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Self-incompatibility of raspberry cultivars assessed by SSR markers

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ABSTRACT

Self-incompatibility in European red raspberry (*Rubus idaeus* L.) is less studied compared to other horticulturally relevant rosaceous crops, although cultivars of this species show differences in self-fertility. In this study, we genotyped progenies of 16 open pollinated raspberry cultivars with SSR markers to determine their natural propensity for self- and cross-fertilization. In further experiments, we genotyped progenies of selected cultivars from a topcross environment and studied seed set after hand pollination. A wide spectrum of cross-fertilized progeny ratio was discovered among the cultivars ranging from 5% of 'Dorman Red' progeny to 100% of 'Rumla' progeny derived by cross-fertilization. This was consistent with results obtained by hand pollination, where a significantly higher number of seed was produced in self-pollinated fruit of 'Dorman Red' and cross-pollinated fruit of 'Rumla'. The difference was particularly large in 'Rumla'; its self-pollinated fruit developed 10.95 drupelets per fruit on average, almost seven times less than its cross-pollinated fruit. The cultivar 'Rumla' showed 100% cross-fertilized progeny in a topcross environment as well, in contrast to the cultivars 'Lucana' and 'Preußen', which both had no cross-fertilized progeny. The results of this study show that there are differences in fertilization behavior between raspberry cultivars. Such information on the fertilization behavior of selected cultivars is useful in planning for cultivar selection in protected growing, where pollination is of special consideration.

1. Introduction

European red raspberry, *Rubus idaeus* L., is an important small fruit crop traditionally grown in temperate regions. Raspberry production worldwide had an increasing trend from 370,000 t in 1998 to over 870,000 t in 2018 (FAOSTAT, 2020). Protection from pests and diseases, the expansion to previously unsuitable climates and a desire to further increase yield has introduced new growing environments and technologies, for instance protected growing (Darnell et al., 2006; Marchi et al., 2019; Palonen et al., 2017). Fertilization has always been an important factor in raspberry fruit production. Drupelets of the raspberry aggregate fruit only develop if their corresponding carpel is pollinated. However, incomplete fertilization results in quality flaws e.g. crumbly fruit, and ultimately low yield. The recent expansion of protected growing has increased the importance of fertilization in raspberry production especially as these environments come with restricted pollen availability. Thus, self-incompatibility of cultivars will pose a serious concern in protected environments.

Self-incompatibility is well known in different species of the Rosaceae plant family. However, raspberry cultivars are believed to have gained self-fertility during their domestication (Jennings, 1988). Nevertheless, modern raspberry cultivars have a complex ancestry with one or more wild raspberry species (e.g. *R. idaeus, R. strigosus* and *R. occidentalis*) in their pedigree. Wild *R. idaeus* and *R. strigosus* are self-incompatible (Keep, 1968), whereas *R. occidentalis* in the wild is most likely self-fertile (Jennings, 1988). *R. arcticus*, which is also a wild relative of *R. idaeus* that is native to North Eurasia and North America, has partially self-fertile populations (Tammisola and Ryynänen, 1970). It is therefore expected that modern cultivars from such a complex ancestry may differ in self-fertility. However, previous works focused on only a few cultivars (Daubeny, 1971; Keep, 1968). Although most raspberry cultivars are considered self-fertile (Keep, 1968), several traits associated with self-incompatibility could still be found in some cultivars. Furthermore, many cultivars suffer from inbreeding depression (Jennings, 1962; Keep, 1968). Nevertheless, these cultivars look like typical outcrossing species, with prominent flowers producing ample nectar that attracts pollinators. The floral structure of raspberries allows for both self- and cross-pollination, with the androecium surrounding the gynoecium and many stamens directly touching carpels (Delaplane and Mayer, 2000).

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Beside fruit production, knowledge about the predominant type of fertilization of raspberry cultivars is also of importance for breeding. Breeders aiming to establish bi-parental populations require information on incompatibility between cultivars in order to determine the number of crosses to reach a sufficient amount of seed. Using SSR markers to assess the progeny of open pollinated cultivars as a method for fertilization type preference has other advantages as well. This genotyping dataset could also be used to discover possible apomicts among the progeny. Apomixis occurs regularly in many species of the genus *Rubus*, although it is rare in the subgenus *Idaeobatus* (Weber, 1996). These rare occurrences has been reported in polyploid *R. strigosus* and a progeny from an interspecific pollination of *R. idaeus*, but not in diploid *R. idaeus* (Antonius and Nybom, 2004; Pratt et al., 1958). Apomictic progeny could be an alternative and economic means of propagation instead of root cuttings or suckers.

