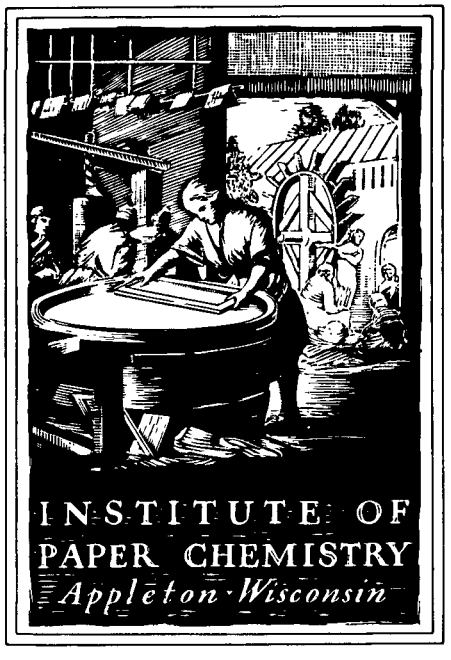
PROJECT ADVISORY COMMITTEE

Subcommittee on

Forest Genetics



IPC STAFF STATUS REPORTS

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Your advice and suggestions on any of the projects will be most welcome.

FOR MEMBER COMPANIES ONLY

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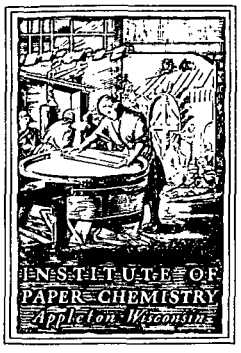

THE INSTITUTE OF PAPER CHEMISTRY
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September 30, 1988

TO: Members of the Forest Genetics PAC

As indicated in my recent letter, we are forwarding a detailed agenda for our fall meeting, October 26-27. Included also is some advance reading material, our most recent status reports. Please use these items in preparing for participation.

Adjusting to and planning for our move to Atlanta has absorbed considerable time and resources, but we nevertheless have completed some interesting research and are busily summarizing results in preparation for the meeting.

We look forward to sharing our findings with you, introducing you to some new employees, and getting to know several new committee members. Please remember to register, if you have not already done so. Be certain also to circulate these materials to other colleagues that plan to participate.

Many thanks and best regards.

Sincerely,

Ronald J. Dinus
Director
Forest Biology Division

RJD/jmm
Enclosure
Copies to: Dr. Robert C. Kellison, North Carolina State University
Dr. David F. Karnosky, Michigan Technological University

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AGENDA

FOREST GENETICS PROJECT ADVISORY COMMITTEE

October 26-27, 1988
The Institute of Paper Chemistry
Continuing Education Center
Appleton, Wisconsin

Wednesday, October 26

8:00 a.m.	Laboratories open and personnel available	
NOON	Lunch, CEC Dining Room	
1:00 p.m.	Opening Remarks Welcome/Introductions Overview Review of PAC Recommendations	Dinus
1:30	Initiation and Maintenance Loblolly Pine, Immature Explants Summary, Prognosis, and Plans	Becwar
2:00	Douglas-Fir and Mature Explants Summary, Prognosis, and Plans	Nagmani
2:20	Development/Maturation, and Conversion to Seedlings Improvements to the Model System Development/Maturation Protocols Media Growth Regulators Genotypes	Nagmani
2:50	Conversion to Seedlings Germination Seedling Yields	Uddin
3:10	Coffee Break	
3:30	Improvements in Loblolly Pine Important Factors Biochemistry of Development	Becwar
3:50	Storage Proteins and Lipids Ultra-Structure Biochemical Analyses	Feirer
4:20	Isozymes and Polar Lipids Zygotic/Somatic Comparisons	Johnson
4:40	Summary and Discussion	Dinus
5:00	Cocktails and Dinner, CEC Dining Room	
7:00	Hardwood Project Position Statement Needs, Priorities, and Plans	Dinus

AGENDA (Contd.)

Thursday, October 27

7:30 a.m.	Breakfast, CEC Dining Room	
8:00	Agenda for Morning	Leach/Dinus
8:15	Discussion	Committee/Staff
9:45	Coffee Break	
10:00	Discussion/Deliberations	Committee/Staff
11:15	Closing Remarks	Leach/Dinus
11:30	Adjournment/Lunch, CEC Dining Room	

NEXT MEETING: March 29 & 30, 1989

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THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

Status Report

to the

FOREST GENETICS

PROJECT ADVISORY COMMITTEE

Project 3223

THE MASS PRODUCTION OF CONIFERS

October 26-27, 1988

PROJECT SUMMARY FORM

DATE: September 15, 1988

PROJECT NO. 3223 - Mass Clonal Propagation of Improved Conifers

PROJECT STAFF: Becwar, Dinus, Nagmani, Uddin, Verhagen

PROJECT OBJECTIVE/GOAL:

Overall - Develop reliable cell and tissue culture systems for mass clonal propagation of improved conifers.

Near-term - Assured and low-cost supplies of quality softwood fiber

PROJECT RATIONALE:

Major increases can be obtained in fiber production, quality, and uniformity via mass cloning of improved trees. Reliable cell and tissue culture systems will also open the way for genetic engineering and production/delivery of new genetic combinations having exceptional growth, increased pest resistance, special fiber properties, and enhanced site and/or climatic adaptability. Screening for and selecting useful variants in culture could also lower costs and accelerate the pace of conventional tree breeding.

Improved growth will reduce raw material costs and increase returns on capital invested in land and equipment. Greater uniformity of clonal plantations can lower both woodlands and mill operating costs as well as enhance end-use properties. Better or new fiber properties can improve end-use performance and foster development of value-added or new products.

CURRENT FISCAL YEAR BUDGET: $500,000

SUMMARY OF RESULTS SINCE LAST REPORT: (March 1988 - September 1988)

Methods previously developed for initiating callus, growing cells in suspension, and maintaining callus and cell lines remain in use or were further

refined. Results from past work on nonconifer model systems, zygotic embryo development, and organogenesis have been integrated, and are being applied to the present model system, Norway spruce, and the target species, loblolly pine and Douglas-fir.

Embryogenesis in the model system is reproducible. Initiation of embryogenic callus from both immature and mature embryos is straightforward. As a result, initiation experiments in Norway spruce were few, and involved only more mature explants.

The major thrust in Norway spruce concerned improvement of protocols for development/maturation and conversion to seedlings. Development remains a difficult step, but changes in media composition and abscisic acid additions yielded improvement in a number of callus lines. Larger numbers of embryos are developing to the cotyledonary stage. Results are being applied to other lines and species as quickly as experiments are completed.

Progress was also made on conversion to seedlings, but "best" results were obtained for only one or so callus lines. "Germination" rates were raised, and now average nearly 40 percent for the best line. Over 500 rooted seedlings have been transferred to soil. The few spruce seedlings recovered in earlier years continue to behave like their zygotic counterparts.

In loblolly pine, developing cones were secured again this summer from the same parents as in earlier years. A series of experiments was established in an effort to increase initiation frequencies. These trials used the best

media developed in past years, with some further modification of growth regulator content. A number of lines yielded embryogenic callus, but some additional time must pass before final results become apparent. Those lines already embryogenic are being maintained and increased.

Over 15 embryogenic lines from earlier years are providing material for experiments on development. Progress on this front has been slow, but some improvement has been noted. Abscisic acid addition has proven helpful, and responses to it have been quantified.

In Douglas-fir, several lines of embryogenic callus were derived from summer cone collections. Numerous early stage embryos are evident in three lines, and two lines are growing better than any produced in earlier years. Much effort is being given to maintain and increase all such lines so that material is available for work on development, and eventually conversion to seedlings. To ensure continued progress with this important species, developing cones will be secured from the Southern Hemisphere this winter.

SHORT TERM GOALS:

Goals for Remainder of FY 88-89

1. Continue refining protocols and raise initiation frequencies in the target species.

2. Improve protocols for increasing development/maturation frequencies, raising efficiency of conversion to seedlings, and generating sufficient material for replicated trials.

3. Increase ability to develop and manipulate somatic embryos in suspension cultures.

4. Investigate protocols for obtaining embryogenic callus from more mature explants.

5. Secure explant sources from the Southern Hemisphere.

6. Accumulate baseline data on anatomy/morphology of somatic/zygotic embryos, especially on nature and frequencies of abnormalities.

7. Execute exploratory research on new approaches, improving techniques, and related topics, e.g., protoplast culture and embryo encapsulation.

8. Provide for prompt presentation/publication of findings.

PROJECT SUMMARY FORM

DATE: September 15, 1988

PROJECT NO. 3223-2 - Biochemistry of Clonal Propagation

PROJECT STAFF: Feirer, Johnson

PROJECT OBJECTIVE/GOAL:

Overall - Develop an improved understanding of biochemical mechanisms controlling embryogenesis and other cloning methods, and devise procedures for raising the effectiveness and efficiency of mass cloning methods.

Near-term - Assured and low-cost supplies of quality softwood fiber

PROJECT RATIONALE:

Improved understanding of biochemical mechanisms controlling embryogenesis and other cloning methods will shorten the time to commercial application of clonal forestry, raise their efficiencies, and facilitate extension to trees mature enough to have been proven genetically superior.

CURRENT FISCAL YEAR BUDGET: $150,000

SUMMARY OF RESULTS SINCE LAST REPORT: (March 1988 - September 1988)

Workers continued to accumulate baseline data on biochemical and molecular characteristics of embryogenesis in zygotic and somatic systems. Special emphasis was given to tracking and promoting embryo development. More than adequate supplies of developing and fully developed zygotic embryos were secured for analyses in both Norway spruce and loblolly pine.

Levels of triglycerides (lipids) were quantified in developing zygotic embryos of Norway spruce and loblolly pine. Parallel analyses of somatic material showed that embryogenic callus contained low levels of triglycerides, but that amount rose as embryos developed. Treatments enhancing

development (e.g., abscisic acid additions) raised accumulation of these important storage compounds. Results were confirmed via electron microscopy. Somatic embryos cultured on media containing abscisic acid had more "fat bodies" than those from media lacking it. Efforts to induce synthesis and accumulation by adding precursors were initiated.

Investigations of protein patterns in developing zygotic and somatic embryos of Norway spruce and loblolly pine moved forward. Work focused on changes in protein synthesis and accumulation as affected by media composition and abscisic acid levels.

Changes in polar lipid composition during development were documented for zygotic embryos of Norway spruce and loblolly pine. Data from somatic embryos are being gathered for comparison of the two systems. These compounds play an important role in membrane synthesis, and may also function in transmitting developmental signals across membranes.

Further assays of peroxidase isozymes and their relationships to development were made in zygotic embryos of Norway spruce and loblolly pine. Numbers of isozymes, and the activities of several, varied with developmental stage, generally rising as development advanced. Numbers and activities showed similar trends in somatic embryos of Norway spruce, but increases were more gradual. Peroxidases may be useful indicators of how well or rapidly development is progressing.

Experiments on the role of glutathione were continued, with emphasis on enhancing development by addition of enzyme inhibitors.

