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PROPAGATION AND POPULATION AUGMENTATION FOR *PLATANTHERA PRAECLARA*, A THREATENED NORTH AMERICAN ORCHID SPECIES

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ABSTRACT. *Platanthera praeclara* is a threatened orchid native to the central region of North America. The orchid faces multiple threats in the western-most reaches of its native habitat. Federal and state permits allowed research on the ex-situ micropropagation of this species, which is difficult to propagate. The study resulted in lab-produced plants that were used for testing population augmentation. Cell biology and seedling development were documented by scanning electron microscopy to detect any nutrient reserves within the microscopic seeds and to illustrate tissue structure. Field trials were preliminary tests only, to be used as a basis for intervention in the event that the species should face immediate threat of extinction in Nebraska. Experimental field trials were conducted on the Valentine National Wildlife Refuge in the sandhills of Nebraska. Seedling survival was low for in-vitro produced plants, with mortality particularly heavy in years characterized by drought.

Key words: micropropagation, reintroduction, augmentation

INTRODUCTION

Platanthera praeclara is a threatened orchid species native to seven states in the USA and the Canadian province of Manitoba. The species faces multiple threats in the Great Plains Region, including habitat destruction and population fragmentation. Inbreeding depression and declining pollen vector populations are two possibilities suspected in the low reproductive rates of the species at the remaining isolated habitats. To further conservation efforts, research was undertaken to develop ex-situ propagation protocols, using asymbiotic methods and histological studies of plant tissues to document cell development, as it relates to the life history of the orchid. The species had resisted asymbiotic germination and survival in previous propagation studies. After successful germination protocols were developed, seedlings were sub-cultured in-vitro for an additional year of growth and subsequently were planted in preliminary field trials for population augmentation at the site of origin near its cohorts. A seedling produced in-vitro can take up to 3 years in aseptic culture to reach a size large enough for planting into the natural environment.

METHODS AND MATERIALS

The objectives of the study were to develop reproducible in-vitro seed germination protocols; to record plant tissue structures by scanning electron microscopy (SEM), to better understand the cell biology, growth cycle, and nutritional requirements of *Platanthera praeclara*;

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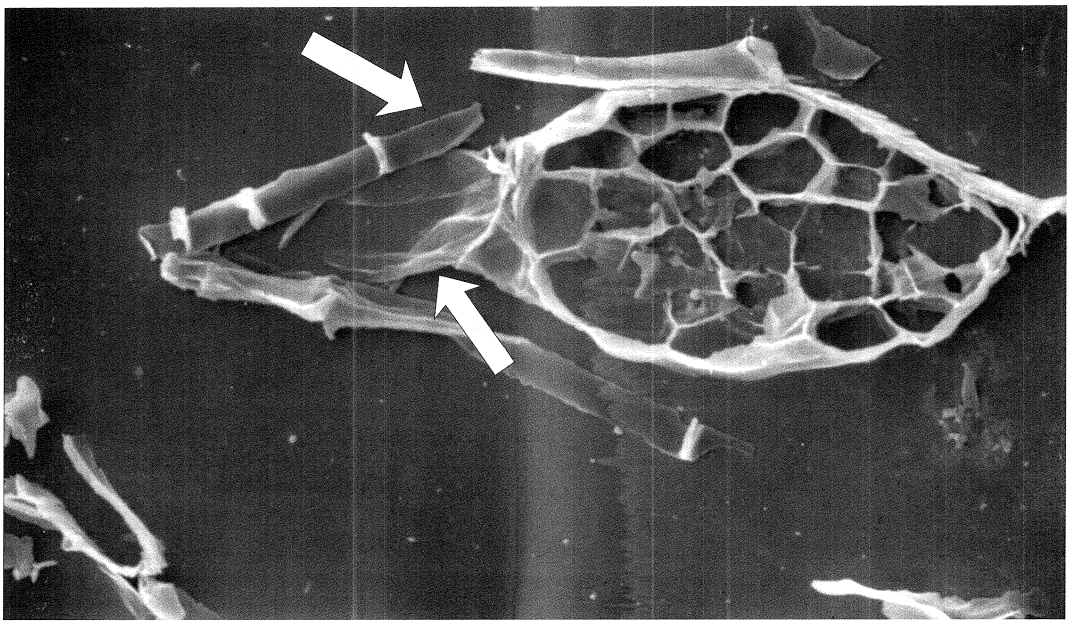

FIGURE 1. Seed semi-thin section showing individual cell walls of *Platanthera praeclara.* The ovoid embryo shows no distinct polarity, and suspensor remnants are still visible (SEM at 312×).

