Preferred citation: D.W. Einspahr and M. Johnson. Tissue culture and the pulp and paper industry. In **Papermaking Raw Materials**, *Trans. of the VIIIth Fund. Res. Symp. Oxford, 1985*, (V. Punton, ed.), pp 1–6, FRC, Manchester, 2018. DOI: 10.15376/frc.1985.1.1.

TISSUE CULTURE AND THE PULP AND PAPER INDUSTRY

D. W. Einspahr[1] and M. Johnson
The Institute of Paper Chemistry
Appleton, Wisconsin, USA

ABSTRACT

Emphasis on the tissue culture propagation of forest trees has increased dramatically. Tissue culture methods available to forestry and the pulp and paper industry are micropropagation, organogenesis, and somatic embryogenesis. Somatic embryogenesis, although more difficult to accomplish, seems to have the most promise for use with forest trees because (1) when appropriately employed it can be a true mass production procedure and (2) the approach can be used efficiently with several genetic engineering techniques. Major genetic gains in growth rate, wood quality, insect and disease resistance, and improved climatic adaptability are anticipated when tissue culture techniques are used in conjunction with genetic engineering. Emphasis in The Institute of Paper Chemistry's tissue culture research is on the development of a somatic embryogenesis procedure for conifers.

INTRODUCTION

Tissue culture, the aseptic culture of cells and tissue, has been widely used in research involving animal cells. Tissue culture in forestry has received a major amount of emphasis the last five to six years, with several papers being published (1,2,3,4) which describe techniques, potential of the approach, and early progress.

---

[1]Senior Research Associate and Research Associate, The Institute of Paper Chemistry, Appleton, WI. The authors gratefully acknowledge the assistance of Dr. John Litvay in developing a number of the concepts described in this paper.

Tissue culture *per se* is not a genetic improvement method but is instead a method of vegetatively propagating (cloning) trees and plants. The tissue culture propagation of nonwoody plants has been used in research and in the commercial propagation of selected high value species for approximately twenty years. Recently, the tissue culture propagation of woody plants has come into its own. Evans *et al.* (5), for example, in a book published in 1983, listed over seventy woody plants that have been regenerated using cell culture techniques. A comprehensive review of recent activity in this area would no doubt result in many additional species being added to the list. The three procedures commonly considered when describing tissue culture methods are (1) micropropagation, the *in vitro* propagation of plants using stem meristems, shoot tips and apical buds, (2) organogenesis, the *in vitro* propagation of plants from explants or callus where organs (roots or shoots) are produced and then are manipulated to produce complete plants, and (3) somatic embryogenesis, the *in vitro* propagation of plants from single cells or small groups of vegetative cells, where the final stages of development produce an embryolike structure that is capable of developing into an intact plant.

Most of the early successful commercial propagation methods for woody species involved micropropagation. More recently, a number of tree species (15 conifers and 17 hardwoods) were reported to have been propagated utilizing the technique of organogenesis (6). Somatic embryogenesis has been the approach that, although desirable, has been difficult to accomplish with woody species. Successful somatic embryogenesis has been reported, however, for at least twelve hardwood species and two conifers. Based on these successes, the current feeling is that the phenomenon exists universally in plants and that only additional fundamental research is required to develop appropriate somatic embryogenesis methods for any tree species.

MICROPROPAGATION AND ORGANOGENESIS

Micropropagation and organogenesis both involve the production of multiple shoots using growth regulators (cytokinins). The shoots produced are in turn rooted individually using auxins. This is an oversimplification because usually several intermediate steps are required to elongate, root, and transfer them into soil. These procedures, although fairly labor intensive, have been used to produce high value ornamentals and have

the potential for use with forest tree species where identical individuals are desired for forest genetics and silviculture research. With the exception of a relatively few easily propagated species (Populus, Prunus, and Acer species, etc.), trees regenerated by organogenesis and micropropagation procedures are expected to be too expensive for use in operational plantings.