Until recently, the effect of the type of pollination and fertilization on fruit quality had been unclear. A few studies explored this issue through the analysis of fruit set and fruit size differences in self- and cross-pollination by hand (Żurawicz, 2016; Żurawicz et al., 2018). These studies showed that cross-pollination increases raspberry fruit size and the number of drupelets. Furthermore, the studies described differences in cross-pollinating efficiency between different cultivars. The authors concluded that cross-fertilization is essential for high yield and improved mass of each berry. As raspberry cultivars are clonally propagated, fertilization between different plants of the same cultivar is in fact self-fertilization. This becomes especially important with cultivation under high tunnels for early harvest, where predominantly single cultivars are grown (Żurawicz et al., 2018).

The present study is a preliminary attempt to examine if there are self-incompatible raspberry cultivars that could pose an issue for growers in a protected environment. We used molecular markers to prescreen 16 raspberry cultivars to ascertain raspberry cultivars with full or partial self-incompatibility. To determine if there is a difference in the extent of self- and cross-fertilization, fruits of these 16 raspberry cultivars grown in an experimental field trial were collected after open pollination. A range of variability was observed between the self-fertilization ratios of cultivars. In an effort to investigate whether the two cultivars on the opposite spectrum of self-fertilization ratio retained their observed differences, their seed set was evaluated after hand-pollination. Finally, a topcross environment was set up in the greenhouse with bumblebees as pollinators to examine if cultivars maintain their low self-fertilization ratio in a restricted pollen environment.

2. Material and methods

2.1. Plant material

Fruits were collected from 16 raspberry cultivars (Table 1) that were open pollinated in an experimental field in Borthen, Germany (lat 50.968778, long 13.826466), where pollinators had access to a wide range of different *Rubus* genotypes. The experimental field was established for cultivar evaluation. Fifty-six raspberry genotypes (cultivars and breeding clones) were grown in a randomized block design consisting of at least two blocks per cultivar with 20 plants per block. The plants were planted 0.3 m from each other initially, with no additional space between blocks and 3 m distance between rows. This field trial was surrounded by a commercial raspberry production field where different standard varieties (mainly 'Tulameen' and 'Glen Ample') were grown. Additionally, the commercial field consisted of a few blocks of different blackberry cultivars and in close distance to the field were hedges where wild raspberries and blackberries were present. The fruits were collected randomly through a cultivar block and from the plant itself and then pooled between the two blocks. Seeds were extracted with a household hand blender and dried over calcium chloride. 200 seeds per cultivar were scarified and stratified according to the protocol of Jennings and Tulloch (1965). After the appearance of the first leaves,

20 seedlings per cultivar were selected randomly from the germination tray. Additional 20 seedlings were selected for the cultivars 'Dorman Red' and 'Rumla', making it a total of 360 progenies. Leaf samples of each plant were sampled for DNA fingerprint analysis. Leaf samples of plants grown at the German Federal Plant Variety Office (Wurzen, Germany), which are known to be true-to-type, were used as positive controls for the female parents. These plants were chosen for positive control as a concurrent test of the mother cultivar plants for trueness-to-type.

2.2. Seed set experiment in the greenhouse

'Dorman Red' and 'Rumla' plants grown in 25 l pots were placed in the greenhouse. Their flower buds were emasculated by removing the anthers using a scalpel before flowers opened. The pollen from these anthers was harvested. Two days after emasculation, open flowers were hand pollinated with pollen of their own or the cultivar 'Tulameen'. Pollinations were repeated until carpels turned brown. Drupelets were counted on harvested ripe fruit.

2.3. Topcross experiment

One plant each of the cultivars 'Lucana', 'Preußen' and 'Rumla', growing in 25 l pots, were placed at 1 m distance from each other in the greenhouse pre-bloom with a commercial box of bumblebees (*Bombus terrestris* L.) mini hive for 100 m^2 for cross-pollination (Katz Biotech AG, Baruth, Germany). Fruits were harvested 33 days after start of bloom. Seeds were treated as described above to germinate progenies. Leaf samples of 20 randomly selected seedlings per cultivar were collected for DNA analysis.