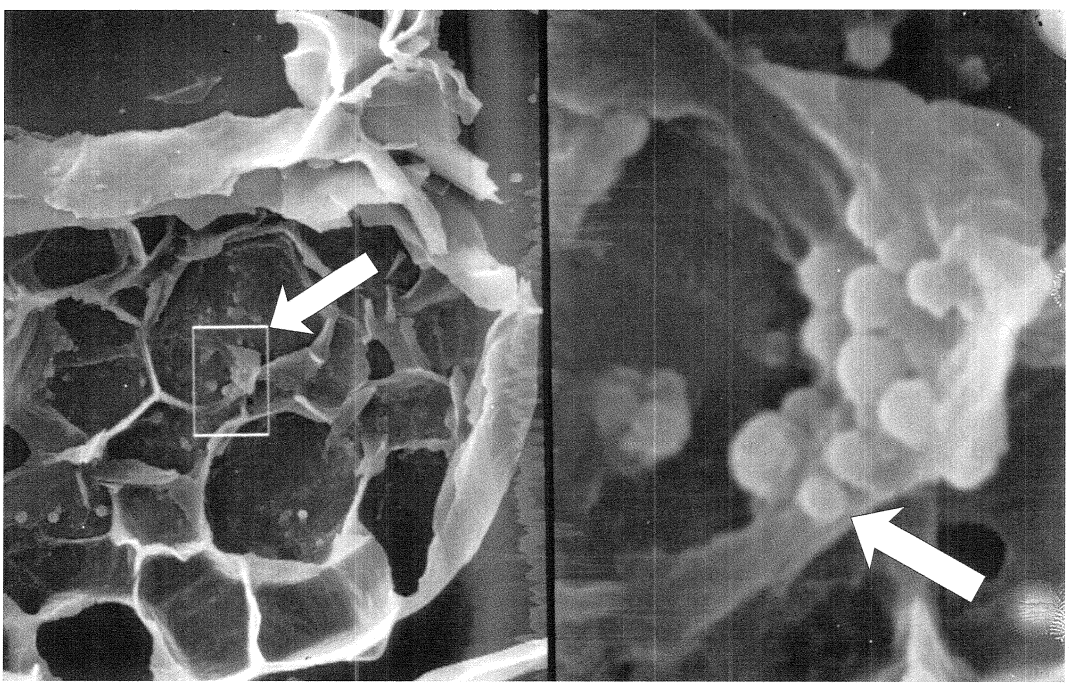

FIGURE 2. The SEM at 625× on the left shows a cluster of spherical bodies inside a single embryonic cell (highlighted rectangle). Those same bodies are magnified to 836× SEM on the right. Elemental analysis of those bodies is shown in FIGURE 7. Nutrient reserves within the seed embryo are extremely small, but any reserves are critical for successful germination and survival.