SHORT TERM GOALS:

Goals for Remainder of FY 88-89

Accumulate baseline data on biochemical and molecular characteristics of zygotic and somatic embryogenesis. Investigations will be executed in Norway spruce, with results applied to loblolly pine and Douglas-fir as data and material become available. Some specific lines of experimentation include:

1. Test addition of precursors as a means of enhancing synthesis and accumulation of storage and polar lipids, and for promoting embryo development.

2. Examine effects of ethylene depletion on embryo development.

3. Evaluate addition of substrates as a mechanism for manipulating appearance, number, and activity of peroxidase isozymes, and thereby improving embryo development.

4. Intensify efforts to quantify endogenous abscisic acid levels in developing zygotic and somatic embryos, and monitor uptake and accumulation of exogenous abscisic acid as a function of embryo development.

5. Extend glutathione assays to somatic embryos of loblolly pine, and attempt to provoke changes in synthesis/degradation parallel to those occurring in zygotic embryos.

6. Secure supplies of developing embryos, if necessary, from the Southern Hemisphere.

7. Begin analyses of more mature explants, and compare features to those of immature tissues known to produce embryogenic callus.

8. Execute exploratory research on new approaches, improving techniques, and related topics.

9. Provide for prompt presentation/publication of findings.

PROJECT SUMMARY FORM

DATE: September 15, 1988

PROJECT NO. 3223-3 - Mass Clonal Propagation of Improved Hardwoods

PROJECT STAFF: Dinus, Nagmani, Uddin

PROJECT OBJECTIVE/GOAL:

Overall - Develop reliable, low-cost systems for mass clonal propagation of genetically improved-engineered hardwoods.

Near-term - Assured and low-cost supplies of quality hardwood fiber

PROJECT RATIONALE:

Major increases can be obtained in fiber production, quality, and uniformity via mass cloning. Reliable cloning systems will also open the way for genetic engineering and production/delivery of new genetic combinations having exceptional growth, greater pest resistance, special fiber properties, and enhanced site and/or climatic adaptability. Screening/selection for useful variants in tissue culture holds promise for raising the pace and efficiency of conventional tree breeding.

Accelerated growth will ensure reliable raw material supplies, reduce their costs, and raise returns on capital invested in land and equipment. Greater uniformity can lower both woodlands and mill operating costs as well as enhance properties related to end-use performance. Better or new fiber properties can improve end-use performance and foster development of value-added or new products.

CURRENT FISCAL YEAR BUDGET: $75,000

SUMMARY OF RESULTS SINCE LAST REPORT: (March 1988 - September 1988)

Considerable work has been done on hardwood tissue culture at the Institute over the years. Results from this work and from other organizations indicate that hardwoods can be manipulated with relative ease. Other work at the Institute and elsewhere has suggested that tissue culture can be used to test for disease resistance. Still other work infers that novel variants produced in culture can be isolated and used to introduce new and different traits into breeding stock and clonal reforestation programs.

Work in this new project centered on developing a "position paper" comparing fiber properties, resource availability, growth rates, suitabilities for plantation management, and propensities for clonal propagation of important species, recommending species to use in future research, and outlining possible research directions.

Colleagues in fiber science and papermaking at the Institute and in member companies were consulted to identify useful fiber properties, preferred species, and resource needs. Discussions with authorities in hardwood management and research were used to evaluate suitabilities for management, examine the utility and benefits of cloning, and identify potential research directions.

SHORT TERM GOALS:

Goals for Remainder of FY 88-89

1. Finalize project goals, objectives, and directions.

2. Choose approaches and develop plans for specific activities.

3. Identify and secure plant material.

4. Establish greenhouse and/or in vitro populations needed for efficient research.

5. Begin evaluating available and new cloning methods, adapting them to designated species, and combining them into cost effective systems for application.

6. Explore novel methods for hastening genetic improvement; e.g., screening for desirable traits and/or producing useful variants in culture.

COOPERATIVE INVESTIGATIONS

1. North Carolina State University - Investigation with Drs. H. Amerson and R. Mott concerning effects of differing seed orchard pollen backgrounds on initiation of loblolly pine embryogenic callus.

2. International Forest Seed Company - Supply of "rejuvenated" loblolly pine material by Dr. S. Foster for experiments on initiation of embryogenic callus from mature explants.

3. University of Florida, Leesburg - Study by Dr. D. Gray of desiccation as a method of preparing Norway spruce somatic embryos for storage and germination.

4. University of Cincinnati - Assay by Dr. J. Caruso of endogenous hormone levels, principally ABA and IBA, in embryogenic and nonembryogenic calli.

RELATED STUDENT RESEARCH:

Completed in 1988 - M.S., Independent Study

Michael Bogenschutz - "Electroporation-mediated genetic transformation of Norway spruce cells." (Becwar)

Scott Fruhwirth - "A comparative study of organosolv-pulped tension and normal wood fibers in the papermaking process." (Conners)

In Progress - M.S., Independent Study

Lisa Dudek - "Encapsulation of zygotic/somatic embryos of conifer species." (Nagmani)

Pat Exarhos - "Electron microscopy study of ultrastructure of Picea abies plants obtained via somatic embryogenesis." (Conners)

Fred Lang - "Application of recombinant DNA technology in construction of a gene library." (Dinus)

Lorrain Logsden - "Patterns of gene expression associated with maturing and germinating tree seeds." (Dinus)

Mary Kay Lynde-Maas - "Fructose utilization by embryogenic and nonembryogenic suspension cultures of Norway spruce." (Johnson)

Colleen Walker - "Optimization and quantification of somatic embryogenic cultures of several conifer species in bioreactors." (Becwar/Dinus)

Ph.D. Program

Dan Bunker - "An investigation of the role of drying strategies in the structure of pigment-adhesive films." (Conners)

Russ Feirer - "Biochemical and molecular studies of plant development." In cooperation with University of Wisconsin, Madison

Jong-Moon Park - "Improving the properties of recycled fibers by chemical and enzymatic treatments." (Johnson)

FOREST GENETICS PROJECT ADVISORY COMMITTEE

HANDOUTS

October 26-27, 1988

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Shirley Verhagen
Gary Wyckoff
Richard Matula
Gwen Redding
Wendall Smith
Ronald Yeske

CODES

Tissue response and the results of many studies may be altered or complicated by the genetic differences between cell lines and/or the length of time in culture. To aid the reader (reviewer) in understanding, and the investigator in reporting/analyzing, it is important to be aware of the tissue source used for each study. An example and explanation of our standard tissue identification coding system is presented below; however, at times only part of the code may appear in a text.

All cell lines in excess of one year old:

Example: 20(NS 384-1)2E

- 20 = subcultured 20 times
- NS = Norway spruce
- 384 = research plan (RP384)[a]
- -1 = time of initiation or treatment identification
- 2 = line or genetic source, e.g., seedling No. 2
- E = Immature embryo; explant type (only used if cell line derived from more than one explant within a research plan).

[a]Each experiment initiated by any team member has an approved research plan with an identifying number. The tissue source origin (clone, seed lot, etc.) and initiation date is recorded under that number in the investigator's IPC research notebook and is available in the Tissue Culture Research Plan files.

Cell lines less than one year old from immature cone collections:

Example: 5(LP6B)E - the RP No. is deleted and the letter within parentheses indicates cone source code.

Species Codes	Explant Codes
LP - loblolly pine	C - cotyledon
DF - Douglas-fir	H - hypocotyl
PP - pitch pine	B - bud
PO - pond pine	E - immature embryo
NS - Norway spruce	M - mature embryo
WP - white pine	N - nucellus
WS - white spruce	G - gametophyte
PL - pitch loblolly	O - ovules

CONE SOURCES - 1988

Species	Tissue Culture Code	Source	Industrial Codes
Douglas-fir	DF O	Weyerhaeuser	WTC-566
	DF P	Federal Way, WA	WTC-567
	DF Q		WTC-568
	DF R		WTC-569
	DF S		WTC-570
	DF T		WTC-571
Loblolly pine	LP A	Union Camp	10-1003 D-22 HQI
	LP B	Rincon, GA	10-1007 F-21 HQI
	LP C		10-1011 C-20 HQI
	LP D		10-1018 B-16 HQI
	LP E		10-1019 C-14 HQI
	LP F	Westvaco	7-34
	LP G	Summerville, SC	7-56
	LP H		11- 9
	LP I		11-10
	LP J		11-16
	LP K	Rigesa	7-34[a]
	LP L	Tres Barras, Brazil	7-56[a]
	LP M		11-10[a]
	LP N		11-16[a]
	LP O		11-19[a]
	LP P		11- 9[a]
	LP R	Westvaco	11-25
		Summerville, SC	
	LP FNC	Georgetown, SC	7-34
	LP JNC		11-16
Norway spruce	NS-1-87	Reid Golf Course	Tree #2
		Appleton, WI	
	NS-1-88		Tree #1
	Sky Pine	Tree Farm, Co.Tk E	--
	Syracuse 1	Syracuse, NY	
	" 9		
	" 16		
	" 18		
	" 19		
	" 20		
Pitch/loblolly Hybrid	PL	Westavaco	65 x LP
		Summerville, SC	

[a]Cones obtained from progeny of the given clone.

STATISTICS

Where statistics beyond means and standard deviations (S.D.) were used in the evaluation of results to be presented, the data were subjected to analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test for multiple comparison of means. Values with a common superscript letter are not significantly different from each other ($P \leqslant 0.05$). The number of replications is indicated by N.

