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Using Peat Pellets in Liquid Media to Root Sunflower Tissue Culture Plants

C.H. Pearson*, K. Cornish, C.M. McMahan, M. Whalen, D.J. Rath, N. Dong, and S. Wong

Traditional plant breeding is often limited by the genetic diversity within a species. The use of biotechnology allows introducing into a plant, specific traits that come from the same or another plant species. In this paper, we focus on tissue culture of sunflower (*Helianthus annuus* L., Asteraceae), particularly related to rooting transgenic sunflower plantlets. Sunflower is notoriously recalcitrant to genetic transformation and regeneration when subjected to tissue culture. Prior to 1985 there had only been four reports, as noted by Paterson and Everett (1985), of successful regeneration of sunflower plants in tissue culture. Although this report is more than 20 years old, sunflower continues to be a challenging species in tissue culture (Baker et al. 1999; Dhaka and Kothari 2002; Hewezi et al. 2002; Radonic et al. 2006) including rooting (Paterson 1984; Gürel and Kazan 1998). The objective of our study was to compare agar and liquid media for rooting transgenic sunflower plantlets.

MATERIALS AND METHODS

A study was conducted at the Colorado State University Western Colorado Research Center at Fruita to compare agar and peat pellet/liquid media used for rooting transgenic sunflower plantlets. Rooting medium was prepared in 3 L quantities as described in Table 1. Liquid rooting medium (100 mL) was poured into a small media bottle and 0.70 g of Caisson agar # A038 (Caisson Laboratories, Inc., North Logan, Utah) was added. The remaining rooting medium of the original 3 L quantity was split into two equal portions and 10.5 g of Caisson agar was added to half of the medium. The other half remained in liquid form. Jiffy-7 peat pellets (No. 730) were positioned in the bottom of 27 of 54 Magenta® boxes. Using a liquid medium in a container such as Magenta boxes requires a lid that is vented (Huang and Chen 2005). Vented lids were made by drilling a 17-mm hole in the center of the lid of the Magenta box. A 30-mm square piece of Milli-Wrap™ (Millipore Corporation, Bedford, Massachusetts) material with a pore size of 0.45 µm was secured over the hole in the lid with autoclave tape. Vented lids were used only on Magenta boxes containing liquid medium. Using vented lids on Magenta boxes containing an agar-based medium typically results in drying and cracking of media (Walker et al. 1989). Media, peat pellets, Magenta boxes, and lids (vented and non-vented) were sterilized by autoclaving.

When liquid medium was cool enough to handle, but still hot, 40-mL of medium was poured into each of 27 boxes with the peat pellets. Forty mL of medium containing agar was poured in each of 27 boxes (without peat pellets). Lids were placed on each Magenta box and the media cooled overnight in a sterile hood.

Explants of cultivar '665' containing the target gene were produced at the USDA-ARS-WRRC at Albany, California and shipped to Fruita, Colorado during December 2004. Fifty-four putative transgenic sunflower

Table 1. Rooting media used for plantlets to compare peat pellet/liquid rooting method and semi-solid agar based method at the Western Colorado Research Center at Fruita, Colorado.

Components	Final concentration
M&S	0.215 % (w/v)
Sucrose	1 % (w/v)
Myo-inositol	0.005 % (w/v)
Thiamine	0.05 µg/ml
NAA	0.1 µg/ml
pH	5.7

Table 2. Shoot development media (based on Baker et al. 1999) used prior to rooting.

Components	Final concentration
M&S	0.43 % (w/v)
KNO_3	0.5 % (w/v)
Casein hydrolysate	0.05 % (w/v)
Sucrose	3 % (w/v)
Myo-inositol	0.01 % (w/v)
NAA	0.01 µg/ml
BA	0.5 µg/ml
pH	5.7
Caisson agar	0.7 % (w/v)

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shoots then were selected. The shoots were grown on shoot development medium (Table 2) in our laboratory until the time the study was initiated.

Shoots were paired based on similar appearance. One shoot of each pair was placed in the agar-based rooting medium and the second shoot of the pair was placed in the liquid medium containing a peat pellet. The peat pellet has a small hole in the center. Each peat pellet hole was plugged with approximately 7 mL of semi-solid agar. The sunflower plantlet was inserted in this medium. This small amount of agar medium served two purposes—it eliminated the air pocket in the center of the peat pellet and anchored the plantlet to the peat pellet. Additional liquid media was added as needed over the course of the study to Magenta boxes containing peat pellets to prevent plantlets from drying out.

