Short report: An in vitro method to rescue embryos of horseradish (*Armoracia rusticana*), a reputedly sterile plant

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Abstract. Horseradish has been reported to be frequently sterile and therefore difficult to improve by traditional sexual crosses. In this paper we report a simple method to perform embryo rescues on Murashige and Skoog medium without growth regulators. We then demonstrate the usefulness of the method by making crosses and self pollinated seeds of several horseradish lines, rescuing the embryos in vitro, and growing the seedlings in soil in a greenhouse. Seedlings obtained in these experiments are being prepared for field plantings in the spring of 2004. We hope that this simple procedure will speed worldwide efforts to improve horseradish germplasm.

INTRODUCTION

For many years horseradish, *Armoracia rusticana* P.Gaertn., B.Mey & Scherb, was believed to be sterile (Courter and Rhodes, 1969) and therefore impossible to improve by traditional sexual crosses. Luther Burbank wrote "The horseradish does, indeed, bloom with the greatest profusion. But the blossoms prove sterile. The plant has entirely and probably forever lost the power of producing seed. I have elsewhere … [made] a joking offer of one thousand dollars an ounce for horseradish seed. Of course I knew that no horseradish seeds were to be had, yet I would gladly have given then, and I would glad pay, at the rate of $1000 an ounce for horseradish seed. But there is not the remotest probability that any one will ever legitimately claim the prize" (Burbank, 1914).

Viable seed was first reported in 1909 (Brezezinski, 1909), but mass seed production remained a problem. Weber (1949) examined seedlings and reported many problems with seed production: high male sterility, self-infertility, abortion, and some crowns lacking the ability to develop an inflorescence. Stokes (1955) studied factors related to seed failure and proposed that lack of viable seeds was due to endosperm maternal tissue incompatibility and embryo abortion. By 1980 Rhodes (oral communication) reported that modern cultivars are fertile. However, delayed harvest can result in pod shatter and seed loss. A fertile pod will contain one to six seeds.

Although horseradish seeds can be produced, their number and viability varies among crosses. Apparently many seeds die before they have a chance to mature sufficiently for germination. In this paper we report a method to germinate both mature and immature seeds of horseradish in vitro.

MATERIALS AND METHODS

Plant materials: Horseradish cultivars ILHR 862A, ILHR 1069, ILHR 22C and ILHR 1005GF from the University of Illinois horseradish collection were used for this study. For the first experiment entire stems of immature siliques were gathered from two of these cultivars (ILHR 862A and ILHR 1069). The stems were placed in plastic bags and stored in a refrigerator (3 to 4 °C) until there were enough seeds were collected to begin the experiment. After seeds were extracted from their pods, they were sorted into two groups (brown and green), and then disinfested in a 10% bleach (0.525% NaOCl) solution mixed with a few drops of a surfactant (Triton x-100) for 10 min, rinsed twice with sterile distilled water, and explanted onto Petri dishes (15 X 100 mm) containing 30 ml standard high salt Murashige and Skoog medium (MS, Murashige and Skoog, 1962) without growth regulators. The pH of the medium was adjusted to 5.6 before the addition of Difco Bacto Agar (6.0 g/l) and autoclaving at 1.06 $kg.cm^{-2}$ for 20 min at 121 °C. All cultures were maintained at 25 ± 2°C in a culture room under cool-white fluorescent light (40-60 $mmol.m^{-2}.s^{-1}$) with a 16 h photoperiod.

Each treatment consisted of 6 replicates and each replicate contained 10 seeds. The number of seeds germinating was assessed by counting at 2 day intervals, and the total germination percentage was determined after 14 days. Seedlings with two true leaves were pricked from the medium, rinsed in warm water to remove clinging agar, transplanted to soil in a greenhouse clear plastic dome-covered flat of soil for about one week. All of the seedlings transferred to soil successfully survived and grew vigorously.

Experimental design and data analysis. Experiments were set up in a completely randomized design and data analyses were performed using the SAS statistical package (SAS

Institute, 2000). Following analysis of variance, grouped means t-test and LSD test were used for mean comparisons at $P \leq 0.05$.

Once an acceptable embryo rescue protocol had been established, crosses and self pollinations were made using three of the horseradish clones available to the authors. For the crosses ILHR 22C was used as a female parent; ILHR 647 and ILHR 1005GF were used as male parents to make putative hybrids (ILHR 22C x ILHR 647 and ILHR 22C x ILHR 1005GF, respectively). The hybrids were made by placing pots of each parents near each other and then bagging individual clusters of each parent together and shaking them periodically to facilitate pollen transfer. The flowers of ILHR 22C, ILHR 1005, and ILHR 647 were bagged and shaken periodically to obtain putative self pollinated seed. To obtain hybrid seeds pollen from the male parental clone was used to pollinate the designated female parents. Seeds were collected from the crossed and selfed siliques and grown in tissue culture then transferred to the greenhouse as previously described.

RESULTS AND DISCUSSION

Because horseradish produces very few seeds, the number of seeds available for these studies was limited. At harvest many siliques appeared to be full and we expected to find many seeds contained in each. However, this was not true and many siliques were empty. Most siliques contained a mixture of green and brown seeds (Table 1). The green seeds appeared to be slightly smaller than the brown ones (data not presented) and were presumed to be less mature.

