Theor Appl Genet (2002) 104:451–456
© Springer-Verlag 2002

L. Crespel · S. Gudin · J. Meynet · D. Zhang

AFLP-based estimation of 2n gametophytic heterozygosity in two parthenogenetically derived dihaploids of *Rosa hybrida* L.

Received: 15 February 2001 / Accepted: 22 May 2001

Abstract Two dihaploid *Rosa hybrida* L. genotypes, derived through parthenogenesis by using irradiated pollen, were crossed with clonally propagated plants of the diploid species *Rosa rugosa* Thunb. and *Rosa wichuraiana* Crép., respectively. Three progeny groups were obtained which contained numerous polyploids, as determined by flow cytometry. Production of fertile 2n female gametes is apparently very common in one of these *R. hybrida* dihaploid derivatives, whereas the other one is able to produce fertile 2n pollen. Hence, an amplified fragment length polymorphism (AFLP) study was performed on the parental plants and the resulting hybrid offspring in order to estimate (1) the respective genomic parental contributions, and (2) the level of heterozygosity transmitted by the 2n unreduced gametes. Comparison of the levels of transmitted parental heterozygosity revealed that two types of 2n gametes were produced simultaneously, presumably resulting from restitution at the first and at the second meiotic division, respectively.

Keywords *Rosa* · Molecular markers · Heterozygosity · Unreduced gametes · Dihaploids

Communicated by F. Salamini

L. Crespel
Meilland Star Rose, Domaine de saint André,
Le Cannet des maures, 83340, Le Luc en provence, France

S. Gudin (✉)
Laboratoire de Morphogenèse végétale,
Université d'Aix-Marseille III,
Avenue Escadrille Normandie-Niémen,
13397, Marseille, Cedex 20, France
e-mail: SERGE.GUDIN@MV.u-3mrs.fr
Tel.: +33-4-91-28-85-21

J. Meynet
INRA, Station d'Amélioration des Plantes Florales,
La Gaudine, 83370, Saint Aygulf, France

D. Zhang
BioGEVES, Domaine du Magneraud, 17700, Surgéres, France

Introduction

The genus *Rosa* subgenus *Eurosa* is a very large genus comprising ten sections and more than 200 species (De Vries and Dubois 1996). The genetic variability in this group of species is very large but poorly used in breeding programs. Maia and Venard (1976) and Berninger (1992) showed that less than ten species belonging to three sections were at the origin of actual cultivated roses. Most of the cultivated roses are tetraploid, whereas the botanic species are mostly diploid. To give an access to the genetic resources of the diploid species, a program of haploidization has been developed since 1992. Dihaploid derivatives from tetraploid cultivated roses have been produced by in situ parthenogenesis induced after pollination by irradiated pollen and in vitro embryo rescue (Meynet et al. 1994). Although some of these parthenogenetically derived dihaploid diploid roses were fertile, their gametogenesis often revealed abnormalities and resulted in the frequent production of 2n gametes (El Mokadem et al. 2000a).

In order to determine the nature of 2n gamete formation, we estimated the level of heterozygosity transmitted by 2n gametes of dihaploid *Rosa hybrida* L. into the progeny resulting from crosses made with diploid species, using AFLPs. Indeed, a large part of this progeny was polyploid. Actually, unreduced gametes are probably responsible for most of the polyploidization events that occur via sexual reproduction (Harlan and De Wet 1975). Unreduced gametes have been shown to mainly derive from either a first division restitution (FDR) or a second division restitution (SDR), as a result of an incomplete first or second meiotic division, respectively (Mendiburu and Peloquin 1971; Mok and Peloquin 1975; McCoy 1982; Hermsen 1984). The gametes resulting from these two phenomena differ in their genetic consequences, by transmitting different levels of parental heterozygosity (Hermsen 1984). Heterozygosity transmitted by 2n ovules and pollen has been determined by using molecular markers in blueberry (Vorsa and Rowland 1997) and perennial ryegrass (Chen et al.

