APPLICATION OF IN-VITRO POLLINATION AND EMBRYO CULTURE TO AUSTRALIAN NATIVE PLANTS

ACRAM TAJI AND R. R. WILLIAMS

Black Hill Native Flora Centre
Department of Environment and Planning
Athelstone, South Australia

Whilst for horticultural plants it is desirable to use vegetative propagation to maintain genetic uniformity, there are times when genetic diversity is desirable, e.g. in plant breeding and selection programmes, or for conservation of species diversity. However, not all plants set viable seed and, in other cases, seed is damaged by insects or released from the plant before it can be collected. Hybrids often do not produce seed, either because of pollen incompatability or because of embryo abortion during seed development. Under these circumstances some means of artificial seed production could be valuable. At the Black Hill Native Flora Centre we have been developing the application of *in-vitro* techniques to such situations.

In-vitro studies of pollination and seed development may also shed light on problems which may ultimately be overcome by more conventional techniques once we understand the problem.

This paper presents examples of the application of *in-vitro* techniques to the conservation of the rare native species, *Swainsona laxa*, and the artificial ripening of *Acacia* seed. Other practical applications will be discussed.

In-vitro pollination. The case example is *Swainsona laxa*, a native legume on the brink of extinction. At the time of this work no wild or cultivated plants were known to exist; all we had were a few stored seed. These seed produced three plants which flowered but did not set seed. This was critical because the species is essentially an annual and, therefore, is dependent on seed production for survival of the species. An urgent investigation began.

The simplest hypothesis was that the flowers were not producing viable pollen. We collected pollen from the open flowers and tested it on agar plates in the laboratory. It did not grow. Since pollen often has a short life span after it is shed more samples were collected just as flowers were opening, i.e. at the first opportunity for cross pollination. Again the pollen did not grow on the agar plates. To establish whether the problem was one of infertile pollen production or of short-lived pollen we dissected flower buds at a stage well before flower opening and tested pollen from this stage. This time the pollen did grow on the agar.

We now have the situation where viable pollen is produced at the bud stage but loses its viability before natural cross-pollination can occur—hence the lack of seed set. But what about self-pollination? Most papilionaceous (pea-flowered) species are self-

fertile. Since we had shown that viable pollen was produced, the flowers of *Swainsona laxa* must be self-incompatible.

A common form of self-incompatability is caused by a deleterious interaction between the pollen grains and the stigmatic surface on which they must germinate to effect fertilization. We wished to see if pollen known to be viable in the laboratory would germinate and produce a pollen tube in the stigma of the flower. This can be observed using fluorescence microscopy and suitable botanical stains. With this technique pollen tubes can be clearly distinguished in stigmas squashed on a microscope slide. We applied the technique and found that no pollen grains grew on the stigmas of *Swainsona* even though we knew they were viable in the laboratory. The point of incompatability was established.

Our next problem was to overcome this incompatability in order to produce seed. First we developed a method of dissecting pistils (the female part of the flower including the stigma and ovary) from flower buds and culturing them on agar in a test tube. This made it more easy to control the process. We then decapitated the pistils to remove the stigma and applied fresh, viable pollen to the remaining explant. The ovaries on these pistils soon began to grow and eventually produced viable seed. The immediate problem of saving the species was solved.

From the above research we now understood why the *Swainsona* plants were not setting seed and we could bypass the problem in the laboratory. However, another approach remained to be explored. Natural cross-pollination was prevented by the lack of viable pollen from open flowers. Was the self-incompatability at the stigma a general response or confined to self-pollination within the same flower? What if viable pollen collected from buds was applied to other open flowers on the plant? We carried out hand cross-pollination of plants in the glasshouse using pollen collected from buds and again obtained seed set. Thus the lack of cross-pollination was due to the lack of a source of viable pollen, and the incompatability was confined to within individual flowers. This hand cross-pollination approach is much more practical than *in vitro* culture and has now been used to produce many seed.

