REPORT FROM UCI

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Despite the new and expensive varieties that grace each year's daffodil catalogs, it can be argued that daffodil hybridizing is reaching a plateau where advances are hard to come by and progress is marked by small steps. There are several reasons for this. Among the first four divisions there is already a long history of hybridizing, and the major advances involving form have already been made. In most of the other divisions sterility is a serious problem that precludes significant additional breeding without going back to the original species. Most standard daffodils are tetraploid while the species are diploid. This results in triploid hybrids, most of which are sterile. These problems are exacerbated by the small numbers of viable seed often produced in a pod and the long generation times which are at least three to five years from seed to flower.

As many daffodil people know, the ADS is partially supporting a research project in my laboratory at the University of California at Irvine (UCI). What follows is a report on one section of the work, following the first two years of the grant. The grant is to conduct research aimed at improving upon traditional daffodil breeding techniques and to apply some of the more modern approaches to daffodils. We proposed to get around the sterility problems by developing and applying techniques that have proven themselves to be valuable for other groups of bulbous plants.

Two main goals were to develop "embryo rescue" techniques that could be used with daffodils and secondly, to work out easy ways of converting sterile hybrids into fertile ones by doubling chromosome number.

Embryo Rescue: Embryo rescue requires removing immature ovules from the ovary after fertilization and growing them on an artificial medium. There are several reasons for doing this but the most important is that it allows one to make crosses that normally fail when traditional seed production techniques are employed.

In many plant groups, the reason why crosses do not take is not because pollen is unable to fertilize the egg cells in the ovule, but rather because endosperm development either fails completely or partially. Endosperm failure results in flattened seeds, most of which never germinate. If the ovules are extracted after fertilization, they can be placed on an artificial medium that can substitute for the nourishment provided by the mother and also the endosperm. We have also found in using other bulbous flowers in the past that we can achieve immense savings in time to maturity using embryo rescue. For example, in *Ornithogalum* (Gries-

bach, Meyer & Koopowitz, 1993) not only could we make hybrids between normally non-fertile species but also they would flower in 9 months instead of 3 years. In *Eucomis*, the pineapple lily, embryo-rescued plants flowered in 18 months instead of 4 years (Koopowitz & Meyer, unpublished). Maybe daffodils could mature at a faster rate too.

The two main problems for doing embryo rescue are finding the correct medium on which to grow the embryos and knowing the time after pollination that fertilization takes place. The first year of the grant was spent trying to find a successful medium and to work out the correct time for harvesting ovaries following pollination. If one succeeded in these two endeavors, then in the second year one could try to embryo-rescue "impossible" crosses that normally fail.

Year 1. We decided to use a medium that had been successful for meristem tissue culture of narcissus (Steinitz & Yahel, 1982). We also tried a second medium that was touted as working for *Clivia*, a notoriously difficult amaryllid in tissue culture (Finnes, 1999). All work had to be performed under sterile conditions using a laminar flow hood to exclude microorganisms.

We selected three different pod parents in order to cover the spectrum of narcissus types and made 3 crosses. They were 1) 'Paperwhite' x 'Paperwhite'; 2).'Little Gem' x 'Gloriosus' and 3) 'Ice Follies' x 'Altun Ha'. The three crosses also spanned the season and gave us material to work with from December to March. We will discuss only the results of the 'Little Gem' crosses here because they produced the most seed. The other crosses were very sparse seed producers and even the controls allowed to mature on the plant produced very little seed.

'Little Gem' x 'Gloriosus' pods were harvested 8, 10, 12, 14, 16, 18, and 20 days after pollination. These dates were selected because previous experience had suggested that this might be an appropriate time for fertilization to have taken place. Pollen had been stored in a refrigerator. At least 10 pods were harvested for each date and the contents of each pod were planted into separate magenta jars. A few of these were contaminated and had to be discarded.

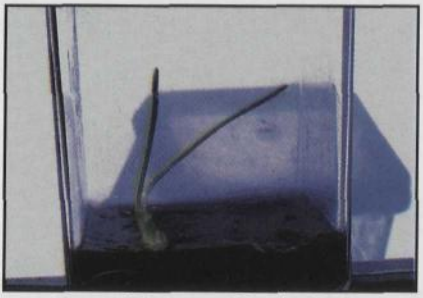
The 'Little Gem' x 'Gloriosus' embryo rescue attempts were very successful and we found that the Steiniz & Yahel medium gave better results with more embryos. The ovules swelled to about normal seed size for 'Little Gem' and then the seed coats, which had partially darkened but not developed to the normal black color burst open and a mass of cellular tissue developed. We found no growth from embryos removed 8 days or less, post-pollination; a very few in those harvested 10 days post-pollination produced embryos that developed into bulblets. Many of the embryos taken 12 to 20 days after pollination developed into plantlets.

Converted 'Hawera' compared to normal 'Hawera' flower between fingers

Embryo-rescued seedlings a few months out of the flask

'Little Gem' x "Gloriosus' seedlings still in flask

'Little Gem' x "Gloriosus' seedlings still in flask



Paper White seedling in flask

This suggests that at least 10 days are required after pollination before fertilization can occur in the 'Little Gem' ovaries.