1997), respectively. Many studies concerning rose cultivar identification, genetic diversity and parentage analysis have been carried out in the last decade by using restriction fragment length polymorphism (RFLP), sequence-tagged microsatellites (STMS) and, mainly, random amplified polymorphic DNA (RAPD) (Gudin 2000). Recently, Debener and Mattiesch (1999), Debener et al. (2000), and Zhang et al. (2000) used AFLPs for the construction of a genetic linkage map and cultivar identification, respectively. This method is nowadays becoming increasingly popular for germplasm analysis (Powell et al. 1996; De Riek et al. 1997; Dirlewanger 1997).

Materials and methods

Plant material

The AFLP analyses were carried out on clonally propagated plants belonging to two diploid ($2n=2x=14$) botanic species, *Rosa wichuraiana* Crép. and *Rosa rugosa* Thunb., two parthenogenetically derived dihaploid *R. hybrida* L. clones, H61 and H3, and three segregating populations: (1) a population of 32 individuals resulting from H61×*R. wichuraiana* comprising eight diploids, 19 triploids and five pentaploids, (2) 38 seedlings resulting from H61×*R. rugosa* comprising six diploids, 28 triploids, two tetraploids and two pentaploids, (3) 28 seedlings resulting from *R. wichuraiana*×H3 comprising 19 diploids and nine triploids. H61 and H3 resulted from the haploidization of the 4*x* cvs FJV6 and Sweet Promise, respectively. They were obtained by in situ parthenogenesis induced by using irradiated pollen (γ rays at 600 Gy) and embryo rescue, as described by Meynet et al. (1994), and could be used in hybridization programs as H61 and H3 are female and male fertile, respectively (El Mokadem et al. 2000a). The ploidy level of the hybrid progenies was determined by flow cytometry, as described by Jacob et al. (1996). Previous test crosses made with a number of diploid species or tetraploid cultivars showed that *R. wichuraiana* and *R. rugosa* always produce haploid reduced gametes, as deduced from the resulting ploidy levels of the offspring, whereas dihaploid derivatives of *R. hybrida* currently produce unreduced gametes (El Mokadem et al. 2000b). The plants were maintained in a greenhouse at the National Agronomy Research Institute in Fréjus. The crosses were carried out, and the plants cultivated, as described by El Mokadem et al. (2000a).

DNA isolation and AFLP analyses

Total DNA was isolated from frozen young leaf tissue as indicated in Zhang et al. (2000). AFLP core reagent and starter primer kits were purchased from Life Technologies (Gibco BRL), and AFLP analyses were performed as described by Vos et al. (1995). Total genomic DNA (125 ng) was digested with 1.25 U of *Eco*RI and 1.25 U of *Mse*I in a 12.5-µl reaction mixtures (Gibco BRL Life Technologies) at 37°C for 2 h. After complete digestion, *Eco*RI and *Mse*I adapters, 0.5 U of T4 DNA ligase, and ligation buffer (Gibco BRL Life Technologies) were added and the mixture was incubated for 2 h at 20°C.

The pre-amplification reaction was performed with 2.5 µl of template DNA (1:10 solution diluted from the restriction-ligation mixture), using a pair of primers based on the sequences of the *Eco*RI and the *Mse*I adaptaters, including one additional selective nucleotide at the 3´ end (E+1, M+1), as described by Vos et al. (1995). The selective amplification reaction was also performed with two primers based on the same sequences as the E+1 and M+1 primers, but with two additional selective nucleotides at the 3´ end of each primer. The *Eco*RI primers were labeled by phosphorylating the 5´ end with [γ-33P]ATP for fragment detection, as described by Vos et al. (1995).

Table 1 AFLP primer combinations used to generate markers in the segregating populations

AFLP primer combination	*Eco*RI	*Mse*I
AFLP1	E-ACA	M-CAC
AFLP2	E-ACA	M-CAT
AFLP3	E-ACA	M-CAG
AFLP4	E-ACA	M-CTG
AFLP5	E-ACT	M-CAC
AFLP6	E-ACT	M-CAT
AFLP7	E-ACT	M-CTT
AFLP8	E-AAC	M-CAA

The PCR products were mixed with an equal volume of tracking dye (95% formamide, 10 mM NaOH, 0.05% bromophenol blue, 12.5% saccharose, and 0.05% xylene cyanol), denatured at 92°C for 3 min. Aliquots (4 µl) of each reaction were loaded onto a denaturing 5% polyacrylamide gel (acrymamide-bisacrylamide 29:1) in 0.5×TBE (50 mM Tris, 50 mM boric acid, 1 mM EDTA, pH 8) and 7.5 mM urea; 0.5×TBE was used as the electrophoresis buffer. Gels were run at a constant power (55 W), fixed, dried, and exposed to a Kodak BioMax x-ray film for 2–4 days before developing. We used eight AFLP primer combinations (Table 1).

