Hand Emasculation and Induced Male Sterility to Improve Field Pennycress (*Thlaspi arvense L.*) Breeding

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ABSTRACT

Field Pennycress (*Thlaspi arvense* L.) is a self-pollinated winter annual weed species that is currently being studied as a new source of industrial products and biodiesel. Conducting controlled crosses between small flowered pennycress varieties is difficult and time consuming. The objective of this study was to develop a reliable method of inducing pollen sterility to assist in conducting controlled crosses in this self-pollinated crop.

A total of 146 controlled crosses between winter and spring lines were conducted during the morning (8-10am) and afternoon (3-5pm) hours resulting in 32.1% and 44.6% fertilization, respectively. The spring to winter line crosses resulted in 46% fertilization. For winter to spring crosses, only 30% were successful. A secondary experiment was initiated to investigate the role of sulphonylurea herbicide, tribenuron-methyl, as a potential aid to induce male sterility in pennycress. Pennycress seedlings were grown in a controlled environment. When all plants reached reproductive stage, applications of 0.1, 0.2, 0.3, and 0.4 µg/ml tribenuron-methyl per plant were applied to the leaves and repeated 10 days later. An application consisted of a single 1 ml mist spray to each of 8 replicated plants in a treatment group. All applications of the herbicide resulted in severe stunting of the plants and a delay in flowering. Control plants flowered within 2 days, while the 0.1 µg/ml application flowered in 10 days. Plants with applications of 0.2, 0.3, and 0.4 µg/ml resulted in severe yellowing of young tissue and did not set seed after 20 days of treatment.

The development of a reliable induced male sterility protocol will greatly improve the efficiency of pennycress breeding and will help lead to the development of new varieties of pennycress.

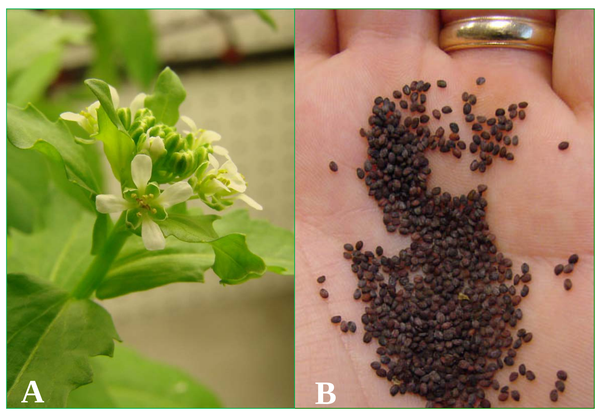

Figure 1. (A) Mature open pennycress flower, (B) Physiologically mature pennycress seed.

INTRODUCTION

Field Pennycress (*Thlaspi arvense* L.) is a winter annual oilseed crop being investigated for its potential as a new crop in the rotation with corn and soybean in the Midwest. Pennycress can be used as a bio-diesel fuel or as an additive in many industrial based products. Pennycress seed is half the size of canola seed and contains 33 percent oil (Figure 1). Wild populations of pennycress can yield as high as 2,000 lbs. of seed per acre. Sixty-two populations of pennycress collected from around the world have demonstrated very limited morphological variation, however, differences in total oil and oil constituents do exist (Phippen, 2011). The individual populations can be characterized as either a dormant winter line or as a non-dormant spring line, each having a unique oil profile. To make pennycress a viable agronomic crop, new crosses need to be developed that are suitable for commercial production and meet oil requirements. Pennycress is a very small flowered, self –pollinated crop with pollen development and fertilization occurring prior to the flower opening. In order to cross pollinate flowers, immature anthers must be removed from floral buds. Pollen from a male donor can then be introduced to create new and unique breeding lines.

The first objective of this study was to determine the success of conducting hand-emasculations between winter and spring lines of pennycress grown under controlled environmental conditions. Experiments on a similar oilseed crop, Camelina have been shown to be successful (Lessman, 1990). Conducting emasculations and subsequent pollinations appears to be highly dependent on time of day. Literature suggests early morning crosses tend to be much more successful than late afternoon (Sabharwal and Doležel, 1993). A second objective was to investigate the role of sulphonylurea herbicide, tribenuron-methyl, as a potential aid to induce male sterility in pennycress. Previous research has suggested the application of sulphonylurea herbicides have been effective in creating male sterility in Brassica species and is now widely used in canola hybrid production (Yu et al. 2006). The development of a reliable induced male sterility protocol will greatly improve the efficiency of pennycress breeding and will help lead to the development of new varieties of pennycress.

