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Inbreeding depression for seed germination and seedling vigor in strawberry (*Fragaria* × *ananassa* Duch.)

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ABSTRACT

Experiments leading to the procurement of subsequent inbred generations were conducted in the years 2006-2013. Seeds obtained from open pollination and after self-pollination of four strawberry cultivars (Teresa, Senga Sengana, Kent and Chandler) and clone 1387 were used. These genotypes were evaluated for their tolerance to strong inbreeding under *in vitro* culture conditions. The aims of this study were to estimate the inbreeding depression of each of the progenies. During the investigation, the germination percentage as well as seedling viability were evaluated. The highest seed germination was shown for populations derived from 'Teresa' × open pollination (82%) and 'Kent' (7) S_4 (78%). Seeds derived from self-pollination resulted in the lowest germination – an average of 16.8%. Generally, seed germination was significantly lower for the five S_1 offspring, whose depression was 0.62, in comparison with the S_4 seedlings, whose depression was 0.31. Inbred offspring showed a depression in relation to the average weight of a single seedling of 0.08 in the case of S_1 progeny, whereas in the case of S_4 progeny it was 0.23. The highest germination energy was shown by 'Kent' (7) S_4 seeds (74%) and hybrids of 'Teresa' derived from open pollination (75%); whereas seeds obtained at the same time from self-pollination germinated 10.8% on average.

Key words: breeding; inbreeding coefficient; offspring; pollination; self-pollination

Abbreviations:

δ – inbreeding depression coefficients, ws – inbred trait value, wo – outbred trait value, OP – open pollination,

INTRODUCTION

Since the strawberry (*Fragaria* × *ananassa* Duch. 2n = 8x = 56) is a vegetatively propagated crop, and thus highly heterozygous in nature, theoretically inbreeding should reveal undesirable combinations of genes which, once eliminated, should leave plants with a high concentration of desirable characteristics. The remaining inbred lines should then be valuable as parents both in crosses between each other and with cultivars, thus producing progenies with a high proportion of elite selections. The value of inbreeding in strawberries has never been fully resolved (Melville et al.1980, Niemirowicz-Szczytt 1989, Rho et al. 2008, Baturin and Ambros 2010, Rho et al. 2012).

Inbreeding depression is widely observed in animals and plants that reproduce by random mating

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(allogamy) when populations are submitted to any natural or artificial inbreeding. This phenomenon is related to an increase in homozygosity at loci with some degree of dominance (Charlesworth and Charlesworth 1987). The effects of inbreeding depression are probably due to a large number of genes with small effects and varying degrees of dominance, or to key genes that affect several other genes of smaller effect (Chaves et al. 2010). However, inbreeding depression is a quantitative character, whose expression is environmentally dependent, and alternative methods to measure it, involving a minimum of artificial disturbance, need investigation. *In vitro* cultures are a perfect method for the observation of seed germination and also of the influence of inbreeding on this process. These observations can be carried out in the small space of an *in vitro* lab and can be screened in strictly controlled conditions.

The basis for assessing the germination is the assumption that the seeds that developed normally shoot at the time and conditions specific to the species and will be capable of further development in field conditions. Germination capacity is the percentage of seeds germinated normally in the conditions and time allowed for the completion of the evaluation (Duczmal 1993). The evaluation of germinating seeds should be carried out in a period of their development when we can determine whether seedlings have normally developed vital organs. Normal seedlings should have: a well-developed root system, a well-developed over – or under – cotyledonous part, one cotyledon in monocotyledon seedlings and two cotyledons in dicotyledonous seedlings (Duczmal and Tucholska 2000).

The aim of the undertaken studies was to estimate the effect of inbreeding on seed germination in the tested populations of strawberry. In this study we evaluated the percentage of germinating seeds and seedling viability of populations S_1 and S_4 derived from four strawberry cultivars and clone 1387.

MATERIAL AND METHODS

Ten strawberry cultivars including Kent, Selva, Elkat, Elsanta, Paula, Ostara, Teresa, Senga Sengana, Chandler and the breeding clone 1387 were examined and self-pollinated, but only five of them (Teresa, Senga Sengana, Kent, Chandler and clone 1387) developed successive inbreeding generations (S_2-S_4).

