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CLONAL PROPAGATION OF PERENNIAL PLANTS FROM FLOWERS BY TISSUE CULTURE[1]

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Abstract. Several herbaceous and woody perennial plants have been clonally propagated by tissue culture using the flowers as explants. Even tetraploid plants regenerated from callus from flowers seem to be genetically stable. Flowers from several *Rhododendron* species and cultivars regenerated plants on Anderson's medium. The epigenetic change from maturity to juvenility may take place in some flower tissues before the formation of an embryo.

The propagation of perennial plants by tissue culture, particularly woody plants, has lagged behind the propagation of herbaceous annual and tropical plants. There are several reasons for this. First, obtaining a sterile explant can be a problem. Then, the problem that propagators have long recognized as loss of juvenility is magnified when we propagate perennial plants by tissue culture. Finally, there is the worry that genetic or even epigenetic changes will cause the plants to vary from the clone of interest. The following discussion will cover some of the advantages of using the flower parts of perennial plants as an explant source for tissue culture propagation in relation to these problems.

We should first examine some of the anatomical similarities and differences between vegetative and flowering growth of the terminal meristem. This phenomenon is covered extensively in Esau's classic anatomy textbook (6) and Gemmell's monograph (8). The vegetative meristem initiates stem and leaves in a very ordered pattern by growth protuberences at regular intervals on the flanks of the meristem. In the axils of

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the leaves new vegetative meristems or side buds are initiated which can grow into side branches.

The vegetative meristem turns into the flower meristem by an epigenetic process (14). This means the meristem has the same genetic material, but a different set of physiological and morphological genes are expressed when the flower organs; sepals, petals, stamens, and pistils are initiated. The flower parts, according to some theories, are modified leaves. The sepals, which are first initiated by the floral meristem, are green and leaf-like and grow up and cover the flower bud. The petals, which are initiated next, are usually devoid of chlorophyll but contain other pigments in some instances, and in others are devoid of all pigments (white). The stamens, the male sexual parts, are formed next. The anthers of these contain cells which undergo meiosis (reduction division) to form male haploid spores or pollen grains. The pistils are initiated last in the center of the flower and have an ovary which encloses one to many ovules. The ovules have cells which become the female spores. The female spores grow into the embryo sac contained in the ovule. All the flower structures are formed by mitotic divisions. This means that any flower part not concerned with the sexual cycle is developed by mitotic divisions and is capable of regenerating a plant similar to the plant that bears the flower. The sexual portion is usually only a small part of a mature flower.

The vegetative meristem, before it turns into a flower meristem, often makes several modified leaves (bud scales) or a single leaf (sheath). These structures grow up over and protect the floral parts until flower development is complete. This is usually done when the meristem initiates several individual flowers before it terminates its growth. These structures or the sepals make the floral parts very easily disinfested, even when the meristem is borne underground as in the case of many of the herbaceous perennials.

Since the regeneration of plants from tissue of mature plants has been found to be more difficult than from seedling plants (9,20), we might expect flowers to be a poor explant for tissue culture propagation purposes. However, there are several reports, including mine, which have found flowers to be an excellent source of explants for tissue culture propagation. I will review the methods and results of a few of these studies on perennial plants. I will then speculate why we can clonally propagate plants from flower explants by tissue culture.

The orchids were the first plants to be successfully propagated as clones by tissue culture techniques. Arditti (4) lists nine genera of orchids which are propagated using the flower

as the explant. Some of these orchids are propagated by stimulation of vegetative growth from nodes on the flower stalk. Other genera will develop callus from the flower parts which will form protocorms similar to the orchid seed germination process.

The ovule, or the nucellar tissue of the ovule, surrounding the sexual embryo sac seems to be a good source for regeneration of embryos by tissue culture. A recent review (23) lists 15 species of *Citrus* and 5 related genera showing this phenomenon. This phenomenon is also apparent in the mango (13). Other papers (11,22) report this regeneration to take place in grape cultivars which are quite old. The nucellar tissue in some flowers will form embryos on the plant without tissue culture and causes polyembryony with apomictic embryos (9). Either process could be considered clonal propagation.

I have had success with several herbaceous perennials (15, 16, 19) by excising flower tissue and culturing it with the appropriate growth regulator treatment in the dark until it develops masses of callus. These callus masses when divided and subcultured in the light will develop large numbers of plants. These techniques have even been used to clonally propagate tetraploid plants (15, 19). It has also been possible to clonally reproduce and separate a chimeral *Hosta* using the flower as an explant (16).

