

RAPID PROPAGATION OF THE SENSITIVE SPECIES *PHYSARIA DIDYMOCARPA* VAR. *LANATA* FOR RECLAMATION¹

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Abstract: In order to ensure sufficient plants for replanting threatened populations of *Physaria didymocarpa* var. *lanata* (Common Twinpod, Woolly Twinpod), *in vitro* propagation of this species was attempted. Seeds from three plants of *Physaria didymocarpa* var. *lanata* were cultured on a Murashige and Skoog salts base medium supplemented with 6-Benzylaminopurine. Both immature and mature seeds survived and germinated in culture. Each seedling culture produced several new shoots every four to six weeks. Each of these shoots was transferred to fresh medium to start new cultures. Roots were produced on excised shoots both *in vitro* on gelled medium and *ex vitro* on moistened coco fiber. Six months after initiating 100 seeds, 2,000 plants were ready for shipment to the nursery greenhouse and enough multiplying cultures were established to produce 1,000 new plants every two months. Though further work needs to be done on acclimatization to greenhouse conditions, survivors grew into normal robust plants, flowering in the pots.

Additional Key Words: tissue culture, micropropagation

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Introduction

Physaria didymocarpa (Hook.) A. Gray var. *lanata* A. Nelson (Common Twinpod, Woolly Twinpod) is a short-lived perennial plant listed as a sensitive species (Heidel and Handley, 2004). Several populations occur in Wyoming and Montana, including areas where habitat destruction has occurred. Though thought to primarily propagate by seed, (Heidel and Handley, 2004), seed source can be insufficient and unreliable. To ensure sufficient plants for reclamation purposes, employing all means of propagation (such as collecting seed, growing seed stock, rooting cuttings, and micropropagating plantlets) may be necessary. In this case, Bighorn Environmental Services (Dillon, MT), contracted with SMK Plants, LLC (Billings, MT), to attempt to micropropagate plants collected from a coal mine stripping area in southeast Montana. The objective of the project described here was to produce 2,000 microplants of *Physaria didymocarpa* var. *lanata* from three site-collected plants.

Micropropagation, also called tissue culture or *in vitro* propagation, involves taking plant material (explants) from a mother plant, placing it in contact with nutrients and growth regulators in a sterile, controlled environment and culturing for several weeks until tiny shoots are produced (George, 2008). At this time the shoot clumps are divided and placed on fresh medium and the process continues, with the cultures usually doubling every four weeks, until the desired number of plants is obtained. Shoots or clumps of shoots are then placed on a root promoting medium until roots begin to develop prior to shipment to the greenhouse, or they can be treated like tender cuttings for rooting directly in the greenhouse. Attempts to micropropagate a species are not always successful due to uncontrollable bacterial and fungal contamination or failure of the explants to thrive in the culture conditions. Culture protocols (explants, nutrients, growth regulators, growing conditions, etc.) vary greatly and have to be determined for each species. The health and growing conditions of the mother plant also affect the culture protocol and success of culturing, as does the seed source origin and adaptive strategy of the species.

A review of tissue culture literature and publications produced no references on *Physaria didymocarpa* var. *lanata* ever being micropropagated. Therefore, the first goal was to determine if this plant would survive and then multiply in *in vitro* cultures. After that, the goal was to produce the desired number and test the rooting capability of the microplants prior to shipment to the nursery.

Materials and Methods

In June 2007, three *Physaria didymocarpa* var. *lanata* plants from southeastern Montana that had been collected and potted by Bighorn Environmental Services, LLC were delivered to SMK Plants, LLC. The plants were healthy and turgid with undeveloped seed pods (Figure 1). The plants remained outside along an eastern facing wall throughout the summer. Plant material collected for culturing (explants) consisted of: side shoots, unexpanded seed pods, immature seeds from newly expanded seed pods, and floral stalks on June 8, 2007 (first collection); and seeds from attached and dehisced fruit on June 28, 2007 (second collection). All explants were initiated into culture on the day of collection.



Figure 1. Collected mother plants of *Physaria didymocarpa* var. *lanata* .

Following commonly used tissue culture procedures (George, 2008), collected plant material was surface disinfested by soaking in a Clorox(10%) plus Tween-20 (20 drops/L) solution for up to 12 minutes, then rinsed in sterile water and aseptically transferred to the propagation medium (Figure 2). The agar gelled culture medium (proprietary, SMK Plants) contained Murashige and Skoog salts (Murashige and Skoog, 1962), sucrose, vitamins, and the shoot promoting cytokinin 6-Benzylaminopurine. For the 2nd collection explants, the seed coat was left intact, removed, or cut prior to placement on the medium. All cultures were kept under lights (12-hr photoperiod) at

room temperature ($25\pm 3^{\circ}\text{C}$). Contamination (bacterial or fungal), survival, and germination were noted.