Seeds from open pollination were collected from these plants grown in the field strawberry germplasm collection. In the obtained seeds, high genetic diversity was expected as a result of random chromosome segregation and crossing-over during megasporogenesis in the mother plants, and of random pollination by the pollen from over 80 cultivars and clones of different origin growing in the proximity of the maternal plants.

To obtain the fourth generation (S_4), the first self-pollination was performed in May 2006. Before flowering time, inflorescences were covered with small bags made of cloth and left undisturbed for spontaneous selfing. 150 seedlings (S_1) derived from each of the cultivars were used in field trials on September 10, 2007. In May 2008, the second self-pollination was performed. Twenty seedlings randomly chosen from the first generation were self-pollinated to generate the second generation (S_2). In 2010, 20 seedlings were retained from these populations and used as parents to obtain the third generation. Seeds of the population S_4 and S_1 were obtained in 2012 (according to the procedure described above). Progeny S_4 – marked with corresponding numbers – was derived from the same S_3 parental plants. The obtained seeds were divided into two parts, and the experiment was repeated twice in 2013.

In vitro germination procedure

Seeds were surface sterilized in sodium hypochlorite for 2 hours, rinsed three times in sterile distilled water (for 5, 10 and 15 min.) and placed on a medium composed of macro- and microelements according to Murashige and Skoog (1962). The medium was enriched in 100 mg dm^{-3} of m-inositol, 20 g dm^{-3} of sucrose, 7 g dm^{-3} of agar, pH 5.65 adjusted with 0.1 N NaOH and 0.1 N HCl prior to autoclaving, then was sterilized for 20 minutes at 121°C in Petri dishes (10 cm in diameter). Dishes of 20 seeds each were placed in the dark, at 5°C, for a period of eight weeks for vernalization. Afterwards the seeds were kept at a constant temperature of 23°C with a 16/8 h day/night photoperiod. Each treatment consisted of five Petri dish replications.

Evaluation of seed germination

Germination was observed in one-week intervals for a period of 10 weeks. Seedlings with two fully developed cotyledons were scored as germinated. Preliminary calculations, after eight weeks from sowing the seeds, allowed us to determine the germination energy (the number of seeds available for rapid germination). This is expressed as a percentage. The time after which the germination energy was calculated was established according to the instructions published by the International Seed Testing Association (ISTA) (Anonymous 2003). A final calculation was carried out when all living seeds germinated. In this manner, the rate of germination can be expressed as a percentage. Seedling viability was estimated as the fresh weight of whole seedlings. Inbreeding depression coefficients for germination ability and average weight of a single seedling for each progeny were calculated as: $\delta = 1 - ws/wo$, where ws and wo were the inbred trait value and outbred trait value, respectively (Agren and Schemske 1993). Duncan's multiple range t-test was used for mean separation at $p = 0.05$.

RESULTS AND DISCUSSION

Out of the 10 tested cultivars, three cultivars – Paula, Selva and Ostara – produced no viable S_1 plants. Two others – Elkat and Elsanta – gave no S_2 progeny. It has been shown that strawberry seeds germinate non-simultaneously and their germination rates depend on the genotypic characteristics of the species. Seeds obtained by cross-pollination (outbreeding) demonstrated a very good germination rate. At the same time, relatively low germination rates and the partial death of seedlings have been reported in several studies (Marta et al. 2004, Baturin and Ambros 2010). The frequency of these symptoms is determined by the action of lethal genotypic factors typical for outbreeding or inbreeding. The multiplicity of systems and ways of seed formation in *F.* × *ananassa* provides a wide genetic variability in seed progeny, which is necessary for primary selection. A number of experiments have been carried out to develop inbred lines in strawberries and to test them in breeding programs (Spangelo et al. 1971, Hulewicz and Hortyński 1979, Niemirowicz-Szczytt 1989, Żurawicz 1990, Shaw 1995, 1997, Kaczmarska 2012). The authors of all the above papers reported that after the first and second self-pollination, plant viability decreased considerably, which made it practically impossible to develop progeny for most cultivars.

