ment where a clean, high humidity condition can be maintained. Jars are washed and dipped in Physan prior to use.

By the third week after grafting, good callus formation can be seen; by the fourth week the buds on the scions begin to elongate and unfold. This is a critical time to watch for jar removal. At the first sign of bud unfolding, the jar should be tipped to provide some air circulation and start the hardening-off process for the graft. The jar may be completely removed two or three days after tilting. If leaves are allowed to unfold in untilted jars, the new scion will usually wilt badly and sometimes even die when the jar is removed. Light hand misting may be necessary on warm days to prevent wilting. Two to three weeks after jar removal, the plants may be taken outside and placed in a shade house where they again may require hand misting on warm days for awhile.

By the following spring, one year later, the grafted plants are ready for shifting to larger containers.

This method has worked well for us for many years. The most important things to remember are to keep the grafts dark, cool and dry, and to be sure to remove the jars before leaves unfold.

RECENT ADVANCES IN THE PROPAGATION OF WOODY PERENNIALS

DON J. DURZAN

Department of Pomology
University of California
Davis, California 95616

Abstract. Major advances have emerged in methods for cloning, creating new genetic variation (directed and misdirected), and in risk reduction associated with the vegetative propagation of woody perennials. These advances are related mainly to the capturing of specific genetic gains. Advances will be illustrated with wood ornamentals, forest (pine, fir, spruce) and fruit trees (peach, cherry, pistachio).

INTRODUCTION

Apart from classical and traditional methods of plant propagation (8,15), the emphasis in this review will be on more recent cloning procedures based on cell and tissue culture (4,5). Recent advances in propagation reflect three categories of trend. First, for cloning procedures, somatic embryogenesis in cell suspensions for mass propagation is increasingly being considered as an alternative or supplemental method to micropropagation (6). The trend with micropropagation and somatic embryogenesis is to apply these precedures to explants from mature trees. Second, methods are now available to create new genetic variation with long-lived woody

perennials, e.g., regeneration of sandalwood from protoplasts, the genetic engineering of cells capable of somatic embryogenesis, and aberrations from true-to-type gene expression (somaclonal variation) (7). With fruit trees and ornamentals, genetic variation is often considerably less than with our native forest trees. For the latter, the trend is towards domestication of the existing genetic variability. Third, computer-assisted mechanization and marketing coupled with improved diagnostic and prognostic methods are sought for product quality control, the reduction of risks, and lower production costs. This trend is reflected by the emergence of enterprises in biotechnology that fully integrate all nursery operations with well-established marketing and sales.

Cloning procedures. Numerous incremental advances in the micropropagation of woody perennials are evident in recent books (2,3,4,5,10). Researchers have attempted to gain greater control of somatic embryogenesis as an alternative to micropropagation because:

1) greater numbers of propagules are based on cellular multiplication rates rather than on separate root and shoot multiplication rates.
2) ease of handling of cells, encapsulation, low temperature storage of somatic embryos, and potentially mechanized production of tissue-culture derived plants.
3) better process control, e.g. pH, gas exchange, temperature, uniformity of environment, early system diagnostics, etc.,
4) ease of scale-up and long-term storage of cell lines with improved prospects for process synchronization, and
5) easier application of principles and novel methods of agricultural biotechnology to uniform cell suspensions (e.g. protoplast fusion, recombinant DNA methodology, etc.)

Somatic embryogenesis in conifers has now been achieved with *Picea abies* (14), and *Larix* (19). More recently, our laboratory has defined the process of conifer-type somatic polyembryogenesis in Douglas-fir, loblolly pine, sugar pine and Norway spruce (13, and unpublished data). These approaches may be especially useful for high-value specimens for the Christmas-tree and ornamental market. Improvements in the cost-effectiveness of this technology are needed to mass propagate forest trees of low individual value.

Another advancement in cloning methods is the work with difficult-to-root mature woody perennials (30-to-100-year-old trees) (12). Our approach recognizes and extends earlier observations in France by AFOCEL scientists. For all conifers, the medium, method of surface sterilization, shoot and root production have been improved to more attractive levels. Micropropagated conifers are now being field-tested in several countries.

Micropropagation, based on explants from mature donors, facilitates the capturing of proven genetic gains of locally adapted populations. Furthermore by cloning mature and proven trees we may reduce our dependence on juvenile-mature correlations for the expression of elite traits. With seeds from controlled crosses (e.g. from seed orchards) the time needed to provide suitable explant material can be reduced by nearly one week by germination in controlled atmospheres.