OPENING REMARKS - RON DINUS

WELCOME AND INTRODUCTIONS

OVERVIEW OF PROJECTS

- BACKGROUND
- RECENT DIRECTIONS

INSTITUTE AND INTERNAL AFFAIRS

PAC RECOMMENDATIONS

GROUNDRULES AND ANNOUNCEMENTS

THANKS AND LET'S HEAR SOME RESULTS

IPC DIVISIONS

CHEMICAL SCIENCES

ENGINEERING

FOREST BIOLOGY

PAPER MATERIALS

INFORMATION SERVICES

FOREST BIOLOGY DIVISION

CELL AND TISSUE CULTURE SCIENCE GROUP

PROJECTS = 3, PRIMARILY DUES FUNDED RESEARCH

SOFTWOOD CLONING

HARDWOOD CLONING

BIOCHEMISTRY

WOOD AND FIBER SCIENCE GROUP

SERVICES, EDUCATION, AND RESEARCH

FOREST GENETICS GROUP

PROJECTS = 2, PRIMARILY COOPERATIVE RESEARCH

ASPEN TREE IMPROVEMENT

LARCH INTRODUCTION AND IMPROVEMENT

CELL AND TISSUE CULTURE SCIENCE GROUP

SOFTWOOD CLONING

(MIKE BECWAR)	SHIRLEY VERHAGEN
NAGMANI RANGASWAMY	LYNNEA ARMSTRONG
RAFIQUE UDDIN	SONJA OZTURK
RON DINUS	TOM MERCHANT

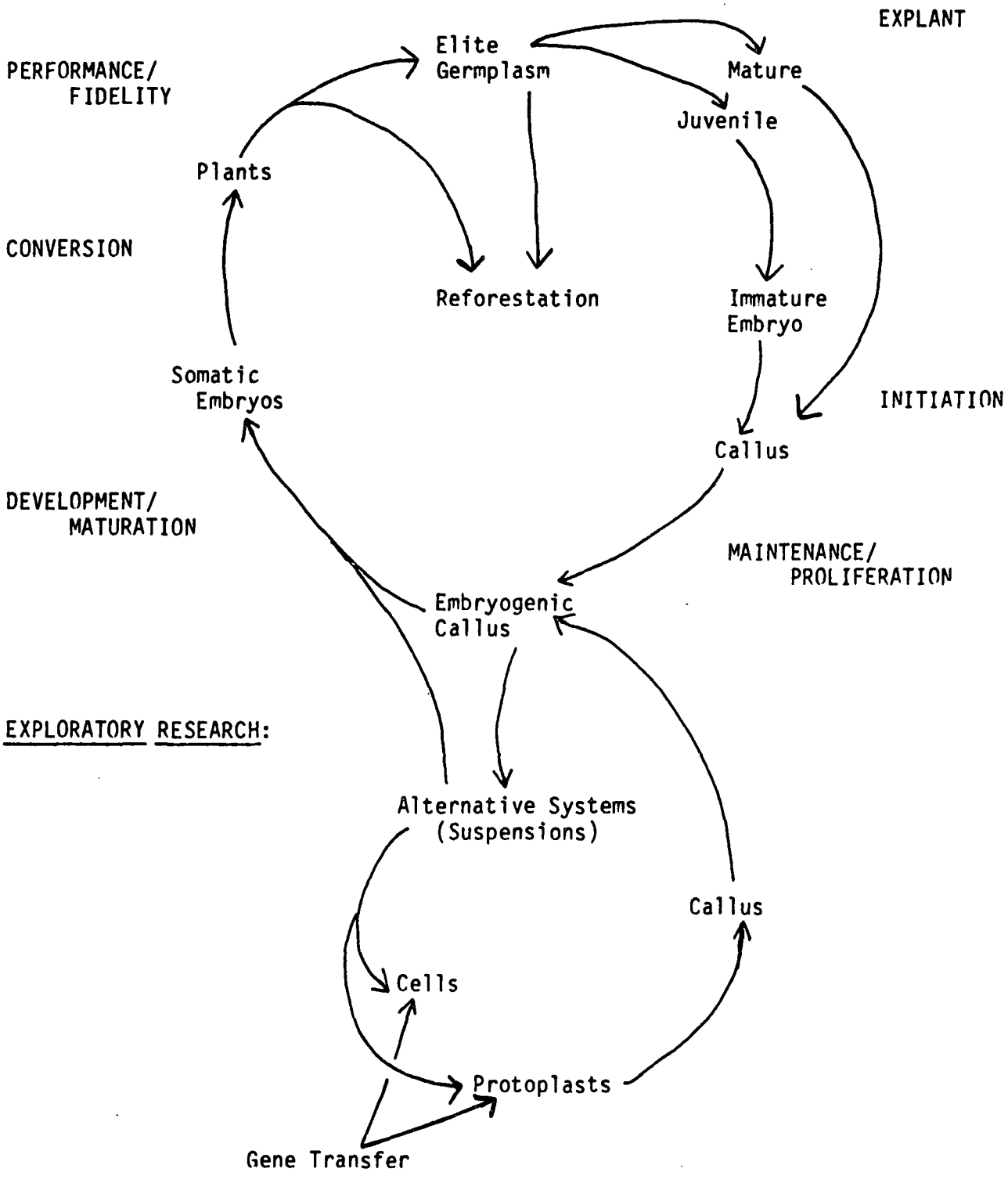
HARDWOOD CLONING

RON DINUS	LYNNEA ARMSTRONG
NAGMANI RANGASWAMY	TOM MERCHANT
RAFIQUE UDDIN	

BIOCHEMISTRY

MORRIS JOHNSON	JOHN CARLSON
RUSS FEIRER	JUDD CONKEY

MASS PROPAGATION OF IMPROVED CONIFERS



INSTITUTE AND INTERNAL AFFAIRS

****

MEMBERSHIP

ATLANTA MOVE

****

VISITORS

STUDENTS

CONFERENCES AND PRESENTATIONS

****

RECENT VISITORS

KIM VON WEISSENBERG - UNIVERSITY OF JOENSUU, FINLAND

DANE ROBERTS - BC RESEARCH CENTRE, CANADA

DAVE ELLIS & JULIE RUSSELL - UNIVERSITY OF WI, MADISON

MICHAEL SHAW - SAPPI FORESTS LTD., REPUBLIC OF SOUTH AFRICA

BOB BAXTER - SHELL RESEARCH LTD., UNITED KINGDOM

MARIE CLAUDE BON - AFOCEL, FRANCE

BOB TEASDALE - BOND UNIVERSITY, AUSTRALIA

DALE SMITH - FOREST RESEARCH INSTITUTE, NEW ZEALAND

VISITING SCIENTIST

KAIJA KEINONEN-METTALA - UNIVERSITY OF JOENSUU, FINLAND

BIOCHEMIST/PLANT PHYSIOLOGIST - MICROPROPAGATION

SUPPORTED BY GRANT FROM FINNISH ACADEMY

EXPERIMENTATION ON:

GERMINATION OF SOMATIC EMBRYOS

EXTRACTION/PURIFICATION OF ABA

PAC RECOMMENDATIONS

ISSUE	ACTION OR PLAN
PRESENTATIONS, EXPERIMENTS, & GOALS	ADJUSTED FORMAT & CONTENT
REPORTS & MEETINGS	DEBATING, CHANGES PENDING
CRITICAL ISSUES	REVIEW & ADJUST FREQUENTLY
RAISE INITIATION FREQUENCIES, TARGET SPECIES	SUMMER EFFORT, LOBLOLLY & DOUGLAS-FIR
INITIATION FROM COTYLEDONS, COMPARE SOMATIC & ZYGOTIC	NORWAY SPRUCE: INCREASE FREQUENCIES THEN PRODUCE "PAIRED" SEEDLINGS
DEVELOPMENT/MATURATION, KEY ISSUE	MAIN EFFORT: SOME PROGRESS, BUT REMAINS DIFFICULT. COMPARING ZYGOTIC & SOMATIC
PERSONNEL	POST DOC WORKING REPLACED 3 TECHS INTERVIEWING SCIENTISTS
EXTERNAL SUPPORT	DOE PROJECT IN PROGRESS ONE D/M PROPOSAL SUBMITTED TO DOE BASIC SCIENCES DIV. PROSPECTS FOR FY '90
PAC MEMBERSHIP	LOST 1 COMPANY, GAINED 5

GROUNDRULES

COFFEE ANYTIME

BREAK AT 3:10; WILL ADJUST IF NEEDED

QUESTIONS AND IDEAS, PLEASE FEEL FREE

PRESENTATION TIMES INCLUDE TALK AND QUESTIONS

EXPECT TO STAY ON SCHEDULE

COCKTAILS AND DINNER AT 5:00

Forest Products
P.O. Box 875, 117 Pace Parkway
Cantonment, Florida 32533
904 968-6614

Champion
Champion International Corporation

May 6, 1988

Dr. Ronald J. Dinus
The Institute of Paper Chemistry
P.O. Box 1039
Appleton WI 54912

Dear Ron:

The meeting of the Project Advisory Committee on March 30 and 31, 1988, was well received by the committee members. It was evident to the committee that a good effort is being made, despite little progress on some of the key steps. The distribution of effort among different areas of research was well in the line with previous PAC recommendations. Some specific comments and recommendations follow:

REPORTS/MEETINGS

1. In some presentations, it was not clear what the hypothesis of the experiment was, and whether the experiment supported it. The logic of undertaking specific experiments must be clear to PAC if we are to view the work as having a well defined path, rather than a lot of empirical experiments.

2. The annual report obviously requires a lot of time to prepare. For most PAC members, a more summarized form would suffice. The PAC does not want to mandate a particular style of annual report; the research team should try what they feel is appropriate, and PAC will provide feedback on their choice. However, it is important that the results of all the experiments are documented in a systematic way someplace, if not in the annual report.

3. To reduce staff preparation time, the spring meeting could be more informal, with concentration on key results rather than a progress report on all ongoing activities.

4. The flow diagrams showing the numbers of propagules of each species going through different steps of somatic embryogenesis were very good. These should be updated for each meeting as a means of documenting progress.

5. A concern voiced by Ron Yeske at the January, 1988, meeting in Atlanta was "micromanagement" by the PAC. The committee suggested that the style and/or content of PAC meetings could be changed so that requests for detailed decision making are not made or perceived by the PAC. However, the format of the latest meeting was the same as in the past. If "micromanagement" is not desired, it should not be requested. The PAC is receptive to a different style if the team wants to try one.

CRITICAL ISSUES

1. Initiation frequencies need to be increased for the target species. So few explants are forming somatic embryos that their development may be atypical also. The "20 year plan" proposed by Steve Wann, which would examine somatic embryos derived from the cotyledon of an excised embryo which is then grown into a seedling, could help answer this question.

2. Development/maturation is the key step where most efforts should be made, but the research team should decide how much effort. An emphasis on understanding the physiology of maturation, including the natural system, should be used when seeking new treatments to test.

3. On issues such as ABA and other hormone concentrations in the cultures, the research team should decide if investigation will help advance the project. The researchers are in a much better position to judge this than the PAC.

4. The short-term benefits which were listed were a good summary of ways to apply the current technology. However, these alone cannot justify the program. It is unnecessary to pursue this further, unless there are specific requests for such information in the future.

5. The team needs to frequently review the "critical issues" which were discussed at length. These are the major roadblocks that need to be overcome to make somatic embryogenesis work. Insuring that experiments are focused at addressing these issues will increase the efficiency and progress of the project.

Mr. Ronald J. Dinus
May 6, 1988
Page Three

PERSONNEL/ADMINISTRATION

1. The new senior level research scientist should be hired so he/she can overlap part of the remaining term of the Industrial Research Fellows. Also, no discussion was made of the status of replacements for the current Industrial Research Fellows. The PAC should be aware of efforts being made to seek replacements, even if no replacements have been found.

2. The PAC was pleased to hear of your recent success in getting a grant from the Department of Energy, even if it does not directly supplement the project. However, more effort must be made to obtain outside funding. It is unlikely that direct industrial support for the project will be significantly increased, so the leveraging of available funding by outside grants/contracts is vital to getting more resources available. This was a main topic at the October, 1987, PAC meeting also, but there has been relatively little effort on it since then. The PAC would like to hear by the October, 1988, meeting that further attempts have been made in this area.

3. Your suggestion to include more representatives on the PAC is encouraged. This can only help strengthen the project and make it more understood and accepted by the broader IPC membership.

The meeting provided a good interchange of ideas between the research team and the committee. The PAC appreciates the time and effort being made on the research and in presenting the results at the meeting.

Sincerely,

Greg

Gregory N. Leach
Research and Development Manager
Western Florida Region

cc. PAC Committee Members

June 29, 1988

Mr. Gregory N. Leach
Research & Development Manager
Champion International Corporation
Southern Timberlands Division
P. O. Box 87
Cantonment, FL 32533

Dear Greg:

Thank you for your letter of response to our spring meeting. We were also pleased with the meeting, and generally concur with your comments. Progress was considerable, but some steps are more difficult than others. In concert with past and current recommendations, we are concentrating on the more difficult steps, especially development/maturation. Some thoughts on specific comments are given below.

