Field Establishment of Tissue Culture-derived *Trillium* grandiflorum[©]

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INTRODUCTION

Trilliums are high-value, garden-worthy plants that are uncommon in the nursery trade due to propagation difficulties. Many herbaceous plants that are conventionally difficult to propagate rely on tissue culture micropropagation. Tissue culture micropropagation involves the clonal production of shoots that are rooted and reestablished under field conditions. Shoot or rhizome production of trilliums in vitro is relatively easy. The purpose of this research was to develop reestablishment protocols for rhizomes of *Trillium grandiflorum*.

MATERIALS AND METHODS

Plant Material. Cultures of *T. grandiflorum* clones 6, 7, and 11 were initiated from seeds.

Tissue Culture. Basal medium contained ½ MS (Murashige and Skoog, 1962) and (in mg liter $^{-1}$) 15,000 sucrose, 0.5 nicotinic acid, 0.5 pyridoxine HCl, 0.4 thiamine HCL,10,000 myo-inositol, 2 glycine, and 8000 Phytagar. Proliferating cultures were maintained on basal medium supplemented with (in mg liter $^{-1}$) 1 BA and 0.3 2,4-D. For root initiation, rhizomes were cultured on basal medium supplemented with 1 mg liter $^{-1}$ IBA. Medium pH was adjusted to 5.7 to 5.8 prior to autoclaving at 124 kPa for 15 min.

Field Establishment. Prior to field establishment, rhizomes were placed on rooting medium and chilled at 4° C for 1 month (Experiments 1 and 3) or 4 months (Experiment 2). Rhizomes were planted under 63% shade either directly into a raised field bed [peat, sand, and silt clay soil (1:1:1, by volume)] or into individual 3% inch \times $5\frac{1}{2}$ -inch tree bands, 25 bands per flat (MetroMix 510). Data were collected April 2000.

Experiment 1. Rhizomes of clones 6, 7, and 11 were planted directly into a raised field bed on 28 Sept. 1996.

Experiment 2. After chilling, rhizomes of clone 11 were placed on a counter for 5 months at 23°C prior to potting into tree bands and being placed in a lath house on 1 Aug. 1997.

Experiment 3. Rhizomes of clones 6, 7 and 11 were potted into tree bands and placed in a lathhouse on 26 Oct., 12 Nov., and 11 Nov. 1997, respectively.

RESULTS

Experiment 1. On average, rhizomes from T. grandiflorum clones 6 and 7 weighed more initially than rhizomes from clone 11 (Table 1). After 42 months in the field, while rhizomes from clones 7 and 11 weighed, on average, twice as much as those from clone 6 (11.5 vs. 5.5 g), a greater percentage of rhizomes from clones 6 and 7 flowered compared to clone 11. There appears to be a direct correlation between initial rhizome mass and time-to-flower; rhizomes that weighed more initially flowered sooner.

Experiment 2. Limited flowering was initiated 33 months after field establishment (Table 1). Rhizome weight at time of planting appears to effect time-to-flowering as nonflowering rhizomes weighed less, on average, that flowering rhizomes (Table 1).

Experiment 3. On average, the initial rhizome weights of clones 6 and 7 were approximately double the weights of rhizomes from clone 11 (Table 1). Clone 11 exhibited the highest survival rate, clone 7 the lowest. Clone 11, with the lowest initial weight rhizomes, exhibited the highest survival rate. Limited flowering (clones 6 and 11) began 30 to 31 months after field establishment.

Variegated and six-leaved forms were generated as a result of being tissue cultured (date not shown).

CONCLUSIONS

Trillium grandiflorum rhizomes generated in tissue culture survived and grew under field conditions. There were clonal differences in initial rhizome weight. There were clonal differences in survival rate. Initial rhizome weight does not appear to affect survival rate. Limited flowering is possible 30 to 33 months (second spring) after field establishment.

Table 1. Effect of initial rhizome weight	t on survival and flowering ^z in <i>Trillium</i>
grandiflorum grown in the field.	

	Initial average rhizome weight (mg)					Flowering	Dead
Clone	N	Live	Flowering	Non- flowering	Dead	(%)	(%)
Experime	nt 1						
11	32	0.31	0.55	0.30	0.22	6	22
6	26	0.53	0.65	0.47	0.43	31	19
7	14	0.59	0.82	0.43	0.74	36	14
Experime	nt 2						
11	121	0.47	0.70	0.43	0.31	8	3
Experime	nt 3						
11	87	0.40	0.74	0.42	0.47	4	13
6	32	0.94	1.00	0.93	1.01	18	36
7	35	0.85	-	0.76	0.71	-	68

^zFlowering as of April 2000.