The control of plant development by plant growth regulators may soon be approached in a very different way with the discovery of oligosaccharins (1). Oligosaccharins, or cell wall fragments, have plant growth regulator activity in cell and tissue culture systems. However, before this new class of substances becomes useful, the factors giving the morphogenetic responses need to be defined chemically, extended to a wider range of plants, and available commercially on an economic scale.

Expressions of totipotency in explant for cell and tissue culture are being exploited in four directions: 1) wide expression of totipotency with limited-to-random control as in micropropagation of fruit trees, 2) narrow expression with specific control as in somatic embryogenesis on conifer-type somatic polyembryogenesis, 3) replacement of the above by easy-to-root clones (azaleas, rhododendrons, forest trees), and 4) production of artificial seeds (18) from somatic embryos for storage at low temperatures to overcome poor seed years and to allow more time for progeny and field-testing (forest trees).

The useful and non-useful methods still have to be sorted out and established as cost-effective. In micropropagation, we can now begin to consider bypassing many of the constraints imposed by mature physiological states of explants. Problems in development remain such as vitrification, rooting, physiological preconditioning, and true-to-type clonal performance. We can expect new approaches to the control of development based on the exploitation of oligosaccharins.

New genetic variation. Attempts at genetically modifying plant cells are now widespread. It now seems surprisingly easy to modify the behavior of cells by microinjection, electroporation, protoplast fusion, induced mutations, etc. (*cf.* Proc. Intl. Hort. Congress, Davis, CA, 1986). However, the question remains: can we obtain useful clonal populations of plants from these genetically modified cells? The commercial answer to this question will take at least another decade. The answer may emerge from studies with the *totipotent cells showing true-to-type somatic embryogenesis.* Whenever possible, fully totipotent cells should be used in genetic modification so that the resultant new germplasm can be compared with industry standards. Unfortunately, we are still years away for developing a good delivery system for new cultivars based on the

above approaches. Nevertheless, progress is encouraging in the sense that new opportunities are seen in basic research where none existed before.

Risk reduction. At the completion of any cloning cycle, efficiency and quality control should be assured to protect the producer, consumer, and society. In clonal propagation, new diagnostic methods and biosensors especially for pathological situations (ELISA, mono- and polyclonal antibodies, radioimmunoassay, etc.) are being developed based on the relations in Figures 1 and 2 (8,16,17). Diagnostic methods, using principles and dogma identified by arrows, can also be applied to insects, pests, and diseases provided that methods are cost-effective, rapid, and reliable. Every indication exists that we will be able to certify trueness-to-type and disease-free stock through high-biotech methodology.

