifying orthologous genes in other genera (*Malus* and *Prunus*). EST-derived markers have also been useful in synteny analysis between *Prunus* and *Fragaria*, where 21 new markers have been developed from *Prunus* EST sequences, and mapped in the diploid reference *Fragaria* linkage map (Vilanova *et al*. 2008).

Four more STS markers were mapped by Sargent *et al*. (2008) during the development of a bin mapping strategy. With the addition of two unlinked RNase loci (loci S and T)

to the *Fragaria* linkage groups FG1 and FG6, both involved in the *Fragaria* RNase-based self-incompatibility system (Bošković *et al.* 2010), the total number of genes of known function in the *Fragaria* genome has been brought to 38 (**Table 2**).

Following a variant methodology, Davis *et al.* (2007) developed a new molecular marker type in *Fragaria*, called gene pair marker. As the genome size of the diploid strawberry is small (about 200 Mbp), genes are closely spaced: about 1 gene per 5.7 kb (Pontaroli *et al.* 2009). As a consequence of the small intergenic distances, PCR primers targeted to conserved exon sequences in two adjacent genes (the gene pair) can be exploited as a source of polymorphisms, by amplifying an entire intergenic region flanked by gene-defining exon sequences. The authors used this strategy to amplify the *CDPK1-BHLH1* strawberry gene pair locus in other taxa, suggesting that gene pair markers can be especially useful in intergenic synteny studies or comparative linkage mapping in the Rosaceae family (Sargent 2009a).

LINKAGE MAPPING IN *Fragaria*

The construction of linkage maps in polyploids has several limitations: There are a large number of possible genotypes for each polymorphism expected in a segregating population, and these genotypes cannot always be identified readily by their banding phenotypes due to possible co-migration of fragments on gel electrophoresis. Furthermore, the genome constitutions (allopolyploidy versus autopolyploidy) in many polyploids are not clearly understood, making it difficult to determine the patterns of inheritance. Despite evidence that *F.* x *ananassa* behaves as an allopolyploid, the evolutionary history of the genus still remains unclear (Bringhurst 1990; Rousseau-Gueutin *et al.* 2008). The development of genetic maps in octoploid *Fragaria* species has lagged behind that of diploid species, where meiotic recombination events can be easily characterized and, consequently, the order of segregating loci can be clearly established.

In the framework of the assessment of the synteny and colinearity between both diploid and octoploid genomes, more genetic maps have been developed using diploid species, since Davis and Yu (1997) developed the first genetic map for *Fragaria*. This study, using an F_2 mapping population derived from a cross between an alpine (*F. vesca* ssp. *vesca* 'Baron Solemancher') and a non-alpine (*F. vesca* ssp *americana* 'WC6') parent, provided the position of 80 loci, most of them RAPD markers, in a 445 cM genetic map covering seven linkage groups. In the map there is also one morphological marker (the runnering locus, *r*), one STS marker for the *ADH* gene and the isoenzymes *Pgi-2* and *Sdh-1*. Some of the markers developed in this work were used by Deng and Davis (2001) to anchor five genes and one transcription factor involved in anthocyanin biosynthesis pathways to their hosting linkage group, using two F_2 populations of 40 individuals each, derived from two crosses of *F. vesca* ssp. *vesca*, var. 'Yellow Wonder' with *F. nubicola*, and *F. vesca* ssp. *bracteata* with *F. vesca* ssp. *vesca*, var. 'Yellow Wonder'.

THE *Fragaria* REFERENCE MAP FVxFB

With the advances in the production of transferable molecular markers for map-based studies of the genus, such as SSR, in both diploid and octoploid species, Sargent *et al.* (2004a) constructed a reference linkage map for the genus, from a cross between *F. vesca* ssp. *vesca* f. *semperflorens* (FDP815) and *F. bucharica* (FDP601). The latter accession, commonly referred to as *F. nubicola* FDP601 (Lin and Davis 2000; Potter *et al.* 2000; Deng and Davis 2001; Sargent *et al.* 2004a, 2004b; Davis *et al*. 2006; Monfort *et al*. 2006; Sargent *et al.* 2006, 2007, 2008; Vilanova *et al.* 2008), was recently reclassified as *F. bucharica*, a closely related species, probably of hybrid origin (Staudt 2006). The map, previously designated as FVxFN, was renamed FVxFB. In its initial version, this map had 68 SSR, one SCAR, six STS markers and three morphological traits: seasonal/perpetual flowering, runner development and pale/dark-green leaf colour.

Even considering the high level of distorted segregation ratios (54%, where 80% of skewed loci were biased towards an excess of alleles from the paternal crossing parent, *F. bucharica*), the map was adopted as a framework for additional marker incorporation for map coverage of the diploid *Fragaria* genome. In a collaboration of several laboratories, a new set of 109 genomic and EST-derived SSR markers, developed in both diploid and cultivated strawberries, were located in this map by Sargent *et al*. in 2006, increasing the number of molecular markers to 182 (175 SSR, 6 STS and one SCAR) to provide a comprehensive coverage of the diploid *Fragaria* genome with an average density of one marker every 2.3 cM. In 2007, Sargent *et al.* developed and mapped 29 functional markers from 24 gene sequences, greatly increasing the number of genes of known function mapped in the genus. Seventeen STS were mapped using the FVxFB population, and the remaining 12 were mapped in a BC1 population derived from a cross between *F. vesca* 815 and *F. viridis* 903 (Nier *et al.* 2006).