Scoring of data

The AFLP profiles observed on the autoradiographs were scored visually. The presence or absence of a band position in a lane were coded 1 or 0, respectively. In order to be able to further evaluate parental contributions, we only scored the bands that were specific to each of the parents used (and absent in their respective sexual partners).

Genomic parental contribution

The Nei and Li (1979) F index was computed to evaluate the similarity between the progeny and the parents. It was computed as $Fxy=2\ Nxy/(Nx+Ny)$, where Fxy=similarity index between varieties x and y, Nxy=number of band positions scored 1 for both x and y, Nx=number of band positions scored 1 for x, and Ny=number of band positions scored 1 for y. The statistical differences between means were tested by a Student's *t* test at the 5% level.

Marker heterozygosity in dihaploid *R. hybrida*

We examined the progeny ratios in the diploid populations of H61×*R. wichuraiana*, H61×*R. rugosa* and *R. wichuraiana*×H3. As bands specific to the dihaploid parents heterozygous alleles (Aa) transferred from dihaploid *R. hybrida* to the diploid progenies should segregate in a 1:1 ratio, the heterozygosity level of this parent could be calculated as corresponding to the number of markers behaving accordingly (segregating) divided by the total number of relevant markers (specific to the dihaploid parent). For each segregating marker in the two diploid populations that issued from H61 (14 seedlings) and in the diploid population that issued from H3 (19 seedlings), a chi^2 test for the fit of progeny ratios adapted by Yates to small strengths, as indicated by Schwartz (1996), was performed ($P\leq0.05$).

Estimation of the heterozygosity transmitted through unreduced gametes of dihaploid derivatives of *R. hybrida*

To estimate the level of heterozygosity maintained by 2n gametes produced by dihaploid derivatives of *R. hybrida* (H61 and H3), we determined both the number of heterozygous loci that was trans-

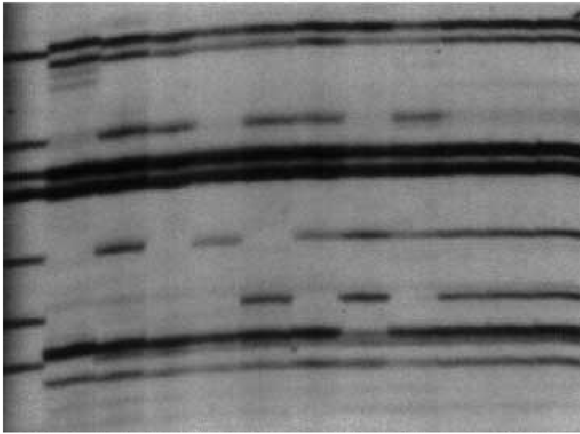

Fig. 1 AFLP marker profiles of a part of the H61×*R. rugosa* progeny, using primer combination E-ACT/M-CTT. The *lanes* are amplification products using template DNA from *R. rugosa* (*A*), H61 (*B*), and a part of the resulting triploid progeny (*C–L*). The *arrow* shows a heterozygous locus present only in H61 but not segregating in the triploid progeny

ferred from the dihaploid parents to the triploid progenies and the number that was lost. Only the markers that segregated in a 1:1 ratio in the diploid progenies were considered in the analysis of how heterozygosity was transmitted from unreduced gametes into the triploid progenies (Fig. 1). Furthermore, heterozygous loci can be lost on the occasion of crossing over during 2n gamete production. If such crossing-over leads to the formation of recessive homozygous loci, the corresponding bands will not appear in the progeny, as AFLPs are dominant markers. Conversely, crossing-over leading to the formation of dominant homozygous loci will still correspond to visible bands, that cannot be differenciated from the heterozygous loci identified earlier. As previously done in heterozygosity estimation studies carried out on *Vaccinium darrowi* Camp. (Qu and Hancock 1995; Vorsa and Rowland 1997), we assumed that equal numbers of cross overs can lead to the formation of recessive or dominant homozygous loci. Hence, we counted the number of unique dominant markers present in the dihaploid parents that were absent in the triploid progenies.