There were several different ways that the embryos developed and sometimes all types occurred in the same jar. In general a mass of cells formed, or a stout root-like structure which then produced leaves formed. The leaves emerging from all these plants seemed to be different from normal seedling leaves. In the culture medium, the initial leaves were fairly stout and channeled on one side. They looked more like second-year or older leaves. They started to make small bulblets while still in the jars and growth continued through that first summer. By September, 2000 a few plantlets had made two leaves. Normally one would only be planting seeds in August or September so the technique allowed a gain of year's growing time.

The next step was adapting the plants to growing outside the laboratory. We waited until the late fall when temperatures started to drop before we planted them out. This appears to be a critical procedure and despite soaking the plantlets in a fungicide we lost half of the plantlets when they were potted up. The survivors, however, grew vigorously, producing several additional leaves. They were forced into dormancy at the end of May 2001.

At the current time of writing, early January, 2002 these plants have successfully broken dormancy and are growing vigorously. Many of them resemble third-year seedlings.

We can summarize the project at that point by saying that we demonstrated that embryo rescue techniques are possible for narcissus, we have a medium that works, and it appears that this technique may even help accelerate seedling growth. So now, what does this mean for the rank and file daffodil grower? This technique should make it possible for breeders to make new kinds of daffodils that were impossible to produce before. For example, *N. dubius* is a tetraploid small white tazetta species that could make a range of new miniature daffodils if it could be bred to miniature trumpet daffodils that are diploid. Last season we harvested over twenty pods of 'Little Beauty' x *N. dubius* and similar numbers of 'Little Gem' x *N. dubius*. All of the pods were chock full of large but aborted seeds, every seed was flat; they all were useless, because of endosperm failure.

Year 2. During the Spring of 2001 we repeated those two *N. dubius* crosses. Ten pods of each cross were harvested approximately 15 days after pollination and embryo-rescued, while a further 10 of each cross were allowed to ripen on the plants. None of the pods allowed to ripen on the plants produced viable seed although they were filled with copious flattened chaff-like seeds. As of this writing we have six embryos devel-

oping from the 'Little Gem' x *N. dubius*-rescued ovules. Two of these have now produced a mass of callus and several bulblets. The results of the 'Little Beauty' x *N. dubius* cross did not develop despite the fact that the ovules from that cross grew to normal seed size but there was no evidence of plantlet formation. Some of the latter seeds were sectioned and examined microscopically but they were devoid of embryos. In the latter case embryo rescue did not work. The utility of embryo rescue seems to vary depending on the parents used to make the crosses. Another series of embryo rescues will be repeated in the coming season.

We are not suggesting that daffodil hobbyists will carry out these laboratory techniques themselves, although they could. They will be able to follow the example of orchid breeders, many of whom are amateurs, who make crosses and then routinely send their pods to one of the various commercial laboratories scattered around the country, to carry out embryo-rescue procedures.

Polyploidy

One of the main reasons there is so much sterility in modern daffodil hybrids after crossing with the various species is because the chromosomes are mismatched. This problem could be overcome by doubling the chromosome numbers. This is what happened spontaneously with 'Quick Step' and its progeny such as 'Limequilla' and 'Regeneration'.

Doubling chromosome numbers is a routine operation, but one needs to work with a very small piece of actively dividing tissue. Normally, the apical bud (called *apical meristem*) in a regular daffodil could be used but getting to that bud is difficult. It is easier to use twin scales and force them into making a new meristem. Ten days after cutting twin scales, swellings between the leaves' bases making up the scales can be seen. At this stage they can be soaked for 24 hours in an agent that stops cell division. The agent is then washed out and the twin scales further incubated to produce bulblets. A certain percentage of the twin scales will make polyploid bulbs, but one generally cannot tell if the process has been successful until they have flowered and been pollinated. While polyploids tend to have larger flowers with heavier substance, this is not always the case.

In 1998 we used an agent called Oryzalin (2%) on a range of different sterile narcissus cultivars. One of these was 'Hawera', a known sterile miniature originally from the cross *N. jonquilla* x *N. triandrus*. About 60% of those bulbs flowered in 2001 and of those flowering, about half had flowers much larger than the size of normal 'Hawera'. All of these flowers were pollinated with viable pollen of *N. longispatha*. But only one of the pollinated plants produced seed. Nine seeds were harvested and of this writing all nine have germinated. The pollen of the seed-

producing plant also looked very good and that was used to pollinate a number of flowers of 'Regeneration'. Several pods were set on 'Regeneration', but when they matured and split open they contained partially formed flat seeds in them. At this time (February, 2002) one quite strong seedling has germinated from that cross. This technique holds promise for making other "sterile" cultivars fertile.

In 2000 we tried to convert an enormous series of sterile miniatures (using 1% Colchicine). This was attempted to increase the range of potential breeding material. In two years time we will know how successful this has been. Our earlier work gives us optimism that we will be successful.

References:

Griesbach, R., Meyer, F. and H. Koopowitz. (1993) "Creation of New Flower Colors in *Ornithogalum* via Interspecific Hybridization." *J. Amer. Soc. Hort. Sci.* 118: 409-414.

Finnes, J.F. (1999) "*In vitro* Culture of *Clivia miniata*." *Clivia Yearbook* 1999.

Steinitz, B and H. Yahel. (1982) "*In vitro* Propagation of *Narcissus tazetta*." *HortScience* 17: 333-334