In 2008, 40 RFLP and 29 EST-derived markers were located in the FVxFB map, in a comparative study of *Prunus* and *Fragaria* genomes (Vilanova *et al.* 2008) and a new SSR, located in an uncovered region of linkage group four, was added to the reference map during the development of a 'bin mapping' strategy for *Fragaria* (Sargent *et al.* 2008). In a phylogenetic study on the relationships among *Fragaria* species, Rousseau-Gueutin *et al.* (2008) described a new STS marker for the DHAR gene. Rouseau-Gueutin *et al.* (2009) added four new SSR loci to the reference map and included some rearrangements in the relative position of several markers from four linkage groups during the assessment of the synteny relationships between diploid and octoploid genomes.

Two loci encoding RNases (*S* and *T*) involved in the self-incompatibility mechanism have been described and mapped in the FVxFB map (Bošković *et al.* 2009). This mechanism includes at least two RNases and a complementary SFB pollen-expressed component. The authors showed that the presence of one of the active alleles at either RNase loci is sufficient to confer self-incompatibility in an appropriate genetic background and that a non-RNase factor, which segregates independently, is necessary for self-incompatibility.

Recently, a set of 74 CAPS and dCAPS markers derived from a T-DNA mutant insertion collection has been developed and incorporated in the FVxFB map (Ruiz-Rojas *et al.* 2010). With the inclusion of these markers, the *Fragaria* reference map (see **Fig. 1**) coverage has been brought to 529 cM, 18% more than that of Davis and Yu (1997), with 310 molecular markers (172 SSR, 16 gene-specific STS, 40 RFLP, 74 CAPS-dCAPS, seven EST and one SCAR) and three morphological traits distributed in seven linkage groups.

BIN MAPING IN *Fragaria*

Normally, linkage mapping populations consist of randomly selected individuals of a large progeny, where the distribution of the meiotic crossover sites (breakpoints) in the genome of the different individuals of the population provides a framework for the assessment of the molecular marker positions. However, when a typical mapping population is well characterized, it is possible to add new markers by selecting a few plants of the population (the most informative set of breakpoints), minimizing time and effort over conventional mapping strategy (Vision *et al.* 2000; Howad *et al.* 2005). This 'selective' or 'bin mapping' approach was recently adapted to the FVxFB map by Sargent *et al.* (2008). The defined bin set, with 6 F_2 individuals plus one parental line and the F1 individual, was shown to be sufficiently

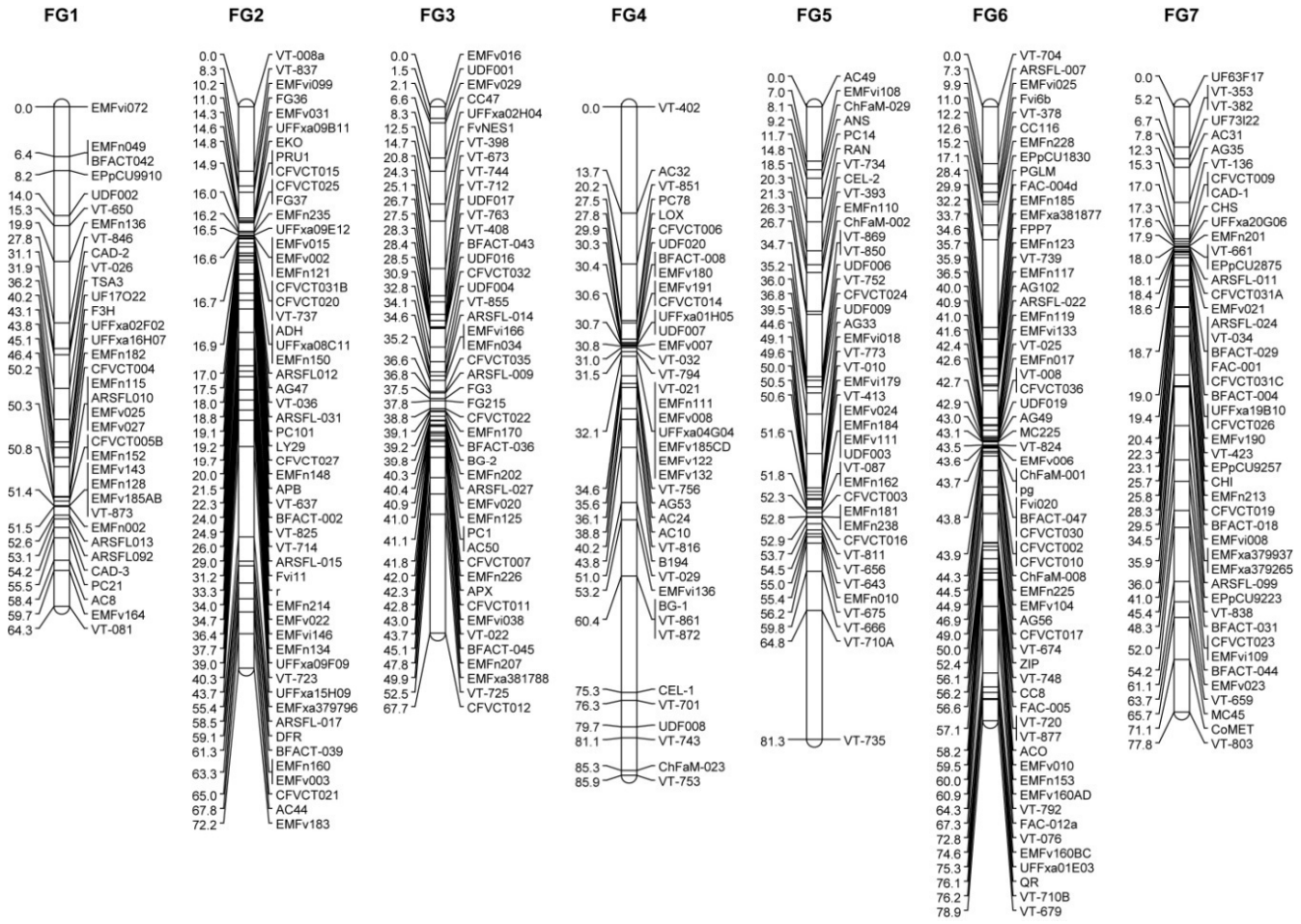

Fig. 1 The diploid *Fragaria* reference linkage map (FVxFB) derived from an F_2 population obtained in an interspecific *F. vesca* 815 x *F. bucharica* cross.

robust to locate 103 new markers (99 genomic and EST-derived SSRs and four gene-specific markers originally described as *Prunus* ripening genes) in one of the 46 mapping bins spanning the *Fragaria* genome, with an average length of 12.6 cM per bin.