SOMATIC EMBRYOGENESIS

One definition of somatic embryogenesis, as briefly described earlier, is a procedure that involves starting with a single cell or a small group of vegetative (somatic) cells and attempts to imitate under cell suspension conditions the development of an embryo in a seed. One potential advantage of this aproach is that this would be a true mass production method. By starting with a relatively few cells, the numbers are first multiplied and then, after the desired number of cells are produced, they are manipulated to divide in an organized manner and develop into embryos (seedlike structures) which are capable of developing like seeds into intact plants. An additional important advantage is that somatic embryogenesis makes possible more efficient use of certain genetic engineering techniques (transformation, protoplast fusion, and selection for somoclonal variation). Genetic engineering techniques are most often and most efficiently conducted at the cellular level. When conducted at the cellular level, it is then very desirable to be able to generate large numbers of plants from the relatively few genetically modified cells. Somatic embryogenesis thus becomes an important step in this approach.

IMPACT ON THE PULP AND PAPER INDUSTRY

Most conventional tree improvement programs involve selection of parent trees, establishment of seed orchards, progeny testing, roguing of the less desirable parent trees, and the establishment of second generation seed orchards. The genetic gains in growth rate, wood quality, etc. result because the seedlings produced from seed orchard seed are superior to seedlings produced from seed collected from native stands. A successful and reliable embryogenesis system would allow operational clonal forestry to become a reality. Utilization of the clonal forestry approach has the immediate potential of

yielding an additional gain of 10 to 30 percent over the presently employed seed orchard method (4,7,8,).

Long-term, somatic embryogenesis could help unlock the potential for major increases in volume growth, wood quality, insect and disease resistance, and site and climatic adaptability through the use of genetic engineering techniques. The gains from genetic engineering *via* somatic embryogenesis are expected to be several times as great as from clonal forestry techniques. The dividends in terms of the factors influenced will be far reaching, but it is obvious that patience and a long-term commitment to such research will be required.

THE INSTITUTE OF PAPER CHEMISTRY PROGRAM

The Institute tissue culture research program has emphasized the development of the techniques for producing somatic embryos from conifer cell suspensions. A model system approach is being used that, to date, has employed a number of model species including natural pine embryogenesis, wild carrot somatic embryogenesis, coffee somatic embryogenesis, and both loblolly pine and aspen organogenesis. Considerable emphasis is being placed upon studying systematically the biochemistry of embryogenesis and developing biochemical and histological markers that can be used to judge cell line quality and determine the progress of embryogenesis.

Presently, methods have been developed that allow us to generate and maintain good quality cell lines from a variety of species and cell tissue origins. Studies on wild carrot cells undergoing somatic embryogenesis and investigations on cells undergoing natural embryogenesis have demonstrated the importance of polyamines in embryo development. Also, for example, we have developed and are presently evaluating biochemical markers that we feel will be useful in monitoring somatic embryogenesis. Data on enzyme activity, free amino acids, polyamines, phenolics, energy levels, redox potential and growth indices are being developed for wild carrot and other model systems and several loblolly pine cell lines.

SUMMARY

The tissue culture propagation of forest trees has been emphasized in the past five or six years. Tissue culture

methods include the techniques of micropropagation, organogenesis, and somatic embryogenesis. Most of the successful commercial propagation methods for woody species have employed micropropagation techniques. Organogenesis has been used successfully on more than thirty species of trees. Propagation of woody species by somatic embryogenesis has been more difficult to accomplish, but there have been enough successes so that the current feeling is that this approach can be successful for any plant species, given adequate fundamental research.

Somatic embryogenesis, when properly employed, has the advantage that it is a true mass production procedure. In addition, since genetic engineering is normally and more efficiently accomplished at the cellular level, a reliable somatic embryogenesis method could result in major gains in volume growth, wood quality, disease and insect resistance, etc., when used in combination with genetic engineering techniques. Use of the clonal forestry approach (multiclonal plantings) is expected to increase volume growth by 10-30% over the widely used seed orchard approach. Even greater gains are anticipated when genetic engineering techniques are used in the production of clones for use in this approach. The major emphasis of The Institute of Paper Chemistry's research in this area has been on the development of a somatic embryogenesis procedure for conifers. A biochemically-oriented model systems approach is being employed.

REFERENCES

1. Karonsky, D. F. Potential for forest tree improvement via tissue culture. BioScience 31(2):114-20(1981).

2. Verma, D. C. and Einspahr, D. W. Conifer tissue culture and how it may impact the pulp and paper industry. Tappi 66(11):25-7(1983).

3. Einspahr, D. W. Tissue culture in forestry, current status. Proceeding Appalachian Soc. of Am. Foresters. Charlotte, NC, Jan. 26-7, 1983:8-16(1984).