REPORTS/MEETINGS

Specifying hypotheses along with objectives and conclusions of individual experiments should raise us all to the same level of understanding. Appropriate statements will be incorporated in future research presentations.

Formats for the annual report, other reports, and the spring meeting are being debated. The team will develop and test appropriate changes. Your feedback will be appreciated. I would be remiss, however, if I did not credit the annual report with generating much favorable attention. Once the last report was issued, I began tallying comments and questions concerning it. I have since received over a dozen calls and four letters. In addition, I have fielded numerous questions while working with member companies on other issues. The annual report as well as our Technical Reports clearly are circulated far and wide within member companies. We have a "good thing going," and must determine how to lessen the associated workload while maintaining or improving quality.

Agreed, flow charts illustrating the process and documenting progress on each step are a good addition. Modified versions were tested at the Executives' Conference, and received a highly favorable response there as well. We will continue to update and use such charts, and seek yet new ways to further improve the approach.

CRITICAL ISSUES

Concerning initiation frequencies and the "20-year plan," we prefer to separate the two issues. Firstly, significant increases must be obtained in initiation frequencies for target species. This certainly is a critical issue, and we continue to devote much effort to it. Optimal initiation protocols should also improve frequencies in subsequent steps. Secondly, the "20-year plan" presently involves the model system, Norway spruce, and deals primarily with development and genetic fidelity of "somatic seedlings." In addition, this work will increase our ability to initiate from "older explants"; ie., cotyledons of newly germinated Norway spruce seedlings. Raising initiation frequencies in this context is also important. Results should answer questions about frequencies of normal and abnormal seedlings, and will facilitate later efforts to initiate from older explants of target species.

We agree that development/maturation is the most critical issue, and are examining it in both somatic and zygotic systems. Our collective efforts are concentrated on this front. This and other critical issues are debated in each of our biweekly review meetings, with approach and intensity adjusted as necessary.

PERSONNEL/ADMINISTRATION

A brief status report seems the most efficient response to comments in this area. We have added a temporary employee to assist with summer workloads, and help Dr. Becwar complete his research and writing. Arrangements have been made with several internal and external organizations to secure Norway spruce, loblolly pine, and Douglas-fir cones. Offers of employment have been extended to candidates for the positions of Postdoctoral Fellow and Technician III. The latter opening resulted from transfer of a coworker to another part of our Division. Interviews for the position of senior scientist are ongoing. As indicated earlier, member company interest in Project 3223 is increasing, and we are seeking to enlist more PAC members and guests. These efforts as well as those devoted to finding new Industrial Research Fellows and external funding will be reviewed at the fall meeting.

We agree that the spring meeting provided for thorough interchange of ideas, and appreciate the efforts of the past chairman and the committee as a whole to raise the level and freedom of debate. The meeting struck us as one of the most profitable, and we look forward to a repeat performance at the fall meeting, October 26 and 27, 1988.

Many thanks and best regards.

Sincerely,

Ronald J. Dinus
Director
Forest Biology Division

RJD/jmm
Copies to: Project Advisory Committee Members

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

Status Report

to the

FOREST GENETICS

PROJECT ADVISORY COMMITTEE

Project 3223

THE MASS PRODUCTION OF CONIFERS

October 26-27, 1988

PROJECT SUMMARY FORM

DATE: September 15, 1988

PROJECT NO. 3223 - Mass Clonal Propagation of Improved Conifers

PROJECT STAFF: Becwar, Dinus, Nagmani, Uddin, Verhagen

PROJECT OBJECTIVE/GOAL:

Overall - Develop reliable cell and tissue culture systems for mass clonal propagation of improved conifers.

Near-term - Assured and low-cost supplies of quality softwood fiber

PROJECT RATIONALE:

Major increases can be obtained in fiber production, quality, and uniformity via mass cloning of improved trees. Reliable cell and tissue culture systems will also open the way for genetic engineering and production/delivery of new genetic combinations having exceptional growth, increased pest resistance, special fiber properties, and enhanced site and/or climatic adaptability. Screening for and selecting useful variants in culture could also lower costs and accelerate the pace of conventional tree breeding.

Improved growth will reduce raw material costs and increase returns on capital invested in land and equipment. Greater uniformity of clonal plantations can lower both woodlands and mill operating costs as well as enhance end-use properties. Better or new fiber properties can improve end-use performance and foster development of value-added or new products.

CURRENT FISCAL YEAR BUDGET: $500,000

SUMMARY OF RESULTS SINCE LAST REPORT: (March 1988 - September 1988)

Methods previously developed for initiating callus, growing cells in suspension, and maintaining callus and cell lines remain in use or were further

refined. Results from past work on nonconifer model systems, zygotic embryo development, and organogenesis have been integrated, and are being applied to the present model system, Norway spruce, and the target species, loblolly pine and Douglas-fir.

Embryogenesis in the model system is reproducible. Initiation of embryogenic callus from both immature and mature embryos is straightforward. As a result, initiation experiments in Norway spruce were few, and involved only more mature explants.

The major thrust in Norway spruce concerned improvement of protocols for development/maturation and conversion to seedlings. Development remains a difficult step, but changes in media composition and abscisic acid additions yielded improvement in a number of callus lines. Larger numbers of embryos are developing to the cotyledonary stage. Results are being applied to other lines and species as quickly as experiments are completed.

Progress was also made on conversion to seedlings, but "best" results were obtained for only one or so callus lines. "Germination" rates were raised, and now average nearly 40 percent for the best line. Over 500 rooted seedlings have been transferred to soil. The few spruce seedlings recovered in earlier years continue to behave like their zygotic counterparts.

In loblolly pine, developing cones were secured again this summer from the same parents as in earlier years. A series of experiments was established in an effort to increase initiation frequencies. These trials used the best

media developed in past years, with some further modification of growth regulator content. A number of lines yielded embryogenic callus, but some additional time must pass before final results become apparent. Those lines already embryogenic are being maintained and increased.

Over 15 embryogenic lines from earlier years are providing material for experiments on development. Progress on this front has been slow, but some improvement has been noted. Abscisic acid addition has proven helpful, and responses to it have been quantified.

In Douglas-fir, several lines of embryogenic callus were derived from summer cone collections. Numerous early stage embryos are evident in three lines, and two lines are growing better than any produced in earlier years. Much effort is being given to maintain and increase all such lines so that material is available for work on development, and eventually conversion to seedlings. To ensure continued progress with this important species, developing cones will be secured from the Southern Hemisphere this winter.

SHORT TERM GOALS:

Goals for Remainder of FY 88-89

1. Continue refining protocols and raise initiation frequencies in the target species.

2. Improve protocols for increasing development/maturation frequencies, raising efficiency of conversion to seedlings, and generating sufficient material for replicated trials.

3. Increase ability to develop and manipulate somatic embryos in suspension cultures.

4. Investigate protocols for obtaining embryogenic callus from more mature explants.

5. Secure explant sources from the Southern Hemisphere.

6. Accumulate baseline data on anatomy/morphology of somatic/zygotic embryos, especially on nature and frequencies of abnormalities.

7. Execute exploratory research on new approaches, improving techniques, and related topics, e.g., protoplast culture and embryo encapsulation.

8. Provide for prompt presentation/publication of findings.

PROJECT SUMMARY FORM

DATE: September 15, 1988

PROJECT NO. 3223-2 - Biochemistry of Clonal Propagation

PROJECT STAFF: Feirer, Johnson

PROJECT OBJECTIVE/GOAL:

Overall - Develop an improved understanding of biochemical mechanisms controlling embryogenesis and other cloning methods, and devise procedures for raising the effectiveness and efficiency of mass cloning methods.

Near-term - Assured and low-cost supplies of quality softwood fiber

PROJECT RATIONALE:

Improved understanding of biochemical mechanisms controlling embryogenesis and other cloning methods will shorten the time to commercial application of clonal forestry, raise their efficiencies, and facilitate extension to trees mature enough to have been proven genetically superior.

CURRENT FISCAL YEAR BUDGET: $150,000

SUMMARY OF RESULTS SINCE LAST REPORT: (March 1988 - September 1988)

Workers continued to accumulate baseline data on biochemical and molecular characteristics of embryogenesis in zygotic and somatic systems. Special emphasis was given to tracking and promoting embryo development. More than adequate supplies of developing and fully developed zygotic embryos were secured for analyses in both Norway spruce and loblolly pine.

Levels of triglycerides (lipids) were quantified in developing zygotic embryos of Norway spruce and loblolly pine. Parallel analyses of somatic material showed that embryogenic callus contained low levels of triglycerides, but that amount rose as embryos developed. Treatments enhancing

development (e.g., abscisic acid additions) raised accumulation of these important storage compounds. Results were confirmed via electron microscopy. Somatic embryos cultured on media containing abscisic acid had more "fat bodies" than those from media lacking it. Efforts to induce synthesis and accumulation by adding precursors were initiated.

Investigations of protein patterns in developing zygotic and somatic embryos of Norway spruce and loblolly pine moved forward. Work focused on changes in protein synthesis and accumulation as affected by media composition and abscisic acid levels.

Changes in polar lipid composition during development were documented for zygotic embryos of Norway spruce and loblolly pine. Data from somatic embryos are being gathered for comparison of the two systems. These compounds play an important role in membrane synthesis, and may also function in transmitting developmental signals across membranes.

Further assays of peroxidase isozymes and their relationships to development were made in zygotic embryos of Norway spruce and loblolly pine. Numbers of isozymes, and the activities of several, varied with developmental stage, generally rising as development advanced. Numbers and activities showed similar trends in somatic embryos of Norway spruce, but increases were more gradual. Peroxidases may be useful indicators of how well or rapidly development is progressing.

Experiments on the role of glutathione were continued, with emphasis on enhancing development by addition of enzyme inhibitors.

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Experiments on the role of glutathione were continued, with emphasis on enhancing development by addition of enzyme inhibitors.

8. Execute exploratory research on new approaches, improving techniques, and related topics.

9. Provide for prompt presentation/publication of findings.

PROJECT SUMMARY FORM

DATE: September 15, 1988

PROJECT NO. 3223-3 - Mass Clonal Propagation of Improved Hardwoods

PROJECT STAFF: Dinus, Nagmani, Uddin

PROJECT OBJECTIVE/GOAL:

Overall - Develop reliable, low-cost systems for mass clonal propagation of genetically improved-engineered hardwoods.

Near-term - Assured and low-cost supplies of quality hardwood fiber

PROJECT RATIONALE:

Major increases can be obtained in fiber production, quality, and uniformity via mass cloning. Reliable cloning systems will also open the way for genetic engineering and production/delivery of new genetic combinations having exceptional growth, greater pest resistance, special fiber properties, and enhanced site and/or climatic adaptability. Screening/selection for useful variants in tissue culture holds promise for raising the pace and efficiency of conventional tree breeding.