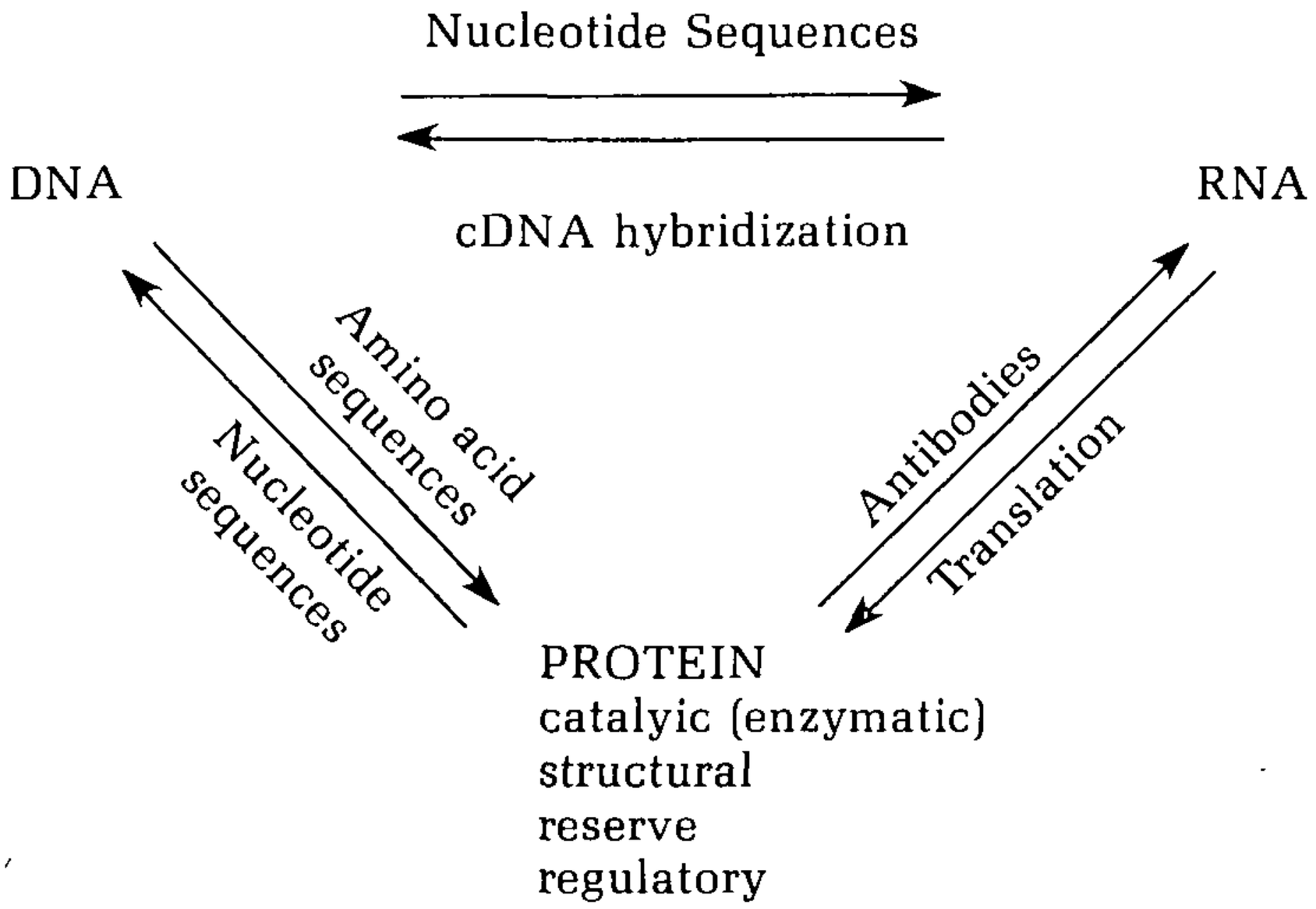

Figure 1. Interrelations among macromolecules (DNA, RNA, PROTEIN) associated with gene expression. Feedforward and feedback steps in process control are based on diagnostic criteria associated with arrows. These interrelationships provide the basis for dynamics shown in Fig. 2.

With woody perennials, the problem remains that the valuable attributes we seek to introduce, clone, and certify are usually found in difficult-to-propagate mature specimens. Gene expression in elite specimens are often based on very complex, long-lived, interactive and dynamic and heterotic genetic systems. In forestry, we are continually faced with the low individual production cost for propagules. Nevertheless, where valuable germplasm can be cloned, novel methods are now available to store encapsulated somatic embryos until performance testing is completed.

Recently, we have characterized gene expression in the developing pistachio fruit as a set of metabolic phenotypes at the experimental laboratory level (9). Our approach is based on the interrelations shown in Figure 2 for all stages of the life cycle.

This diagnostic method should provide sharper definitions of developmental processes in specific steps of cloning procedures. Definitions involve mathematical representations of physiological processes and strategies (algorithms) used by the clone in true-to-type gene expression. Notions of "process control" are based on time (physiological states and development), metabolic networks, and a quantitative description of the behavioral dynamics of the system in the solid, liquid, and gaseous phases.