4. McKeand, S. E. and Weir, R. J. Tissue culture and forest productivity. J. Forestry 82(4):212-18(1984).

5. Evans, D. A., Sharp, W. R., Ammirato, P. V., and Yasuyuki, Y. (ed.). Handbook of Plant Cell Culture. Vol. 1, Techniques for Propagation and Breeding. McMillan Publishing Co., NY, 1983. 970 p.

6. Flick, C. E., Evans, D. A., and Sharp, W. R. Organogenesis in Handbook of Plant Cell Culture. Evans et al. (see above) Editors. Vol. 1. Techniques for Propagation and Breeding. McMillan Publishing Co., NY, 1983:13-81.

7. Kellison, R. C. and Sprague, J. Genetics programs increase pine disease resistance, growth rate. Pulp Paper 55(6):187-91(1981).

8. Kleinschmidt, T. A program for large scale cutting propagation of Norway spruce. N.Z. J. for Sci. 4:359-66(1974).

Transcription of Discussion

SESSION 1 PULP
GROWTH STRUCTURE AND POLYMORPHY
Chairman D. Attwood

Tissue Culture and the Pulp and Paper Industry
by D.W. Einspahr and M. Johnson

Dr. N.K. Bridge Pira, Leatherhead, England

Your paper was very interesting and quite exciting. What time-scale do you envisage for the improvements in non-genetically engineered systems?

Dr. D.W. Einspahr We think it will be three or four more years before we have plantlets from cell suspensions. We are fairly close, in the case of Douglas Fir, to having plantlets that will come off a callus but that is not quite the system we want. So if we take it from the origin of our work it will have taken us approximately ten years to accomplish somatic embryogenesis.

Dr. D.W. Clayton PPRIC, Pointe Claire, Canada.

The creation of trees that are disease resistant is an excellent goal. Do you know if other characteristics, such as fibre properties, changed at the same time as the tree acquired the disease resistance? It would be a pity if, in acquiring disease resistance, the tree developed undesirable fibre qualities.

Einspahr In this case we have not modified the plant at all. We have only pulled from a seedling population already existing resistant individuals. So in this case we have not genetically engineered the plants but merely used a tissue culture system to select out those that show resistance at the cellular level, but of course in the future we will have to bear fibre qualities in mind.

Dr. R.H. Marchessault Xerox Research, Mississauga, Canada

Could you give us a prognosis on whether or not the genetic engineering approach will come to replace that of tissue culture and what is the time scale?

Einspahr There are techniques in genetic engineering, such as protoplast fusion where you enzymatically remove the cell wall and then fuse the two cell nuclei and produce unusual types of hybrids and this is progressing with other species. However, what you need is a technique for going from single cells to plantlets to take advantage of this approach. So in other words, while this technique is progressing in other species, I think it will be three or four years before we see it applied in forestry.

Prof. J. Marton Westvaco Corp, Laurel, USA.

I would like to make a comment on your interesting presentation, also related somewhat to Dave Clayton's question. As you know, our forestry group has been working in this area with your people and with others. Over the last four years we have planted out plantlets of Loblolly pine obtained through organogenesis by a tissue culture technique. These have developed very nicely and survived well, but grow more slowly than regular seedlings. We believe this may be due to their having a weaker root system. Consequently, we think it is very important that a stronger root system should be developed in the plantlets to be outplanted, otherwise their rate of growth will not be competitive.

Einspahr The plants you refer to have been produced using a technique developed by North Carolina State University. They used what might be called an organogenesis approach. They will take seedling explants, place them on a growth medium and produce shoots and then those shoots are rooted. They have experienced the development of a more fibrous root system and thus a slower growth rate than one would normally expect to have found from regular Loblolly pine seedlings that have a tap root. I think the somatic embryogenesis approach has an inherent advantage in that it may preserve the original growth characteristics of the species.

Dr. J. Cassidy Wiggins Teape R & D, Beaconsfield, England

Can you please tell us why you use callus material rather than normal cell material?

Einspahr We actually use normal dividing cells to produce callus and the callus is used to produce cell suspensions. It is actually more difficult to scale up from callus material to the same degree that you can scale up using single cells or groups of cells. So there is no difficulty in using callus and producing somatic embryos. These would be perfectly acceptable materials. The biggest problem is that there isn't the same degree of flexibility of the scale up so that this is one of the main advantages of using a cell suspension approach.