Tissue culture and plant regeneration from sunflower 
(*Helianthus annuus*) and interspecific hybrids (*H. 
tuberosus × H. annuus*)

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Abstract. A method is described for the culture and regeneration of plants from callus of 
sunflower (*Helianthus annuus*) and *H. annuus × H. tuberosus* hybrids. Immature embryos 
proved to be the only explant which consistently gave regenerable cultures in all genotypes. 
The most responsive embryos were approximately 12 mm² in area. Genotype had a significant 
effect on the capacity of cultures to regenerate. Some regeneration was also obtained from 
cultures of tuber tissue but only from one genotype, *H. tuberosus × H. annuus* cross 200. 
None of the *H. annuus* accessions gave regenerable callus from root tissue. Difficulties included 
the premature initiation of flowering of regenerating shoots and the frequent occurrence of 
"vitreous" plantlets which could not be transplanted successfully to soil. Some amelioration 
of both these problems was achieved by replacing inorganic nitrogen partially with amino 
acids. More effective reduction of these difficulties was accomplished by the addition of 10, 30 
and 100 µM phloridzin, esculin or naringin.

Abbreviations: BAP, 6-benzylaminopurine; zeatin, trans-6-(4-hydroxy-3-methyl-but-2-enyl) 
aminopurine; kinetin, 6-furfurylaminopurine; IAA, indole-acetic acid; NAA, naphthyl acetic 
acid.

Introduction

The sunflower, *Helianthus annuus* L. is a significant oil-seed crop with 
high-yielding and uniform hybrids available commercially. However since 
pests, diseases, and other environmental influences can reduce yield and 
reliability, there is a need for the development of improved genotypes.

The potential of wild relatives of sunflower to contribute new resistance 
genes has already been recognized in this regard. Resistance to rust (*Puccinia 
helianthi*) has been incorporated from wild forms of *H. annuus* [17]. *H. 
tuberosus* has been reported to carry resistance to *Sclerotinia sclerotiorum* 
[14]. Resistance to *Alternaria helianthi* has been found in a population of *H.*
argophyllus naturalized in Australia [8]. Although hybridization among Helianthus species has potential usefulness, sterility barriers cause difficulties [5]. Development of a regenerating tissue culture system for Helianthus species would enable techniques such as embryo rescue, somatic hybridization, somaclonal variation and culture-induced non-homologous recombination to contribute to broadening the genetic variation available for crop improvement.

There have been few reports on cultured sunflower tissues capable of regeneration. Trifi et al. [20] demonstrated shoot multiplication from cultured shoot apices. Greco et al. [4] induced callus on hypocotyls, leaves, cotyledons and shoot apices using benzylaminopurine (BAP) alone and could regenerate plantlets from these cultures. Bohorova et al. [2] produced callus on stem and shoot apex tissue which could regenerate at low frequency. They also cultured sterile hybrids of H. annuus × H. decapetulus, H. annuus × H. tomentosus, H. annuus × H. hirsutus and indicated the potential of culture to induce chromosomal exchange resulting in the introgression of alien genes into sunflower. Six of the H. annuus × H. decapetulus regenerants were sufficiently female fertile for backcrosses to be achieved. Patterson & Everett [15] were also successful in regenerating from hypocotyl callus and report that somatic embryogenesis may be occurring.

Sunflower protoplasts have also been successfully cultured to colonies [11] and to plants [1]. Researchers have consistently referred to the difficulties caused by premature flower initiation on culture-derived sunflower plantlets and high losses when transplanted to soil [1, 2, 4, 20]. This present paper demonstrates the utility of immature embryos as an explant in producing regenerable cultures.

We also describe the use of compounds such as phloridzin, naringin and esculin hydrate to reduce and eliminate early flower induction and “vitreous” plantlets which do not transplant to soil.

Materials and methods

Inbred lines of Helianthus annuus: BHA89, RHA274 and R299 from the USDA, RHA880 from the company Ag-Seed Pty Limited, Dubbo, N.S.W. 2830 were cultured from stem and leaf explants as well as immature embryos. In addition, hybrids between A124 and R299 were cultured. Plants were glasshouse-grown and stem and leaf explants taken at the 4–6 leaf stage.

Hypocotyl and cotyledon explants were taken from seedlings when the first true leaves appeared, which was about 11 days after placing aseptic seed