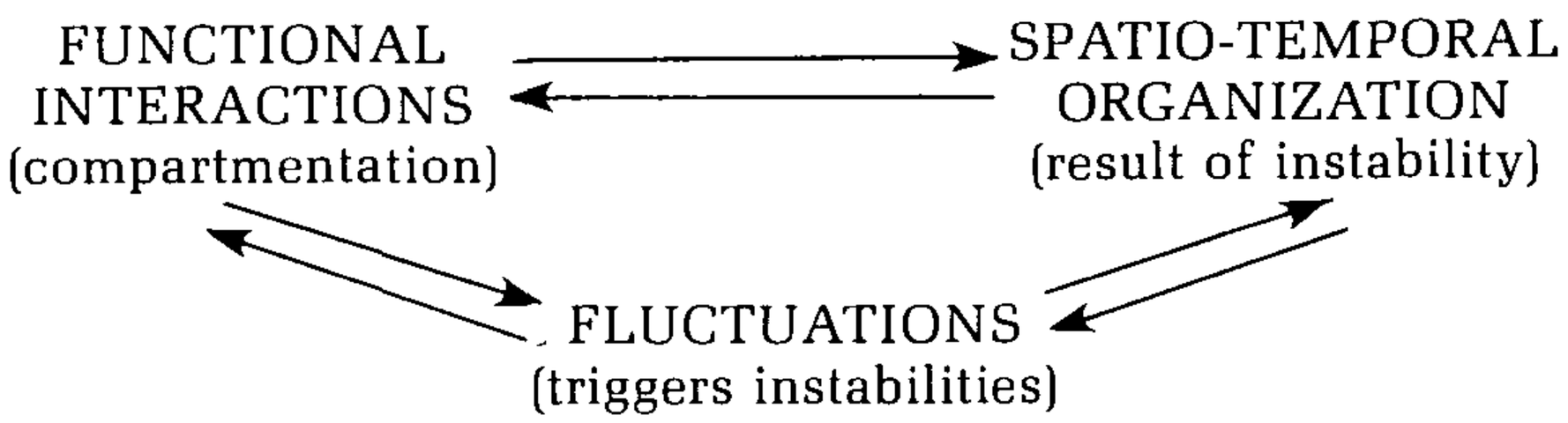

Figure 2. In cell and tissue culture, risk reduction should be based on a better understanding of gene expression and dynamics of growth and development of clonal materials. Dynamics arise from functional interactions among components of the system their spatio-temporal organization and their fluctuations. Functional interactions may include relation between exogenous (synthetic) and endogenous (naturally occurring) growth regulators calling into play new sets of gene activities that result in fluctuations and in the reorganization of cell potentials in the explant. Attempts have been made to capture these interactive properties and identify them on maps of metabolic phenotypes.

Metabolic phenotypes for clonal materials may someday be produced routinely to understand why some species remain recalcitrant and some processes unworkable. Unfortunately, metabolic phenotypes are yet of little value to the nursery where simple, single variable, specific, rapid cost-effective diagnosis is required. Phenotypes are an experimental tool more suitable for complex situations, involving many variables and dynamic interactive production systems critically focused on some aspect of quality control of a highly valued product (*cf.* Table 1).

Fortunately, metabolic phenotypes involving large-scale data arrays can now be compared, subtracted from one another or modified by computers to show more effectively the dynamics and efficiency of processes underlying gene expression. Phenotypic maps could include known factors and even factors based on yet unidentified, but useful, marker compounds for elite genes. Appropriate diagnostic methods for the computer assisted mechanization of the propagation process are not yet available.

Table 1. Values and attributes of diagnostic methods (Fig. 1) and metabolic phenotypes (Fig. 2) that relate to risk reduction (certification and quality control). Criteria for quality assurance may be realized with the aid of computer-aided chemistry and diagnostic reasoning.

Certification	*Attributes of Metabolic Phenotypes*
Insect-free	Distribution of key indicators
Pest-free	Kinematics and dynamics of problem
Disease-free	Precatastrophic indicators
True-to-type traits	Stability analysis
	Probabilistic scenarios
Quality Control	General vs. high specificity
Aberrant phenotypes	Ability to quantify physiological preconditioning
Synchronization	Process control logic
Scale-up	Plausible developmental algorithms
Cost-effectiveness	Plant-machine compatibility in clonal propagation

SUMMARY

While much of the recent progress in clonal propagation involves methods to capture genetic gains, mature trees and to improve quality control, they are not yet immediately or widely useful for plant propagation. Nevertheless, trends are in keeping with the practical needs of the propagator. In the long run, new genetic variation and biotechnological fixes will emerge based on recent advances that could lead to cost-effective, reliable, rapid and novel quality control technologies, especially for companies producing 5 to 10 million plants annually. Evidence is also emerging that some existing technologies in propagation can rapidly become obsolete because of improved biotechnology. We still need more examples and evidence for widely applicable, stable, and long-term procedures especially for woody perennials. Some examples should emerge from newly established biotechnology companies dealing with horticultural and forestry products or "green goods" in North American and in developing countries. All are continually threatened by limits in natural resources and by an economy challenged by currency, energy crises, and by natural and man-made catastrophes that lead inexorably to the high-grading of germplasm.

