Tissue Culture of Roses: Past, Present, and Future

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Roses have been the subject of myriad tissue culture studies for the last fifty years. The justification for most of the studies has been based on either crop improvement (breeding) or propagation. Early work focused on seed germination and embryo culture; artificial culturing (*in vitro*) of embryos followed. Micropropagation, *in vitro* morphogenesis, and genetic engineering have been the most popular areas of study in the last twenty years.

THE PAST

In the 1940s, Lammerts (1942) developed a technique for the culture of rose embryos. This was not done *in vitro*, but the work did show that rose embryos could be excised from seeds and germinated. A decade later Asen and Larson (1951) developed a technique for the artificial culturing of rose embryos. Their major contribution was in the use of a seed coat softening solution known as Cross and Bevan's Reagent. This reagent was made by dissolving 30 g of $ZnCl_2$ in 50 ml concentrated hydrochloric acid. After varying times (2-16 hr) in this reagent the seed coats of various rose species were softened to the point where they could be cut away and the embryos removed. They also found that immature embryos could be removed from hips and cultured immediately using this seed coat softening technique.

Hill (1967) successfully developed a system for the regeneration of shoot primordia from stem tissue. This was important work since it showed that roses had the capability to form adventitious organs *in vitro*. Hill's most successful culture medium included (in mg/liter) 0.5 α-naphthaleneacetic acid, 0.2 kinetin (6-furfurylaminopurine) and 20 gibberellic acid. Other work also in the 1960s, 1970s and 1980s focused on the use of apical and axillary meristems as primary explants to establish *in vitro* cultures for rapid micropropagation (Bressan et al., 1982; Davies, 1980; Elliot, 1970; Hasegawa, 1979; 1980; Hyndman et al., 1982a,b; Jacobs et al., 1969; 1970a,b; Jacobs et al., 1968; Khosh-Khui and Sink, 1982a,b,c; Skirvin and Chu, 1979a;b).

THE PRESENT

Our work has focused on embryo rescue. In cooperation with Bear Creek Gardens, Inc. (Somis, CA), we cultured immature embryos resulting from crosses between *Rosa* Bridal Pink® and six separate pollen parents. It had been observed that these crosses resulted in no or few progeny probably due to abortion sometime during embryo development. Questions requiring an answer included: How would the various crosses (genotypes) respond to tissue culture?, What was the optimum time of removal of the immature embryo?, What was the optimum tissue culture medium?, and What was the optimum culture environment? The original goal was to simply learn how to germinate excised immature embryos in vitro. This goal was not realized since germination was never observed. The response of the excised embryos was to develop an organogenic callus that, after 6-9 months, was capable

of forming adventitious shoots that could be excised and rooted on a different culture medium (Burger et al., 1990). In short, we found that:

There were significant differences in how the six genotypes responded to tissue culture. One genotype (code #174) responded well by forming adventitious shoots that rooted well and were easily transplanted to greenhouse conditions. Three genotypes regenerated plantlets, but to a much lesser degree than #174, and two genotypes were not capable of regeneration under our experimental conditions. Since all six genotypes had the same maternal parent ('Bridal Pink'), it's remarkable that there was such wide variation in their capability for regeneration and points to an important genetic component when selecting plant materials for tissue culture studies.

Table 1. Summary table of in vitro responses from various rose cultivars.

Author(s)	Explant	Response
Hill	stem pith	callus, *shoot primordia
Jacobs et al.	stem pith	callus, *buds
Mollard et al.	stem pith	callus
Nesius et al.	stem pith	callus
Weinstein et al.	stem pith	callus
Amorim et al.	stem pith	callus
Burger et al.	peduncle, callus	roots
Tabaeezadeh and Khosh-Khui	anther	callus
Davies	axillary buds	callus, shoot elongation, roots
Elliot	shoot apex	callus, shoot elongation, roots
Hasegawa	shoot apex	shoot development, roots
Hyndman et al.	shoot apex	roots
Jacobs et al.	shoot apex	callus, leaf development, roots
Khosh-khui and Sink	shoot apex	shoot elongation, roots
Skirvin and Chu	shoot apex	shoot elongation, roots

* no further development observed.

Embryos had to be at least 25 days post-pollination for them to develop an organogenic callus capable of regeneration. The seed coat of rose become very hard after about 40 days post-pollination. After this time embryos are quite difficult to remove from the seed.

The culture medium had to include a cytokinin and an auxin for regeneration. Our choices for a cytokinin and auxin were 1 μM 6-benzyladenine (BA) and 0.05 μM α-naphthaleneacetic acid (NAA), respectively, in a half-strength Murashige and Skoog Medium (1962).

Embryos formed the organogenic callus and regenerated shoots only on a semi-solid medium (0.6% agar) in the light (50 $\mu moles \cdot m^{-2} \cdot sec^{-1}$ or about 150 fc).

The regeneration system has been exploited for mutation breeding purposes.

Calli were exposed to Cobalt 60 for varying lengths of time to give varying doses of irradiation. Doses above 4 kRad killed the callus tissues. Irradiated calli were then placed in tissue culture conditions stated earlier to undergo regeneration. Mutant plants obtained from callus that had been irradiated formed flowers that had fewer petals and the petals had lower levels of red pigmentation. The benefit of irradiating organogenic callus can be appreciated when the mutants maintained their unique characteristics for more than three years, showing a stability that had not been possible when axillary buds had been irradiated (Wang and He, 1990).

THE FUTURE

The capability for regeneration of whole plants is necessary for modern genetic engineering techniques to be useful in rose improvement. We are working on two new regeneration systems: leaf disc method and fragmented shoot tip culture. The leaf disc method has been used successfully with many other species. It utilizes leaf discs as the primary explant and depends on the formation of adventitious organs from the wounded margin of the disc. Fragmented shoot tip culture was developed by Barlass working with grape (Barlass and Skene, 1978). In this technique, shoot tips, 1 mm long, are excised from the plant, macerated with a scalpel, and plated onto tissue culture media. The meristematic cells of the shoot tip provide excellent explants for regeneration.

Several laboratories around the world are working on using *Agrobacterium tumefaciens* as a means of incorporating new genetic information into roses. This bacterium is a causal agent for crown gall in roses. It has the capability of inserting part of its DNA into wounded plant cells once it becomes associated with the cell, thus transforming it. Techniques have been developed whereby genes of interest (e.g. RoundUp resistance) can be engineered into that portion of the DNA that *Agrobacterium* inserts into the plant's DNA. Ultimately, the usefulness of *Agrobacterium*-mediated transformation depends on the ability to regenerate whole plants from transformed cells.

In summary, the interest in using *in vitro* techniques for rose improvement continues today. Several rose cultivars have been successfully regenerated using tissue culture techniques and the list will surely grow. There is great variation in the ability of various cultivars to undergo organogenesis or embryogenesis, even those that are very closely related. Regeneration is a necessary process to make use of the genetic improvement techniques such as *Agrobacterium*-mediate transformation.

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