During the study a large genotypic difference between the examined objects was observed. Each of the tested cultivars reacted to the selfing in a different way. Figure 1 demonstrates that, in the case of the 'Chandler' and 'Teresa' hybrids, the best germination was displayed by seeds obtained after open pollination. Whereas in the case of 'Kent' and clone 1387, the seeds of the S_4 generation germinated most successfully. The progeny 'Chandler' S_1 showed a high reduction in seed germination at the beginning of the experiment followed by an increase of 21%.

As reported by Melville et al. (1980), four inbred strawberry selections were selfed, intercrossed and outcrossed to two non-inbred selections. The latter were also intercrossed and selfed. Most progenies germinated as well as or better than the control outcross between two vigorous non-inbred clones. The germination of an S_3 progeny was lower than the control. In general, inbreeding reduced seedling vigor while intercrossing or outcrossing restorer vigor.

The average weight of a single seedling for all S_1 and S_4 progenies was not significantly different from those obtained by open pollination. The highest weight of a single seedlings was observed in the case of progeny 'Chandler' S_1 and 'Kent' (7) S_4 (data not shown). The lowest weight of seedlings was from the progeny 'Chandler' (123) S_4 and clone 1387 S_1. The inbreeding coefficient estimated for this trait amounted to 0.08 in the case of the S_1 progeny, whereas it was 0.23 in the case of the S_4 progeny.

The highest energy of germination, or the number of seeds capable of rapid germination, was exhibited by the seeds of 'Teresa' hybrids from open pollination (75%) and 'Kent' (7) S_4 (74%) (Tab. 1). In the S_1 progeny, the energy of germination was low – on average 10.8%, ranging from 1.0% (clone 1387 S_1) to 20.0% ('Senga Sengana' S_1).

As shown in Table 1, germination ability was the highest in the population of 'Teresa' derived from open pollination (82%) and the 'Kent' (7) S_4 population (78%). Observations of four cultivars and one advanced breeding clone have shown evident differences in sensitivity to inbreeding during *in vitro* germination. These differences have a genetic background and might be exploited in breeding. Most of the variation observed was due to the general ability of the seeds to germinate.

An experiment conducted by Rho et al. (2008) attempted to solve the problem associated with inbred line breeding of strawberry. For this purpose, inbreeding depression and percent germination, categorized by the generation of individuals with non-inbreeding depression to be selected and individuals with inbreeding depression to be eliminated, was researched. When the percent germination of the inbreeding depression plant and non-inbreeding depression plant in lines originated from the Akihime cultivar were compared, there was no difference. However, in lines originated from the Johong and Sachinoka cultivars, the percent