Thus, the heterozygosity ratio transferred from the dihaploid parents to the triploid progenies could be calculated as corresponding to the number of heterozygous loci specific to the dihaploid parent transferred to the triploid offspring minus the number of heterozygous loci detected in the dihaploid, but absent in the triploid offspring, divided by the total number of heterozygous loci identified in the dihaploid genotype concerned.

Results

All seedlings belonging to the studied progenies showed individually unique AFLP profiles, different from the parents. They could also be easily differentiated by their morphological aspect. Thus, it can be asserted that apomixis did not interfere here, although its occurrence in roses has often been suspected (Täckholm 1920; Kroon and Zeilinga 1974; Wissemann and Hellwing 1997; Werlemark et al. 1999), as recently evidenced by Werlemark (2000) on *Rosa* section *caninae*, and Crespel et al. (2001), on *R. hybrida*, including dihaploid derivatives. Furthermore, the ploidy levels in the progenies varied from 2 to 5*x*, as determined by flow cytometry, and included an average of 56% triploids on which the heterozygosity level study could be carried out.

Genomic parental contributions

In the three studied crosses, when the involved dihaploid genotype was used as the female parent (H61×*R. wichuraiana*, H61×*R. rugosa*), as well as when it was used as the male parent (*R. wichuraiana*×H3), the similarity estimates of the resulting diploid hybrids varied between 0.47 and 0.60, and 0.47 and 0.61, with the dihaploid and diploid parent, respectively. For the triploid hybrids, similarity estimates ranged from 0.56 to 0.80 when compared to the dihaploid parent, and from 0.40 to 0.52 when compared to the diploid parent. The average similarity estimates of the diploid hybrids with the dihaploid and diploid parents were thus comparable, while within the triploid hybrids they were noticeably higher with the dihaploid than with the diploid parent (Table 2).

Heterozygosity in dihaploid *R. hybrida*

As previously stated, only the diploid progenies resulting from the three studied crosses were considered for the following calculations.

The eight primer combinations produced a total of 72 and 106 fragments that were present in the dihaploids H61 and H3, respectively, and absent in their respective associated sexual partners, corresponding to diploid species (*R. wichuraiana* and *R. rugosa* used as males for H61, and *R. wichuraiana* used as a female for H3). Among these specific markers, 64 (88.8%) and 82

Table 2 Average genetic similarity estimates between parents and triploid progenies

Progeny origin and ploidy level	H61×*R. wichuraiana*		H61×*R. rugosa*		*R. wichuraiana*×H3	
	2*x*	3*x*	2*x*	3*x*	2*x*	3*x*
Mean similarity estimate with the seed parent[a]	0.54a	0.76a	0.57a	0.78a	0.55a	0.47a
Mean similarity estimate with the pollen parent[a]	0.53a	0.45b	0.57a	0.48b	0.54a	0.69b

[a] Means followed by different letters in the same column are significantly different at $P \leq 0.05$ (Student's *t* test)

(77.3%) were heterozygous for H61 and H3, respectively. For both dihaploid progenitors, the segregation ratios of the heterozygous markers varied considerably, as tested by an adapted chi^2 test. Among the heterozygous markers found in the H61 and H3 respective progenies, 53 (82.8%) and 57 (69.5%) best fitted a 1:1 ratio, while 11 (17.2%) and 25 (30.4%) displayed distorted segregation ratios, respectively.

Levels of heterozygosity transmitted by 2n gametes in triploid progenies

The levels of estimated heterozygosity transmitted by H61 and H3 in their progenies ranged from 37.0 to 100%, and from 7.1 to 95.1%, with an average heterozygosity of 94.0% and 72.2%, respectively. However, two descendants of each, H61 and H3, had less than 50% estimated heterozygosity. Hence, the triploid seedlings corresponding to the different progenies could be grouped into two classes, according to the levels of inherited heterozygosity: (1) the ones that had less than 50%, (2) the ones that had 70% to 100% estimated heterozygosity. Thus, 94.7, 96.4, and 77.8% of H61×*R. wichuraiana*, H61×*R. rugosa*, and *R. wichuraiana*×H3 triploid progenies, respectively, belonged to the second class (Fig. 2).

