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Germination of red raspberry seeds as affected by origin and chemical scarification

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Abstract

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In 2014, this research was conducted at the Research Institute of Horticulture in Skierniewice, Poland. It was based on different red raspberry seed treatments with sulfuric acid during the scarification process. The seeds were obtained from crosses among ten parental forms, producing 55 hybrid families. During scarification, the seeds were subjected to concentrated sulfuric acid for 20, 30 and 40 minutes. Assessment of the germinated seeds, performed 2.5 months after the sowing, revealed different effects of the seed origin/pedigree and the applied scarification treatments. The best seed germination was recorded for the hybrid families where 'Radziejowa' (56.5% of germinated seeds), 'Laszka' (63.9%) and 'Sokolica' (73.5%) were the maternal forms, and the poorest one – where 'Polana' (6.2% of germinated seeds), 'Glen Ample' (32.5%) and 'Canby' (33.1%) were used as the maternal cultivars. The highest germination, on average for all the hybrid families, was obtained for seeds treated with H_2SO_4 for 30 min (45.3% of germinated seeds), and the lowest when the seeds were treated with H_2SO_4 for 40 min (35.6%).

Keywords: cultivars; seed scarification; mating design; cross-pollination; self-pollination

One of the problems in red raspberry breeding is the very slow and uneven germination of seeds and the small number of seedlings obtained. The reason is the very deep seed dormancy, determined by many mechanical, physical, biochemical and physiological factors, such as the thick seed coat, impermeable to air and water, mechanical resistance of the seed coat to the swelling of the embryo, and the requirement of the embryo of certain physiological changes that occur under proper conditions of air moisture and temperature (Ourecky 1975). The obstacles preventing germination of seeds due to the inability of oxygen and water to have access to the embryo can be reduced or eliminated by removing or mechanically damaging the seed coat. The method most often used for this purpose is treatment with concentrated sulfuric acid (Scot, Ink 1957; Jennings, Tulloch 1965; Dale, Jarvis 1983; Wada, Reed 2011a,b; Grzesik et al. 2015). Scoring the seed coat (the endocarp, testa, endosperm) or rubbing seeds with sandpaper can also be used (Nesme 1985; Grzesik et al. 2015). The biochemical and physiological obstacles to seed germination, on the other hand, disappear as a result of biochemical and biophysical processes that occur after-ripening at 0–5°C, called stratification (Wada, Reed 2011a,b; Hołubowicz, Hołubowicz 2015). Therefore, to obtain good germination of red raspberry seeds, both scarification and stratification are used (Jennings, Tulloch 1965; Dale, Jarvis 1983; Daubeny 1986; Jennings 1988; Wada, Reed 2011a,b). The problem with raspberry seed germination was

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also raised by Clark et al. (2007). These authors reported that in the breeding of plants of the species of the genus *Rubus* (red raspberry and blackberry), the major problem was not only the need for scarification and stratification of seeds, but also the varied response of seeds of the different species to the seed treatment procedure used before sowing them. This thesis was also confirmed by subsequent studies by Wada and Reed (2011a,b). It should be added, however, that Jennings (1971, 1988) had already pointed out much earlier that in red raspberry both the seed set and seed germination were influenced by the seed parent. This means that there is no universal procedure for obtaining good germination of red raspberry seeds derived from different combinations of crosses. In fact, it should be expected that the results for the germination of seeds from various crossing (pollination) combinations of cultivated varieties will vary considerably, even if the seeds are subjected to scarification and stratification.

The aim of the study was to evaluate the effects of various combinations of crosses among ten commercially grown raspberry varieties and of the duration of exposure of the obtained seeds to chemical scarification with concentrated sulfuric acid on the speed and uniformity of germination.