Figure 2. Disinfesting of explant during initiation.

After four weeks, surviving actively growing cultures were divided and transferred to fresh media every four to six weeks for six months.

During this time, rooting trials were conducted on some of the cultures. Over 400 elongated shoot tips or short-shoot clumps were excised and placed on a rooting medium similar to propagation medium but without 6-Benzylaminopurine and with or without the root promoting auxin Indole-3-Butyric Acid(0.1mg/L). *Ex vitro* rooting ability of shoots was also tested in the laboratory by excising 250 shoot tips of various sizes, dipping cut ends in rooting powder (Hormex No.1), sticking into moistened sterile coco fiber, and culturing under the same conditions as the *in vitro* cultures for six weeks.

Results and Discussion

From the first collection, the only surviving explants were the immature seeds. Of these, the earliest germination was one week after initiation (Figure 3). At the end of four weeks, five of the 30 immature seeds had germinated and were beginning to produce multiple shoots that could be divided to start new cultures. *Physaria didymocarpa* var. *lanata* is thought to reproduce primarily by seeds (Heidel and Handley, 2004), which may partially explain the success of the

seeds and failure of the collected shoots to grow in culture. Fast germination from a portion of the immature seeds indicated viable seeds with no physiological germination barriers.

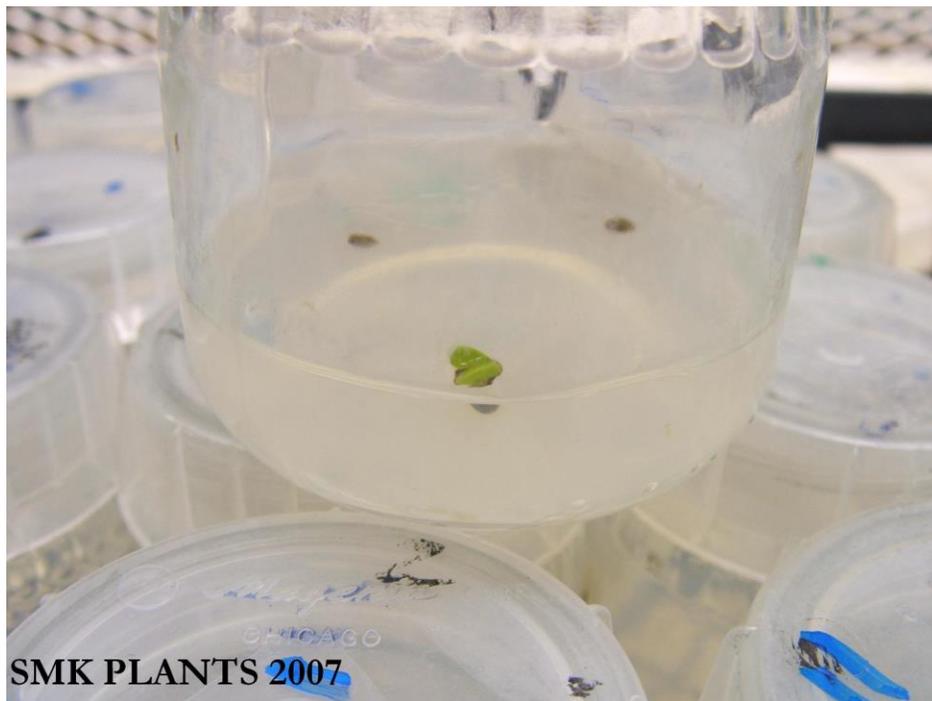


Figure 3. Germinating seed in culture.

Since it was not necessary for the resulting plantlets to be genetically identical to the three collected plants, the second collection for initiation consisted of maturing seeds from the collected plants. Seeds from this initiation began to germinate within one week. Removal or cutting of the seed coat was not necessary for *in vitro* germination and growth.

Contamination was less than 10% for both initiations. By July 25, 2007, 56 of the initiated seeds (54%) had germinated with many producing multiple shoots (Figure 4). Germination studies could not be found for this species, so it is unknown if this rate is similar to that in nature. Each seedling culture produced several new shoots every four to six weeks. Each of these shoots was transferred to fresh medium to start new cultures.

Two forms of shoots were noted: tight clumps with no or a few small internodes and short petioles, and loose clumps of elongated shoots with visible internodes and petioles (Figure 5). Both tight and loose clump forms have been noted in nature (Heidel and Handley, 2004) which may be manifesting itself *in vitro*. However, form variation in nature is attributed to different environmental factors whereas the *in vitro* environment was the same for both forms. Hyper-

hydricity, a waterlogged appearance of the plant cells, was noted in some cultures. Further research on optimizing the medium would be needed to address this problem.