2.4. DNA isolation and SSR marker analysis

DNA was extracted from 0.05 g of young leaf materials using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. For marker analysis, 18 simple sequence repeat (SSR) markers (Castillo et al., 2010; Graham et al., 2002, 2004) were used in this study. Of these 18 markers, 15 were previously used by Girichev et al. (2015) and Pinczinger et al. (2020a) as well as five by Pinczinger et al. (2020b). The remaining three SSR markers, Rub244a, Rubus2a and Rubus12a were developed by Graham et al. (2004, 2006). To optimize marker information output, multiplexes were arranged individually for the progeny group of every cultivar with markers that were polymorphic for the cultivar in previous experiments by Girichev et al. (2015) and Pinczinger et al. (2020a). Two to four markers were used per multiplex in one PCR reaction (File S1). All samples were visualized and evaluated with the CEQ 8800 Genetic Analysis capillary electrophoresis system (Beckman Coulter, Krefeld, Germany) and the results were analyzed through the CEQ 8800 software of the same supplier. The resulting peaks were assessed visually. Failed reactions were repeated with samples that worked in previous runs as positive controls.

2.5. Data analysis and statistical analysis

Results of the extent of cross-fertilization of the 16 cultivars were presented with the software R (R Core Team, 2017). Statistical significance of the seed set experiment was analyzed by one-way ANOVA followed by a Tukey HSD multiple pairwise-comparison using the software R (R Core Team, 2017). For a descriptive statistical analysis, allele and genotype frequencies as well as Hardy-Weinberg distribution of 38 self-fertilized 'Dorman Red' progenies was investigated with Genepop version 4.7.5 (Raymond and Rousset, 1995; Rousset, 2008). Chi square test was calculated with the following equation:

Table 1

Allele sizes of the 16 raspberry cultivars used as positive controls for the SSR marker analysis of open pollinated progenies obtained with up to 18 SSR markers. Markers with no allele listed were not tested on the cultivar or progeny group in this study, as they delivered monomorphic results in previous studies for the cultivar. Underlined markers did not fit the progenies' allele sizes. gray markers were tested on the female parent, but not used in the evaluation of the progenies because of weak signal strength in samples. The SSR markers were developed by: [1]Castillo et al. (2010), [2]Graham et al. (2004), [3]Graham et al. (2002) and [4]Graham et al. (2006).

	RhM011[1]	RhM043[1]	RiM017[1]	RiM019[1]	RhM021[1]	RhM001[1]	RhM003[1]	RiM036[1]	RiM015[1]	RiG001[1]	Rubus 123a[2]	Rubus 285a[2]	Rubus 223a[3]	Rubus 270a[2]	Rubus 275a[2]	Rubus 2a[2]	Rubus 12a[2]	Rub 244a[4]
Dorman Red	280 284		204 206	172 212	283 295		198 202	306 309	350 356		169 181	181 206	139 145	176 234	134 171			
Elida	283 287	377	195	168 182	281 285	239	202 217		350	349	148 150	172 199	149 152	156 207	176	159 166	143 148	148 170
Glen Ample	287 291	374 377	195	180	281	241	198 202		350	348	148 161	172 175	155	156 164	130 182	157 166	102 148	157
Lucana	289 291	374 377	195	180 182	281	239 241	206 217		350	347	144 148	172 178	154 156	142 180	114 144			
Malling Promise			195	180 184	281	239 241	206 210	314 316	350 353		149 253	180 197	154 170	156 164	130 182			
Meeker	287	377	195	168 180	281	239	202 210	314 316	350 362		147 149	172 196	154 176	156	114 182	146 184	130 143	156
Oktavia	283 287	374 377		172 180	281	239 241	198 202	314 316		348	144 161	174 178	152 154	164 207	122 182			
Preußen	287 289		195	176 184		239 241	198 217		350 359		144 149	172 180	152 154	164 207	148 174	166 182	130 143	147 168
Royalty	287 291	377	192 195	184	281	239 241	210 219	314 316	350	347 350				156 180		164 166	102 125	154 170
Rumla				168 195	281	239 241	198 206			348 350	147	172 213	154	156 164	124 130	159 177	125	150 157
Rutrago			194 195	168 184	281 285	239	200 206	234 316	350		149 245	189 197	147 154	156 207	130	157 162	102 125	148 155
Sanibelle			194 195	195 197	281 285	239	198 206	314 316	350		149 245	188 196	152 154	156 160	130	159 165	102 125	149 155
Schönemann	287 316	377		168 184	281	239 241	202 206			347	144 167	178 190	152 154	156 207	130 174			
Tulameen	283 291	377		168 184	281	239 241	198 217	314 316		347 348	144 149	178 197		156	148 182	165	130 143	147 170
Valentina	282 289	374 377	195 198	180	281 291	239 241	198	314	350	348		172 178	139 152	186 207	114 182			
Willamette			195	168 184			202 210		350 353		148 149	172 197	154 156	156 207	124 182	157 159	143 148	148 156

$$\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where O_i is the number of observations of type i, E_i the expected count of type i and n the number of genotypes.