The correlation coefficient between the F index (reflecting the similarities between the triploid offspring and their dihaploid parents) and the level of heterozygosity transmitted by 2n gametes corresponded to 0.90 ($P<0.05$). These factors are thus highly correlated.

The triploid seedlings resulting from the three crosses with less than 50% estimated heterozygosity transmitted by the dihaploid parent had an average similarity estimate of 0.64 with that parent, whereas this value was equal to 0.77 for the ones with 70 to 100% estimated transmitted heterozygosity.

Hence, according to the F index and the level of transmitted heterozygosity, the existence of two distinct classes of 2n gametes, at the origin of the triploid progenies, could be proposed. It has indeed been shown that FDR results in significantly more transmitted heterozygosity (±80%) than SDR (±40%), because FDR gametes contain the non-sister chromatids of each homologous pair of chromosomes, whereas SDR gametes contain only sister chromatids (Hermsen 1984).

Discussion

Levels of heterozygosity transmitted by diploid (2n) gametes

The high level of estimated heterozygosity transmitted by H61 2n female gametes in triploid hybrids suggests that FDR-type gametes are produced by this dihaploid genotype. However, 5.3 and 3.6% of the triploid progenies of H61×*R. wichuraiana* and H61×*R. rugosa*, respectively, had less than 50% estimated heterozygosity. This supports the co-existence of SDR mechanisms as well. Indeed, in *Solanum*, it has been calculated that 2n gametes produced by FDR transmit 80% to 100% of the parental heterozygosity to the progeny (Werner and Peloquin 1991a). In contrast, about 40% of the parental heterozygosity is expected to be transmitted by a SDR 2n gamete (Mendiburu and Peloquin 1977; Werner and Peloquin 1991a, 1991b). FDR and SDR 2n female gametes have also been identified in a diploid blueberry (*Vaccinium darrowi* Camp) clone (Vorsa and Rowland 1997), and in haploidized *Solanum tuberosum* L. (Werner and Peloquin 1990). In our experiments, H61 FDR 2n gametes transfered a large amount of the parental heterozygosity to the triploid hybrids, which ranged from 84.3 to 100%. These values are close to the theoretical rate of heterozygosity transfered by 2n gametes re-