While this example of preserving a rare species is a special case, the same techniques could have wider application. In-vitro culture of pistils offers a useful research tool for the study of pollination biology of other species. It could also be useful where controlled pollination is required in a plant breeding program. Pistils could be collected well before natural pollination occurs thereby avoiding uncontrolled pollination. Where desirable crosses are prevented by incompatability arising at the stigma, decapitation and pollination of cultured pistils may be the solution. In this way these techniques could play a practical role in the development of new cultivars of horticultural plants.

Embryo or immature seed culture. Acacias, along with many other native species, often release their seed soon after it matures, usually after a burst of hot weather. This makes the collection of mature seed difficult unless the plants are watched closely. On the other hand if seed is collected before it matures it may not germinate. We face this problem when trying to propagate species of wild plants from remote areas. Embryo or immature seed culture can provide the solution.

Green (immature) seed pods are present on plants over a relatively long period of time, therefore it is not necessary to be there at just the right time. The earlier the collection the less developed will be the embryos or seed within the pod. It is possible to extract these embryos or immature seed and culture them on suitable media so that they complete their development. Depending on the culture conditions and the stage of development of the embryos, one may get the embryos developing and growing directly into plants, or mature seed may be produced. Plants can be propagated either way.

The development of mature seed in culture is of particular interest. We have shown that immature but well developed seed can readily be brought to maturity in the laboratory, then dried and stored as normal seed for later sowing. This technique could be of practical benefit because it should be possible to use bulk liquid cultures for mass production. Given appropriate culture conditions this may be a way of producing high quality seed of some species.

Where embryos are collected at a very immature stage a more complex medium may be required. It is also more likely that the embryos will grow into plants rather than forming seed. This makes handling of the plants more difficult and they need to be grown-on directly rather than stored as seed. However, it may be possible to establish suitable conditions for suspension of embryo growth to permit their storage until required.

Embryo culture may also be used for plant breeding. Sometimes certain crosses result in successful pollination and fertilization but the embryos abort early in their development. It may be possible to extract and culture these embryos to maturity.

Another exciting possibility is the creation of artificial seed. Whether embryos are produced as suggested above or are produced through tissue culture involving somatic embryogenesis, it may be possible to provide an artificial coat enclosing individual embryos with a small quantity of nutrient medium thereby creating an artificial seed. Such a development would open the way for economic mass production of cultured seed for field crops where more conventional tissue culture is uneconomic.

Conclusions. The actual examples discussed above are part of our research to solve specific problems of native plants but it should be clear that there is considerable potential for the broader applica-

tion of the techniques being developed. Much of the technology referred to will only be used by the specialist but I hope I have helped make you aware of the possibilities. You might also be able to see other potential applications of relevence to your particular area of interest.

SALINITY MEASUREMENTS IN POTTING MEDIA

R. J. WORRALL AND G. CRESSWELL

New South Wales Department of Agriculture
P.O. Box 720, Gosford, 2259, N.S.W.

One of the most common problems encountered in nurseries is salinity, although the grower is often not aware of it. Excessive salinity may be the result of over-fertilization, combined with lack of leaching, and/or high levels of salts in the water supply. It will dramatically reduce growth rates, often before there are any visible symptoms. Low levels of soluble salts in the potting mix can also be a useful indicator of fertilizer deficiency.

The salt level in pots may also change rapidly, even on a daily basis. For example, in heat-wave conditions there may be rapid fertilizer release from controlled-release fertilizers, especially if it has been recently applied. A single heavy watering can also dramatically reduce the salt level. Since salinity readings can be "out of date" quickly, measurement at the actual nursery is very desirable.

A number of techniques for measuring the salinity of potting media are being evaluated by the Department. The techniques are:

(a) *Saturated paste extract method* (SP). This is a widely used standard technique. A paste is made of the potting medium, the water is extracted under vacuum and the electrical conductivity (EC) of the solution is measured. This technique is time consuming, requires specialised equipment, and may not be suitable for very coarse potting media.

(b) 1:1.5 medium/water dilution technique (1:1.5). Moist potting medium is mixed with 1.5 times its own volume of water. The EC of this slurry is then measured. Because of its simplicity this technique is gaining in popularity and gives reasonable results. However, it does not take into account fully the bulk density of the media.

(c) *1:5 medium/water dilution technique* (1:5). Potting medium is mixed with 5 times its weight of water. The