MATERIAL AND METHODS

Experimental material. The study was conducted in 2014 in the Fruit Plant Breeding Department (now Department of Horticultural Crop Breeding) of the Research Institute of Horticulture in Skierniewice, Poland. The experimental material consisted of the seeds of red raspberry obtained by controlled cross-pollination and self-pollination of 10 cultivars of red raspberry. The cultivars come from different geographical regions of the world and differ in terms of several genetically determined biological characteristics, including ripening time and fruit quality, and the susceptibility of the plants and fruit to the dangerous *Raspberry Bushy Dwarf Virus* (RBDV) that causes bushy dwarfism in raspberry. A list of the cultivars used in the pollination programme, their brief description and the method of controlled pollination are given in Tables 1 and 2. Cuttings of all the cultivars (well-rooted shoots, headed to a height of 50–60 cm), 50 of each variety, were purchased in the autumn of 2013 from a certified nursery. Until the time of planting, the plants were kept in cold storage, where the humidity was 95% and the air temperature +2°C. To extend the flowering period and ensure the ability to carry out all the pollination combinations, the plants were planted on two dates (i.e. in two series) – January 27 and February 18, 2014, with 25 plants of each combination transplanted on each of these dates into 3.8 l pots. The growing medium was a mixture of peat substrate, compost soil and washed sand at a 1:1:1 ratio (by volume). After planting, the potted plants were placed on a windowsill in a heated greenhouse at a temperature of 20–22°C during daytime and 14–16°C at night, using artificial lighting to ensure a 16-hour day. No protective measures against diseases or pests were used, but sticky traps were arranged densely throughout the greenhouse to capture flying *Diptera* and other insects.

To make sure that the mother plants were free from serious viral diseases that could affect the germination of seeds, all of them were tested by DAS-ELISA (Clark, Adams 1977) for the presence of the *Raspberry Bushy Dwarf Virus* (RBDV). Tests for the *Raspberry leaf mottle virus*, the *Raspberry vein chlorosis virus* and the *Raspberry leaf blotch virus* were carried out for only 10 mother plants randomly selected from a pool of 50 plants of each of the 10 genotypes, using the method of reverse transcription-polymerase chain reaction (McGavin et al. 2011, 2012).

Pollination and seed acquisition. To implement the pollination programme, the plants were grown until they had reached the stage of well-developed shoots with inflorescences and floral buds developing on them. For pollination, fully developed flower buds at the closed white bud stage were used. Buds from the first, earliest developing inflorescences were removed to obtain anthers with pollen from them for the pollinations. The collected anthers were dried at room temperature for 24 h; viability of the collected pollen was not tested. The preparation of flowers for pollination consisted in emasculating fully developed but closed buds by removing the sepals and the stamens. Pollination of the emasculated buds was performed once, immediately after emasculation, according to the scheme given in Table 2 (Griffing's method II). The pollen was applied onto the stigma with a finger. After the completion of pollination, the plants were covered with insulators. Within each pollination combination, about 20 of the best developed flower buds on the mother plants were pollinated.

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Table 1. List and short description of the cultivars used in the pollination program (Skierniewice, 2014)

Cultivar name	Country of origin	Pedigree	Ripening time	Productivity	Fruit size	Fruit color	Susceptibility to RBDV	Shoot thorns***
Canby*	USA	Viking × Lloyd Georg	fairly early	high	medium	vivid red	susceptible	+
Glen Ample*	UK	SCRI 7326E1 × SCRI 7412H16	medium early	high	large	bright red	low susceptible	–
Laszka*	POL	80408 × 80192	early	high	very large	bright red	susceptible	+
Polana**	POL	Heritage × Zeva Herbsternte	middle of August	fairly high	medium	vivid red with high gloss	low susceptible	+
Polka**	POL	Autumn Bliss + Lloyd George + *R. crataeifolius*	end of July	high	medium and large	vivid red with high gloss	very susceptible	+
Radziejowa*	POL	92271 × 96221	early (2nd half of June)	fairly high	large	vivid red	susceptible	+
Schönemann*	GER	Lloyd George × Preussen	very late	high	very large	dark red	low susceptible	+
Sokolica*	POL	96131 × 96221	medium early	high	large	bright red	susceptible	+
Veten*	NOR	Asker × Lloyd George	early	medium to high	medium and large	red and dark red	low susceptible	+
Willamette*	USA	Lloyd George × Newburgh	late	high	large	vivid red	tolerant	+

