

8B-Trigiano-Bob-2018

Whorled Sunflower (*Helianthus verticillatus*) – A Potential Landscape Plant[©]

R. N. Trigiano^{1,a}, S.B. Wilson², and C.N. Steppe²

¹University of Tennessee, Department of Entomology and Plant Pathology, Knoxville, Tennessee, 37996-4560, USA; ²University of Florida, Department of Environmental Horticulture, Gainesville, Florida, 32611-0675, USA.

^aE-mail: rtrigian@utk.edu

Keywords: Clonal propagation, seed propagation, cuttings, tissue culture, endangered species, plant breeding

INTRODUCTION

Helianthus verticillatus or whorled sunflower is a recently designated endangered species found only on a few prairie remnants in Tennessee, Georgia, and Alabama. It is a perennial species that spreads via rhizomes (Mathews et al., 2002) and produces copious amounts of small, black or gray seeds if compatible genotypes are present. This sunflower species is self-incompatible (Ellis and McCauley, 2009). Plants emerge in late winter in Tennessee as a basal rosette (Fig. 1) and reach 3-4 meters by August (Fig. 2). It flowers profusely in August and September, and through the middle of October or until a killing frost. The plants bear up to 20, 4-6 cm diameter yellow ray flowers with brown to black discs at the top of the stalks. The flowers attract over 30 species of potential pollinators, which include mostly native bees (Fig. 3) and flies, and a variety of moths and butterflies. Inflorescences are covered with pollination bags when brown and shriveled, and seeds collected in November after the flowers are thoroughly dried (Fig. 4). This sunflower also appears to be drought tolerant.

The specific aims of this research were to determine germination conditions for seeds and to develop clonal propagation methods that utilize tissue culture and rooted cuttings.

MATERIALS AND METHODS

Seed viability and germination. Seeds were collected from a 200 stem stand growing in Maryville, Tennessee in November 2017. Seeds were separated from somatic tissues, stored in glass vials at room temperature, and shipped via postal service to the University of Florida in early February 2018. Seed viability was assessed with 100 seeds using a tetrazolium staining method (Peters, 2005) over 18-24 hours. Seeds with embryos that evenly stained dark pink- to - red were considered viable (Fig. 5). Germination of seeds was evaluated using four replications of 100 seeds (AOSA, 2016) each at the following four temperature regiments: 22/11⁰C; 27/17⁰C; 29/19⁰C; and 33/24⁰C with a 12- hour photoperiod. Tests were conducted over 28 days. Post-germination tetrazolium tests were performed on un-germinated seeds after 28 days and percent dormancy, total viability, and germination of viable seeds determined.

Clonal Propagation - Leaf culture. Young, not fully expanded leaves of less than 0.5-meter tall plants were harvested in April and surface disinfested in 25% NaOCl for 10 min and then rinsed thoroughly with sterile distilled water. Dissected leaves were placed on Murashige and Skoog (MS) (1962) medium amended with a range of concentrations of various cytokinins with and without auxin and cultured at 22⁰C with a 12- hour photoperiod. Explants were transferred to MS medium without growth regulators after two weeks and incubated under the same conditions for the next four weeks. Explants were assessed for callus, shoot, and root formation after six weeks.

Clonal Propagation - Nodes/Axillary buds. Nodes, each with two or three axillary buds, were harvested from less than one-meter plants. Leaves were removed and the nodal

segments surface disinfested in 25% NaOCl for 15 min and then rinsed in sterile distilled water. Nodes were transferred to 60 mm diameter plastic test tubes containing MS medium supplemented with a range of concentrations of various cytokinins. Cultures were incubated at 22⁰C with a 12-hour photoperiod. Shoots were harvested after four weeks and rooting attempted on MS medium amended with auxin.