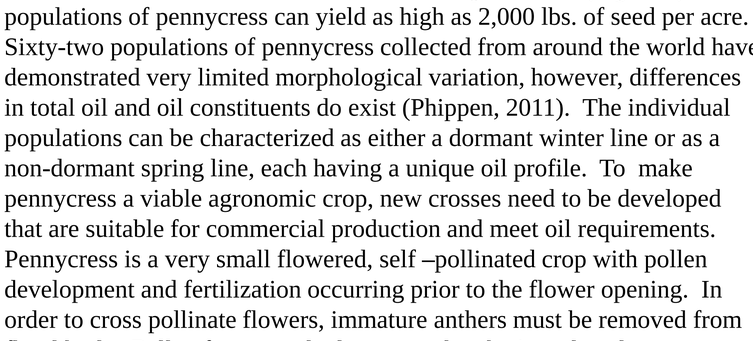

Figure 2. (A) Conducting hand emasculations on winter and spring lines of field pennycress, (B) close up of flower dissection.

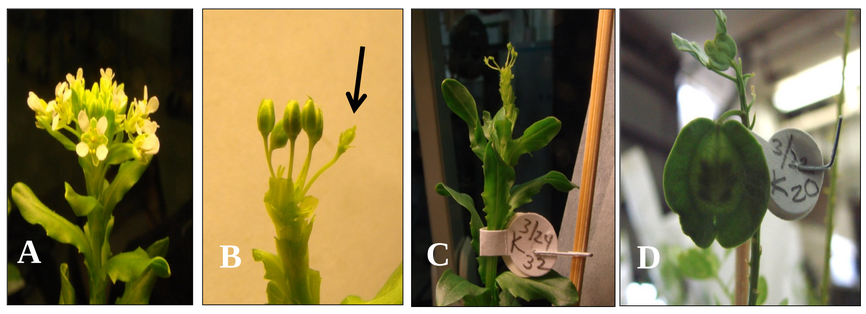

Figure 3. Steps in hand-emasculation process, (A) mature floral buds, (B) prepared buds and emasculated flower (arrow), (C) labeled cross, and (D) fertilized seed pod.

Table 1. Results of hand emasculations conducted between winter and spring lines of pennycress.

		Crosses conducted		Successful fertilizations	
Female	**Pollen Donor**	Morning (8-10am)	Afternoon (3-5pm)	Morning (8-10am)	Afternoon (3-5pm)
Spring 32	**Winter 20**	14	20	5	11
Spring 32	**Winter 12**	19	13	4	5
Spring 32	**Winter 10**	9	9	1	3
Winter 20	**Spring 32**	10	7	4	2
Winter 12	**Spring 32**	16	11	5	5
Winter 10	**Spring 32**	13	5	7	3
Total		81	65	26	29
Grand Total		146		55	

MATERIALS AND METHODS

Hand-Emasculations:
Field pennycress (*Thlaspi arvense* L.) breeding lines ‘NY10’, ‘W-12’, ‘OH 20’, and spring line ‘Spring 32’ were used throughout these experiments. To synchronize flowering of winter lines to spring lines, the winter varieties were germinated under warm (24°C) and long day (18hr) conditions. Upon germination, single seedlings were transplanted into individual 7.5 cm square pots. A total of 12 seedlings were transplanted for each line and held under warm conditions for a period of three days. Transplanted seedlings were watered with a wicking system throughout the entire experiment . After the 3 day acclimation period, all winter line pots were placed under cold (4°C) and short day (12 hr) conditions for 21 days. Plants were transferred back to warm conditions to initiate bolting and flowering. Ten days prior to removing the winter lines from the cold, ‘Spring 32’ seeds were germinated under warm conditions and transplanted to individual pots. Within 30 days, both winter and spring lines were initiating floral buds. Hand-emasculations were performed in the morning (8 to 10am) and in the afternoon (3 to 5pm) for 2 weeks (Figure 2). All open flowers and immature buds were removed from the apical meristem leaving only partial expanded floral buds (Figure 3). Sepals, petals, and anthers were removed from the female plant utilizing magnifiers and sterilized forceps. Pollen was placed on the exposed stigma by a small paint brush or from a donor flower. Plants were labeled and covered with a glycine bag (Figure 4).