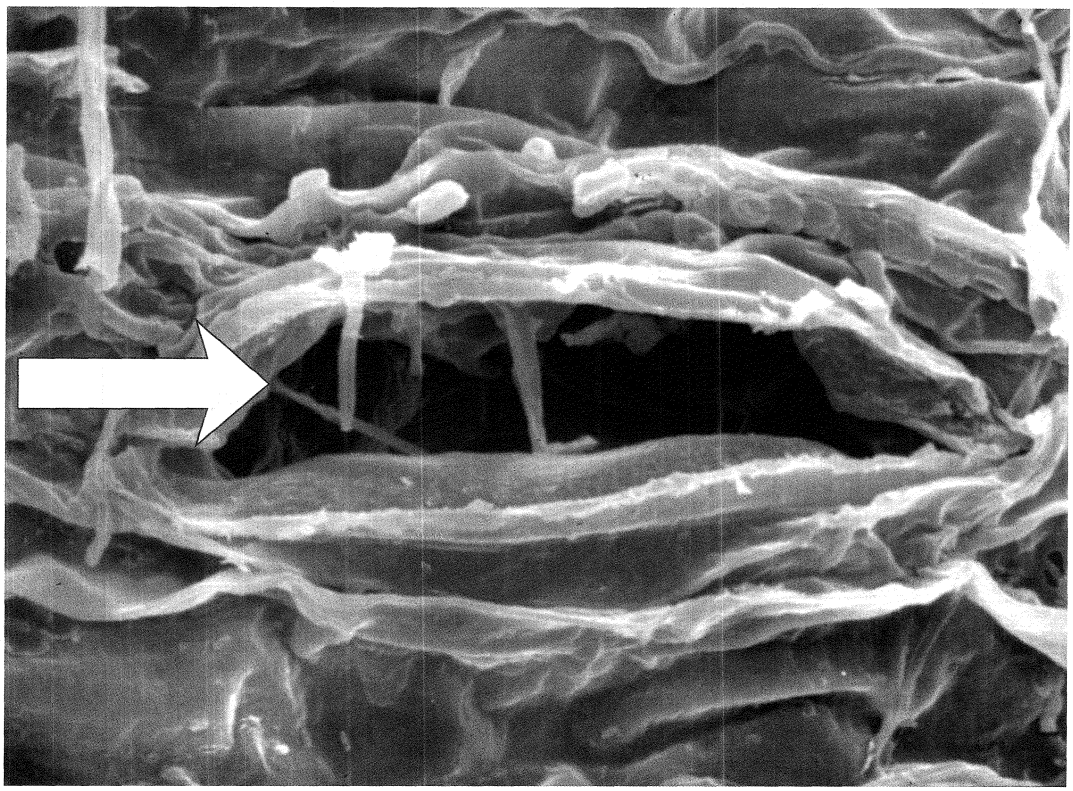

FIGURE 3. A leaf surface magnified to 1010× SEM demonstrates that seedlings produced in-vitro can successfully develop stomatal pores on their leaf tissues.

to detect and analyze nutrient reserves present in seeds by SEM spectrum graph; and to conduct field trials for population augmentation.

Mature seeds were disinfested with a 10% commercial bleach (5.25% v/v NaOCl) solution for 15 min., rinsed in sterile distilled water, and aseptically cultured on three different media. Cultures were incubated at 23 ± 2°C for 6 weeks, followed by 60 days at 5 ± 2°C in darkness. Germination is defined here as embryo exit from the testa, development of hair-like rhizoids, and shoot and root initiation.

Histological Studies

Seeds and plant tissues were treated with an ethanol series, sputter-coated with gold and examined by SEM, using a novel scanning process (Cano et al. 1986). The following tissues were documented: seed interior structures, nutrient reserves within an embryo, stomatal pore structure on leaf surface, root parenchyma cells, hollow stem tissue with vascular bundles, and rhizoid formation. For cell structures and nutrient reserves, see FIGURES 1–7.

Field Trials

Micropropagated seedlings, 2–3 years old, were planted in 2000 on the Valentine National Wildlife Refuge. It is generally believed that the species develops a symbiotic association with specific soil microorganisms for some part, if not all, of its life cycle. Seedlings were planted in proximity to their wild cohorts in an effort to provide the conditions that the species may require for successful establishment.