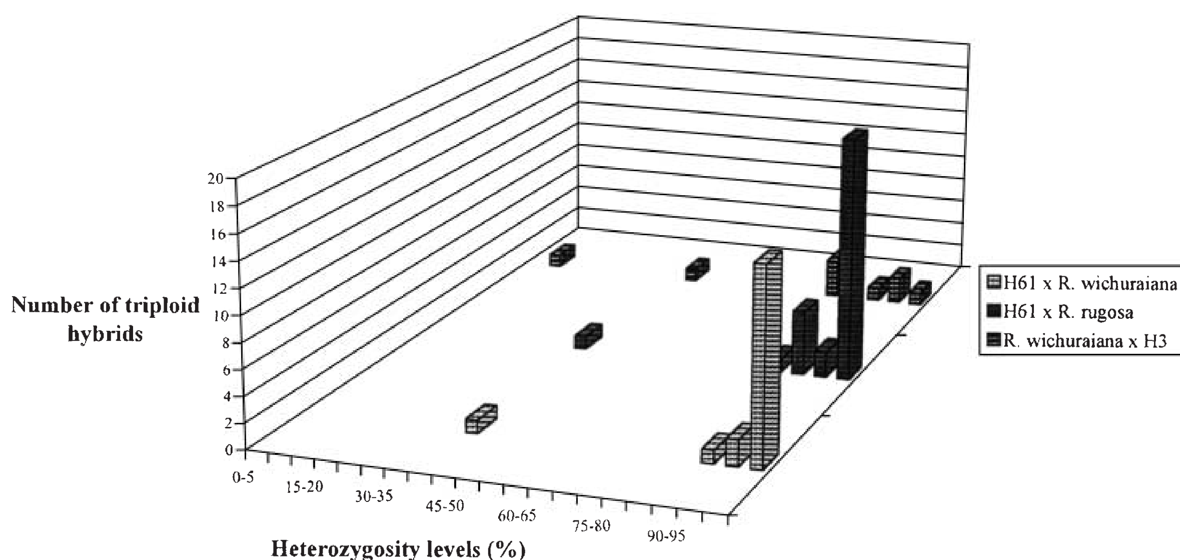

Fig. 2 Classification of H61×*R. wichuraiana*, H61×*R. rugosa*, and *R. wichuraiana*×H3 triploid hybrids, according to the level of heterozygosity transmitted by 2n female gametes

sulting from FDR with or without crossing-over (FDR-CO or FDR-NCO, respectively), which correspond to 80 and 100%, respectively (Buso et al. 1999). Eighty nine point four and 75% of the triploid H61×*R. wichuraiana* and H61×*R. rugosa* progenies had more than 90% estimated heterozygosity transmitted by the seed parent. It is thus likely that most of the 2n gametes in this dihaploid genotype are formed via FDR-NCO.

The level of estimated heterozygosity transmitted by H3 2n pollen in triploid progeny suggests that FDR-type 2n male gamete-forming mechanisms are also predominantly operating in H3. However, as 22.2% of the triploid hybrids in the *R. wichuraiana*×H3 population had less than 50% estimated heterozygosity, the co-existence of SDR mechanisms can here too be suspected. They would then be operating more frequently than in the previous case of H61 2n female gamete formation. FDR and SDR 2n pollen have previously been identified in perennial ryegrass (Chen et al. 1997). In our experiments, H3 FDR 2n pollen transfered a large amount of the parental heterozygosity to the triploid hybrids, which ranged from 76.1 to 95.1%. Moreover, 44.4% of the triploid progeny had between 76.1 and 89% of estimated heterozygosity transmitted by the pollen parent. It is thus likely that, contrary to the case of the unreduced female gametes produced by H61, most of the H3 2n pollen is formed via FDR-CO.

Cytological aspects of unreduced gamete production

H61 2n, 3n and 4n female gamete production, presumably responsible for the occurrence of triploid, tetraploid and pentaploid hybrids in the studied progenies, probably results from abnormal (or absence of) cytokinesis. This has indeed previously been shown for 2n pollen and ovules of *Medicago sativa* (Pfeiffer and Bingham 1983) and for 3n and 4n pollen in the single clone of the same species (Mariani et al. 1991; Tavoletti et al. 1991). The mode of nuclear restitution may be SDR or FDR (Bretagnolle and Thompson 1995) in the case of 2n gametes, and it has been shown that in the absence of cytokinesis, post-meiotic fusion of the three or four telophase nuclei produced a 3n or 4n pollen grain in *M. sativa* (Pfeiffer and Bingham 1983; Mariani et al. 1991; Tavoletti et al. 1991).

Chromosome pairing and/or crossing-over at the pachytene stage may be influenced by the action of mutant synaptic genes. Synaptic mutants are characterized by an increased frequency of univalent chromosomes at metaphase-I. These may result from either the falling apart of normally paired, homologous chromosomes in prophase-I, due to their inability to generate or retain chiasmata (desynapsis), or the lack of chromosome pairing and thus crossing-over in prophase-I (asynapsis). In these cases SDR 2n and reduced gametes, when produced, are expected to be predominantly sterile due to chromosome imbalance, whereas FDR 2n gametes are expected to be mostly balanced and thus functional (Jongedijk 1983; Ramanna 1983). If there is no crossing-over, the FDR 2n gametes preserve the parental genotype and 100% of the estimated heterozygosity is transmitted. In desynaptic mutants of potato a severe reduction in crossing-over was observed for two marker loci, ym and y, 83.7 and 90%, respectively (Jongedijk et al. 1991). This could explain the wide range of heterozygosity transmitted by FDR H61 2n gametes in triploid hybrids.