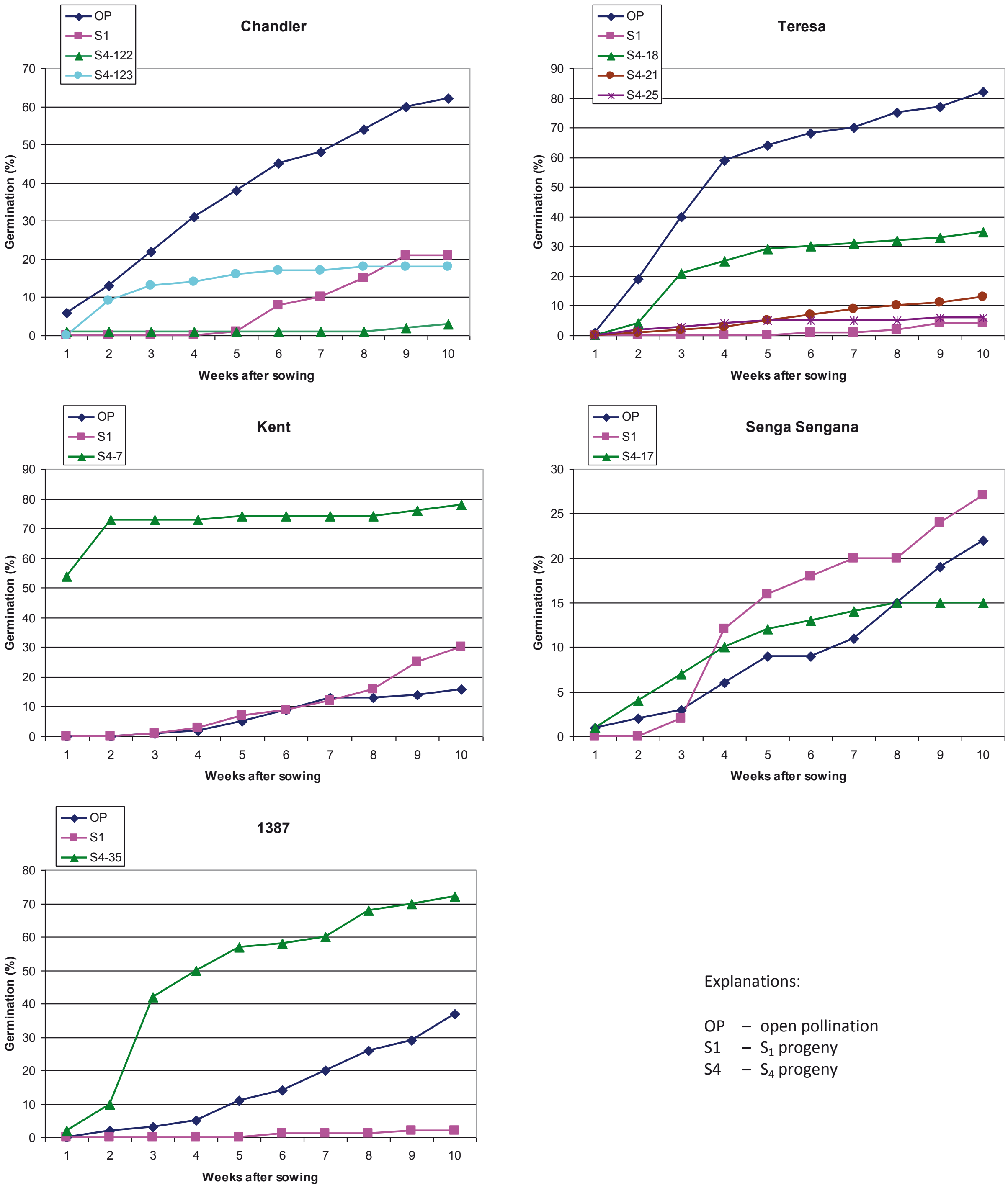

Figure 1. Seed germination of 4 cultivars and breeding clone 1387 of strawberries and their inbred offspring under *in vitro* culture conditions

germination of inbred plants was remarkably lower. Inbreeding depression occurred in the next generations of lines, although its degree varied.

In subsequent studies, Rho et al. (2012) reported that the germination percentages in the S_1-S_9 generations of inbred lines derived from three strawberry cultivars – Akihime, Johong and Sachinoka – showed an increase in the S_5 generation and remained at 75-80% after the S_6 generation. Continued self-pollination of octoploid strawberry over several generations resulted in a progressive decrease in yield, plant height and leaf area.

The highest inbreeding depression (δ = 0.95) occurred in the offspring 'Teresa' S_1, 1387 S_1 and 'Chandler' (122) S_4. The average inbreeding depression coefficient for generation S_1 (0.62) was twice as high as in generation S_4 (0.31). These findings are in agreement with a report

Table 1. Seed germination and weight of seedlings of four cultivars and one breeding clone of strawberries and their inbred offspring (S_1 and S_4)

