PROBLEMS IN MICROPROPAGATION: CHANGE IN MORPHOGENETIC POTENTIAL AND DIFFICULTY OF PREDICTION OF SHOOT MULTIPLICATION BEHAVIOUR

MARGARET E. NORTON AND COLIN R. NORTON

Department of Biological Science and
Department of Plant and Soil Sciences
University of Idaho
Moscow, Idaho 83843

We have now reached an impasse in the use of micropropagation and tissue culture techniques. We know that these techniques are useful to us as growers and yet we still cannot take a given genus and immediately propagate it without extensive research work. However, we have gained sufficient knowledge of these practices that we should be able to make predictive models. We have been working at The University of Idaho on the problem of prediction of behaviour of plants and plant parts when cultured *in vitro*.

Current commercial micropropagation practice involves the use of shoot culturing techniques, with subcultures taken regularly (perhaps every 4 to 6 weeks). Sometimes we are advised to obtain fresh culture material regularly and yet it is much easier to repeatedly work with our already cultured, and therefore, sterile material. Our work has shown some interesting changes in shoots of Rosaceous plants after repeated subculture.

The test plants were species from the genera *Chaenomeles, Crataegus, Potentilla, Prunus* and *Spiraea*. Shoots were cultured on a modified Murashige and Skoog nutrient medium with the addition of benzyladenine and were subcultured every four weeks (3). Changes in morphogenesis and morphology of shoots and roots were recorded over a period of nine months.

It was found that shoot number increased over the first few generations and then decreased gradually in later generations. Shoot length and also leaf size decreased with each successive generation. Eventually, in some cases, the material became less shoot-like, more disorganized and callus growth increased. Root morphogenesis followed a similar pattern to that of shoots; root initiation declined gradually after the first few generations. Alteration of the balance and concentration of growth regulators supplied to the shoots did not significantly reverse this decline.

Changes in long term cultures of callus have frequently been reported, one such change being decreased organogenesis (4,5). However, such behaviour in shoot cultures has not been reported by other workers although Jones and Murashige (1), reported an increased number of deviant plants with repeated subculturing of shoots in *Aechmea fasciata* and Murashige (2) warned that culti-

vars of known instability should not be subcultured more than three to four times. From our work, it can be concluded that perhaps a number of species may be subject to both decline in vigour and changes in morphogenetic potential when maintained in culture in an actively growing state for a long period of time.

A second approach to our work in looking at the possibility of predicting the behaviour of shoot cultures has been to study plants by family. It is generally more straightforward to develop a micropropagative technique for a species in a plant family which has previously been studied in the same laboratory. In our laboratory we have specialized in the plant families Rosaceae and Ericaceae.

Although economics dictate that generally a plant needs to be fairly important commercially before it can feasibly be developed for micropropagation, we have found that if we know a general behavioural pattern for a plant family the development of a method for propagation of an additional species in that family can be hastened. In our laboratory we can develop techniques for new species in the studied plant families rapidly.

LITERATURE CITED

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2 Murashige, T, 1977 Clonal crops through tissue culture In Plant Tissue Culture and its Bio-technological Application. Barz, W E Reinhard, and M H Zenk, eds p 392-403

3 Norton, M E. and A A Boe, 1980 Change in morphogenetic potential with repeated subculture of shoots of Rosaceous plants *Plant Physiol* 65(6) 35

4 Smith, S.M. and H E. Street, 1974 The decline of embryogenic potential as callus and suspension cultures of carrot (*Daucus carota* L) are serially subcultured *Ann. Bot* 38 223-241

5 Torrey, J G 1967 Morphogenesis in relation to chromosomal constitution in long-term plant tissue cultures *Physiol Plant* 20 265-275

MODERATOR ROGER DUER: Now is the time for questions for our panelists.

RALPH SHUGERT: Dr. Norton, I didn't catch the name of the genera you are working on in your micropropagation.

COLIN NORTON: With the Roseaceous plants that were referred to, we worked with *Potentilla, Spiraea, Crataegus, Chaenomeles. Prunus,* and *Pyracantha.*

BRUCE BRIGGS: When working on the items that are normally evergreens, like rhodendrons, and working on items which are deciduous, did you notice any difference in the decline of the shoot length — when they began to reduce in length during subculturing?

MARGARET NORTON: My answer to that is no. I don't

think there is really very much difference between evergreen and deciduous species. Both seem to show reduced shoot length gradually with each successive subculture.