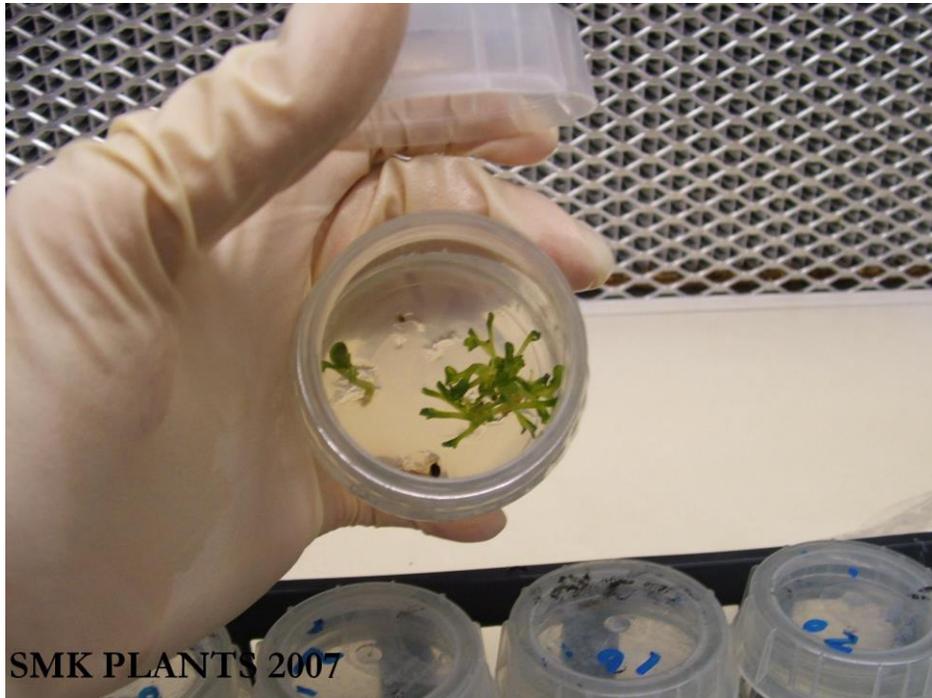


Figure 4. In vitro germination and production of multiple shoots.



Figure 5. Elongated shoot form with roots in gel.

Once the cultures were established, multiplication averaged 4x every five weeks. Three months after initiation, over 1,100 plants were growing in culture from the original 56 seeds. In other words, each seed produced an average of 20 plants in three months. At this time, shoots were removed from the propagation medium and transferred to rooting media.

Rooting occurred in four weeks on 30% of the shoots, regardless of the presence of an auxin. Though root primordia too small to observe may have been on more shoots, the low rooting rate suggests the need for more work in this area. Two types of root morphology were noted: very thin fragile and fibrous roots resembling root hairs, and thicker linear roots with some branching similar to 'normal' roots. This second root type may be more representative of the tap root *Physaria didymocarpa* var. *lanata* is noted to possess, though no strongly defined single tap roots were observed. This second root form was also more prevalent on elongated shoots (Figure 5) than short shoots and clumps. Both root forms were somewhat fragile making the plants difficult to remove from the gel and pot up without breaking.

To eliminate the *in vitro* rooting step and avoid root damage from removing from the culture gel, the rooting ability of the elongated shoots was tested on coco fiber (Figure 6). Rooting occurred in four weeks on over 50% of the shoots (Figure 7). The roots produced were similar to linear roots noted *in vitro*. Though more work is needed to improve rooting success, treating shoots as tender cuttings appears to be a viable option.



Figure 6. Microplants rooting in coco fiber.



Figure 7. Rooted microplants.

By February, 2008, 2000 microplants had been produced, given an *in vitro* rooting treatment, and shipped to the nursery for potting out. Standard nursery acclimatization and growing procedures for these plants proved inadequate as less than 20% (<400) of the microplants survived. Other methods, such as the coco fiber *ex vitro* rooting, need to be developed and tested in the greenhouse to increase survival. However, survivors grew into normal looking plants with similar morphology to seedlings grown concurrently in the same greenhouse. These plants were flowering in the containers by July, 2008 (Figure 8). *Physaria didymocarpa* var. *lanata* in nature is thought to flower within the first year (Heidel and Handley, 2004).

Conclusion

The primary objective of this project was to produce 2,000 microplants. This was accomplished within six months of obtaining the three mother plants using 56 viable seeds. Though the project ended at this point, enough cultures were produced and could have been maintained for continual plant production. Based on the multiplication rate of these cultures, maintenance of four nursery flats of *in vitro* cultures (400 cultures) could easily produce 1,000 microplants every two months year round.

Nursery survival rates were low (i.e. <20%). More work needs to be done on the rooting and acclimatization of the microplants to increase nursery survival to an acceptable level. Once these

problems are worked out, this tissue culture method for plant production could be valuable when viable seed or stock plants for seed production are limited.



Figure 8. Potted microplants (background with tags) and seedlings (foreground).

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