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Seed Cryostorage and Micropropagation of Georgia Aster, *Symphyotrichum georgianum* (Alexander) Nesom: A Threatened Species from the Southeastern United States

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***Abstract. Symphyotrichum georgianum* (Asteraceae), commonly known as Georgia aster, is a candidate for listing under the Federal Endangered Species Act in the four southeastern U.S. states where it lives. Rarity of this species is thought to be attributable in part to small population sizes and limited seed production. Protocols for in vitro germination, sustainable shoot micropropagation, shoot establishment in soil, and seed cryopreservation are presented that will assist in the safeguarding and augmentation of dwindling natural populations. Germination in vitro on growth regulator-free half-strength Murashige and Skoog (MS) medium after sterilization in H_2O_2 initiated the development of shoot cultures. Shoot multiplication and elongation occurred on half-strength MS salts containing 0.1 mg·L^{-1} benzylaminopurine and 0.2 mg·L^{-1} gibberellic acid, producing an average of 18 new shoots over a 6- to 8-week subculture cycle. Shoots rooted easily when planted into cutting mix after treatment with rooting powder containing indole-3-butyric acid (IBA) or in vitro rooting in medium with or without N-acetyl-L-aspartic acid (NAA). Plant survival after 1 month was 90% or higher for all treatments. Cryopreservation tests with seeds from three populations averaged 46.7% germination compared with control seed (no cryostorage) germination of 43%; differences were not statistically significant. Fresh seeds and seeds equilibrated for 1 to 4 weeks at room temperature and 12% relative humidity did not differ significantly in germination post-cryopreservation. Initial observations suggest that Georgia aster rapidly loses seed viability over 1 to 2 years when stored at room temperature. The ability to increase seed longevity through cryopreservation storage may be a critical step in the conservation of this species.**

Symphyotrichum georgianum (Alexander) Nesom (syn. *Aster georgianus*), commonly known as Georgia aster, is a perennial member of the sunflower family (Asteraceae). Georgia aster is native to open, grassy meadow habitats and has been steadily losing its natural habitat mainly as a result of forest fire prevention and extirpation of large native grazing animals. It is now classified as a vulnerable species with a moderate and imminent threat of extinction (Federal Register, 2009; USFWS, 2010). The species is currently found in south–central North Carolina, South Carolina to central Georgia, west to central Alabama, and on the Coastal Plain of southwest Georgia. There are 127 known populations, many of which have not been seen in 10 years and 23 of which have been either extirpated or not observed for over 20 years (USFWS, 2010; Weakley, 2011). Historic populations in the eastern Panhandle of Florida are considered extirpated. Extant populations typically have 10 to 500 stems often containing only one or a few genotypes (Federal Register, 2009; USFWS, 2010).

Georgia aster grows up to 100 cm in height and flowers in early October to mid-November with 5-cm diameter heads having dark purple rays surrounding white (occasionally purple) disc flowers with purplish tips, purplish anthers, and whitish pollen (Chafin, 2007; Georgia Department of Natural Resources Wildlife Resources Division, 2008; Weakley, 2011). Successful pollination results in the production of achene-like fruits (cypselae) that each contain a single seed and embryo. However, the species reproduces mostly asexually from rhizomes (USFWS, 2010). The habitat it now most often occupies include roadsides, utility rights-of-way, and openings where land management mimics the roles formerly played by fire and a dry upland habitat. Unfortunately, these areas also leave Georgia aster at risk to invasive species such as kudzu, herbicides, road expansion, and development. Herbicide use can further fragment populations, potentially reducing population persistence and survival in small isolated patches.

In vitro propagation from seed, shoot tips, and rhizomes can multiply the number of individuals of an endangered species rapidly and continually (Fay, 1992; Reed et al., 2011). Once enough plants are available, they can be used to repopulate old populations or to establish new populations for conservation, research, education, or recreational purposes.

Cryopreservation is emerging as a reliable process for seed conservation and long-term storage of many desiccation-tolerant seeds and is likely to maintain seed viability for longer periods than conventional storage at –18 °C (Englemann, 2011; Li and Pritchard, 2009; Pritchard, 2007; Reed et al., 2011). Seed cryopreservation may play a particularly important role in the conservation of threatened plants because safeguarding or propagating material from seed maintains new genetic combinations resulting from sexual reproduction (Pence, 1991).

The objectives of this study were to develop a reliable micropropagation protocol for threatened Georgia aster using seeds to start cultures and to investigate the feasibility of long-term seed storage through cryopreservation.

Materials and Methods

Plant materials, experimental design, and evaluation

A small number of *S. georgianum* seeds collected in Nov. 2009 from two locations designated as CP and PM in Cobb County, GA, were stored at 22 to 25 °C until germination tests occurred in 2010. Seeds were also collected in Dec. 2010 from three Union County, SC, sites (SC5, SC7, SC8), stored at 22 to 25 °C, and used during winter and Spring 2011.

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