In the first experiment, brown and green seeds were explanted onto MS medium separately as described earlier. The number of seeds that germinated was determined at two day intervals. Seeds germinated rapidly on the MS medium. More than half of the seeds that

germinated for these two cultivars did so within two days of explanting (Figure 1). In the first two days about three times as many brown seeds germinated as the green seeds (36.7% vs 11.6% and 68.3% vs 21.6%, respectively, Figure 1). Seeds continued to germinate over the full 14 days of the experiment, but most seeds germinated in the first 10 days (Figures 1 and 2). For both cultivars, the percentage of brown seeds that germinated was higher than that for green seeds (93% and 72% vs. 53% and 52%, respectively, Table 1). This observation supports our hypothesis that the green seeds were less mature than the brown seeds and, hence, less likely to germinate.

Some putative hybrids (ILHR 22C x ILHR 647 and ILHR 22C x ILHR 1005GF) and all three of the selfed plants produced siliques with seeds (Table 2). Seeds from selfed and putative hybrid siliques were germinated on MS medium (Table 2). Albinos were noted among the seedlings, however, all of these died soon after germinating. The seeds of the presumed hybrids germinated well on MS medium (80% and 67%, respectively) to yield putative hybrids (20% and 53%, respectively, Table 2). These were transferred to soil where they are now growing in a greenhouse (Figure 2). The seeds of one of the selfed plants (ILHR 647) failed to germinate (0%); the seeds of another (ILHR 1005GF) produced seedlings but none survived long enough for transfer to greenhouse conditions.

In conclusion, horseradish improvement using traditional sexual systems has been limited by real and perceived problems with seed production and germination. In this paper we report a simple method to perform embryo rescues on MS medium. We then demonstrate the usefulness of the method by making crosses and self pollinated lines of several horseradish lines, rescuing the embryos in vitro, and growing the seedlings on in soil in a greenhouse. Seedlings obtained in these experiments are being prepared for field plantings in the spring of 2004. To verify whether

hybridizations have actually taken place, we are currently performing DNA/PCR tests on the three self pollinated lines and the putative hybrids to determine the hybrid status of the seedlings resulting from the crosses. We hope that this simple procedure will speed the efforts to improve horseradish germplasm.

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REFERENCES

Burbank, L., 1914. Luther Burbank, his methods and discoveries and their practical application. Vol. VII. Luther Burbank Press, New York. p 172.

Courter, J.W. & A.M. Rhodes, 1969. Historical Notes on Horseradish. Econ Bot 23:156-164.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473-476.

SAS Institute Inc., 2002. SAS User=s Guide: Statistics Cary, NC.

Weber, W. W., 1949. Seed production in horseradish. J Hered 40: 223-227.

Cultivar	Seed type	Germination (%)
ILHR 862A	brown	93 a [z/]
	green	53 b
ILHR 1069	brown	72 c
	green	52 b

[z/] Percentages with different letters are statistically different with a $P < 0.05$ by LSD.

Table 1: Total germination percentages of each of two types of seed from two horseradish cultivars 14 days after explanting in MS medium.

Crosses	No of seeds	germination (%)	No. of albino seedlings	% surviving seedlings (n)
ILHR 22C x ILHR 647	15	80	9	20(3)
ILHR 22C x ILHR 1005	15	67	0	53(8)
ILHR 1005 selfed	15	47	3	0(0)
ILHR 647 selfed	15	0	0	0(0)
ILHR 22C selfed	15	67	1	53(8)

Table 2: Successful crosses and self pollinated lines of three horseradish cultivars and their ensuing germination and seedling survival ex vitro.

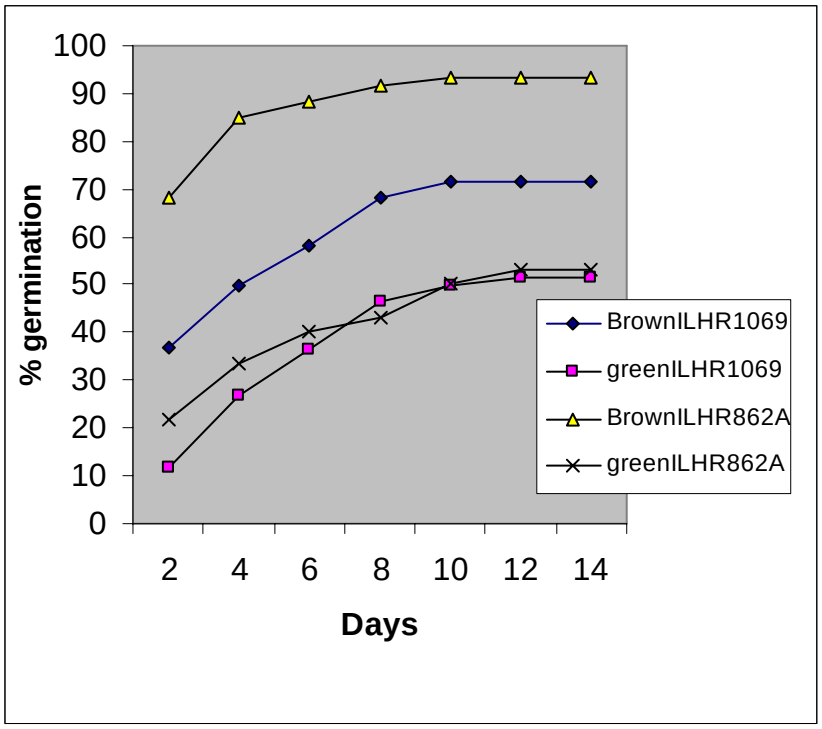

Figure 1: Germination curves for two types of seed found in siliques of two horseradish cultivars.

Figure 2. Horseradish ILHR 862A seeds and seedlings. *Top:* germinating seeds 2 days (left) and 7 days (right) after explanting on Murashige and Skoog (MS) tissue culture medium. *Bottom*: Seedlings harvested from MS medium 25 days after transplanting to soil in a greenhouse