LITERATURE CITED

1. Albersheim, P. and A. G. Darvill. 1985. Oligosaccharins. *Sci. Am.* 253:58–64.
2. Ammirato, P. V., D. A. Evans, W. R. Sharp, and Y. Yamada. 1984. Handbook of plant cell culture, Vols. 1 to 3, Macmillan, New York.
3. Bjaj, T. S. P. (ed.). 1986. Biotechnology in agriculture and forestry. Vols. 1 and 2, Springer-Verlag, New York.
4. Bonga, J. M. and D. J. Durzan (eds.). 1982. Tissue culture in forestry. Martinus Nijhoff/Dr. W. Junk, The Hague, 420 pp. ISBM-247-2660-3.
5. Bonga, J. M. and D. J. Durzan (eds.). 1986–87. Cell and tissue culture in forestry. Martinus-Nijhoff/Dr. W. Junk, Derdrecht, Vols. 1, 2 and 3 (in press).
6. Durzan, D. J. 1980. Progress and promise in forest genetics. *In* Paper/Science

and Technology: The Cutting Edge, 50th Anniversary, May 8–10, 1979, The Institute of Paper Chemistry, Appleton, Wisconsin, pp. 31–60.

7. Durzan, D. J. 1984. Potential for genetic manipulation of forest trees: Totipotency, somaclonal aberration, and trueness to type. *In* Proceedings International Symposium on Recent Advances in Forest Biotechnology, July 10–13, 1984, Michigan Biotechnology Institute, Traverse City, Michigan, pp. 104–125.
8. Durzan, D. J. 1985. Tissue culture and improvement of woody perennials: An overview. Proceedings in Tissue Culture in Forestry and Agriculture. R. Henke, K. Hughes, M. Constantin, and A. Hollaender (eds.), September 9–14, 1985, University of Tennessee, Knoxville, Plenum Press, New York 32:233–256.
9. Durzan, D. J. and K. Uriu. 1986. Metabolic networks in developing pistachio embryos (*Pistacia vera* cv. Kerman). *In* Progress in Developmental Biology, H. C. Slavkin, ed. Proceedings of the Tenth International Congress of the International Society of Developmental Biologists, August 4–9, 1985, Los Angeles, California. pp. 199–202.
10. George, E. F. and P. D. Sherrington. 1984. Plant propagation by tissue culture. Exegetics, Ltd., Eastern Press, Reading, Berks.
11. Gupta, P. K. and D. J. Durzan. 1985. Shoot multiplication from mature trees of Douglas-fir (*Pseudotsuga menziesii*) and sugar pine (*Pinus lambertiana*). *Plant Cell Reports* 4:117–179.
12. Gupta, P. K. and D. J. Durzan. 1986. *In vitro* establishment and multiplication of juvenile and mature Douglas-fir and sugar pine. Symposium on *In Vitro* Problems Related to Mass Propagation of Horticultural Plants, September 16–20, 1985, Gembloux, Belgium. *Acta Hort.* (in press).
13. Gupta, P. K. and D. J. Durzan. 1986. Somatic polyembryogenesis from callus of mature sugar pine embryos. *Bio/Technology.* 4:643–645.
14. Hakman, I., L. C. Fowke, S. Von Arnold, and T. Ericksson. 1985. The development of somatic embryos in tissue cultures initiated from immature embryos of *Picea abies* (Norway spruce). *Plant Sci. Lett.* 38:53–59.
15. Hartmann, H. T. and D. E. Kester. 1983. Plant Propagation: Principles and Practices, 4th Ed., Prentice Hall, Englewood Cliffs, New Jersey.
16. Jarvis, M. T. 1985. Biosensors: Today's technology, tomorrow's producers. SEAL Tech. Publ., Madison, Georgia.
17. Lewin, B. 1985. Genes. Second Ed., J. Wiley and Sons. New York.
18. Linden, T. 1984. Planting seeds for the future. Encapsulation. In *Western Grower and Shipper*, Jan. pp. 12–14.
19. Nagmani, R. and J. M. Bonga. 1985. Embryogenesis in subcultured callus of *Larix decidua*. *Can. Jour. For. Res.* 15:1088–1091.