Accelerated growth will ensure reliable raw material supplies, reduce their costs, and raise returns on capital invested in land and equipment. Greater uniformity can lower both woodlands and mill operating costs as well as enhance properties related to end-use performance. Better or new fiber properties can improve end-use performance and foster development of value-added or new products.

CURRENT FISCAL YEAR BUDGET: $75,000

SUMMARY OF RESULTS SINCE LAST REPORT: (March 1988 - September 1988)

Considerable work has been done on hardwood tissue culture at the Institute over the years. Results from this work and from other organizations indicate that hardwoods can be manipulated with relative ease. Other work at the Institute and elsewhere has suggested that tissue culture can be used to test for disease resistance. Still other work infers that novel variants produced in culture can be isolated and used to introduce new and different traits into breeding stock and clonal reforestation programs.

Work in this new project centered on developing a "position paper" comparing fiber properties, resource availability, growth rates, suitabilities for plantation management, and propensities for clonal propagation of important species, recommending species to use in future research, and outlining possible research directions.

Colleagues in fiber science and papermaking at the Institute and in member companies were consulted to identify useful fiber properties, preferred species, and resource needs. Discussions with authorities in hardwood management and research were used to evaluate suitabilities for management, examine the utility and benefits of cloning, and identify potential research directions.

SHORT TERM GOALS:

Goals for Remainder of FY 88-89

1. Finalize project goals, objectives, and directions.

2. Choose approaches and develop plans for specific activities.

3. Identify and secure plant material.

4. Establish greenhouse and/or in vitro populations needed for efficient research.

5. Begin evaluating available and new cloning methods, adapting them to designated species, and combining them into cost effective systems for application.

6. Explore novel methods for hastening genetic improvement; e.g., screening for desirable traits and/or producing useful variants in culture.

COOPERATIVE INVESTIGATIONS

1. North Carolina State University - Investigation with Drs. H. Amerson and R. Mott concerning effects of differing seed orchard pollen backgrounds on initiation of loblolly pine embryogenic callus.

2. International Forest Seed Company - Supply of "rejuvenated" loblolly pine material by Dr. S. Foster for experiments on initiation of embryogenic callus from mature explants.

3. University of Florida, Leesburg - Study by Dr. D. Gray of desiccation as a method of preparing Norway spruce somatic embryos for storage and germination.

4. University of Cincinnati - Assay by Dr. J. Caruso of endogenous hormone levels, principally ABA and IBA, in embryogenic and nonembryogenic calli.

RELATED STUDENT RESEARCH:

Completed in 1988 - M.S., Independent Study

Michael Bogenschutz - "Electroporation-mediated genetic transformation of Norway spruce cells." (Becwar)

Scott Fruhwirth - "A comparative study of organosolv-pulped tension and normal wood fibers in the papermaking process." (Conners)

In Progress - M.S., Independent Study

Lisa Dudek - "Encapsulation of zygotic/somatic embryos of conifer species." (Nagmani)

Pat Exarhos - "Electron microscopy study of ultrastructure of Picea abies plants obtained via somatic embryogenesis." (Conners)

Fred Lang - "Application of recombinant DNA technology in construction of a gene library." (Dinus)

Lorrain Logsden - "Patterns of gene expression associated with maturing and germinating tree seeds." (Dinus)

Mary Kay Lynde-Maas - "Fructose utilization by embryogenic and nonembryogenic suspension cultures of Norway spruce." (Johnson)

Colleen Walker - "Optimization and quantification of somatic embryogenic cultures of several conifer species in bioreactors." (Becwar/Dinus)

Ph.D. Program

Dan Bunker - "An investigation of the role of drying strategies in the structure of pigment-adhesive films." (Conners)

Russ Feirer - "Biochemical and molecular studies of plant development." In cooperation with University of Wisconsin, Madison

Jong-Moon Park - "Improving the properties of recycled fibers by chemical and enzymatic treatments." (Johnson)

INITIATION AND MAINTENANCE OF EMBRYOGENIC CALLUS IN LOBLOLLY PINE

MICHAEL R. BECWAR

INITIATION AND MAINTENANCE OF EMBRYOGENIC CALLUS IN LOBLOLLY PINE

TOPICS

1. REVIEW OF EMBRYOGENIC CALLUS INITIATED AND MAINTAINED FROM 1987

2. SUMMARY OF EMBRYOGENIC CALLUS INITIATED SUMMER 1988

SUMMARY OF EMBRYOGENIC CALLUS LINES OF LOBLOLLY PINE
INITIATED IN SUMMER 1987

CLONE			EMBRYOGENIC CALLUS LINES		
IPC CODE	ID NO.	EXPLANTS CULTURED	INITIATED	MAINTAINED	SE's DEVELOPED
A	10-1003	950	4	1	1
B	10-1007	960	1	0	1
C	10-1011	965	2	1	1
D	10-1018	995	7	6	3
E	10-1019	980	0	0	0
F	7-34	3311	14	11	6
G	7-56	1357	4	3	1
H	11-9	3482	16	16	3
J	11-16	1392	1	1	0
R	11-25	1375	0	0	0
	TOTALS (%):	15,767	49	39 (80)	16 (41)