Clonal Propagation – Cuttings. The terminal 15 cm of young plants were harvested in late May 2017 and either treated with IAA or water for 10 min. Shoots were then placed in Promix B. In a second experiment, the upper 45 cm of plants were harvested from plants in late June 2017. Three node cuttings were placed in Promix B after a 10 min treatment with either IAA or water. Cuttings were rooted in the greenhouse with mist. Rooting was determined four weeks after treatment.

RESULTS AND DISCUSSION

Seed viability and germination. Greater than 98% of the seeds were viable (Fig. 6). Seeds did not lose viability within 7 months of dry ambient storage. Germination of seeds occurred in all treatments, but only about 77% of the seeds in the high-temperature treatment germinated within 28 days. In the other three treatments over 96% of the seeds germinated. Fifty percent of the seed in the coldest treatment germinated within 7-8 days, whereas 50% of the seed in the three warmer treatments germinated within 1-2 days. Seeds are easy to germinate and will be critical in breeding efforts that combined resistance to powdery mildew and flower morphology.

Tissue culture. More than 50% of cultures became contaminated within ten days of culture initiation. Very few shoots were formed from explants cultured on medium containing cytokinin (Fig. 7). However, leaves cultured on MS without growth regulators formed numerous

roots and some callus. More than 75% of the nodes cultured became contaminated with bacteria and fungi after two weeks and were discarded. On the remaining 25% of the cultures, axillary buds elongated on medium with or without cytokinins, but excised shoots failed to produce roots. Additional shoots did not form on nodes transferred to fresh medium. Preliminary results suggest that tissue culture propagation methods do not seem to be well suited to this sunflower, but additional studies are necessary. Besides controlling contamination, shoot proliferation appears to be very problematic.

Cuttings. Almost all (98%) of the cuttings harvested in late May formed robust root systems after four weeks when treated with auxin or water (Fig 8). These plants grew well and flowered normally in September. Only about 20% of the three node cuttings harvested in late June produced roots in response to either water or auxin treatment. Only one surviving whole plant flowered in September. Propagation of plants by terminal cuttings harvested in late May is very efficient. Rooted cuttings typically produce as many as four additional shoots via rhizomes during the growing season. All of these are clonal, which is essential for determining compatibility and controlled (not open pollination) breeding for desirable traits.

SUMMARY

We concluded from these preliminary experiments that seed propagation is easy and efficient, although because of self-incompatibility, the attributes of the seedlings may vary widely. *H. verticillatus* does not seem to be amenable to clonal propagation using tissue culture techniques. Obtaining axenic cultures and shoot and root formation are very problematic. Cuttings taken from young plants in late May form robust root systems in four weeks without an auxin treatment. Cutting propagation appears to be the best method to produce clonal materials.

ACKNOWLEDGEMENTS

Authors thank J. Jablonski for technical assistance and E. van Santen for statistical expertise.

Literature Cited

Association of Official Seed Analysts (AOSA). (2016). Rules for testing seeds. 16:1-113.

Ellis J.R. and D.E. McCauley. (2009). Phenotypic differentiation in fitness related traits between populations of an extremely rare sunflower: conservation management of isolated populations. *Biol. Conservation* 142: 1836-1843.

Matthews J.F., Allison J.R., Ware R.T., Sr., and Nordman C. (2002). *Helianthus verticillatus* Small (Asteraceae) rediscovered and redescribed. *Castanea* **67**: 13-24.

Murashige T. and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.

Peters, J. (2005). Tetrazolium testing handbook. Association of Official Seed Analysts, Las Cruces, NM.



Figure 1. *Helianthus verticillatus* emerging in the spring.

EARLY AUGUST**MID-TO-LATE SEPTEMBER**

Figure 2. Rapid vegetative growth (early August) and prolific flowering on tall stalks of *Helianthus verticillatus* in September.



Figure 3. In the center of the flower is a native bee (*Bombus* sp.), a potential pollinator of *Helianthus verticillatus*.



Figure 4. Pollination bags covering multiple heads of *Helianthus verticillatus*. Seeds were harvested in November 2017.



Figure 5. Seed germination and viability studies of *Helianthus verticillatus*.

