

SEED GERMINATION OF RASPBERRY (*Rubus idaeus* L.) DEPENDING ON THE AGE OF SEEDS AND HYBRIDIZATION PARTNERS

Short communication

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ABSTRACT

Research was carried out on the germination of seeds obtained from crossings between the cultivars of red raspberry (*Rubus idaeus* L.) ‘Sokolica’, ‘Willamette’, ‘Veten’, ‘Canby’ and ‘Glen Ample’ depending on the age of the seeds. The crossings were performed in the winter–spring of 2015–2018 in a heated greenhouse. The obtained seeds were stored dry in paper bags at a temperature of 4–5°C. In January 2019, seeds were scarified and then cold stratified for 50 days and seeded in pots in a greenhouse. The seedling emergence was assessed 60 days after sowing. The seeds produced in 2015 had the lowest germination percentage (14% on average for all combinations of crosses), while the seeds produced in 2018 had a germination rate of 44.9%. Significant differences were also observed in the emergence of seedlings from different combinations. On average, the fewest seedlings were obtained from the combination ‘Glen Ample’ × ‘Willamette’ and the most from ‘Sokolica’ × ‘Willamette’.

Key words: age of seeds, raspberry breeding, seed germination, combinations of crosses

INTRODUCTION

Red raspberry (*Rubus idaeus* L.) belongs to an important group of berry plants. It is cultivated on a large commercial scale in many countries of the world on different continents, both in the open field and under covers, and grown by gardening enthusiasts on allotments and in home gardens. It owes its popularity to the relative ease of its cultivation and the high value of the fruit, which are ideal for fresh consumption and also for preserves and frozen food. There are many cultivars on the market, but new ones are constantly being sought to better meet the needs of fruit growers, consumers, and the processing industry. Breeding is therefore carried out in many countries to obtain new genotypes that will be better than those cultivated now in production terms (Daubeney et al. 1982; Danek 1989, Danek & Pasiut

1991; Stahler et al. 1995; Danek 1999, 2002; Jones & McGavin 2004; Moore & Martin 2008; Muster 2008; Moore & Hoashi-Erhardt 2012; Żurawicz 2016; Żurawicz et al. 2018).

One of the problems in breeding red raspberry is the slow and uneven germination of seeds. The reason is the very deep dormancy of the seeds, which is determined by mechanical, physical, biochemical, and physiological factors, including a thick endocarp. The endocarp is impermeable to air and water, thus affecting the physiological changes of the embryo, which can only occur at a specific humidity and temperature (Ourecky 1975). The obstacles preventing oxygen and water access to the embryo can be reduced or eliminated by inflicting chemical or mechanical damage to the hard seed coat. This is most often achieved by using concentrated sulphuric acid (Scott & Ink 1957;

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Jennings & Tulloch 1965; Dale & Jarvis 1983; Wada & Reed 2011a, b; Grzesik et al. 2015; Żurawicz et al. 2017), scoring the seed coat, or rubbing the seeds with sandpaper (Nesme 1985; Grzesik et al. 2015). In turn, biochemical and physiological barriers to seed germination can be eliminated as a result of biochemical and biophysical processes that occur during postharvest maturation of seeds at 0–5°C, called stratification (Wada & Reed 2011a, b). Therefore, both scarification and cold stratification are used to achieve good germination of red raspberry seeds (Jennings & Tulloch 1965; Dale & Jarvis 1983; Daubeney 1986; Jennings 1988; Daubeney 1996; Clark et al. 2007; Wada & Reed 2011a, b; Żurawicz et al. 2017).

In traditional red raspberry and blackberry breeding, the problem is not only the need for scarification and long, cold seed stratification, but also the different responses to the above procedures of seeds' genotypes, which is determined by their pedigree. Jennings (1971, 1988) stated that in the red raspberry, both fruit setting and germination of seeds are highly influenced by the maternal genotype. This means that there is no universal procedure for achieving seeds of high germination rate from different crossing combinations. This statement has also been confirmed by Wada and Reed (2011a, b) and Żurawicz et al. (2018). However, there have been very few studies on the influence of the age of red raspberry seeds on their germination. Although Ourecky (1975) reported that the seeds of most *Rubus* species, stored at 1–5°C, remain viable for several years, he did not provide detailed data to support this. In turn, Clark and Moore (1993) studied the viability of seeds of various genotypes of *Rubus* stored at 4–5°C for 22–26 years. The seeds were first scarified and stratified over a 4-month period at 4°C before sowing. Among the seeds of three red raspberry genotypes, obtained through free-pollination, emergence of seedlings was observed in only one genotype in which 1% of seeds germinate.