The SSR fingerprints and familial relationships of the 'Rumla' progenies originating from the topcross experiment were visualized with Pedimap version 1.2 (Voorrips, 2004) and visually enhanced with Inkscape version 0.92 (Inkscape Project, 2017) and Adobe Illustrator 25.0.1 (Adobe Inc., 2020).

3. Results

3.1. Pre-screening to identify genotypes differing in their fertilization propensity

Genotyping the 320 progenies originating from open pollination of 16 different raspberry cultivars (20 progenies per cultivar) resulted in large differences for cross-fertilization ratio between the cultivars. The cross-fertilization ratio varied between 100% obtained for 'Rumla' and 5% obtained for 'Dorman Red'. To confirm these differences, 20 additional seedlings of 'Rumla' and 'Dorman Red' were genotyped. The genotyping results of all 360 progenies are shown in Fig. 1. Even after adding more progenies, 'Dorman Red' had the lowest cross-fertilization ratio with all but two progenies out of 40 (5%) resulting from self-fertilization. 'Rumla' retained its high cross-fertilization ratio as well with all 40 progenies resulting from cross-fertilization (100%). There were 213 out of 360 progeny classified as resulting from cross-fertilization. These progenies had between 1 and 10 marker alleles originating from their paternal parent, on average 4.11 paternal alleles per plant. Although the marker allele patterns were analyzed for possible apomicts, no apomictic seedling was found among any of the 360 progenies. A plant was determined to be an apomictic candidate if

all its marker alleles matched its maternal parent. The self-fertilized progeny had between one and nine alleles missing compared to their maternal parent, representing 4.9 alleles per progeny on average.

3.2. Association between seed set and pollen parent

The highly differing cross-fertilization propensity of 'Dorman Red' and 'Rumla' was further analyzed by hand pollination in a controlled environment with their own pollen and pollen of the cultivar 'Tulameen'. Plants of each cultivar ('Dorman Red' and 'Rumla') were grown in the greenhouse. The flowers opened incrementally on the mother plants, accumulating to 40 flowers on 'Dorman Red' and 164 flowers on 'Rumla'. Hand pollination was done with the aim to obtain an equal number of self- and cross-pollinated flowers for each cultivar. The seed set was determined by counting the number of drupelets per fruit. The seed set of the fruit among all progeny groups was significantly different following ANOVA (Table 2). After performing a post-hoc Tukey

Table 2
Seed set of two hand pollinated raspberry cultivars. The first cultivar name denotes the female parent, whereas the second cultivar name denotes the male parent. The arithmetic mean was calculated from single drupelets on n number of hand-pollinated flowers. The software R was used to calculate the one-way ANOVA.

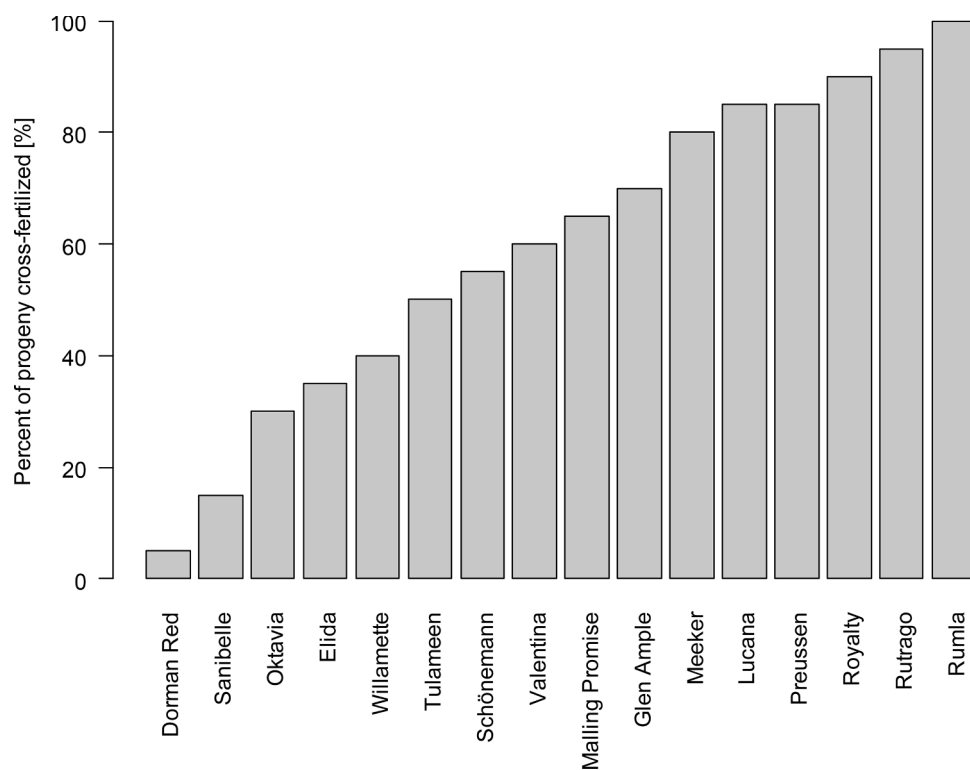|	'Dorman Red' x 'Dorman Red'	'Dorman Red' x 'Tulameen'	'Rumla' x 'Rumla'	'Rumla' x 'Tulameen'
Drupelets per fruit, mean	32.11	25	10.95	76.45
N	19	21	98	66
Standard deviation	10.08	9.08	15.84	23.92
One-way ANOVA		$p < 0.0001$		