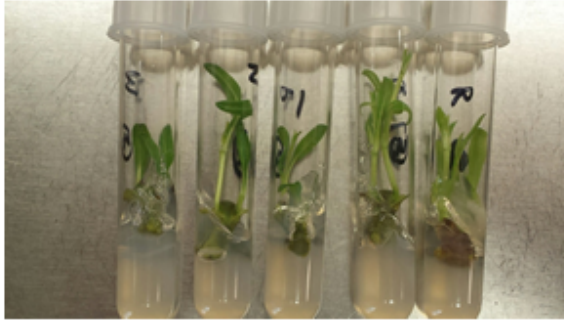


Results

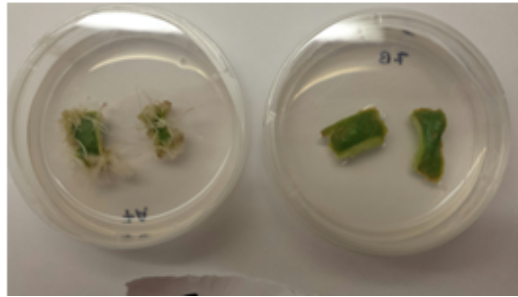
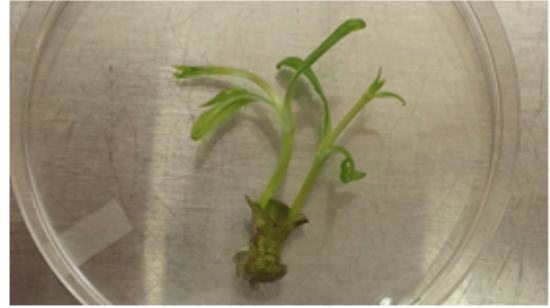
- **Pre-germination (n=100)**
 - TZ viability = 91% positively stained
 - X-ray analysis= 95% filled seed
- **Germination (n=400)**
 - High (96-99%) at 22/11, 27/17 and 29/19°C
 - Reduced (76%) at warmer temp (33/24 °C)
 - Fast germination rate: 50% germination was achieved by day 2 (27/17°C, 29/19, or 33/24°C) or day 7 (22/11°C)

Figure 6. Results of seed germination studies of *Helianthus verticillatus*.

Clonal Production via Tissue Culture



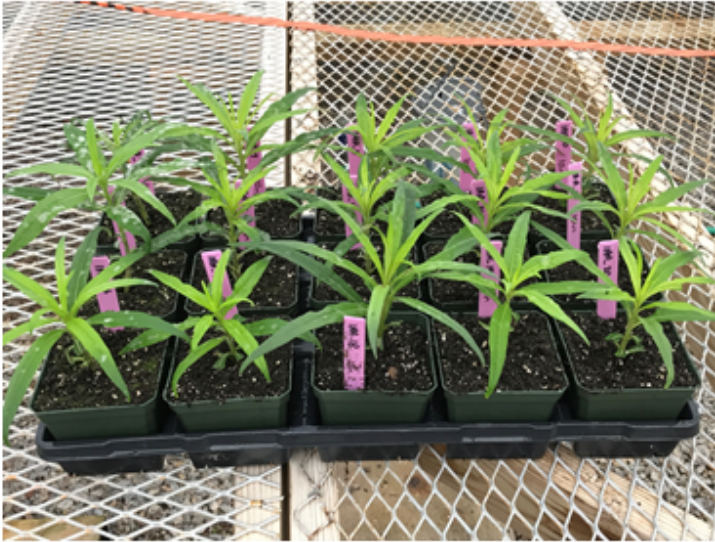
From Axillary Buds



From Leaves

Figure 7. Clonal propagation by tissue culture using explants from axillary buds and leaves of *Helianthus verticillatus*.

Clonal Production by Cuttings – Late May
(Late June is poor)



Three to Four Weeks



Figure 8. Clonal propagation by cuttings of *Helianthus verticillatus*