A baseline evaluation was conducted for plant height, leaf number, and plant width as plantlets were started on rooting media. Plantlets were evaluated thereafter at two week intervals on three sampling dates. Plant height, leaf number, plant width, and rooting score (Table 3) were determined for plantlets grown on both agar and peat pellet/liquid media during each of the three sampling dates. Thus, on Sampling Date 1 plantlets had been growing on both rooting media for two weeks, on Sampling Date 2 plantlets had been growing on both media for four weeks, and on Sampling Date 3 plantlets had been growing on both rooting media for six weeks.

Plant height was measured from the base of the media to the highest point of green tissue on the plantlet. Plant width of the sunflower plantlets was measured using a micrometer. Two measurements were obtained. The first measurement was taken across the widest point of the plantlet and the second measurement was taken across the plant at 90° from the first. Plant width was calculated as the average of these two measurements. Additional observations were noted at each sampling date for plantlet appearance, noting overall health, necrosis, yellowing, vitrification, root hairs, number of roots, and length of roots.

Once plant heights and widths were measured, media were gently removed from the plant tissue in the agar-based medium by washing with distilled water from a squirt bottle and roots were evaluated and scored as described in Table 3. Plants grown in liquid medium and peat pellets were harvested by removing the pellets from the liquid medium, the peat pellet was carefully dissected, and the rooting system was rated as described in Table 3. Plant material, including roots if present, was dried in a drying oven at 56°C until reaching a constant weight.

Data were analyzed by analysis of variance as a completely randomized design (Statistix 8, Analytical Software, Tallahassee, Florida). Data were collected from nine replications with each plant being a replicate. If P was ≤ 0.10, means were considered to be significantly different. Rooting scores were rated from 1 to 5 as previously discussed. Rooting score data were transformed using a square root transformation prior to analysis (Steel and Torrie 1980). Standard errors of means for plant height, leaves, plant width, plant dry weight, and rooting scores were calculated.

RESULTS AND DISCUSSION

Overall, 26% of the plantlets in this experiment developed roots. Of the plantlets that developed roots, 71% were in agar and 29% were in peat pellet/liquid medium. Representative transgenic sunflower plants growing in peat pellet/liquid medium with vented Magenta boxes are shown in Fig. 1. The only significance difference between plantlets rooted in agar and those rooted in peat pellet/liquid medium for plant height, number of leaves, plant width, plant dry weight, and rooting score occurred for rooting score at Sampling Date 3 (Table 4). At Sampling Date 3, plantlets rooted in agar were scored at 2.1 while those rooted in peat pellet/liquid medium received a rating of 1.0, which means that none of the plantlets grown in peat pellet/liquid medium in the third

Table 3. Rooting score rating scale.

Key	Description
1	No roots
2	Up to 2 roots with up to 15 mm average root length, roots may have some necrosis
3	Up to 4 roots with up to 30 mm average root length, roots are not necrotic
4	Up to 4 roots with up to 75 mm average root length, roots are not necrotic
5	5 or more roots, roots are not necrotic

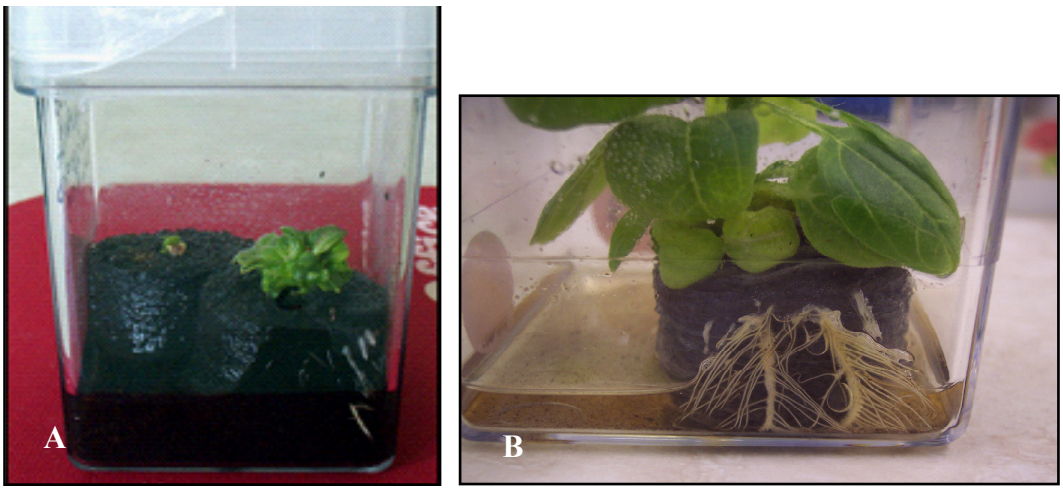

Fig. 1. A) Shoots growing in liquid rooting media in peat pellets. Vented lid and roots growing out of the peat pellet can be seen here. B) A tissue culture grown sunflower plant rooted in a peat pellet has a well developed root system.