El Mokadem et al. (2000b) showed that anaphase II spindles are parallel in many of the H3 cells that are probably at the origin of 2n pollen formation. Such abnormal spindle geometry during second meiotic division has thus been shown to lead to FDR 2n pollen formation in potato and apple (Mok and Peloquin 1975; Zhang et al. 1988).

Acknowledgements The authors thank Mr. Alain Meilland for his kind authorisation to publish this paper. The experiments presented here comply with the current laws of France.

References

Berninger E (1992) Le rosier de serre pour fleurs à couper. In: Gallais A, Bannerot H (eds) Amélioration des espèces végétales cultivées, objectifs et critères de sélection. INRA Editions, Paris, pp 490–504

Bretagnolle F, Thompson JD (1995) Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. New Phytol 129:1–22

Buso JA, Reifshneider FJB, Boiteux LS, Peloquin SJ (1999) Effects of 2n-pollen formation by first meiotic division restitution with and without crossovers on eight quantitative traits in 4*x*–2*x* potato progenies. Theor Appl Genet 98:1311–1319

Chen C, Sleper DA, Chao S, Johal GS, West CP (1997) RFLP detection of 2n pollen formation by first and second division restitution in perennial ryegrass. Crop Sci 37:76–80

Crespel L, Zhang D, Meynet J, Jacob Y, Gudin S (2001) Identification of apomictic plants in *Rosa hybrida* L. by AFLPs. Acta Hort 547:51–55

Debener T, Mattiesch L (1999) Construction of a genetic linkage map for roses using RAPD and AFLP markers. Theor Appl Genet 99:891–899

Debener T, Janakiram T, Mattiesch L (2000) Sports and seedlings of rose varieties analysed with molecular markers. Plant Breed 119:71–74

De Riek J, Dendauw J, Roldan-Ruiz I, van Bockstaele E, De Loose M (1997) Identification strategy using RAPD and AFLP markers for Belgian pot azaleas. Proceedings of the UPOV – Working Group on Biochemical and Molecular Techniques and DNA-profiling in particular, 4th session. Cambridge, March 11–13, 1997

De Vries DP, Dubois LAM (1996) Rose breeding: past, present and future. Acta Hort 424:241–248

Dirlewanger E (1997) Identification of peach cultivars using RAPD and AFLP markers. Proceedings of the UPOV – Working Group on Biochemical and Molecular Techniques and DNA-profiling in particular, 4th session. Cambridge, March 11–13, 1997

El Mokadem H, Meynet J, Jacob Y, Gudin S (2000a) Utilisation of parthenogenetic diploid plants of *Rosa hybrida* L. in interspecific hybridizations. Acta Hort 508:185–190

El Mokadem H, Crespel L, Meynet J, Gudin S, Jacob Y (2000b) Gametic behaviour of parthenogenetic plants of *Rosa canina* L. and *R. hybrida* L. Acta Hort 547:289–296

Gudin S (2000) Rose: genetics and breeding. Plant Breed Rev 17:159–189

Harlan JR, De Wet MJ (1975) On O.Winge and a prayer. The origins of polyploidy. Bot Rev 41:361–391

Hermsen JG (1984) Mechanisms and genetic implications of 2n gamete formation. Iowa State J Res 58:421–434

Jacob Y, Teyssier C, Reynders-Aloisi S, Brown SC (1996) Use of flow cytometry for the rapid determination of ploidy level in the genus *Rosa*. Acta Hort 424:273–278

Jongedijk E (1983) Selection for first division restitution 2n-egg formation in diploid potatoes (abstract). Potato Res 26:399

Jongedijk E, Hutten RCB, Van Der Wolk JMASA, Schuurmans Stekhoven SIJ (1991) Synaptic mutants in potato, *Solanum tuberosum* L. III. Effect of the Sd-1/ds-1 locus (desynapsis) on genetic recombination in male and female meiosis. Genome 34:121–130

Kroon GH, Zeilinga AE (1974) Apomixis and heterogamy in rose rootstocks (*Rosa canina* L.). Euphytica 23:345–352

Ma N, Venard P (1976) Cytotaxonomie du genre *Rosa* et origine des rosiers cultivés. In: Fédération Nationale des Producteurs de l'Horticulture et des Pépinières (ed) Travaux sur le rosier de serres menés au C.R.A. d'Antibes. INRA, Antibes, France, pp 7–20