The aim of the study was to assess the germination capacity of red raspberry seeds, differing in age and stored at 4–5°C, derived from five crossing combinations of commercially important cultivars.

MATERIALS AND METHODS

The seeds were obtained from the crossing of five cultivars of red raspberry that have been or are still grown commercially in many countries. The list of these cultivars and their brief characteristics are given in Table 1, and the combinations of crosses in Table 2. Crossings were performed annually from 2015 to 2018 on shrubs growing in a greenhouse in plastic 5.0 dm³ pots. The shrubs were planted in mid-January, and cross pollination was carried out in March–April. Until planting, the shrubs had been kept in a cold store that maintained high air humidity (95%) and a temperature of +2°C. The substrate was a 1:1:1 (by volume) mixture of peat, compost soil, and washed sand. After planting in mid-January, the pots with the plants were placed on a windowsill in a heated greenhouse at 20–22°C during the day and 14–16°C at night, using supplementary artificial lighting to ensure a 16-hour day. The plants were watered as needed. No plant protection against disease or pests were applied, but sticky traps were densely placed in the greenhouse to trap dipterous flies and other insects. Tests for the presence of viral infections in tissues of the plants, carried out in the Department of Phytopathology of the National Institute of Horticultural Research, confirmed the absence of the Raspberry leaf mottle virus (RLMV), Raspberry vein chlorosis virus (RVCV), Raspberry leaf blotch virus (RLBV), and the Raspberry bushy dwarf virus (RBDV).

The pollen for cross-pollination was obtained from the swollen flower buds of the paternal genotypes. Pollination of emasculated flowers of the maternal forms was performed with a brush twice – the first time immediately after emasculation, and the second time two days later. After the emasculation, the mother plants were fitted with isolators. 'Sokolica' flowers were also self-pollinated and not emasculated. Within each pollination combination, about 30 of the earliest developing flowers were pollinated on two mother plants.

The fruits from the crosses were successively harvested as they matured, packed in plastic bags, and placed in a refrigerator at a temperature of about 5°C.

After the harvest, lasting about 3 weeks within individual combinations of crosses, seeds were extracted. Each year, each combination of crosses produced at least 1000 seeds. After rinsing thoroughly in running water, the seeds were dried at room temperature for 24 hours, packed into white paper bags, and stored in a refrigerator at 4–5°C.

At the beginning of January 2019, all the seeds were scarified according to the method described by Żurawicz et al. (2017), in which the seeds were treated with concentrated sulphuric acid (95% H₂SO₄) for 30 minutes. After that, the seeds, separately for each combination of crosses and each year, were mixed with moist sand roasted beforehand at 200°C and stratified in plastic bags in a 'MIR-554' seed stratification incubator (SANYO, Moriguchi, Japan) at a temperature of 5°C for 50 days. After this time, 200 seeds were randomly taken from each bag. This batch of seeds was divided into four parts (50 seeds each), and each part was sown separately into pots filled with a 3:1 v/v mixture of peat substrate and sterile sand, covered with a thin layer of this sand, and placed on a windowsill of a heated greenhouse in which the air temperature was maintained at 16–18°C at night and 20–22°C during the day, with supplementary artificial lighting (16-hour day). After 18 days, emergence of single seedlings was observed. The seedlings were counted and removed at the stage at which the cotyledons were clearly visible. The assessment of seedling emergence was repeated weekly and completed after 60 days from the day the seeds were sown. The described procedure is commonly used in the breeding of *Rubus idaeus*. It only takes into account the general rules of ISTA, as there are no specific recommendations for this species.

Statistical analysis of the results was performed using two-factorial analysis of variance: factor A – age of seeds (year of seed production), factor B – crossing combinations of the parental cultivars. Due to deviations from normality of distribution and homogeneity of variances verified by Shapiro–Wilk and Levene tests, data were transformed according to Bliss procedure before analysis. The differences between means were assessed using Duncan's t-test at a significance level of 5%.