Cultivar / progeny	Average number of seedlings per plate	Total weight of seedlings (g)	Germination energy (%)	Germination ability (%)	Inbreeding coefficient of germination ability (δ)
Teresa OP*	16.4 a**	1.118 ab	75.0 a	82.0 a	-
Senga Sengana OP	4.4 d	0.237 b	15.0 d	22.0 d	-
Kent OP	3.2 d	0.296 ab	13.0 d	16.0 d	-
Chandler OP	12.4 abc	0.814 ab	54.0 abc	62.0 abc	-
1387 OP	7.4 bcd	0.450 ab	26.0 dc	37.0 bcd	-
Mean OP	8.8 A	0.583 A	36.6 A	43.8 A	-
Teresa S_1	0.8 d	0.038 b	2.0 d	4.0 d	0.95
Senga Sengana S_1	5.4 cd	0.275 b	20.0 dc	27.0 cd	-0.23
Kent S_1	6.0 cd	0.354 ab	16.0 d	30.0 cd	-0.87
Chandler S_1	4.2 d	0.199 b	15.0 d	21.0 d	0.66
1387 S_1	0.4 d	0.009 b	1.0 d	2.0 d	0.95
Mean S_1	3.4 C	0.175 C	10.8 C	16.8 C	0.62
Teresa (18) S_4	7.0 bcd	0.340 ab	32.0 bcd	35.0 bcd	0.57
Teresa (21) S_4	2.6 d	0.102 b	10.0 d	13.0 d	0.84
Teresa (25) S_4	1.2 d	0.059 b	5.0 d	6.0 d	0.93
Senga Sengana (17) S_4	3.0 d	0.173 b	15.0 d	15.0 d	0.32
Kent (7) S_4	15.6 a	1.453 a	74.0 a	78.0 a	-3.87
Chandler (122) S_4	0.6 d	0.039 b	1.0 d	3.0 d	0.95
Chandler (123) S_4	3.6 d	0.073 b	18.0 cd	18.0 d	0.71
1387 (35) S_4	14.4 ab	0.772 ab	68.0 ab	72.0 ab	-0.95
Mean S_4	6.1 B	0.376 B	27.9 B	30.0 B	0.31

*Open pollination (OP); **Values marked with the same letter do not differ significantly at $\alpha = 0.05$

by Niemirowicz-Szczytt (1989) showing that the germination percentage slightly increases with the process of selfing generations. However, the germination percentage showed significant differences within lines derived from a cultivar, even when compared to lines derived from other cultivars.

Baturin and Ambros (2010) compared the seed germination dynamics of parthenogenetic progenies of the strawberry cultivar Purpurovaya during inbreeding, outbreeding and agamospermy. Seeds from the outbreeding germinated within 46 days, seeds from self-pollination within 68 days at a slightly higher proportion of germinated seeds (80.0%) compared to the variant with outbreeding (76.4%). It was shown that the revealed differences are caused by genotypic conditions. The seed germination of progenies is much higher during inbreeding and outbreeding than that of the original cultivar from which they were breeding.

The obtained results indicate that in the case of strawberries, inbreeding depression for the germination of seeds decreased in the successive inbreeding generations and was lower in the fourth generation compared to the S_1 generation, which is consistent with data presented by other authors. Kaczmarska (2012) reported that the average fruit yield per plant in inbred lines of strawberry appeared to be much lower than that of the standard cultivars (inbreeding depression for this trait decreased in successive inbred generations and amounted to 52.5 for S_1, 36.6 for S_2 and 22.0 for S_3). Moreover, a significant correlation exists between inbreeding depression and selfing/outcrossing species, i.e. repeated selfing reduces the magnitude of depression. In contrast, outcrossing usually increases heterozygosity, which tends to produce genotypes better adapted to different environmental conditions. It has been depicted that different cultivars of strawberry differ in terms of the degree of inbreeding depression and percent germination. This problem in the inbred line breeding of strawberry can be solved by selecting vigorous and productive plants from the early selection.

CONCLUSIONS

1. Achenes of strawberry obtained by inbreeding and by open pollination showed different germination rates owing to their genotypic differences.
2. The average weight of a single seedling derived from open pollination did not differ significantly from seedlings obtained by self-pollination.
3. Generally, the germination of seeds from self-pollination was significantly lower for S_1 offspring, whose depression was 0.62, in comparison with the S_4 seedlings, whose depression was 0.31.

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