Table 4. Growth characteristics of transgenic sunflower plantlets when grown on two different media rooting systems.

Sampling date	Plant height (mm)	Leaf no.	Plant width (mm)	Plant dry weight (mg)	Rooting score[z]
Initial					
Agar	6.5±0.5	8.2±0.7	13.5±0.9		
Pellet/liquid	7.5±0.7	7.8±0.8	14.2±0.6		
Date 1					
Agar	12.4±2.1	13.5±1.3	16.8±1.2	21.4±4.2	2.1±0.5
Pellet/liquid	7.7±0.7	11.9±1.1	16.2±0.8	15.0±2.6	1.2±0.2
Date 2					
Agar	18.0±4.2	17.1±2.1	23.0±3.1	70.9±19.7	1.9±0.4
Pellet/liquid	10.0±1.4	13.2±1.9	19.3±2.0	72.4±20.3	1.6±0.4
Date 3					
Agar	24.1±9.7	18.9±3.9	26.2±5.1	121.5±47.2	2.1±0.6a
Pellet/liquid	9.4±1.4	13.0±2.0	27.0±3.7	49.8±13.1	1.0±0.0b

[z]Rooting score is described in Table 3.

sampling date rooted. In a subsequent study, using the same peat pellet/liquid medium and peat pellets, 80% of the plants rooted (data not shown). Why we did not experience a similar amount of rooting in the study presented in this report is not yet known. It is possible the pH was not agreeable to rooting in the present study.

Plantlets in agar tended to grow taller over time than plantlets grown in peat pellet/liquid medium (Table 4). Leaf production was similar between plantlets grown in agar and peat pellet/liquid media although numerically there were a few more leaves on plantlets grown in agar on Sampling Dates 2 and 3 than on plantlets grown in peat pellet/liquid medium. Plant width between plantlets grown in agar-based and peat pellet/liquid media were similar. Dry weight of plantlets grown on agar at Sampling Date 3 was more than twice that of dry weight of plantlets grown in peat pellet/liquid medium although the difference between these two treatments was not statistically significant.

Vented Magenta lids allow for increased gas exchange and less vitrification of tissue-cultured plants (Huang and Chen 2005). All of the vitrified plants in our study were rooted in agar while none of the plants rooted in liquid medium and peat pellets vitrified. Because sunflower plantlets that become vitrified typically do not

recover (Witrzens et al. 1988) and 50% of the agar-rooted plantlets in our study were vitrified we would expect no more than 50% of the agar-rooted plants at a maximum would live to maturity. Thorough cleaning of agar off roots without causing damage to the root system is difficult. Preventing mold development is problematic when transferring plantlets grown in rooting medium containing agar to soil. Lucas et al. (2000) found it difficult at best to root plantlets grown on medium containing agar. Transplanting plants rooted in liquid medium with peat pellets to soil is less destructive and more likely to result in healthy plants that will reach maturity than plants grown in agar.

SUMMARY

Traditional plant breeding is limited by the genetic diversity within a species. The use of biotechnology allows introducing into sunflower, specific traits from other plant species that could result in improved plant performance that is not possible to achieve using traditional plant breeding.

Sunflower is notoriously recalcitrant to genetic transformation and regeneration when subjected to tissue culture. The study described in this paper compared agar and liquid media for rooting transgenic sunflower plantlets. Despite a smaller plant size and fewer plants that rooted, sunflower plantlets grown in peat pellets are less likely to become vitrified and more likely to develop to maturity.

The next step for this research would be to determine how transgenic sunflowers mature when rooted on peat pellets in liquid medium. It may be that peat pellets contain a chemical component or characteristic that does not promote plant growth of sunflowers. Additional research is needed to determine how to improve plant growth when sunflowers are rooted in liquid medium using peat pellets.

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