OCTOPLOID MAPPING

Breeding for many traits in the cultivated strawberry could be enhanced through the use of molecular markers, but the octoploid nature of the genome provides a challenge to the development of traditional molecular breeding tools. Until 2008, the absence of a public consensus linkage map meant marker assisted selection in strawberry was delayed with respect to other crops, with only reports of partial maps or markers linked to single traits. With meiotic behaviour clearly established and multi-allelic transferrable markers anchored to the octoploid and diploid *Fragaria* genome (Rousseau-Gueutin *et al.* 2008; Spigler *et al.* 2008; Sargent *et al.* 2009b), the assessment of accurate trait-marker linkage relationships should be feasible in the near future.

The first map-based association study in cultivated strawberry was carried out to identify markers for the everbearing locus in the octoploid strawberry (Sugimoto *et al.* 2005). An F_1 progeny from a cross between 'Ever Berry' (a Japanese everbearing variety) and 'Toyonoka' (a Junebearing variety) was used to identify the locus governing the flowering pattern. The flowering test gave the expected 1: 1 ratio for everbears to Junebears, suggesting that the inheritance of the trait is controlled by a monogenic dominant gene. In order to locate this gene, the authors used 175 RAPD primer pairs. With five out of 89 polymorphic fragments relating to the everbearing locus, a 39.7 cM long linkage group was constructed, with two markers flanking the everbearing gene, at 11.8 and 15.8 cM.

In the first linkage map of the cultivated strawberry, derived from an F_1 population of 113 individuals from the cross of the variety 'Capitola' and the clone CF1116 ['Pajaro' x ('Earliglow' x 'Chandler')], using AFLP analyses, Lerceteau-Köller *et al.* (2003) applied a two-step mapping procedure, previously reported by Grivet *et al*. (1996), to assign the linkage between the coupling- and repulsion-phase markers. The authors identified a large cluster of markers segregating only in the coupling phase, and deduced that the meiotic behaviour of the octoploid *Fragaria* genome may not be as completely diploidized as assumed by several authors (Arulsekar and Bringhurst 1981; Ahmadi *et al.* 1990; Ashley *et al.* 2003). Their maternal map had 235 markers distributed among 30 linkage groups covering 1,604 cM and the paternal map was built with 280 markers assigned to 28 linkage groups, yielding a map size of 1,496 cM.

Transferable markers and new individuals were later added to this map (Rousseau-Gueutin *et al.* 2008), allowing the integration of most of the paternal and maternal linkage groups of Lerceteau-Köller *et al*. (2003) and demonstrating that the meiotic behaviour of the strawberry genome is mainly disomic. However, the absence of repulsion-phase markers in some linkage groups can only be explained by the existence of residual polysomic pairing that may still occur within the F x ananassa genome (Sargent *et al.* 2009a). In this version, this map covers a genetic distance of 5,017 cM, defined by 162 transferable loci across both maps, 23 of which, shared between the two maps, were used for map integration.

To identify the genetic control of day-neutrality in the commercial strawberry, an AFLP-based map was constructed from a cross between 'Tribute', a day-neutral, and 'Honeoye', a short-day variety. In an F_1 population of 127 individuals, 429 single dose restriction fragments (SDRFs)

were located on an integrated map of 1,541 cM with 43 linkage groups. SDRFs are particularly useful for constructing genetic maps of polyploids when meiosis behaviour is unclear (Wu *et al.* 1992). Eight significant QTLs were identified, none of which explained more than 36% of the variation, as expected for a polygenic-inherited trait (Weebadde *et al.* 2008).

The genetic mechanisms involved in sex determination were also investigated by a map-based association approach in the wild octoploid *F. virginiana* (Spigler *et al*. 2008). Using SSRs, the authors mapped sex determination as two qualitative traits, male and female function, both located in linkage group 41, separated by 6 cM. The existence of recombination events between the sex-determining loci, an important hallmark of incipient sex chromosomes, suggested *F. virginiana* as a novel model system for the study of sex chromosome evolution. Their map covered a total distance of 2,373 cM on 42 linkage groups, much higher than the 28 expected linkage groups, including 212 transferable markers, 39 of which segregate in both the maternal and paternal maps, and were used for map integration. Skewed segregation was apparent in almost a third of the mapped markers, and many of the primer pairs used in this study produced alleles that mapped to more than one locus on the same linkage group. The authors suggested that this may have been due to changes within and between genomes of the highly heterozygous, polyploid genome of *F. virginiana*, so representing alleles from the same genetic locus. This would reduce the number of transferable markers of the map to 161 (Sargent *et al.* 2009b).

A new integrated octoploid-diploid *Fragaria* genome map was developed by Sargent *et al.* (2009b) from an F_1 population of 174 seedlings derived from a cross between two *F.* x *ananassa* cultivars, 'Redgauntlet' x 'Hapil', segregating for interesting agronomic traits. The resultant map has 315 molecular markers (218 SSR, 11 gene-specific markers and 86 AFLP and RAPD markers) spanning 3,116 cM in 69 different linkage groups that could be associated to one of the 56 theoretical scaffolds by at least one anchor marker. It contains 230 transferable markers, 28 of which are shared between the two parental maps and were used for map integration.