1988 INITIATION EXPERIMENT OBJECTIVES

1. INCREASE INITIATION FREQUENCY
2. EVALUATE CLONAL DIFFERENCES

1988 INITIATION EXPERIMENTS

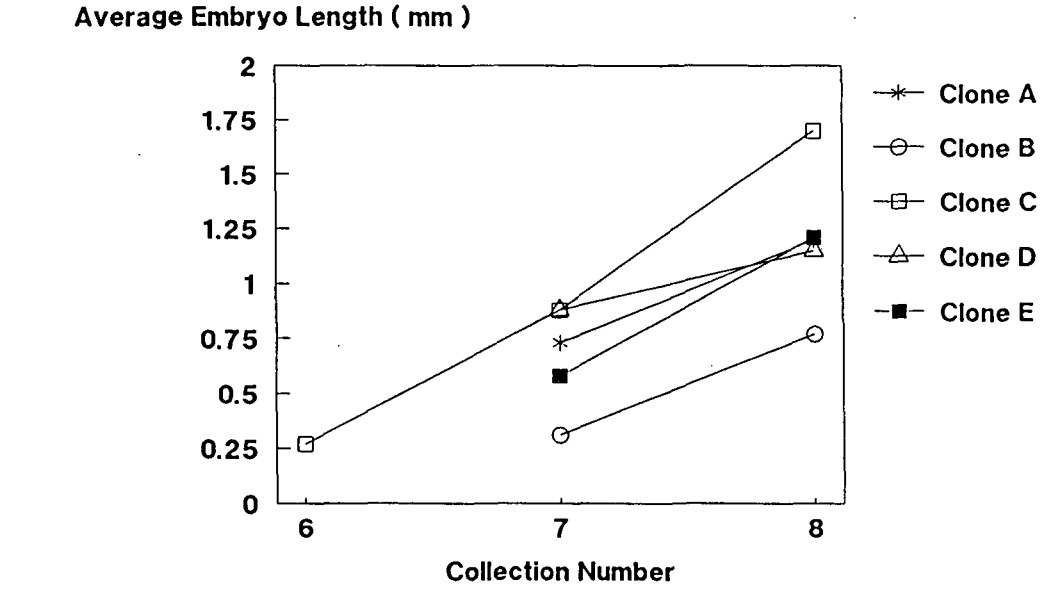
EXPT. 1.....CULTURE OF OVULES

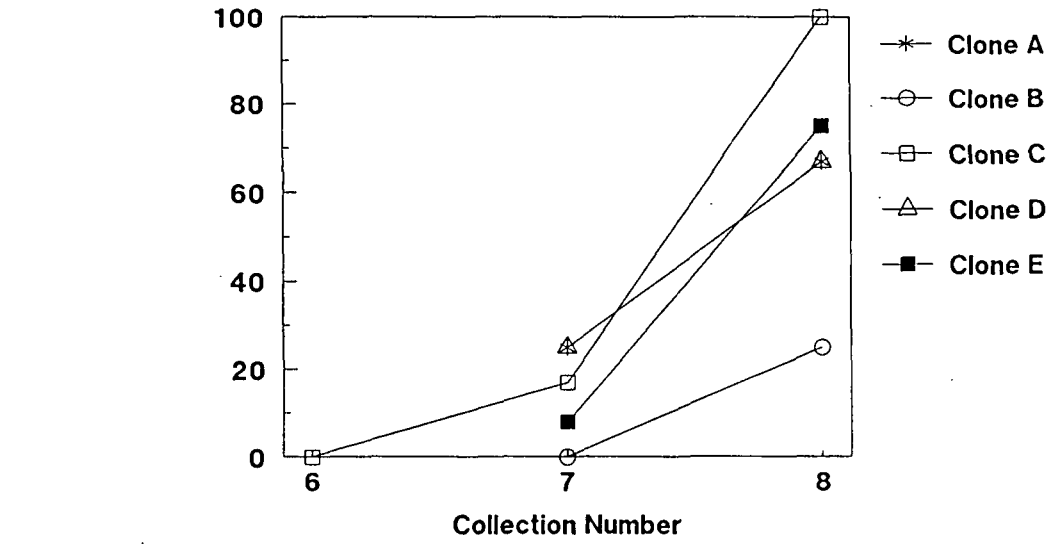
EXPT. 2.....CULTURE OF IMMATURE EMBRYOS

EXPT. 3.....COMPARISON OF EXPLANTS FROM DIFFERENT RAMETS OF THE SAME CLONE

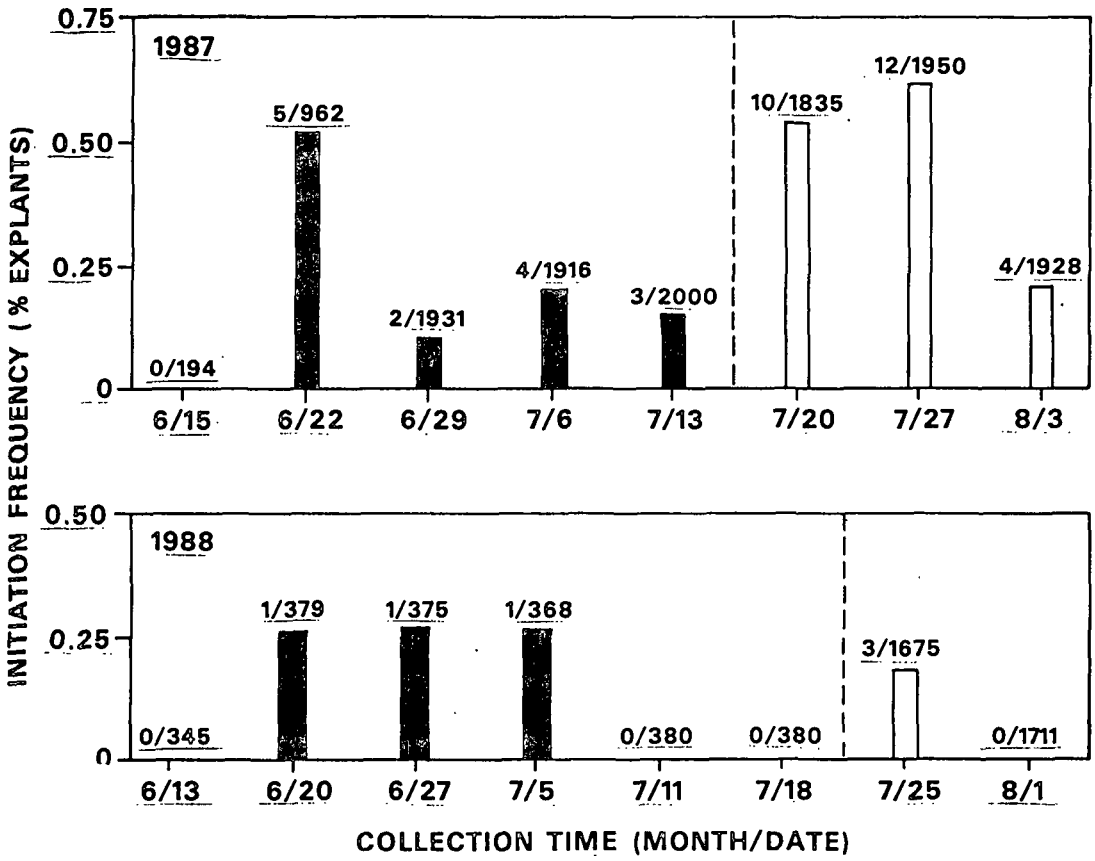
INITIATION EXPERIMENT DESIGN

FACTOR	NO. LEVELS	VALUE OF LEVELS
MEDIA	2	DCR 3/.5 & MSCG 3/.5
EXPLANTS	2	OVULE & IMMATURE EMBRYO
CLONES		
EXPT. 1	2	F & H
EXPT. 2	10	A,B,C,D,E,F,G,H,J & R
DATES		
EXPT. 1	5	JUNE 13 - JULY 18
EXPT. 2	2	JULY 25 & AUG 1
RAMETS (F & J)		
EXPT. 3	2	WV & IP SEED ORCHARDS





Stage of embryo development in seeds of five clones of loblolly pine collected in 1988. Top graph is embryo length and bottom graph is percentage of embryos with cotyledonary primordia. Collection number 6, 7, and 8 = July 18, July 25, and Aug. 1, respectively.



Comparison of frequency of initiation of embryogenic callus in loblolly pine in 1987 and 1988. Solid bars are ovule explants and open bars are immature embryo explants. Values above each date indicate the number of initiations in the numerator and the total no. of explants cultured in the denominator.

SUMMARY OF INITIATION FREQUENCY OF EMBRYOGENIC CALLUS AMONG LOBLOLLY PINE CLONES SURVEYED

CLONE	EXPT 1[A]	EXPT 2[B]	EXPT 3[B]
A	--	0/360	--
B	--	0/186	--
C	--	0/370	--
D	--	2/350	--
E	--	0/170	--
F_{IPC}	1/1147	0/400	--
F_{NCS}	--	--	0/200
G	--	0/350	--
H	2/1080	1/400	--
J_{IPC}	--	0/400	--
J_{NCS}	--	--	0/200
R	--	0/400	--
TOTALS:	3/2227	3/3386	0/400

[A] = OVULE EXPLANTS
[B] = IMMATURE EMBRYO EXPLANTS

SUMMARY OF INITIATION OF EMBRYOGENIC CALLUS ON DCR AND MSCG MEDIUM

EXPERIMENT	DCR 3/.5	MSCG 3/.5
1	3/1182	0/1105
2	2/1698	1/1688
3	0/200	0/200
TOTALS:	5/3020	1/2985

SUMMARY & CONCLUSIONS

1. Only the most highly embryogenic lines initiated in 1987 have been maintained for somatic embryo development studies.

2. A total of 6013 explants were cultured this summer and 6 embryogenic callus lines were initiated.

3. Five of the six cultures initiated were cultured on DCR 3/.5. Thus, similar to 1987 initiation results, DCR was the most effective initiation medium.

4. Although initiation frequency was not increased, the responsive clones (F, D, & H) were also the source of the most responsive explants in 1987. These results support the hypothesis of clonal differences in initiation of embryogenic callus in loblolly pine.

INITIATION AND MAINTENANCE OF EMBRYOGENIC CALLUS FROM IMMATURE EXPLANTS OF DOUGLAS-FIR

NAGMANI RANGASWAMY

INITIATION AND MAINTENANCE OF EMBRYOGENIC CALLUS FROM IMMATURE EXPLANTS OF DOUGLAS-FIR

OBJECTIVES

TO DETERMINE OPTIMUM WINDOW OF INITIATION

DEFINE EXPERIMENTAL PROTOCOL FOR INITIATION

SUCCESSFUL MAINTENANCE OF EMBRYOGENIC CALLUS

HYPOTHESES: MEDIA FORMULATIONS IN MSCG 5/0 AND MSCG 5/2.5 BASED ON MEDIA USED FOR EMBRYOGENESIS IN EUROPEAN LARCH, 1985; MODIFIED TO OMIT NH_4NO_3 REDUCE KNO_3 (NAGMANI & BONGA, 1985; IPC, 1987).

DCR 3/.5 AND DOM MEDIA BASED ON SUGAR PINE AND DOUGLAS-FIR PROTOCOLS FOR EMRBYOGENESIS (GUPTA & DURZAN, 1986,87)

Table 1: Variations In Media Used For Initiation of Embryogenic Callus In Douglas-Fir

Components $mg \cdot l^{-1}$	MS	MSCG 5/0	MSCG 5/2.5	DCR 3/.5	DOM*
NH_4NO_3	1650	----	----	400	550
KNO_3	1900	100	100	340	2337
$MgSO_4 \cdot 7H_2O$	370	370	370	370	370
KH_2PO_4	170	170	170	170	170
$CaCl_2 \cdot 2H_2O$	440	440	440	85	440
$Ca(NO_3)_2 \cdot 4H_2O$	----	----	----	556	---
KCl	----	745	745	----	---
KI	0.83	0.83	0.83	0.83	0.83
H_3BO_3	6.2	6.2	6.2	6.2	6.2
$MnSO_4 \cdot H_2O$	16.9	16.9	16.9	22.3	16.9
$ZnSO_4 \cdot 7H_2O$	8.6	8.6	8.6	8.6	8.6
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25	0.25	0.25	0.25
$CuSO_4 \cdot 5H_2O$	0.025	0.025	0.025	0.25	0.025
$CoCl_2 \cdot 6H_2O$	0.025	0.025	0.025	0.025	0.025
$NiCl_2 \cdot 6H_2O$	----	----	----	0.025	---
$FeSO_4 \cdot 7H_2O$	27.8	27.8	27.8	27.8	27.8
Na_2EDTA	37.3	37.3	37.3	37.3	37.3

* Durzan & Gupta's Protocol (1987) ½ MS

Table 1: Variations In Media Used For Initiation of Embryogenic Callus In Douglas-Fir

Components $mg \cdot l^{-1}$	MS	MSCG 5/0	MSCG 5/2.5	DCR 3/.5	DOM*
Inositol	100	100	100	200	100
Glycine	----	----	----	2	---
Nicotinic Acid	0.5	0.5	0.5	0.5	0.5
Pyridoxine	0.1	0.1	0.1	0.5	0.1
Thiamine HCl	0.1	0.1	0.1	1.0	1.0
Sucrose	30,000	30,000	30,000	30,000	30,000
Glutamine (G)	----	500	500	250	450
Casein Hydrolysate(C)	---	----	1000	500	500
Agar	0.8%	0.8%	0.8%	0.8%	0.8%
<u>Growth Regulators</u>					
2,4-D	----	5	5	3	11
BA	----	----	2.5	0.5	4
Kinetin	----	----	----	----	4.3

* Durzan & Gupta's Protocol (1987) ½ MS

TABLE 2: INITIATION PROTOCOL FOR PRE- AND POST-FERTILIZED OVULES

CLONE	COLLECTION MONTH/DATE	MSCG 5/0	MSCG 5/2.5	DOM	TOTAL NO	EC LINES
WTC-566 (O)	JUNE 7- JULY 15	550	550	550	1,650	0
WTC-567 (P)	JUNE 10- JULY 15	475	475	475	1,425	0
WTC-568 (Q)	JUNE 3- JULY15	470	480	475	1,430	0
WTC-569 (R)	JUNE 10- JULY 15	425	425	425	1,275	0
WTC-570 (T)	JUNE 7- JULY 8	425	425	425	1,275	0
				TOTAL =	7,055*	

*251 EXPLANTS CONTAMINATED
503 EXPLANTS PRODUCED NON-EMBRYOGENIC WHITE OR YELLOW CALLUS (TYPE C) CALLUS FROM THE PROLIFERATION OF GAMETOPHYTE.

TABLE 3: INITIATION PROTOCOL FOR ISOLATED IMMATURE EMBRYOS: LIGHT

CLONE	COLLECTION MONTH/DATE	MSCG 5/0	MSCG 5/2.5	DOM	DCR 3/.5	TOTAL NO	EC LINE
WTC-566 (O)	JULY 25-AUGUST 11	175	175	175	175	700	0
WTC-567 (P)	" "	70	70	65	70	275	0
WTC-568 (Q)	JULY 21-AUGUST 11	35	35	35	35	140	0
WTC-569 (R)	JULY 25-AUGUST 11	150	150	155	155	610	1 (9R)
WTC-570 (T)	JULY 15-AUGUST 11	125	125	120	120	495	0
					TOTAL =	2,220*	1

*804 EXPLANTS FORMED GREEN CALLUS (TYPE C CALLUS)

113 EXPLANTS FORMED WHITE CALLUS (TYPE B CALLUS)

1 EXPLANT FORMED EMBRYOGENIC CALLUS ON MSCG 5/0 (TYPE A CALLUS)

TABLE 4: INITIATION FROM ISOLATED IMMATURE EMBRYOS: DARK

CLONE	COLLECTION MONTH/DATE	MSCG 5/0	MSCG5/2.5	DOM	DCR 3/.5	TOTAL NO	EC LINE
WTC-566 (O)	JULY 25-AUGUST 11	190	190	190	190	760	0
WTC-567 (P)	" "	143	144	142	144	573	0
WTC-568 (Q)	JULY 21-AUGUST 11	75	75	75	75	300	1? (9Q)
WTC-569 (R)	JULY 25-AUGUST 11	200	200	200	200	800	0
WTC-570 (T)	JULY 15-AUGUST 11	240	250	240	240	970	3 (8T)
					TOTAL	3,403*	3±2

*3 EC LINES ON MSCG 5/2.5; 1 ON MSCG5/0; 1 ON DCR3/.5

591 WHITE CALLUS TURNING BROWN (TYPE B CALLUS)

353 WHITE OR GREEN CALLUS NON EMBRYOGENIC CALLUS (TYPE C)

CALLUS PHENOTYPE AND FREQUENCIES

TYPE A	TYPE B	TYPE C
WHITE	WHITE	WHITE
EMBRYOGENIC	?	NON-EMBRYOGENIC
MUCILAGINOUS	MUCILAGINOUS	NON-MUCILAGINOUS
MAINTAINED	TURNS BROWN	TURNS YELLOW/GREEN
(4 ± 2)/5,623 0.07 %	(704)/5623 12.5 %	(1157)/5,623 20.5 %

SUMMARY OF INITIATION PROTOCOL USED FOR ISOLATED IMMATURE EMBRYOS AND RESULTS

- USED EXPLANTS FROM 5 CLONES = 5,623
- TIME OF EXPLANT COLLECTION = JULY 15 - AUGUST 11
- NUMBER OF MEDIA TREATMENTS = 4
- COMPARED LIGHT vs DARK

CLONE	MEDIUM	D/L	EC LINE
8T	MSCG 5/2.5	D	2
8T	MSCG 5/0	D	1
9R	MSCG 5/0	L	1
9Q	MSCG 5/0	D	1?
9P	DCR 3/.5	D	1?