Fig. 1. The ratio of cross-fertilized progeny of 16 open pollinated raspberry cultivars identified by SSR fingerprinting. The percentage was calculated from 20 progenies per cultivar except for 'Dorman Red' and 'Rumla'. For these two cultivars, 40 progenies each were genotyped. Progenies with marker alleles exclusively from its female parent were classified as self-fertilized.

HSD multiple-comparison, the seed set of 'Rumla' was significantly higher in cross-pollinated than in self-pollinated fruit ($p < 0.01$), confirming the results from the field experiment that the cultivar 'Rumla' is self-incompatible. Such cultivars are only suitable for protected cultivation to a limited extent, as suitable pollinator cultivars have to be planted as well. In contrast, differences between the seed set of 'Dorman Red' fruit was not significant (Table 2). This shows that the cultivar 'Dorman Red' accepts foreign pollen but can also be self-pollinated in shortage situations. Such varieties are very suitable for protected cultivation. No seed set or fruit development was obtained for 18 flowers of 'Rumla', of which 16 flowers were self-pollinated and two flowers were cross-pollinated. No failure in seed set and fruit development was found for 'Dorman Red' irrespective of the pollen source.

3.3. Heterozygote deficiencies calculated from genotype frequencies of self-fertilized progenies

Hardy-Weinberg equilibrium (HWE) test was used to assess allele frequencies of the 11 polymorphic SSR markers in the 38 'Dorman Red' progenies originating from self-fertilization in the field. Two markers, RiM017 and RhM021, showed a statistically significant deviation from HWE, with both markers showing heterozygote deficiency (Table 3). However, the remaining markers fit HWE. When allele distribution fits the Hardy-Weinberg equilibrium, an undisturbed inheritance of the alleles is assumed, as was the case for most of the markers used.

3.4. Verifying the fertilization behavior of selected genotypes in a topcross environment

The cultivars 'Lucana', 'Preußen' and 'Rumla' were chosen for the topcross experiment since all three showed high cross-fertilization rate in the open pollination environment. However, their cross-fertilization rate in the artificial topcross environment only partly corresponded to the results obtained after open pollination. All 'Rumla' progenies were cross-fertilized. This is consistent with the results obtained for progenies originating from open pollination. Based on the SSR marker data, 19 out of the 20 'Rumla' topcross progenies matched to the SSR marker data of 'Preußen' and one to 'Lucana' as the male parent (Fig. 2).

In contrast, 'Lucana' and 'Preußen', which both had 85% cross-fertilized progenies after open pollination, had no progenies resulting from cross-fertilization in the topcross environment. It is unclear whether the lack of cross-pollination of these two varieties is due to preferences of the bumblebees or other reasons. However, it is certain that the cultivars 'Preußen' and 'Lucana' are not self-incompatible.