COMPARATIVE MAPPING

Genome comparisons based on the map position of homologous sequences between closely related species can be used to construct robust phylogenies (Rokas *et al*. 2003) or to identify changes in genome structure (Deleu *et al.* 2007). When well-characterized sequence data are available, the conservation of synteny between different plant species can be detected even comparing members of different families. Genome comparisons based on the map position of homologous markers between distantly related species is quite limited (Rong *et al.* 2005), but this approach has been used to identify genome rearrangements and syntenic and extensive colinear chromosomal regions in different plant species within a family (McClean *et al.* 2010; Wu and Tanksley 2010). These comparisons can be very useful for breeding purposes.

Diploid *Fragaria* species seem to be essentially colinear at the macrosynteny level when comparing the available maps constructed by interspecific crosses, such as *F. vesca* x *F. bucharica* or *F. vesca* x *F. viridis* (Nier *et al.* 2006). Sargent *et al.* (2007) used PCR-based methods for comparative mapping between *Fragaria*, *Malus* and *Prunus*, indicating that DFR and EKO are orthologous genes and that their hosting linkage group (LG2) could be homeologous to *Malus* LG15 and *Prunus* LG1. This relationship has been confirmed for *Fragaria* and *Prunus* genus by hybridization methods. *Prunus* and *Fragaria* reference maps were compared by analyzing the position of 71 anchor markers covering both genomes, and it was found that, in spite of the conservation of large chromosomal fragments as expected for confamilial species, many chromosomal rearrangements, occurring since divergence of the two genera, separate the *Fragaria* and *Prunus* genomes, supporting their distant position within the Rosaceae family. However, there is sufficient synteny between the genomes to allow the information on marker, gene or quantitative trait locus (QTL) position from one species to be used in studies in the other (Vilanova *et al.* 2008). Further comparisons between different Rosaceae genus is being carried out (Illa E, pers. comm.), and new DNA sequence data from high-throughput sequencing will permit a next generation of microsynteny studies in the Rosaceae very soon.

Using transferrable markers for mapping purposes enables the identification of homologous linkage groups between maps, allowing the selection of further markers from any other study to enrich the existing maps in areas not covered. Recently, the synteny relationship between the diploid and octoploid *Fragaria* genome has been established by Rousseau-Gueutin *et al*. (2008) and Sargent *et al.* (2009b). Anchoring syntenic regions with transferable markers (most of them SSR), all the octoploid *Fragaria* linkage groups were identified on the diploid *Fragaria* reference map, according to their homologous groups, and the homeologous groups within the octoploid genome were identified by mapping marker loci derived from the same primer pair to different linkage groups on each of the parental maps. The authors observed almost complete conservation of marker order between the octoploid and diploid genetic maps. Except for an apparent duplication of the Fvi6b locus on homologues of diploid *Fragaria* linkage groups one and six, which may indicate an ancient chromosomal duplication or translocation event in *Fragaria* (Sargent *et al.* 2009b), no evidence of any chromosomal rearrangements between the diploid and octoploid maps has been reported.

FUTURE PERSPECTIVES

Although the architecture of the strawberry genome has been investigated over the last thirty years, the amount of genomic data has dramatically increased since 2005, with the advent of the High Throughput Sequencing Technology. It has been used for the construction of the first draft of the strawberry genome, which was released recently (Shulaev *et al.* 2011), marking another milestone in the extraordinarily fast moving field of plant genomics. Despite all the sequencing advances, very little is known about how to read the "book of life that is opening before us", says Michael Egholm, one of the people responsible for next generation sequencing technology (Wadman 2008).

New genomic resources in *Fragaria* are being constructed worldwide: a collection of near isogenic lines (NILs) in diploid strawberry is coming out soon (Bonet J, PhD thesis 2010); a collection of T-DNA insertional mutants is available (Ruiz-Rojas *et al.* 2010); transgenic lines for many interesting traits are being investigated in different laboratories (Qin *et al.* 2008). The new resources are unexplored sources of phenotypic variation. Information on genome-wide and transcriptome-wide variation in diverse germplasms can be used in different studies, ranging from mapping interesting mutations to studying genome structure and function. Identification of alleles (including QTLs and eQTLs (expression QTLs) controlling phenotypes, genetic mechanisms such as genome-wide patterns of recombination, will benefit from the availability of saturate marker maps, to give a sound basis for marker-assisted selection procedures in strawberry production.

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REFERENCES

Agarwal M, Shrivastava N, Padh H (2008) Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Reports* **27**, 617-631

Ahmadi H, Bringhurst RS, Voth V (1990) Modes of inheritance of photoperiodism in *Fragaria*. *Journal of the American Society for Horticultural Science* **115**, 146-152

Albani MC, Battey NH, Wilkinson MJ (2004) The development of ISSR-derived SCAR markers around the *SEASONAL FLOWERING LOCUS* (*SFL*) in *Fragaria vesca*. *Theoretical and Applied Genetics* **109**, 571-579

Arulsekar S, Bringhurst RS (1981) Genetic model for the enzyme marker PGI in diploid California *Fragaria vesca* its variability and use in elucidating the mating system. *Journal of Heredity* **72**, 117-120

Arulsekar S, Bringhurst RS, Voth V (1981) Inheritance of PGI and LAP isozymes in octoploid cultivated strawberries. *Journal of the American Society for Horticultural Science* **106**, 679-683