*floricane cultivar; **primocane cultivar; RBDV – *Raspberry Bushy Dwarf Virus*; ***Shoot thorns – (+) present, (–) absent

The fruits were harvested successively as they reached full maturity (developed red colour), and counted. The harvesting of fruit lasted from April 24 to May 28, 2014. To extract the seeds, the fruits were wrapped in densely woven gauze and crushed, the pulp was washed out with running water, and then, on the same gauze, the seeds were dried at room temperature for 24 hours. After drying and counting, the seeds were packed into paper bags (letter envelopes) and stored as such at room temperature until scarification. Scarification began on June 3, 2014.

Scarification and stratification of seeds. The scarifying agent used was concentrated sulfuric acid (95% H_2SO_4), having the ability to damage severely the very thick and hard seed coat of drupes (raspberry seeds). Scarification was performed in three ways (three experimental combinations), with different duration of seed treatment with concen-

Table 2. Scheme for the pollination of parental forms of red raspberry (Griffing's Method II) (Skierniewice, 2014)

♂ ♀	Canby	Glen Ample	Laszka	Polana	Polka	Radziejowa	Schönemann	Sokolica	Veten	Willamette
Canby	xx	x	x	x	x	x	x	x	x	x
Glen Ample		xx	x	x	x	x	x	x	x	x
Laszka			xx	x	x	x	x	x	x	x
Polana				xx	x	x	x	x	x	x
Polka					xx	x	x	x	x	x
Radziejowa						xx	x	x	x	x
Schönemann							xx	x	x	x
Sokolica								xx	x	x
Veten									xx	x
Willamette										xx

♀ – maternal form; ♂ – paternal form; x – controlled crossing; xx – self-pollination

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trated sulfuric acid: (1) Seed treatment for 20 min, (2) Seed treatment for 30 min, (3) Seed treatment for 40 minutes. The scarification procedure involved the following successive steps: (1) Placing of test tubes with seeds in a mixture of ice and water, then pouring concentrated sulfuric acid over the seeds and keeping them fully submerged for 20, 30 or 40 min, mixing the contents every 3–5 min; (2) Pouring out the sulfuric acid and rinsing the seeds under running water for 10 min, surface-drying the seeds on a paper towel and then immersing them in an aqueous solution of $NaHCO_3$ (1 teaspoon $NaHCO_3$ per 1 l of water); (3) Pouring over the seeds 25 ml of 1% solution of calcium hypochlorite ($Ca(OCl_2)_2$) with the addition of 5 ml calcium hydroxide ($Ca(OH)_2$), changing the bath 3 times every 2 days; (4) After pouring out the solution, washing of the seeds in running water for 10–15 min, surface-drying on a paper towel, then immersing them in a solution of Captan (0.2%), followed by drying on paper at room temperature for 12 hours. Afterwards, on June 10, 2014, the seeds were subjected to stratification. The stratification involved mixing the seeds with disinfected peat and placing the mixture in plastic bags in an incubator at 3°C for 50 days. Each experimental combination consisted of 250 seeds; altogether, 41,250 seeds were subjected to scarification and stratification (55 combinations of mother plant pollination × 3 durations of seed scarification × 250 seeds).

Seed germination. The stratification treatment ended on July 30, 2014, and the seeds were sown into 3.8 litre plastic pots filled with a mixture of peat substrate and sand at a 3:1 ratio (by volume) and covered with a thin layer of sterile sand. The seeded pots were placed on a windowsill in a greenhouse at a temperature of about 20–22°C by day and about 16–18°C at night. To assess the growth dynamics and uniformity of seed germination within each experimental combination, the germinated seeds were counted every 7 days, starting 18 days after sowing (i.e. after 18, 25, 32, 39, 46, 53, 60, 67 and 75 days after sowing). The seeds considered as germinated were little seedlings with two small cotyledons and a rudimentary juvenile leaf.