4. Discussion

There is little known about the specifics of self-incompatibility (SI) in *Rubus idaeus* cultivars. However, it is known that some cultivars are able to self-fertilize and seed set is often improved by cross-pollination

(Keep, 1968; Żurawicz, 2016). The results of this study provide evidence for the existence of a mechanism for self-incompatibility in red raspberry. At least the cultivar 'Rumla' was found to be self-incompatible to a large extent. The self-incompatibility of this cultivar was demonstrated in three independent experiments with (i) seedlings obtained after open pollination in the open field, (ii) seed set obtserved after hand pollination in the greenhouse, and (iii) seedlings derived from a topcross experiment. The other 15 cultivars used in this study showed a variance in their self-fertilization ratios after open pollination in the field. The causes for this variance can be genetic or purely coincidental. The approach used for pre-screening, especially the limited number of seedlings tested, is not suitable for making statistically significant statements about fertilization behavior of different cultivars. However, this pre-screening was shown to be suitable to identify genotypes that may differ strongly in this trait. The existence of self-incompatibility in red raspberry is important, as a sufficient amount of self-fertility is necessary in modern raspberry production, where protected growing limits possible pollen sources and thus requires planning for cultivar compatibility.

Reports about the self-fertility of raspberry cultivars differ, with Keep (1968) seeing it as a rule, whereas Daubeny (1971) found a lack of full self-fertility among cultivar groups from certain origins. There is quite a few evidence of gametophytic self-incompatibility mechanism in raspberry consisting of a pollen S-gene and a stylar S-RNase (Franklin-Tong and Franklin, 2003; Keep, 1968). Studies on the inheritance of self-compatibility posited that 'Lloyd George', a main founder for many cultivars and ancestor of 87% of all European and American raspberry cultivars (Dale et al., 1993), is heterozygous for a mutated pollen S-allele (Keep, 1968; Lewis, 1940). It is therefore plausible that this mutated S-allele is widespread in raspberry cultivars.

Our findings reinforce the results of Żurawicz et al. (2018) on self-fertilization. The authors reported a mean number of seeds per fruit in self-pollinations of 19.43 (39%), 26.40 (52%) and 33.70 (57%) for 'Glen Ample', 'Schönemann' and 'Willamette', respectively. The ratio of self-fertilized progeny of these cultivars in our study was 30%, 45% and 60%, respectively. Although the percentages do not match perfectly, the order of cultivars remains the same. Since the number of progenies tested was comparably low and the seeds were retrieved from open pollination in the field, other factors (pollinator behavior, weather conditions and differences in flowering time) could also have influenced the amount of self- and cross-fertilization. In order to minimize these factors, a follow-up hand-pollination and topcross experiment was done on selected cultivars.

In all three pollination experiments, the cultivar 'Rumla' consistently showed a significant impediment in self-fertilization and preference to cross-fertilization. Although there were some fertilized drupelets in the seed set experiment, pseudo-self-compatibility has been proposed to be the cause of seed set in self-incompatible pairings (Keep, 1968). Surprisingly, only one out of 20 'Rumla' progeny had 'Lucana' as the male parent in the topcross experiment. Further work would be needed to

Table 3
Allele and genotype frequencies, Hardy-Weinberg distribution and chi square test of 38 'Dorman Red' progenies originating from self-fertilization in the open field.* significant effect, Hardy-Weinberg frequency does not fit at 0.05 significance level for 1 df if chi square test is over 3.84.