Ashley MV, Wilk JA, Styan SMN, Craft KJ, Jones KL, Feldheim KA, Lewers KS, Ashman TL (2003) High variability and disomic segregation of microsatellites in the octoploid *Fragaria virginiana* Mill. (Rosaceae). *Theoretical and Applied Genetics* **107**, 1201-1207

Bassil NV, Gunn M, Folta K, Lewers K (2006) Microsatellite markers for *Fragaria* from 'Strawberry Festival' expressed sequence tags. *Molecular Ecology Notes* **6**, 473-476

Battey NH, Le Miere P, Tehranifar A, Cekic C, Taylor S, Shrives KJ, Hadley P, Greenland AJ, Darby J, Wilkinson MJ (1998) Genetic and environmental control of flowering in strawberry. In: Cockshull KE, Gray D, Seymour GB, Thomas B (Eds) *Genetic and Environmental Manipulation of Horticultural Crops*, CAB International, Wallingford, pp 111-131

Bentley S, Bassam BJ (1996) A robust DNA amplification fingerprinting system applied to analysis of genetic variation within *Fusarium oxysporum fsp cubense*. *Journal of Phytopathology* **144**, 207-213

Bonet GJ (2010) Desarrollo y Caracterización de herramientas genómicas en Fragaria diploide para la mejora del cultivo de fresa. PhD thesis, Universidad Autonoma de Barcelona. Barcelona, Spain

Bonet J, Lopez-Girona E, Sargent DJ, Muñoz-Torres MC, Monfort A, Abbott AG, Arús P, Simpson DW, Davik J (2009) The development and characterisation of a bacterial artificial chromosome library for *Fragaria vesca*. *BMC Research Notes* **2**, 188

Bošković RI, Sargent DJ, Tobutt KR (2010) Genetic evidence that two independent *S*-loci control RNase-based self-incompatibility in diploid strawberry. *Journal of Experimental Botany* **61 (3)**, 755-763

Bringhurst RS, Arulsekar S, Hancock JF, Voth V (1981) Electrophoretic characterization of strawberry cultivars. *Journal of the American Society for Horticultural Science* **106**, 684-687

Bringhurst RS (1990) Cytogenetics and evolution in American *Fragaria*. *HortScience* **25**, 879-881

Capomaccio S, Barone P, Reale L, Veronesi F, Rosellini D (2009) Isolation of genes from female sterile flowers in *Medicago sativa*. *Sex Plant Reproduction* **22**, 97-107

Chagné D, Gasic K, Crowhurst RN, Han Y, Bassett HC, Bowatte DR, Lawrence TJ, Rikkerink EH, Gardiner SE, Korban SS (2008) Development of a set of SNP markers present in expressed genes of the apple. *Genomics* **92 (5)**, 353-358

Cipriani G, Testolin R (2004) Isolation and characterization of microsatellite loci in *Fragaria*. *Molecular Ecology Notes* **4**, 366-368

Cipriani G, Pinosa F, Bonoli M, Faedi W (2006) A new set of microsatellite markers for *Fragaria* species and their application in linkage analysis. *Journal of Horticultural Science and Biotechnology* **81**, 668-675

Congiu L, Chicca M, Cella R, Rossi R, Bernacchia G (2000) The use of random amplified polymorphic DNA (RAPD) markers to identify strawberry varieties: A forensic application. *Molecular Ecology* **9**, 229-232

Davis TM, Yu H (1997) A linkage map of the diploid strawberry, *Fragaria vesca*. *Journal of Heredity* **88**, 215-221

Davis TM, DiMeglio LM, Yang RH, Styan SMN, Lewers KS (2006) Assessment of SSR transfer from the cultivated strawberry to diploid strawberry species: Functionality, linkage group assignment, and use in diversity analysis. *Journal of the American Society for Horticultural Science* **131**, 506-512

Deleu W, González V, Monfort A, Bendahmane A, Puigdomènech P, Arús P, García-Mas J (2007) Structure of two melon regions reveals high microsynteny with sequenced plant species. *Molecular Genetics and Genomics* **278**, 611-622

Deng C, Davis TM (2001) Molecular identification of the yellow fruit color (c) locus in diploid strawberry: A candidate gene approach. *Theoretical and Applied Genetics* **103**, 316-322

Folta KM, Staton M, Stewart PJ, Jung S, Bies DH, Jesdurai C, Main D (2005) Expressed sequence tags (ESTs) and simple sequence repeat (SSR) markers from octoploid strawberry (*Fragaria* × *ananassa*). *BCM Plant Biology* **5**, 12-23

Gil-Ariza DJ, Amaya I, Botella MA, Muñoz Blanco J, Caballero JL, López-Aranda JM, Valpuesta V, Sánchez-Sevilla F (2006) EST-derived polymorphic microsatellites from cultivated strawberry (*Fragaria x ananassa*) are useful for diversity studies and varietal identification among *Fragaria* species. *Molecular Ecology Notes* **6**, 1195-1197

Grivet L, D'Hont A, Roques D, Feldmann P, Lanaud C, Glaszmann JC (1996) RFLP mapping in cultivated sugarcane *(Saccharum* spp.): Genome organization in a highly polyploid and aneuploid interspecific hybrid. *Genetics* **142**, 987-1000

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* **113**, 163-185

Hadonou AM, Sargent DJ, Wilson F, James CM, Simpson DW (2004) Development of microsatellite markers in *Fragaria*, their use in genetic diversity analysis and their potential for genetic linkage mapping. *Genome* **47**, 429-438

Hancock JF, Bringhurst RS (1978) Inter-populational differentiation and adaptation in perennial, diploid species *Fragaria-vesca* L. *American Journal of Botany* **65**, 795-803

Hancock JF, Bringhurst RS (1979) Ecological differentiation in perennial, octoploid species of *Fragaria*. *American Journal of Botany* **66**, 367-375