RESULTS

The maternal and paternal plants used for pollination in our study showed no evidence of the presence of any of the viruses they were tested for; thus viral diseases had no negative effect on the setting of fruit by the pollinated flowers and on the germination of the seeds produced. It was found, however, that despite subjecting all the seeds to different durations of scarification, and also to 50-day cold stratification, the seeds germinated very slowly and unevenly after sowing. Regardless of the duration of scarification, when the first observation was made 18 days after sowing, only single seeds were found to have germinated. The impact of parental genotypes, especially of the maternal variety, was clearly evident. In this respect, positively notable were especially the seeds obtained from those pollination combinations in which the cultivars 'Laszka', 'Radziejowa' and 'Sokolica' served as the maternal form. In this group, many seeds had already germinated while the seeds from the other combinations of crosses had not germinated yet. The dynamics of seed emergence increased within all the crossing combinations with the passage of time after sowing (detailed results of these findings are not presented in this paper).

The results for the number of germinated seeds for all the hybrid families after 75 days from sowing, i.e. on the last day of observations, are shown in Table 3 as a percentage of the number of seeds sown. As can be seen, after a 2.5-month period from sowing, only 40.1% of the seeds had germinated, on average for all the combinations of crosses and scarification durations. The lowest number of seeds, only 35.6%, germinated after they had been scarified with concentrated sulfuric acid for 40 min, and the highest – 45.3%, after they had been scarified with the acid for 30 minutes. Therefore, the number of germinated seeds depended much more on the pedigree of the seeds than on the method of scarification, i.e. the duration of the treatment with concentrated sulfuric acid. By far the worst at germinating were the seeds obtained from those pollination combinations in which 'Polana' was the maternal form, pollinated with its own pollen and from various paternal forms. In this case, only 6.2% of the seeds sown had germinated, on average for all the families and scarification times. Markedly better at germinating were the seeds obtained from crosses in which the maternal form were the cultivars 'Canby' (an average of 33.1% of germinated seeds for different scarification durations), 'Glen Ample' (32.5%), 'Veten' (35.9%), 'Schönemann' (43.3%) and 'Polka' (43.8%).

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Table 3. Dynamics of red raspberry seed germination (% germinated seeds) depending on the duration of scarification with concentrated H_2SO_4, assessed 75 days after sowing