RESULTS

As can be seen in Table 2, the percentage of seedlings obtained within 60 days of sowing the seeds, varied widely, and depended mostly on the age and pedigree of the seeds. In general, the fewest seedlings were obtained from the oldest (four-year-old) seeds produced in 2015. On average, for all crossing combinations, the number of seedlings obtained from the four-year-old seeds was only 14.0% of the number of seeds sown, with the lowest number of seedlings obtained from seeds produced by self-pollinated 'Sokolica' (only 3.0%), and the greatest number of seedlings from seeds produced by crossing 'Canby' × 'Sokolica' (25.0%). In contrast, the highest average number of seedlings was obtained from one-year-old seeds, produced in 2018. On average for all the crossing combinations, 44.9% of those seeds successfully produced seedlings. However, significant pedigree-related differences between the seeds were found here. The fewest seedlings were obtained from seeds produced by crossing 'Glen Ample' × 'Willamette' (31.5%), and the most from seeds produced by crossing 'Sokolica' × 'Willamette' (72.5%).

On average for the four years of the study, the fewest seedlings were obtained from seeds of the hybrid 'Glen Ample' × 'Willamette', which was only 17.5% of the number of seeds sown. On the other hand, seeds derived from the crossing combination 'Sokolica' × 'Willamette' germinated at the highest rate, which was, also on average for the four years, 47.4%. In addition, seeds from this combination of crosses were characterized by a high capacity for germination in each individual year of the study; the percentage of seeds successfully producing seedlings was 17.0% for four-year-old seeds, 49.0% for three-year-old seeds, 51.0% for two-year-old seeds, and 72.5% for one-year-old seeds. It should also be noted that there was no marked inbreeding depression in terms of emergence from seeds (number of seedlings obtained) from the crossing combination 'Sokolica' × 'Sokolica'. Although the oldest (four-year-old) seeds from this combination of crosses germinated at the lowest rate, which was only 3.0%, this was not significantly different compared to seeds of the same age from the combination 'Sokolica' × 'Veten'.

Table 1. Short description of the cultivars used in the crossing program

Cultivar name	Country of origin	Pedigree	Ripening time	Productivity	Fruit		Susceptibility to RBDV	Spines on shoots
					size	color		
‘Canby’	USA	‘Viking’ × ‘Lloyd George’	early	high	medium	intense red	susceptible	+
‘Glen Ample’	UK	SCRI 7326E1 × SCRI 7412H16	medium early	high	large	bright red	low susceptibility	–
‘Sokolica’	POL	96131 × 96221	medium early	high	large	bright red	susceptible	+
‘Veten’	NOR	‘Asker’ × ‘Lloyd George’	early	medium to high	medium to large	red and dark red	low susceptibility	+
‘Willamette’	USA	‘Lloyd George’ × ‘Newburgh’	late	high	large	intense red	resistant	+

Table 2. Influence of age and pedigree of the red raspberry seeds on the ability for germination within 60 days from the date of sowing the seeds (Skierniewice 2019)

Seed age (year of crossing)	Pedigree of seeds (crossing combinations of cultivars)					
	‘Sokolica’ × ‘Sokolica’	‘Sokolica’ × ‘Willamette’	‘Sokolica’ × ‘Veten’	‘Canby’ × ‘Sokolica’	‘Glen Ample’ × ‘Willamette’	Average for years
4 years (2015)	3.0 a*	17.0 b–d	7.5 ab	25.0 c–f	17.5 b–d	14.0 a
3 years (2016)	50.5 i	49.0 hi	9.0 ab	27.5 c–g	8.0 ab	28.8 b
2 years (2017)	42.0 f–i	51.0 i	50.0 i	19.5 b–e	13.0 bc	35.1 c
1 year (2018)	39.5 f–i	72.5 j	45.0 g–i	36.0 e–i	31.5 d–h	44.9 d
Average for combination	33.8 b	47.4 c	27.9 b	27.0 b	17.5 a	

* values marked with the same letter do not differ significantly (Duncan’s test, $P \leq 0.05$); SE for the factors studied: A – age of seeds (year of seed production), B – crossing combinations of the parental cultivars and interaction A*B equal 2.18, 2.52, and 4.37, respectively