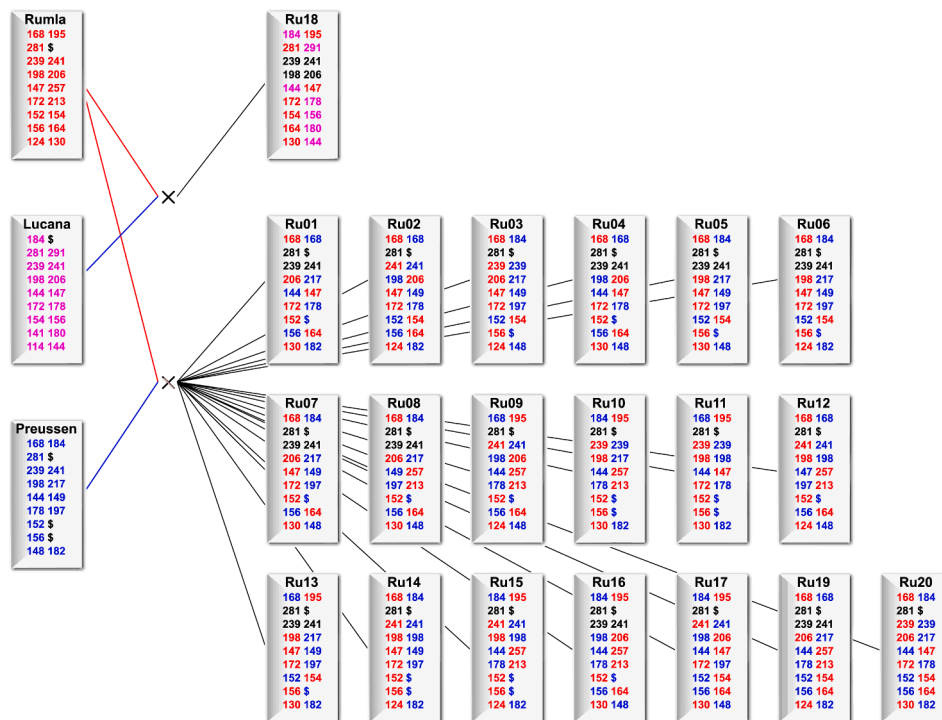| Marker | Allele frequency | | Genotype frequency | | | | | | Hardy-Weinberg distribution | X^2 test |
	A	B	AA observed	AA expected	AB observed	AB expected	BB observed	BB expected		
RhM011	0.645	0.355	18	15.680	13	17.640	7	4.680	0.154	2.7138
RiM017	0.408	0.592	13	6.200	5	18.600	20	13.200	0.000	20.9052*
RiM019	0.592	0.408	14	13.200	17	18.600	7	6.200	0.738	0.2893
RhM021	0.776	0.224	28	22.813	3	13.373	7	1.813	0.000	24.0614*
RhM003	0.474	0.526	7	8.400	22	19.200	9	10.400	0.514	0.8301
RiM015	0.461	0.540	6	7.933	23	19.133	9	10.933	0.325	1.5944
Rubus123a	0.500	0.500	9	9.123	19	18.753	9	9.123	1.000	0.0066
Rubus285a	0.500	0.500	9	9.373	20	19.253	9	9.373	1.000	0.0587
Rubus223a	0.697	0,.303	17	18.373	19	16.253	2	3.373	0.443	1.1259
Rubus270a	0.461	0.540	6	7.933	23	19.133	9	10.933	0.325	1.5944
Rubus275a	0.526	0.474	11	10.400	18	19.200	9	8.400	0.752	0.1525