Mariani A, Tavoletti S, Veronesi F (1991) Cytology of 2n gamete formation in the *Medicago sativa* complex. In: Veronesi F, Bullita S, Carelda S (eds) Proc 17th Meeting of the Fodder Crops Section of Eucarpia, Alghero (Italy), October 14–18, 1991, pp 60–67

McCoy JJ (1982) The inheritance of 2n pollen formation in diploid alfalfa *Medicago sativa*. Can J Genet Cytol 24:315–323

Mendiburu AO, Peloquin SJ (1971) High yielding tetraploids from 4*x*-2*x* and 2*x*-2*x* matings. Am Potato J 48:300–301

Mendiburu AO, Peloquin SJ (1977) The significance of 2n gametes in potato breeding. Theor Appl Genet 49:53–61

Meynet J, Barrade R, Duclos A, Siadous R (1994) Dihaploid plants of roses (*Rosa*×*hybrida* cv Sonia) obtained by parthenogenesis induced using irradiated pollen and in vitro culture of immature seeds. Agronomie 2:169–175

Mok DW, Peloquin SJ (1975) The inheritance of three mechanisms of diplandroid (2n pollen) formation in diploid potatoes. Heredity 35:295–302

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc Natl Acad Sci USA 76:5269–5273

Pfeiffer TW, Bingham ET (1983) Abnormal meiosis in alfalfa, *Medicago sativa*: cytology of 2n egg and 4n pollen formation. Can J Genet Cytol 25:107–112

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2:225–238

Qu L, Hancock JF (1995) Nature of 2n gamete formation and mode of inheritance in interspecific hybrids of diploid *Vaccinium darrowi* and tetraploid *V. corymbosum*. Theor Appl Genet 91:1309–1315

Ramanna MS (1983) First division restitution gametes through fertile desynaptic mutants of potato. Euphytica 32:337–350

Scharwtz D (1996) Méthodes statistiques à l'usage des médecins et des biologistes. Coll. Statistique en biologie et en médecine. Flammarion, Paris

Täckholm G (1920) On the cytology of the genus *Rosa*. Svensk Bot Tidskr 14:300–311

Tavoletti S, Mariani A, Veronesi F (1991) Cytological analysis of macro- and micro-sporogenesis of a diploid alfalfa clone producing male and female 2n gametes. Crop Sci 31:1258–1263

Vorsa N, Rowland LJ (1997) Estimation of 2n megagametophyte heterozygosity in a diploid blueberry (*Vaccinium darrowi* Camp) clone using RAPDs. J Hered 8:423–426

Vos P, Hogers R, Bleeker M, Rijans 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 Res 23:4407–4414

Werlemark G, Uggla M, Nybom H (1999) Morphological and RAPD markers show a highly skewed distribution in a pair of reciprocal crosses between hemisexual dog-rose species, *Rosa* sect. *Caninae*. Theor Appl Genet 98:557–563

Werlemark G (2000) Evidence of apomixis in hemisexual dog-roses, *Rosa* section *Caninae*. Sex Plant Reprod 12:353–359

Werner JE, Peloquin SJ (1990) Inheritance and two mechanisms of 2*n* egg formation in 2x potatoes. J Hered 81:371–374

Werner JE, Peloquin SJ (1991a) Yield and tuber characteristics of 4*x* progeny from 2*x*×2*x* crosses. Potato Res 34:261–267

Werner JE, Peloquin SJ (1991b) Significance of allelic diversity and 2n gametes for approaching maximun heterozygosity in 4*x* potatoes. Euphytica 58:21–29

Wissemann V, Hellwig FH (1997) Reproduction and hybridization in the genus *Rosa* Section *Caninae* (Ser.). Rehd Bot Acta 110:251–256

Zhang YX, Lespinasse Y, Salesses G (1988) Mise en évidence de quelques anomalies mé conduisant à la formation de gamètes mâles non réduits chez le pommier cultivé (*Malus*×*domestica* Borkh.). Cytologia 53:749–755

Zhang D, Besse C, Cao M, Gandelin MH (2000) Evaluation of AFLPs for variety identification in modern rose. Acta Hort 546:351–357