Crossing combinations (origin of seeds)		Scarification of seeds (duration of treatment with concentrated H_2SO_4)			
		I 20 minutes	II 30 minutes	III 40 minutes	average
		% germinated seeds			
1	Canby × Canby	24.0	27.2	30.0	27.1
2	× Glen Ample	0.0	56.8	21.2	26.0
3	× Laszka	2.0	41.2	31.6	24.9
4	× Polana	0.0	27.6	29.2	18.9
5	× Polka	32.0	26.8	24.4	27.7
6	× Radziejowa	46.0	33.6	26.0	35.2
7	× Schönemann	42.0	54.4	28.8	41.7
8	× Sokolica	68.8	58.8	46.0	57.9
9	× Veten	48.8	50.0	23.2	40.7
10	× Willamette	28.0	30.0	35.2	31.1
	Average for Canby families	29.2	40.6	29.6	33.1
11	Glen Ample × Glen Ample	19.2	29.2	6.4	18.3
12	× Laszka	25.6	30.0	18.8	24.8
13	× Polana	26.8	18.0	16.8	20.5
14	× Polka	35.2	43.2	7.6	28.7
15	× Radziejowa	40.4	20.0	15.6	25.3
16	× Schönemann	44.8	52.4	21.6	39.6
17	× Sokolica	76.4	53.6	31.2	53.7
18	× Veten	13.6	38.4	24.4	25.5
19	× Willamette	49.6	76.4	42.4	56.1
	Average for Glen Ample families	36.8	40.1	20.5	32.5
20	Laszka × Laszka	47.2	46.4	33.6	42.4
21	× Polana	44.8	63.2	50.0	52.7
22	× Polka	50.0	55.6	72.4	59.3
23	× Radziejowa	64.8	87.6	54.4	68.9
24	× Schönemann	79.6	68.4	54.0	67.3
25	× Sokolica	95.2	93.2	74.0	87.5
26	× Veten	68.0	49.2	58.8	58.7
27	× Willamette	68.8	85.6	69.2	74.5
	Average for Laszka families	64.8	68.6	58.3	63.9
28	Polana × Polana	16.8	17.2	10.8	14.9
29	× Polka	12.0	2.8	3.6	6.1
30	× Radziejowa	5.6	2.4	6.0	4.7
31	× Schönemann	9.6	2.8	4.8	5.7
32	× Sokolica	1.6	6.0	12.0	6.5
33	× Veten	3.6	0.0	8.8	4.1
34	× Willamette	2.4	0.0	1.6	1.3
	Average for Polana families	7.4	4.5	6.8	6.2

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Table 3 to be continued

Crossing combinations (origin of seeds)		Scarification of seeds (duration of treatment with concentrated H_2SO_4)			
		I 20 minutes	II 30 minutes	III 40 minutes	average
		% germinated seeds			
35	Polka × Polka	26.8	48.4	6.0	27.1
36	× Radziejowa	45.2	65.2	63.2	57.9
37	× Schönemann	56.8	56.8	50.8	54.8
38	× Sokolica	64.4	70.4	66.4	67.1
39	× Veten	23.6	34.4	42.8	33.6
40	× Willamette	5.6	44.8	16.0	22.1
	Average for Polka families	37.1	53.3	40.9	43.8
41	Radziejowa × Radziejowa	69.6	27.6	22.0	39.7
42	× Schönemann	54.4	55.2	47.2	52.3
43	× Sokolica	48.4	64.0	60.8	57.7
44	× Veten	71.6	71.6	58.4	67.2
45	× Willamette	59.2	70.8	66.4	65.5
	Average for Radziejowa families	60.6	57.8	51.0	56.5
46	Schönemann × Schönemann	51.2	25.6	26.0	34.3
47	× Sokolica	68.4	89.6	76.8	78.3
48	× Veten	30.0	59.6	20.8	36.8
49	× Willamette	0.0	54.4	17.2	23.9
	Average for Schönemann families	37.4	57.3	35.2	43.3
50	Sokolica × Sokolica	72.4	71.6	66.0	70.0
51	× Veten	64.8	66.8	66.4	66.0
52	× Willamette	80.8	88.8	84.0	84.5
	Average for Sokolica families	72.6	75.7	72.0	73.5
53	Veten × Veten	8.0	10.0	24.4	14.1
54	× Willamette	59.2	49.6	64.4	57.7
	Average for Veten families	33.6	29.8	44.4	35.9
55	Willamette × Willamette	15.2	16.0	16.4	15.9
	Average for Willamette families	15.2	16.0	16.4	15.9
Average for all families		39.4	45.3	35.6	40.1