Rumla
168 195
281 $
239 241
198 206
147 257
172 213
152 154
156 164
124 130

Ru18
184 184
281 291
239 241
198 206
144 147
172 178
154 156
164 180
130 144

Lucana
184 $
281 291
239 241
198 206
144 147
172 178
154 156
141 180
114 144

Preussen
168 184
281 $
239 241
198 217
144 149
178 197
152 $
156 $
148 182

Ru01
168 168
281 $
239 241
206 217
144 147
172 178
152 $
156 164
130 182

Ru02
168 168
281 $
241 241
198 206
147 149
172 178
152 154
156 164
124 182

Ru03
168 184
281 $
239 239
206 217
147 149
172 197
152 154
156 $
124 148

Ru04
168 168
281 $
239 241
198 206
144 147
172 178
152 $
156 164
130 148

Ru05
168 184
281 $
239 241
198 217
147 149
172 197
152 154
156 $
130 148

Ru06
168 184
281 $
239 241
198 217
147 149
172 197
152 154
156 $
124 182

Ru07
168 184
281 $
239 241
206 217
147 149
172 197
152 154
156 164
130 148

Ru08
168 184
281 $
239 241
206 217
149 257
197 213
152 $
156 164
130 148

Ru09
168 195
281 $
241 241
198 206
144 257
178 213
152 $
156 $
124 148

Ru10
184 195
281 $
239 239
198 217
144 257
178 213
152 $
156 $
130 182

Ru11
168 195
281 $
239 239
198 198
144 147
172 213
152 $
156 $
130 182

Ru12
168 168
281 $
241 241
198 198
147 257
197 213
152 $
156 164
124 148

Ru13
168 195
281 $
239 241
198 217
147 149
172 197
152 154
156 $
130 182

Ru14
168 184
281 $
241 241
198 198
147 149
172 197
152 $
156 $
124 182

Ru15
184 195
281 $
241 241
198 198
144 257
178 213
152 $
156 $
124 182

Ru16
184 195
281 $
239 241
198 206
144 257
178 213
152 $
156 164
130 148

Ru17
184 195
281 $
241 241
198 206
144 257
172 197
152 154
156 164
130 148

Ru19
168 168
281 $
239 241
206 217
144 147
178 213
152 154
156 164
124 182

Ru20
168 184
281 $
239 239
206 217
144 147
172 178
152 154
156 164
130 182

Fig. 2. SSR fingerprints of the 'Rumla' progenies originating from the topcross experiment. The alleles of the progenies are indicated with different colors according to the parent the progeny originates from. The alleles inherited from the female parent, 'Rumla', are depicted in red, the alleles from the male parents 'Lucana' and 'Preußen' in pink and blue color, respectively. The alleles where the parental source is undetermined are depicted in black. The '$' symbol indicates an allele combination that can be homozygous or heterozygous with a null allele. The crosses depict a crossing event between parents, where the red lines connect to the female parent, the blue lines to the male parent and the black lines to the progenies.

determine if this is due to the small sample size or a pollen incompatibility between 'Rumla' and 'Lucana'. Hand-pollination experiments with different raspberry cultivars combined with a pollen tube growth analysis could further elucidate the incompatibility of 'Rumla'.

'Lucana' and 'Preußen' did not retain their high cross-fertilizing ratio in the topcross experiment. This suggests that only 'Rumla' has a genetic cause for its high cross-fertilizing ratio. The other two cultivars can self-fertilize in a pollen-restricted environment. Interestingly, genotyping 'Lucana' and 'Preußen' progeny revealed no cross-fertilized progeny at all. There can be various reasons for this, including cultivar-specific incompatibility, random occurrence of this distribution due to an insufficient sample size, or cleistogamy.

Results of the seed set experiment suggest that 'Dorman Red' has no significant preference to self-fertilization. 'Dorman Red' has a floral morphology conducive for self-pollination, as it has small flowers half the size of other raspberry cultivars with petals curved inwards. The petals close off the flower to foreign pollen from pollinators for days while containing ripe pollen. This could explain why all but two out of 40 of 'Dorman Red's progenies were self-fertilized.

During marker analysis, a progeny sample was identified as cross-fertilized if one of their two alleles was different from their female parent cultivar's in at least one marker. However, there were samples with discrepancies in both alleles. As results stayed the same in replications, technical error can be excluded. There were two types of these unexpected marker results, the first where the marker alleles of the whole progeny group do not fit both of the female parent cultivar's. This occurred with five markers in 'Preußen' and 'Lucana' (Table 1). As the samples used for positive controls were taken from the Federal Plant Variety Office in Wurzen and not from the original field the fruit was collected from, a cultivar mix-up, spontaneous mutation or somaclonal variation could have occurred with the maternal parent in the field. This is a documented occurrence in raspberry, with several publications reporting that commercially sold cultivars are not true-to-type (Bassil et al., 2012; Pinczinger et al., 2020a). Furthermore, there are raspberry cultivars with multiple well-known types, like 'Schönemann' with types 'Kraege', 'Meyer' and 'Penkhues', which originated from different propagators.

The second marker scenario we observed involved one individual progeny not having alleles of the female parent cultivar. This was the case in seven progenies; one in 'Dorman Red', two in 'Rumla', one in 'Rutrago', one in 'Tulameen' and two in 'Willamette'. This type of discrepancy could be explained with a spontaneous mutation, which is common in raspberry, with an occurrence of visible mutation of 0,05% in a planted field reported by Janick (2009). As SSR markers are in untranslated regions of tandem repeats, slippage is easier to occur than in translated regions, causing a size difference in the marker (Kalia et al., 2011). The seven marker discrepancies we report here represent 0.129% of the 5440 marker alleles evaluated. This can still fall under the species-specific high mutation rate if the properties of SSR markers are considered. Nevertheless, the presence of these mutation discrepancies should be noted for further studies on raspberry progeny pools.

As more of raspberry production is moving into protected growing in Europe, the tolerance for self-fertilization of individual cultivars becomes an economically relevant question. According to the findings of this study, there are raspberry cultivars with higher self-compatibility, which could be used as mono-cultivars in protected growing environments. If cultivars with lower self-compatibility were chosen based on other traits, a mix of cultivars would be advised to guarantee better seed set.

Availability of data and material: File S1: table of the different multiplex PCRs with their individual SSR marker combinations used for each 16 cultivar progeny groups. Any other specific data not in the article is available from the corresponding author on reasonable request.

Author contribution statement

HF, M-VH and MvR contributed to the study conception and design. MvR collected plant material. Material preparation, data collection and analysis were performed by DP. The first draft of the manuscript was written by DP and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2021.110384.

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