RALPH SHUGERT: Where are your micropropagated plants now? From the lab, did they then go to the field; are they growing outside now?

MARGARET NORTON: Originally the explants were taken from plants which were mainly field-grown and then we subcultured them over a series of generations. Now some of those have got to the stage of being transferred into greenhouse conditions. But I haven't gone further than that.

RALPH SHUGERT: In other words, they are still not out of a controlled environment; they are not in the nursery area?

MARGARET NORTON: The results I presented were just purely relating to the state of the cultures, the state of the shoots in culture. They are in controlled conditions, yes. But I have transferred some of them to the greenhouse also.

RALPH SHUGERT: The ultimate performance is the thing of importance to the commercial nurseryman.

MARGARET NORTON: Yes, it is, but if you get a considerable decrease in shoot number and shoot length every time you subculture, then this is of importance to the commercial grower as well.

RALPH SHUGERT: We grow *Potentilla* cultivars. *Potentilla* is a very important flowering shrub, particularly in the eastern U.S. When it goes through microculture, is this going to disturb a cultivar down the line in my nursery that I have got to sell to the ultimate consumer?

MARGARET NORTON: I think it might . . . but I think the important thing really is that we probably shouldn't grow our shoots under many subcultures for a long period of time. We should go back to our original stock material very frequently to get new explants to be sure that our material is staying true-to-type.

COLIN NORTON: Could I just add a point in there. We believe that we are going to get material true-to-type in most instances. But in micro-culture, if you get shoots arising from callus-like material there is an increased likelihood of those not being true-to-type.

RALPH MOORE: Dr. Norton, you spoke about the ill effects of soaking the seeds in water too long, as I understood it. We have never gone back to repeat this but — we are breeders of roses — three or four years ago, we accidentally had some seed stored in the refrigerator, and the plastic bag was not folded over and sealed down as we generally do with a rubber band. The

refrigerator leaked water into this bag. How many days it was in there, we don't know, but it was icy water when I discovered it. I know it was there for days. My first impulse was — those seeds are no good and let's throw them away. But I said no, just let's go ahead and plant them; they probably won't come up very well, so we planted very thickly. Some was of *Rosa multiflora* origin, one of the dwarf forms, and some was hybrid rose seed. The hybrid seed came up about normal but the other came up as thick as hair on a dog's back and I never had such good germination in my life. I don't know if soaking in the icy water had anything to do with it; how many hours you should do it, how many days, I don't know.

COLIN NORTON: Well, maybe you are quite lucky working with roses that you have a seed with a fairly thick seed coat, so it is probably a reflection of the fact that the seeds were dormant. Maybe you just softened the seed coat with the water and it enhanced germination.

RALPH MOORE: One of the former students at Cal Poly, in Pomona, California, commented to me that they had studies in which they were soaking the seeds in a fish tank. They bubbled air through this water constantly and it gave them good germination.

COLIN NORTON: Yes, I can't really give a comment on that not knowing the species of seed.

RALPH MOORE: There were several species of seeds that they used.

COLIN NORTON: Well, all I can say is that in some instances this does work and in some instances it is detrimental, but it is better to have oxygen bubbling through than no oxygen bubbling through.

VOICE: What time of year were your *Sequoiadendron* cuttings taken?

LAUREN FINS: In the results that I presented the cuttings were taken at different times of the year. We have done some other experiments in which we looked at time of year to take cuttings; it seems that the fall is the best time to do that, from donor plants that are grown outdoors. We were taking cuttings in November when we had the best rooting success.

DALE KESTER: Coming back to the question about micropropagation. Many of our deciduous plants and strawberries and bulbs do deteriorate with time in consecutive propagations without chilling. I suspect that this is probably the thing that we need to look for. There is no reason why this little rooted growing point should act like a little runner of the strawberry that has been worked out. Nursery production of strawberries in Califor-

nia depends on having this period of chilling. Have you done anything on that aspect of micropropagation? I really suspect genetic breakdown may be something physiological.

MARGARET NORTON: Experiments that I conducted were done over a period of about a year which is not a long time, really. But I suppose the plants are going through a very rapid development phase.

There is one other point that I should have made in relation to other possible changes in relation to a question over here. I was interested in Bob Ticknor's comments about rhododendrons. He had not observed them producing flowers very readily after they had come out of tissue culture propagation. In fact, I have observed the same thing with many Roseacous species as well. I am not sure, but I think, perhaps, flowering might be decreased with an increasing number of passages through culture. So that is another possible interesting aside.