Decidedly better at germinating were those seeds that came from the pollination combinations in which the maternal forms were the cultivars 'Radziejowa' (an average of 56.5% of germinated seeds for all the combinations of crosses and scarification durations), 'Laszka' (63.9%) and 'Sokolica' (73.5%). In the case of these combinations of crosses, a much smaller impact on the germination of red raspberry seeds was exerted by the paternal forms, except the cultivar 'Sokolica'. The seeds from the pollination combinations in which the paternal form was 'Sokolica' germinated very well, and the percentage of germinated seeds, on average for the three scarification durations, was 53.7% for the pollination combinations 'Glen Ample' × 'Sokolica', 57.7% for 'Radziejowa' × 'Sokolica', 57.9% for 'Canby' × 'Sokolica', 67.1% for 'Polka' × 'Sokolica', 78.3% for 'Schönemann' × 'Sokolica', and as much as 87.5% for the pollination combination 'Laszka' × 'Sokolica'. It should be added that the seeds obtained by self-pollination of the cultivar 'Sokolica' also germinated very well, best of all the self-pollinations of maternal forms. In this case, as much as 70% of seeds had germinated, on average

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for all the scarification durations. Compared with the germination of seeds obtained by self-pollination of the other maternal varieties, this result was at least twice as high.

DISCUSSION

In this study, the seeds of red raspberry were subjected to both scarification and stratification, and yet the germination of these seeds was slow and uneven. After 2.5 months from sowing, less than 50% of the seeds sown had emerged, on average for all the combinations of crosses and methods of post-harvest treatment of seeds. The pollinated cultivars were free from the tested viruses, thus viral diseases had no negative effect on the setting of fruit by the pollinated flowers and on the germination of the seeds produced. The results, therefore, show that the number of germinated seeds was dependent to a greater extent on the pedigree of the seeds than on how they had been scarified, i.e. how long they had been treated with concentrated sulfuric acid. Poor germination of seeds is, however, a problem known to red raspberry growers. The issue had been pointed out earlier by Scott and Ink (1957), Jennings and Tulloch (1965), Jennings (1971), Dale and Jarvis (1983), Daubeny (1986) and Jennings (1988). The latter author also found that the setting of fruit and the germination of seeds in red raspberry were both affected by parental genotypes. This is fully confirmed by the results of this study, which also indicate that the dynamics of red raspberry seed germination depends on the combination of the crossed parental forms. However, although Jennings and Tulloch (1965) recommend that the scarification of raspberry seeds in concentrated sulfuric acid be carried out for 20 min, in our study we obtained considerably better germination when the seeds had been treated with sulfuric acid for a period of 30 minutes. The shorter (20 min) treatment of seeds with this acid contributed to obtaining a number of germinated seeds smaller by 13.0%, whereas after the longer (40 min) treatment of seeds with concentrated sulfuric acid, 21.4% fewer seeds had germinated. It can be thus assumed that with the shorter treatment of seeds with concentrated sulfuric acid the seed coat had not been damaged to a degree sufficient for good germination. On the other hand, during the longer treatment of seeds with this acid it was not only the seed coat that got damaged, but probably also the seed embryo.

Among the tested red raspberry seeds, the worst at germinating were definitely the seeds derived from the maternal cultivar 'Polana', regardless of the paternal variety used to pollinate its flowers (from 0% to 12% of seeds germinated) and of the duration of scarification with concentrated sulfuric acid. This is likely to indicate that the cause of poor germination of these seeds was not only the thickness of the seed coat, but also other mechanisms, such as the presence of more germination inhibitors, or incomplete development of embryos. Perhaps different conditions of scarification and stratification of the seeds of this cultivar would have improved their ability to germinate. 'Polana' is a valuable red raspberry variety in Poland, fruiting in the second half of summer until autumn. Under Polish conditions, it is a productive variety, resistant to low sub-zero temperatures, and tolerant to the *Raspberry Bushy Dwarf Virus* (Danek 1991). However, due to the low germination capacity of its seeds, in the breeding of new varieties, 'Polana' should be used primarily as a paternal and not a maternal form.

CONCLUSION

For good germination, the red raspberry seeds tested in the study require both scarification and stratification.

In terms of the duration of scarification, the best at germinating are those red raspberry seeds that have been treated with concentrated sulfuric acid for 30 minutes.

Germination capacity of red raspberry seeds depends to a greater extent on their pedigree (the crossed parental forms) than on the duration of the scarification treatment.

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