Haymes KM, Henken B, Davis TM, van de Weg WE (1997) Identification of RAPD markers linked to a *Phytophthora fragariae* resistance gene (Rpf1) in the cultivated strawberry. *Theoretical and Applied Genetics* **94**, 1097-1101

Haymes KM, van de Weg WE, Arens P, Maas JL, Vosman B, Den Nijs APM (2000) Development of SCAR markers linked to a *Phytophthora Fragariae* resistance gene and their assessment in European and North American strawberry genotypes. *Journal of the American Society for Horticultural Science* **125**, 330-339

Herrmann MG, Durtschi JD, Voelkerding KV, Wittwer CT (2006) Instrument comparison for DNA genotyping by amplicon melting. *Journal of the Association for Laboratory Automation* **11**, 273-277

Howad W, Yamamoto T, Dirlewanger E, Testolin R, Cosson P, Cipriani G, Monforte AJ, Georgi L, Abbott AG, Arús P (2005) Mapping with a few plants: Using selective mapping for microsatellite saturation of the *Prunus* reference map. *Genetics* **171**, 1305-1309

James CM, Wilson F, Hadonou AM, Tobutt KR (2003) Isolation and characterization of polymorphic microsatellites in diploid strawberry (*F. vesca* L.) for mapping, diversity studies and clone identification. *Molecular Ecology Notes* **3**, 171-173.

Jones CJ, Edwards KJ, Castaglione S, Winfield MO, Sala F, van de Weil C, Bredemeijer G, Vosman B, Matthes M, Daly A, Brettschnieder R, Bettini P, Buiatti M, Maestri E, Malcevschi A, Marmiroli N, Aert R, Volckaert G, Rueda J, Linacero R, Vazquez A, Karp A (1997) Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding* **3**, 381-390

Kamei A, Tsuro M, Kubo N (2010) QTL mapping of clubroot resistance in radish (*Raphanus sativus* L.). *Theoretical and Applied Genetics* **120**, 1021-1027

Andrew K, Hopkins CJ, Jewell E, Morrison B, Spangenberg GC, Edwards D, Batley J (2006) Identification and characterization of simple sequence repeat (SSR) markers from *Fragaria×ananassa* expressed sequences. *Molecular Ecology Notes* **6**, 319-322

Kim IJ, Lee BH, Jo J, Chung WI (2001) Sequence variability of nine cytosolic ascorbate peroxidases in polyploid strawberry. *Mitochondrial DNA* **11**, 475-484

Konieczny A, Ausubel FM (1993) Procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. *Plant Journal* **4**, 403-410

Korbin M, Kuras A, Zurawicz E (2002) Fruit plant germplasm characterisation using molecular markers generated in RAPD and ISSR-PCR. *Cellular and Molecular Biology Letters* **7 (2B)**, 785-794

Kunihisa M, Fukino N, Matsumoto S (2005) CAPS markers improved by cluster-specific amplification for identification of octoploid strawberry (*Fragaria x ananassa* Duch.) cultivars, and their disomic inheritance. *Theoretical and Applied Genetics* **110**, 1410-1418

Kunihisa M, Ueda H, Fukino N, Matsumoto S (2009) Genotyping of strawberry (*Fragaria x ananassa* Duch.) cultivars by DNA markers: Interlaboratory study. *Journal of AOAC International* **92 (3)**, 896-906

Kuras A, Korbin M, Zurawicz E (2004) Comparison of suitability of RAPD and ISSR techniques for determination of strawberry (*Fragaria* × *ananassa* Duch.) relationship. *Plant Cell, Tissue and Organ Culture* **79**, 189-193

Lerceteau-Köhler E, Guerin G, Laigret F, Denoyes-Rothan B (2003) Characterization of mixed disomic and polysomic inheritance in the octoploid strawberry (*Fragaria×ananassa*) using AFLP mapping. *Theoretical and Applied Genetics* **107**, 619-628

Lerceteau-Köhler E, Guerin G, Denoyes-Rothan B (2005) Identification of SCAR markers linked to *Rca2* anthracnose resistance gene and their assessment in strawberry germplasm. *Theoretical and Applied Genetics* **111**, 862-870

Lewers KS, Styan SMN, Hokanson SC, Bassil NV (2005) Strawberry GenBank-derived and genomic simple sequence repeat (SSR) markers and their utility with strawberry, blackberry, and red and black raspberry. *Journal of the American Society for Horticultural Science* **130**, 102-115

Lin J, Davis TM (2000) S1 analysis of long PCR heteroduplexes: Detection of chloroplast InDel polymorphisms in *Fragaria*. *Theoretical and Applied Genetics* **101**, 415-420

McClean PE, Mamidi S, McConnell M, Chikara S, Lee R (2010) Synteny mapping between common bean and soybean reveals extensive blocks of shared loci. *BMC Genomics* **11**, 184

Michaels SD, Amasino RMA (1998) A robust method for detecting single nucleotide changes as polymorphic markers by PCR. *Plant Journal* **14**, 381-385

Monfort A, Vilanova S, Davis TM, Arus P (2006) A new set of polymorphic simple sequence repeat (SSR) markers from a wild strawberry (*Fragaria vesca*) are transferable to other diploid *Fragaria* species and to *Fragaria* × *ananassa. Molecular Ecology Notes* **6**, 197-200

Neff MM, Neff JD, Chory J, Pepper AE (1998) dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: Experimental applications in *Arabidopsis thaliana* genetics. *Plant Journal* **14**, 387-392