Germination Results

Platanthera praeclara seeds were pre-treated with the bleach solution and cultured on three medium formulae in experiments spanning three trial dates over a 2-month period in 1997. The media used were ⅓ strength MS (Murashige & Skoog 1962), VW (Vacin & Went 1949), and MFAF (Fast 1982) with modifications (Anderson 1990). In TABLE 1, the values represent the percentage of seeds that exhibited ovoid embryos within the testae, which subsequently formed

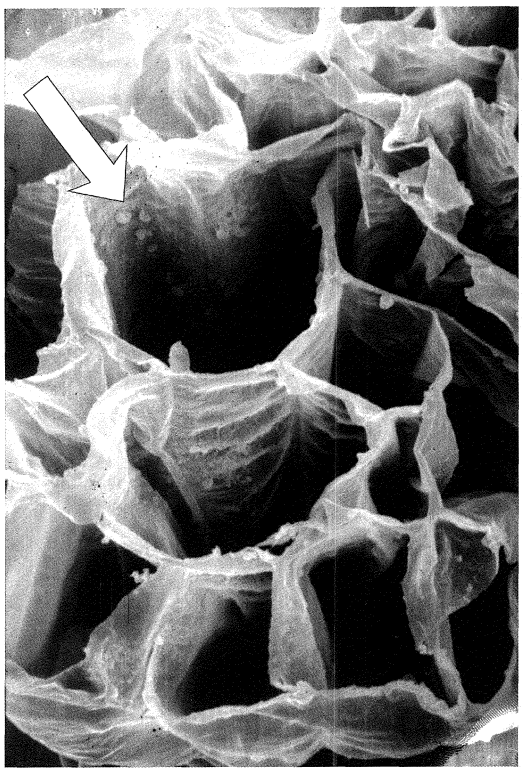

FIGURE 4. Thin-walled parenchyma cells make up the root cortex for *Platanthera praeclara* and display a typical polyhedral shape. Some cells possess a peg-walled interior surface that may be part of the vascular system (SEM at 3240×).

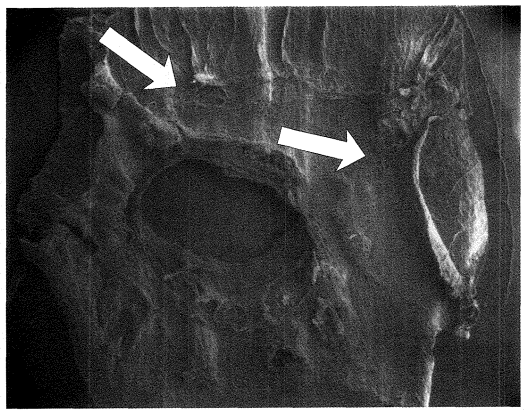

FIGURE 5. Vascular bundles are evident in the cross section of a stem. The image also shows that the interior of the stem is hollow (SEM at 390×).

protocorms and eventually produced leaf-bearing plants. Seed testae that did not possess embryos were excluded from the total seed counts.

ANOVA was performed on the Randomized Complete Block Design (TABLE 2). There was no significant difference among the three media tested at $P < 0.05$ (TABLE 1). Media differed at $P < 0.10$, however, with the most germination on MS after 12 months in culture. The F-test was 5.03 for medium, obtained by dividing the mean square for treatment by the mean square for date × treatment to detect media differences in the replications.

CONCLUSIONS

Platanthera praeclara was successfully propagated asymbiotically in this early study, with protocorm survival for >12 months. No significant difference among asymbiotic media at the $P < 0.05$ level may indicate that the species is capable of germinating on more than one substrate. Seed condition and scarification pretreatments are factors that need to be carefully determined prior to culture. Visual observation supported the assessment that protocorm enlargement and survivability were good on ⅓ MS medium. The species may show a trend toward MS as a preferred medium. MS is a defined medium with a broader range of macronutrients and micronutrients as compared to MFAF and VW,

TABLE 1. Germination percentage for *Platanthera praeclara* on three medium formulae. Each value is the mean for three replications ± SE, after 12 months in culture.

Date of replication	Germination % on three media		
	MFAF	VW	MS
Date 1	2.25	3.65	5.46
Date 2	1.37	0.00	2.02
Date 3	1.42	2.46	3.80
Means	1.68 ± 0.49	2.04 ± 0.91	3.76 ± 1.04

Note: Media follow MFAF = Fast (1982) with modifications by Anderson (1990), VW = Vacin & Went (1949), MS = Murashige & Skoog (1962). Means were not significantly different at the $P < 0.05$ level, LSD = 1.93 at $P = 0.05$, LSD = 1.48 at $P = 0.10$.