CONCLUSIONS

CHOICE OF EXPLANTS:	ISOLATED PRE-COTYLDONARY EMBRYOS ARE RESPONSIVE
EXPLANT COLLECTION: TIME	EMBRYOS FROM CONES COLLECTED BETWEEN JULY 15 - 25 MOST RESPONSIVE
MEDIA TREATMENT:	MSCG 5/0 AND MSCG 5/2.5 MOST EFFECTIVE; DCR 3/.5 AND DOM LEAST EFFECTIVE
LIGHT/DARK:	3 OF 4 LINES INITIATED IN DARK; RESULTS NOT CLEAR CUT
•	3 EC LINES THAT WERE INITIATED COULD BE SUCCESSFULLY MAINTAINED

SOMATIC EMBRYO DEVELOPMENT AND MATURATION IN NORWAY SPRUCE: IMPROVEMENTS TO THE MODEL SYSTEM

NAGMANI RANGASWAMY

SOMATIC EMBRYO DEVELOPMENT AND MATURATION IN NORWAY SPRUCE: IMPROVEMENTS TO THE MODEL SYSTEM

OBJECTIVES:

INCREASE DEVELOPMENT AND MATURATION FREQUENCIES

IMPROVE ORIGINAL DEVELOPMENT PROTOCOL

ORIGINAL DEVELOPMENT PROTOCOL

HM(Hakman et.al; 1985) medium
+ 2 mg/l 2,4-D
+ 1 mg/l BA
(2 wk subculture)

↓

HM + 1 % activated charcoal
(no growth regulators)
1 week

↓

HM + 1 μM IBA & 1 μM ABA
2-3 weeks

↓

Mature somatic embryos
(cotyledonary)

↓

¼ HM - (for germination)

RESULTS WITH ORIGINAL PROTOCOL

EXPT RP NO	CALLUS LINE	MATURATION FREQUENCY
476	NS1-5	2.9%
489	NS1-5	2.3%
518-1	NS1-5	1.9%
518-2	NS1-5	1.1%

FACTORS CONSIDERED IN IMPROVING THE DEVELOPMENT PROTOCOL

1. CHANGE OF BASAL MEDIA COMPOSITION
2. INCREASE IN ABA LEVELS
3. TIMELY SEPARATION OF DEVELOPED EMBRYOS FROM THE SURROUNDING CALLUS

HYPOTHESIS 1

REPLACEMENT OF AMMONIUM NITRATE (HM) WITH GLUTAMINE (BLG) FAVORS MERISTEMATIC ACTIVITY IN CELLS OR TISSUES SIMILAR TO THAT IN GROWING REGIONS OF THE PLANT (P.R. WHITE, 1966)

BASAL MEDIA FORMULATIONS - COMPARISON

Components $mg \cdot l^{-1}$	HM	BLG
NH_4NO_3	1200	---
KNO_3	1900	100
$MgSO_4 \cdot 7H_2O$	370	320
KH_2PO_4	340	170
$CaCl_2 \cdot 2H_2O$	180	440
KCl	---	745
KI	0.75	0.83
H_3BO_3	0.63	6.2
$MnSO_4 \cdot H_2O$	2.2	16.9
$ZnSO_4 \cdot 7H_2O$	2.87	8.6
$Na_2MoO_4 \cdot 2H_2O$	0.025	0.25
$CuSO_4 \cdot 5H_2O$	0.0025	0.025
$CoCl_2 \cdot 6H_2O$	0.0025	0.025
$FeSO_4 \cdot 7H_2O$	13.9	27.8
Na_2EDTA	18.65	37.3

BASAL MEDIA FORMULATIONS - COMPARISON

Components $mg \cdot l^{-1}$	HM	BLG
Inositol	100	100
Nicotinic Acid	2	0.5
Pyridoxine	1	0.1
Thiamine HCl	5	0.1
Sucrose	34200	20000
Glucose	180	---
Xylose	150	---
Arabinose	150	---
Glutamine	---	1500
Asparagine	---	100
Agar	0.5 %	0.7 %
pH	5.5	5.8

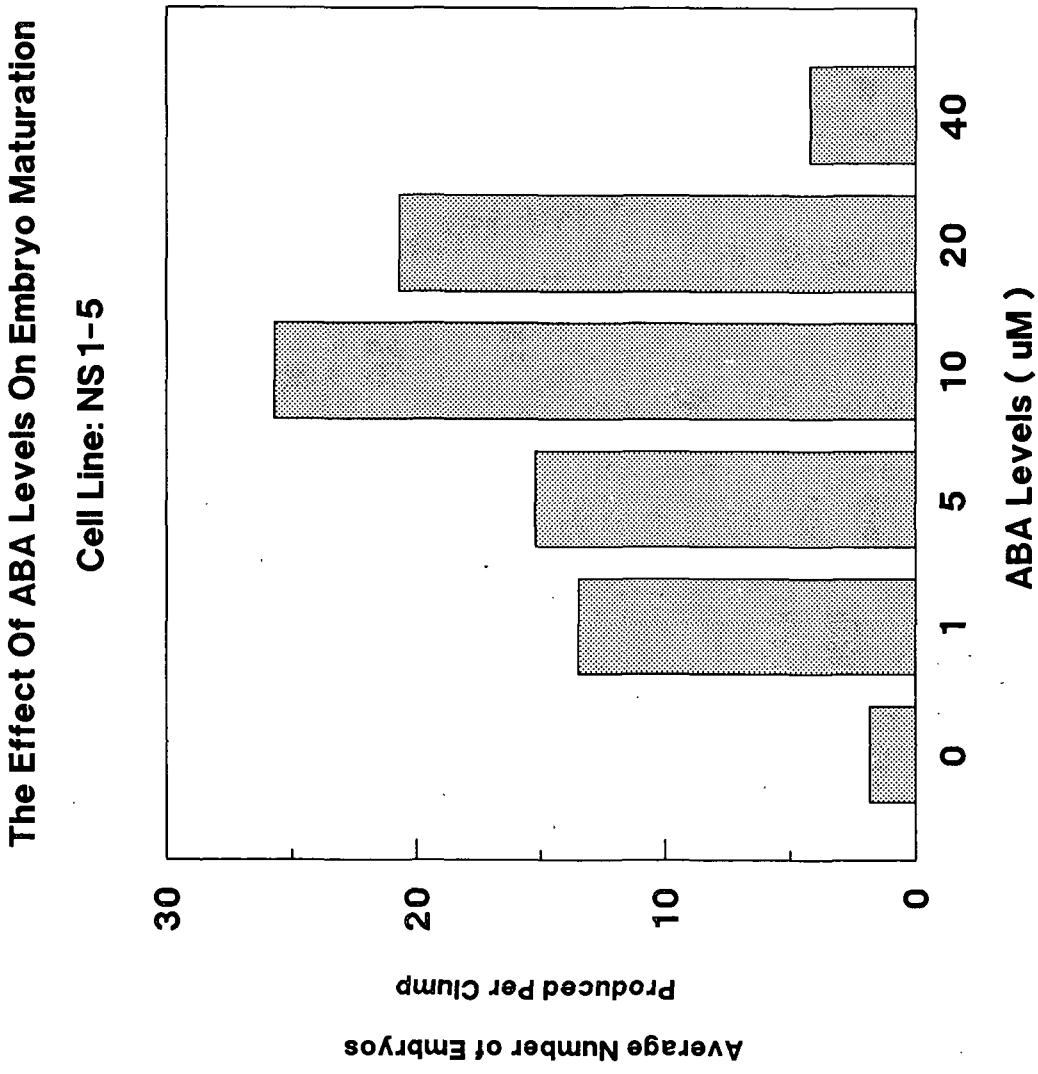
EFFECT OF CHANGES IN BASAL MEDIA

MEDIA	TOTAL NO OF CLUMPS	NUMBER OF MATURE EMBRYOS TOTAL	MEAN
HM I/A	40	14	0.35
BLG I/A	40	485	12.10

HYPOTHESIS 2

ABA PROMOTES SOMATIC EMBRYO DEVELOPMENT IN ANGIOSPERMS AND IN NORWAY SPRUCE. (AMMIRATO, WILLIAMSON, ET.AL, 1985; 1987)

ABA AT HIGHER LEVELS (7.6 μM) ENHANCES DEVELOPMENT IN NORWAY SPRUCE (VON ARNOLD, 1988)


The Effect Of ABA Levels On Embryo Maturation
Cell Line: NS1-5
Average Number of Embryos Produced Per Clump
0
10
20
30
0
1
5
10
20
40
ABA Levels (uM)

EFFECT OF ABA LEVELS ON DEVELOPMENT IN BLG MEDIA

ABA μM	EC LINE	TOTAL NO OF CLUMPS	MATURE SOMATIC EMBRYOS TOTAL NO	PER CLUMP
0	NS1-5	30	55	1.8333..C
1	NS1-5	38	515	13.55263.B
5	NS1-5	38	578	15.21053.B
10	NS1-5	38	977	25.71053 A
20	NS1-5	30	623	20.76667 AB
40	NS1-5	38	163	4.28947..C

* Mean value of 30-38 clumps on 3 or 4 petri dishes per treatment. Means followed by a common superscript are not significantly different as determined by ANOVA

REVISED DEVELOPMENT PROTOCOL

HM (Hakman, et.al; 1985) medium
+ 2 mg/l 2,4-D
+ 1 mg/l BA
(2 week subculture)

↓

BLG + 1 μM IBA + 20 μM ABA
(2-3 weeks)
Development and maturation
of embryos

↓

Individual picking of
cotyledonary embryos

CONCLUSIONS

CHANGE IN BASAL MEDIA COMPOSITION BY REMOVAL OF AMMONIUM NITRATE AND REPLACEMENT WITH GLUTAMINE ENHANCES SOMATIC EMBRYO DEVELOPMENT AND MATURATION

INCREASED LEVEL OF ABA (10 AND 20 μM) IN THE MEDIUM PROMOTES MATURATION

TIMELY SEPARATION AND PICKING OF MATURE SOMATIC EMBRYOS FROM THE SURROUNDING CALLUS PREVENTS CALLUS FORMATION AND PROMOTES DEVELOPMENT