Induced Male Sterility:
Seeds from pennycress line ‘Spring 32’ were germinated as described above and transferred to individual pots and allowed to grow until the reproductive stage under warm (21°C) and long day (18hr) conditions. Applications of 0.1, 0.2, 0.3, and 0.4 µg/ml tribenuron-methyl per plant were applied to the leaves of 8 plants per treatment and repeated 10 days later on only 4 plants per treatment. An application consisted of a single 1 ml mist spray. Tribenuron-methyl is the active ingredient at 50% in Express™ herbicide from Dupont.

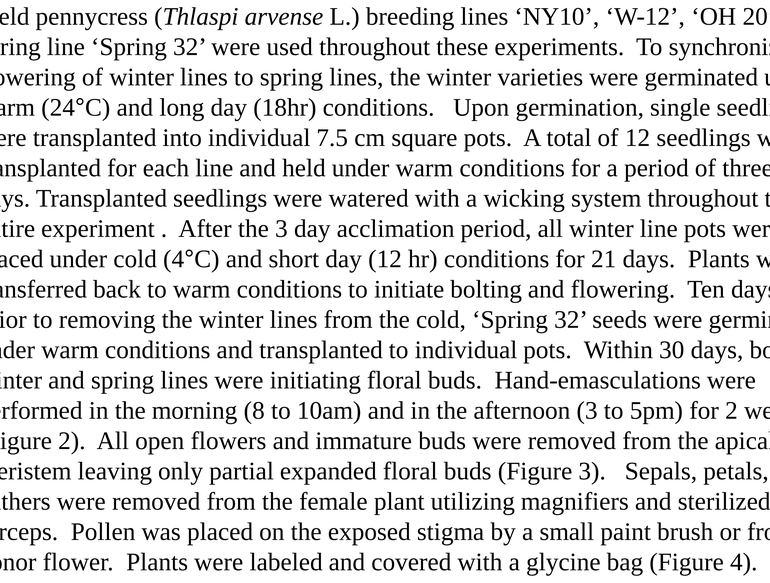

Figure 4. Completed crosses covered with glycine bags.

Table 2. Percentage of successful hand emasculations conducted in the morning and afternoon between winter and spring lines of pennycress.

Time of day	Crosses conducted	Successful fertilization	% fertilization
Morning	81	26	32.1%
Afternoon	65	29	44.6%

Pollen donor	Crosses conducted	Successful fertilization	% fertilization
Winter	62	29	46.0%
Spring	84	26	30.1%

Figure 5. Applications of 0.1, 0.2, 0.3, and 0.4 µg/ml tribenuron-methyl to pennycress plants. Photo taken two weeks after treatment.

RESULTS AND DISCUSSION

Results of the hand-emasculation experiment can be seen in Table 1 and Table 2. A total of 146 controlled crosses between winter and spring lines were conducted during the morning (8-10am) and afternoon (3-5pm) hours resulting in 32.1% and 44.6% fertilization, respectively. The spring to winter line crosses resulted in 46% fertilization, while only 30% of the winter to spring crosses were successful.

All applications of the sulphonylurea herbicide resulted in severe stunting of plants and a delay in flowering. Control plants flowered within 2 days and had excellent fertilization (Figure 5.), while the 0.1 µg/ml application flowered in 7 days with nearly 100% sterility. Applications of 0.2, 0.3, and 0.4 µg/ml resulted in severe yellowing of young tissue and had not flowered after 14 days of treatment.

CONCLUSIONS

Our experimental results indicate that hand-emasculations are possible but are extremely labor intensive and inconsistent. Crosses conducted in the afternoon appear to have a higher success rate; however, this may be an artifact of the person conducting the crosses. Application of 0.1µg/ml tribenuron-methyl appears to be an effective method of inducing male sterility with minimal delay in flowering. The development of a reliable induced male sterility protocol will greatly improve the efficiency of pennycress breeding and will help lead to the development of new varieties of pennycress.

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