Nier S, Simpson DW, Tobutt KR, Sargent DJ (2006) Construction of a genetic linkage map of an interspecific diploid *Fragaria* BC1 mapping population (*F. vesca 815 x [F. vesca 815 x F. viridis 903*]) and its comparison to the *Fragaria* reference map (FVxFN). *Journal of Horticultural Science and Biotechnology* **81**, 645-650

Palmieri L, Saviane A, Sordo M, Virzi A, Grando MS, Giongo L (2008) Characterization of *Fragaria vesca L.* genotypes and through the use of SSR, SCAR and SNP markers. In: *Book of Abstracts. VI International Strawberry Symposium ISHS.* Huelva, Spain, 3-7 March 2008. CAP Junta de Andalucía. 2008, 1079 pp 173

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theoretical and Applied Genetics* **85**, 985-993

Polans NO, Weeden NF, Thompson WF (1985) Inheritance, organization and mapping of *rbcS* and *cab* multigene families in pea. *Proceedings of the National Academy of Sciences USA* **82**, 5083-5087

Pontaroli AC, Rogers RL, Zhang Q, Shields ME, Davis TM, Folta KM, SanMiguel P, Bennetzen JL (2009) Gene content and distribution in the nuclear genome of *Fragaria vesca. The Plant Genome* **2 (1)**, 93-101

Potter D, Luby JJ, Harrison RE (2000) Phylogenetic relationships among species of *Fragaria* (Rosaceae) inferred from non-coding nuclear and chloroplast DNA sequences. *Systematic Botany* **25**, 337-348

Qin Y, Teixeira da Silva JA, Zhang L, Zhang Z-L (2008) Transgenic strawberry: State of the art for improved traits. *Biotechnology Advances* **26**, 219-232

Rokas A, Williams B, King N, Carroll S (2003) Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature* **425**, 798-804

Rong J, Bowers JE, Schulze SR, Waghmare VN, Rogers CJ, Pierce GJ, Zhang H, Estill JC, Paterson AH (2005) Comparative genomics of *Gossypium* and *Arabidopsis*: Unraveling the consequences of both ancient and recent polyploidy. *Genome Research* **15**, 1198-1210

Rousseau-Gueutin M, Lerceteau-Köhler E, Barrot L, Sargent DJ, Monfort A, Simpson DW, Arús P, Guérin G, Denoyes-Rothan B (2008) Comparative genetic mapping between octoploid and diploid *Fragaria* species reveals a high level of colinearity between their genomes and the essentially disomic behavior of the cultivated octoploid strawberry. *Genetics* **179**, 2045-2060

Rousseau-Gueutin M, Gaston A, Aïnouche A, Aïnouche ML, Olbricht K, Staudt G, Richard L, Denoyes-Rothan B (2009) Tracking the evolutionary history of polyploidy in *Fragaria* L. (strawberry): 3 New insights from phylogenetic analyses of low-copy nuclear genes. *Molecular Phylogenetics and Evolution* **51**, 515-530

Rousseau-Gueutin M, Richard L, Le Dantec L, Caron H, Denoyes-Rothan B (2011) Development, mapping and transferability of *Fragaria* EST-SSRs within the Rosodae supertribe. *Plant Breeding* **13**, 248-255

Ruiz-Rojas JJ, Sargent DJ, Shulaev V, Dickerman AW, Pattison J, Holt SH, Ciordia A, Veilleux RE (2010) SNP discovery and genetic mapping of T-DNA insertional mutants in *Fragaria vesca* L. *Theoretical and Applied Genetics* **121**, 449-463

Sargent DJ, Hadonou AM, Simpson DW (2003) Development and characterisation of polymorphic microsatellite markers from *Fragaria viridis*, a wild diploid strawberry. *Molecular Ecology Notes* **3**, 550-552

Sargent DJ, Davis TM, Tobutt KR, Wilkinson MJ, Battey NH, Simpson DW (2004a) A genetic linkage map of microsatellite, gene-specific and morphological markers in diploid *Fragaria*. *Theoretical and Applied Genetics* **109**, 1385-1391

Sargent DJ, Geibel M, Hawkins JA, Wilkinson MJ, Battey NH, Simpson DW (2004b) Quantitative and qualitative differences in morphological traits re-vealed between diploid *Fragaria* species. *Annals of Botany* **94**, 787-796

Sargent DJ, Clarke J, Simpson DW, Tobutt KR, Arús P, Monfort A, Vilanova S, Denoyes-Rothan B, Rousseau M, Folta KM, Bassil NV, Battey NH (2006) An enhanced microsatellite map of diploid *Fragaria*. *Theoretical and Applied Genetics* **112**, 1347-1359

Sargent DJ, Rys A, Nier S, Simpson DW, Tobutt KR (2007) The development and mapping of functional markers in *Fragaria* and their transferability and potential for mapping in other genera. *Theoretical and Applied Genetics* **114**, 373-384

Sargent DJ, Cipriani G, Vilanova S, Gil-Ariza D, Arús P, Simpson DW, Tobutt KR, Monfort A (2008) The development of a bin mapping population and the selective mapping of 103 markers in the diploid *Fragaria* reference map. *Genome* **51**, 120-127

Sargent DJ, Davis TM, Simpson DW (2009a) Strawberry (*Fragaria* spp.) structural genomics. In: Folta KM, Gardiner SE (Eds) *Genetics and Genomics of Rosaceae*, Springer, New York, NY, pp 437-456

Sargent DJ, Fernández-Fernández F, Ruiz-Rojas JJ, Southerland BG, Passey A, Whitehouse AB, Simpson DW (2009b) A genetic linkage map of the cultivated strawberry (*Fragaria x ananassa*) and its comparison to the diploid *Fragaria* reference map. *Molecular Breeding* **24**, 293-303