Other workers have developed callus from flowers into shoots from more woody species. Bennet and McComp (5) reported success with *Eucalyptus marginata* from stamen filament callus. Hearne (10) reported a successful technique with passion flower. The immature female flowers of *Salix tetrasperma* were found by Angrish and Nanda (3) to make vegetative shoots directly from the flowers. Some of the leaves of these vegetative shoots had stigmatic surfaces on the tips.

Plants of the genus *Rhododendron* have been propagated more widely than any other landscape plant. These have been mainly by shoot tip proliferation with the medium developed by Anderson (1) and commercially developed as reported in a paper by Kyte and Briggs (11). I have found that Anderson's medium works well using the flowers as explants (17, 18). Several species and cultivars of *Rhododendron* at several times during the dormant season have been propagated in substantial quantities (Table 1). The flowers are very easily extracted in a sterile condition from the dormant flower bud, as the resinous bud scales form a protective covering for several individual flowers.

The individual flowers with as much pedicel as possible are placed on Anderson's medium in the dark until there is

substantial callus-like growth. Flowers of two clones of 'Northern Lights' deciduous hybrid azaleas seem to respond better to 12 mg/l zeatin than to the 15 mg/l of 6-(gamma, gamma, dimethylallyamino)purine (2iP) of Anderson's medium. Zeatin was also found by Fordham *et al.* (7) to be superior for Exbury azalea shoot tips. The growth occurs from the base of the flower and the pedicel. When the callus-like masses are subcultured in the light on Anderson's medium with the growth regulator lowered, substantial quantities of shoots are formed. These can be rooted using standard transfer techniques (2). The plants formed are juvenile in nature with small rounded leaves and will grow continuously if kept under long days and high moisture and fertility (18).

Table 1. Tissue culture propagation of several species, cultivars and hybrids of hardy *Rhododendron* from flowers.

Species and Cultivar	Dates of Init.	Ease*
R. 'Nova Zembla'	4/28, 10/10	++
R. 'Sefton'	2/5, 3/27	+
R. 'Roseum Elegans'	2/18, 3/19, 9/17	−
R. catawbiense 'Album'	4/17	−−
R. 'Sefton' × *R.* 'Purple Splendour'	8/24	+
R. 'Abraham Lincoln'	8/24	−
R. 'Vulcan'	10/10	−
(R. carolinanum × *R. dauricum)* × *R. dauricum* 'White'	9/14	−
R. kosteranum × *R. prinophyllum* 'Northern Lights S'	1/21, 3/23	+**
R. 'Northern Lights M'	3/23	−−

* Relative ease of propagation: ++, easy; −−, difficult.
** Zeatin 12 mg/l better than 15 mg/l 2iP.

One of the first scientific papers brought before this Society was by F. L. S. O'Rourke (21) on the effects of juvenility on plant propagation. This juvenility phenomenon has been even more severe for propagation by tissue culture (9, 20). In botanical terms the end product of the ontogenetic change from juvenile to mature form is the formation of flowers. The above discussion gives several perennial plants that can be clonally propagated by tissue culture from flowers and this would seem to be a contradiction. It is a tacit assumption that the reinitiation of juvenility is the combination of the egg and sperm to form a zygote which grows into a plant embryo. However, this does take place in the flower and the flower tissue must transport all the metabolites needed for the development of the zygote to the embryo stage or to develop the endosperm for this purpose. Since the flower formation involves a considerable epigenetic change over mature vegetative growth, the flower may not be the end of the maturing process but the

beginning of the reinitiation of juvenility. Perhaps the flower is the transitional phase in the mature to juvenile change. It would appear this way from the above tissue culture work. *Rhododendron* flowers would be a good example of this where the petal and ovary base and even the pedicel reinitiates shoots with juvenile characteristics. The flower may be the easiest explant source for formation of juvenile tissue needed to propagate clones by tissue culture that lose juvenility at a young age, as in many woody plants.

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GRAFT INCOMPATIBILITY IN WOODY PLANTS

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Grafting is an old method of plant propagation and since ancient times propagators have been aware of the problem of scions failing to make satisfactory growth when budded or grafted to an understock. Compatibility is defined as the ability of two different plants, when grafted together, to produce a successful union and develop satisfactorily into one composite plant (5). The opposite, failure to develop satisfactorily, is called incompatibility. Several excellent reviews on stock-scion incompatibility have been published by Argles (1), Hartmann and Kester (5), Mosse (10), and Nelson (11). The publication by Nelson (11) in our Proceedings is particularly important for plant propagators because of the extensive number of ornamental graft combinations surveyed and reported on in tabular form. However, just what constitutes graft-incompatibility has presented difficulties because many of the symptoms are nonspecific and similar to those which can be caused by unfavorable environmental conditions, viral infection, desiccation of the tissues, or poor techniques. Also, incompatibility can take numerous forms from slight symptoms of ill-health to complete graft failure when no union is formed.