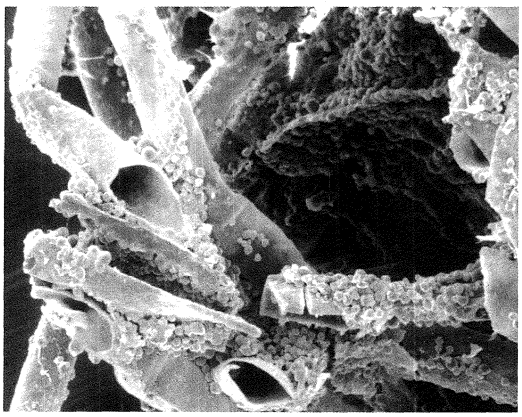

FIGURE 6. Rhizoids are simple, unicellular structures that are extensions of epidermal hairs, which help create a greater interface with the substrate (SEM at 745×).

and it also is high in potassium and nitrogen. The nitrogen is in the ammonium nitrate form (NH_4NO_3). More protocol refinements continue to be made that support the hypothesis that seed germination can be improved further than was the case in the initial experiments reported above.

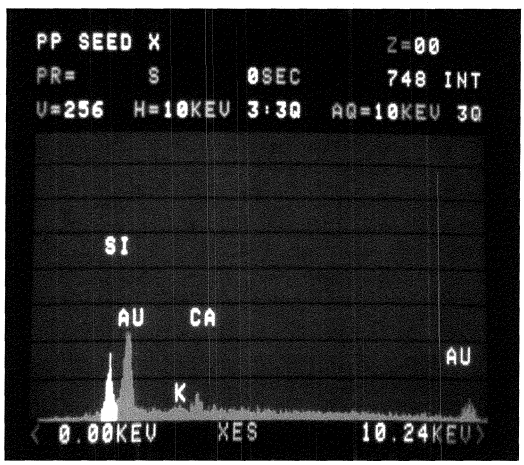

FIGURE 7. Elemental analysis of nutrient bodies contained in a single orchid embryonic cell (see FIGURE 2). Calcium (Ca) and potassium (K) produced the highest peaks. The spectrograph qualifies the elements present but does not quantify them. Gold (Au) and silicon (Si) register peaks because of the surfaces on the microscope slide holding the seed that were gold-coated in a process developed by Cano et al. (1986). Calcium is a component of cell walls, and potassium is an early component of plant sugars needed by a developing embryo to support metabolic processes.

FIGURE 8. An adult plant of *Platanthera praeclara* bloomed 3 months after being planted out from in-vitro culture.

Individual seedlings were photographed prior to planting out to document which seedlings survived under ex-vitro conditions. Seedling survivability was low in the first test trials, generally <25%. Some seedlings that were planted out were visible as a perennating bud above the soil surface later in the growing season, and an adult plant (FIGURE 8) bloomed three months after planting out from in-vitro culture. The seedlings are particularly sensitive to root disturbance or damage, both in-vitro, or when being planted in soil. Damage to the brittle, fleshy roots often results in browning or physical damage to the root tip's meristematic region, which in turn causes almost 100% mortality. Further study is warranted to monitor the lab-produced seedlings through several more annual cycles. Recording annual temperatures and precipitation levels, and how those factors affect the seedlings' overall survivability, may be necessary for at least ten years in order to draw accurate con-

TABLE 2. ANOVA with mean squares for germination of *Platanthera praeclara* on three media formulae.

Source	df	*SS*	*MS*	*F*	*P*
Date	2	42.369	21.185	—	—
Medium	2	26.793	13.396	5.03	0.081*
Experimental error	4	10.650	2.662	—	—
Sampling error	24	183.530			

Note: df = 2 for date, and df = 2 for medium; *SS* = 42.369 for date, and *SS* = 13.369 for medium; $F = 5.03$; $P = 0.081$.

* Media differed at $P < 0.10$ but not at $P < 0.05$.

clusions about the merits of further population supplementation at the site.

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