DISCUSSION

Hybridization between different parental genotypes and the production of seeds is a basic stage of breeding work in traditional new cultivar breeding. The seeds of the red raspberry are not sown immediately after harvesting because they require both scarification and a cold stratification (Jennings & Tulloch 1965; Dale & Jarvis 1983; Żurawicz et al. 2017). It is quite common, however, for breeders to not use all of the obtained seeds in the year of their production. This may be due to technical and organizational constraints, but it may also be the result of an adopted strategy in which the breeders leave for themselves a batch of seeds as a breeder’s reserve. This reserve allows the breeder to preserve seeds from a specific, valuable crossing combination that might be difficult or impossible to repeat, or can be used to broaden genetic variation in subsequent years of a breeding program.

In our study, despite the scarification and cold stratification, which were the same for all the tested red raspberry seeds, the seeds germinated slowly, unevenly, generally in small numbers, with the results largely dependent on age and pedigree. This is not surprising, as poor seed germination is a problem known to red raspberry breeders. Earlier, this problem was pointed out by Scott and Ink (1957), Jennings and Tulloch (1965), Jennings (1971, 1988), Dale and Jarvis (1983), Daubeney (1986), and Żurawicz et al. (2017). It is difficult to explain unambiguously the reason for such poor germination of seeds in our study because it may have been related to their age, pedigree, method of scarification, length of the stratification period, as well as the adopted duration of the period from the time of sowing the stratified seeds to the completion of the assessment of seedling emergence.

Our results, however, are consistent with those reported by Jennings (1971, 1988), who found that the germination of red raspberry seeds was largely dependent on the maternal genotype and recommended, therefore, that when it is desired to obtain a specific combination of genes, reverse crosses should also be performed. The results presented here are also consistent with the results of our previous study (Żurawicz et al. 2017), in which we evaluated germination of one-year-old red raspberry seeds obtained from 55 crossing combinations of various parental genotypes. In that study, using the same duration of seed scarification with concentrated sulphuric acid, the number of germinated seeds (seedlings obtained) 75 days after sowing depended on seed pedigree (combination of crosses) and was from 0.0% ('Polana' × 'Willamette') up to 93.2% ('Laszka' × 'Sokolica').

Age had a significant influence on the germination of the seeds we tested because the oldest (four-year-old) seeds clearly germinated at the lowest rate. Three- and two-year-old seeds germinated considerably better, while one-year-old seeds generally performed the best. Considering that a ripe red raspberry fruit contains (depending on the genotype and pollen source) around 30–100 seeds (Żurawicz et al. 2018), even with a 20% seed germination rate, roughly 20 additional fruits are enough to obtain seeds for keeping in the breeder's reserve for 3–4 years. The results of seed germination obtained in our study would probably have been higher if we had extended the time of seed stratification from 50 days to 75 days to make it the same as in our earlier study (Żurawicz et al. 2017). It should be added, however, that although Clark and Moore (1993) had stratified seeds of this raspberry species over a period of 4 months, the 6-week period of red raspberry seed stratification is sufficient according to Daubeney (1996). It can therefore be assumed that the longer the seed stratification time, the more seedlings will be obtained, although the latter authors stratified very old seeds (over 26 years old). The same applies to the period of time required for seedlings to emerge from the seeds sown.

The longer this period is, the more germinated seeds there will be, and the more seedlings will be obtained. In our study, this period was 50 days, but Clark and Moore (1993) had continued their assessment of the emergence of red raspberry seedlings for as long as 5 months after sowing the seeds. However, for organizational reasons, excessively prolonging both the time of seed stratification and the time to obtain small red raspberry seedlings (over 2 months) may pose problems in producing good quality plant material for open-field planting before the cold autumn weather arrives.

CONCLUSIONS

Seedling emergence (seed germination rate) in red raspberry depends both on the age of seeds and their pedigree. In general, one-year-old red raspberry seeds germinate at the highest rate, but the breeder's reserve may also include three- or even four-year-old seeds, provided that their amounts are slightly larger than those of one- and two-year-old seeds, and that they are stored at a temperature of around 5°C.

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