Shimomura K, Hirashima K (2006) Development and characterization of simple sequence repeats (SSR) as markers to identify strawberry cultivars (*Fragaria x ananassa* Duch.). *Journal of the Japanese Society for Horticultural Science* **75**, 399-402

Shulaev V, Sargent DJ, Crowhurst RN, Mockler TC, Folkerts O, Delcher AL, Jaiswal P, Mockaitis K, Liston A, Mane SP, Burns P, Davis TM, Slovin JP, Bassil N, Hellens RP, Evans C, Harkins T, Kodira C, Desany B, Crasta OR, Jensen RV, Allan AC, Michael, Setubal JC, Celton J-M, Rees DJG, Williams KP, Holt SH, Ruiz Rojas JJ, Chatterjee M, Liu B, Silva H, Meisel L, Adato A, Filichkin SA, Troggio M, Viola R, Ashman T-L, Wang H, Dharmawardhana P, Elser J, Raja R, Priest HD, Bryant Jr DW, Fox SE, Givan SA, Wilhelm LJ, Naithani S, Christoffels A, Salama DY, Carter J, Lopez Girona E, Zdepski A, Wang W, Kerstetter RA, Schwab W, Korban SS, Davik J, Monfort A, Denoyes-Rothan B, Arus P, Mittler R, Flinn B, Aharoni A, Bennetzen JL, Salzberg SL, Dickerman AW, Velasco R, Borodovsky M, Veilleux RE, Folta KM (2011) The genome of woodland strawberry (*Fragaria vesca*). *Nature Genetics* **43**, 109-116

Smith HO (1970) Nucleotide sequence specificity of restriction endonucleases. *Science* **205**, 455-462

Spigler RB, Lewers KS, Main DS, Ashman TL (2008) Genetic mapping of sex determination in a wild strawberry, *Fragaria virginiana*, reveals earliest form of sex chromosome. *Heredity* **101**, 507-517

Staudt G (2006) Himalayan species of *Fragaria* (Rosaceae). *Botanische Jahrbucher für Systematik* **126**, 483-508

Sugimoto T, Tamaki K, Matsumoto J, Yamamoto Y, Shiwaku K, Watanabe K (2005) Detection of RAPD markers linked to the everbearing gene in Japanese cultivated strawberry. *Plant Breeding* **124**, 498-501

Tautz D, Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Research* **12**, 4127-4138

Varshney RK, Graner A, Sorrells ME (2005) Genic microsatellite markers in plants: Features and applications. *Trends in Biotechnology* **23**, 48-55

Vilanova S, Arús P, Sargent DJ, Monfort A (2008) Synteny conservation between two distantly-related Rosaceae genomes: *Prunus* (the stone fruits) and *Fragaria* (the strawberry). *BMC Plant Biology* **8**, 67

Viruel MA, Sánchez D, Arús P (2002) An SSR and RFLP linkage map for the octoploid strawberry (*Fragaria* × *ananassa*). In: Plant, Animal and Microbe Genomes. 10th Conference, San Diego, California, USA. Available online: http://www.intl-pag.org/10/abstracts/PAGX_P660.html

Vision TJ, Brown DG, Shmoys DB, Durrett RT, Tanksley SD (2000) Selective mapping: A strategy for optimizing the construction of high-density linkage maps. *Genetics* **155**, 407-420

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research* **23 (21)**, 4407-4414

Wadman M (2008) James Watson's genome sequenced at high speed. *Nature* **452**, 788

Waldron J, Peace CP, Searle IR, Furtado A, Wade N, Graham MW, Carroll BJ (2002) Randomly amplified DNA fingerprinting (RAF): A culmination of DNA marker protocols based on arbitrarily primed PCR. *Journal of Biomedicine and Biotechnology* **2,** 141-150

Weber JL (1990) Informativeness of human (dC-dA)n. (dG-dT)n polymorphisms. *Genomics* **7**, 524-530

Weebadde CK, Wang D, Finn CE, Lewers KS, Luby JJ, Bushakra J, Sjulin TM, Hancock JF (2008) Using a linkage mapping approach to identify QTL for day-neutrality in the octoploid strawberry. *Plant Breeding* **127**, 94-101

Williamson SC, Yu H, Davis TM (1995) Shikamate deshydrogenase allozymes: Inheritance and close linkage to fruit color in the diploid strawberry. *Journal of Heredity* **86**, 74-76

Wu F, Tanksley SD (2010) Chromosomal evolution in the plant family Solanaceae. *BMC Genomics* **11**, 182

Wu KK, Burnquist W, Sorrells ME, Tew TL, Moore PH, Tanksley SD (1992) The detection and estimation of linkage in polyploids using single-dose restriction fragments. *Theoretical and Applied Genetics* **83**, 294-300

Wu SB, Tavassolian I, Rabiei G, Hunt P, Wirthensohn M, Gibson JP, Ford CM, Sedgley M (2009) Mapping SNP-anchored genes using high-resolution melting analysis in almond. *Molecular Genetics and Genomics* **282**, 273-281

Wu SB, Wirthensohn MG, Hunt P, Gibson JP, Sedgley M (2008) High resolution melting analysis of almond SNPs derived from ESTs. *Theoretical and Applied Genetics* **118**, 1-14

Yu H, Davis TM (1995) Genetic linkage between runnering and phosphoglucoisomerase allozymes, and systematic distortion of monogenic segregation ratios in diploid strawberry. *Journal of American Horticultural Sciences* **120**, 687-690

Zorrilla-Fontanesi Y, Cabeza A, Torres A, Botella MA, Valpuesta V, Monfort A, Sánchez-Sevilla JF, Amaya I (2011) Development and bin mapping of strawberry genic-SSRs in diploid *Fragaria* and their transferability across the Rosoideae subfamily. *Molecular Breeding* **27**, 137-156