HIGH FREQUENCY ROOTING INDUCTION IN NORWAY SPRUCE SOMATIC PLANTLETS

RAFIQUE UDDIN

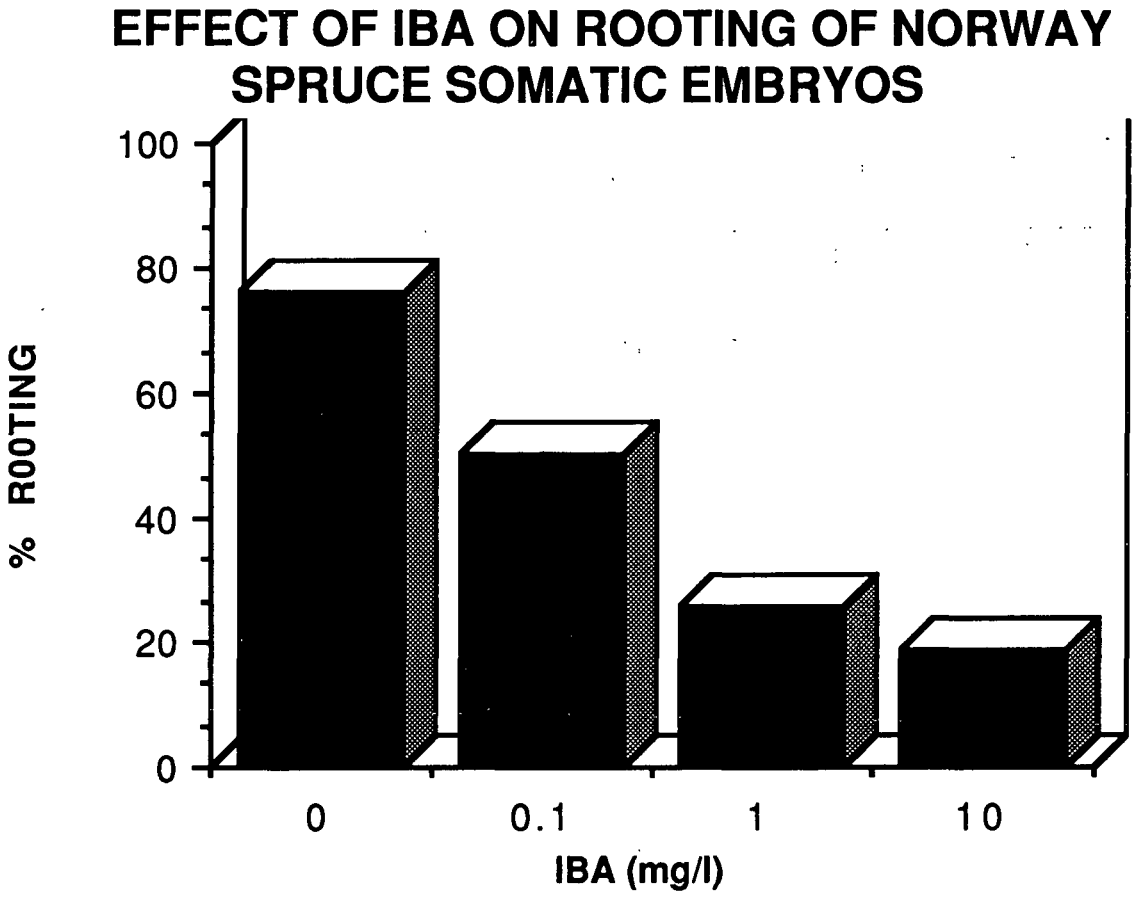
HIGH FREQUENCY ROOTING INDUCTION IN NORWAY SPRUCE SOMATIC PLANTLETS

OBJECTIVE

TO DETERMINE THE LEVELS OF IBA SUITABLE FOR MAXIMIZING THE ROOTING FREQUENCY OF NORWAY SPRUCE SOMATIC PLANTLETS

HYPOTHESIS

AT A CERTAIN CONCENTRATION OF IBA, ROOTING OF SOMATICALLY DERIVED PLANTLETS IS SIGNIFICANTLY HIGHER THAN IS CURRENTLY ACHIEVED WITHOUT IBA


EFFECT OF IBA ON ROOTING OF NORWAY SPRUCE SOMATIC EMBRYOS
% ROOTING
100
80
60
40
20
0
0
0.1
1
10
IBA (mg/l)

EFFECT OF IBA ON ROOTING OF NORWAY SPRUCE SOMATIC EMBRYOS

IBA (mg/l)			
0	0.1	1.0	10.0
76.1	50.5	25.5	18.5

% ROOTING

SUMMARY:

1/2 DCR SUPPLEMENTED WITH 25 mg/l NH_4NO_3, 25 mg/l GLUTAMINE AND 3% SUCROSE IS THE MOST SUITABLE ROOTING MEDIA.

ELEVATED IBA LEVEL HAS AN INHIBITING EFFECT ON THE ROOTING OF NORWAY SPRUCE SOMATIC EMBRYOS.

NORWAY SPRUCE SOMATIC EMBRYOGENESIS PROTOCOL

HM 2/1

↓

BLG (-) + 1% CHARCOAL (1 WEEK)

↓

BLG + 1 uM IBA/l + 20 uM ABA/ l (2-3 WEEKS)

↓

1/2 MS (-) (1-2 WEEKS)

↓

1/2 DCR + 25 mg/l NH_4NO_3 + 25mg/l GLN + 3% SUCROSE

↓

ROOTING

↓

1:1:1 v:v:v SAND : VERMICULITE : PEAT

CONVERSION

PERCENTAGE CONVERSION OF SOMATIC EMBRYOS TO PLANTLETS (GERMINATION)

RESEARCH PLAN	CONVERSION	PERCENTAGE
639-1	247/415	59.5
639-2	180/621	29.0
656	13/35	37.0
665-2	357/1397	25.6
667	883/2923	30.2
	1680/5391	31.2

ACCLIMATIZATION

TRANSFER TO TECHNICULTURE PEAT PLUG

PLANTING CAVITY IN PLASTIC CONTAINER

NUTRITION

(HALF STRENGTH HOAGLAND SOLUTION)

GROWTH & INHIBITION OF EARLY BUD SET

ESTABLISHMENT

DEVELOPMENT AND MATURATION OF LOBLOLLY PINE SOMATIC EMBRYOS

MICHAEL R. BECWAR

DEVELOPMENT AND MATURATION OF LOBLOLLY PINE SOMATIC EMBRYOS

OBJECTIVES

1. OPTIMIZE CONDITIONS FOR MATURATION OF SOMATIC EMBRYOS
2. OBTAIN QUANTITATIVE DATA AND STATISTICAL EVIDENCE FOR THE RELATIVE IMPORTANCE OF SEVERAL PARAMETERS OF DEVELOPMENT PROTOCOL

EMBRYO DEVELOPMENT EXPERIMENTS

1. EMBRYOGENIC CALLUS: LINE LP8F-1
2. EMBRYOGENIC LIQUID SUSPENSION: LINE LP7F-1

EMBRYO DEVELOPMENT FROM CALLUS FACTORIAL EXPERIMENT DESIGN [a]

FACTOR	NO. LEVELS	VALUE OF LEVELS
DEV. MEDIUM	2	MSG & DCR
LIGHT	2	DARK & LIGHT
ABA CONC.	4	0, 1, 10, & 30 μM

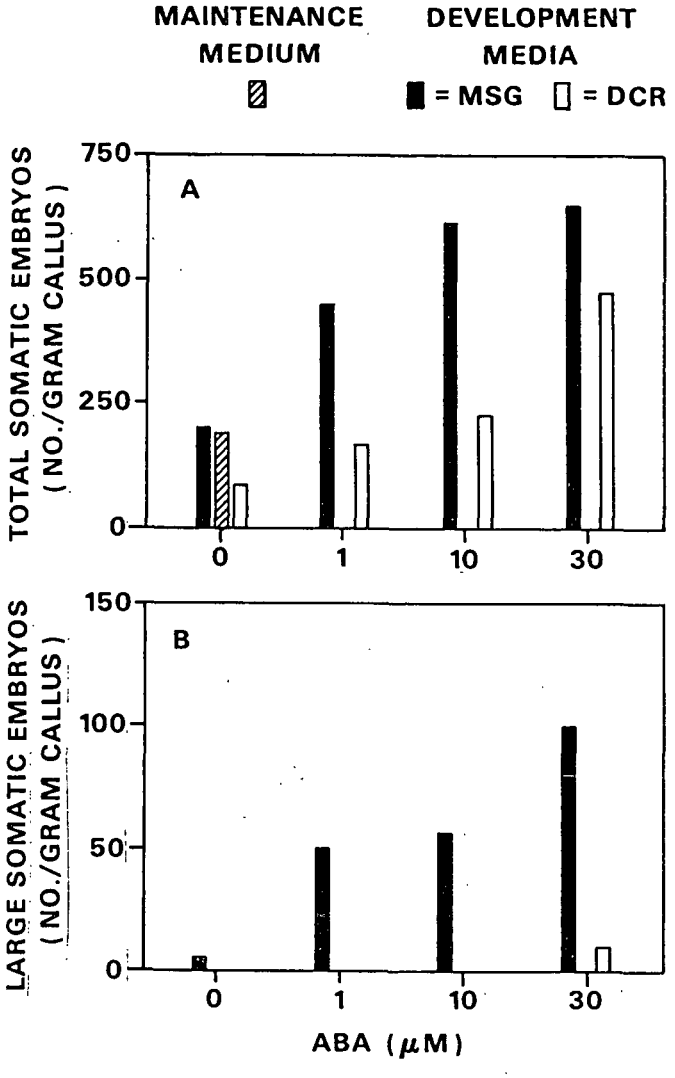
[a] Four replications per treatment

ANALYSIS OF VARIANCE RP659: TOTAL SOMATIC EMBRYOS

SOURCE	DF	MEAN SQUARE	F RATIO	PROB. > F
A (DEV. MED.)	1	891542.2	30.49	0.000
B (DARK/LIGHT)	1	65712.0	2.25	0.140
AB	1	178.5	0.01	0.938
C (ABA CONC.)	3	507073.3	17.34	0.000
AC	3	57638.0	1.97	0.131
BC	3	17218.0	0.59	0.625
ABC	3	60411.0	2.07	0.117
ERROR	47	29236.2		
TOTAL	62			

ANALYSIS OF VARIANCE RP659: LARGE SOMATIC EMBRYOS

SOURCE	DF	MEAN SQUARE	F RATIO	PROB. > F
A (DEV. MED.)	1	40053.8	14.17	0.000
B (DARK/LIGHT)	1	1220.1	0.43	0.514
AB	1	1254.9	0.44	0.508
C (ABA CONC.)	3	7244.2	2.56	0.066
AC	3	4744.5	1.68	0.184
BC	3	1638.4	0.58	0.631
ABC	3	1501.3	0.53	0.663
TOTAL	62			



Effect of ABA and development medium on somatic embryo maturation in loblolly pine. A: The total number of somatic embryos counted per gram of embryogenic callus. B: The number of "large" well-formed somatic embryos with smooth dense embryonal heads (stage 2 somatic embryos) per gram of embryogenic callus. Slashed bar is value on maintenance medium, DCR 3/.5. Solid and open bars are values on MSG and DCR development media, respectively, with ABA.

EMBRYO DEVELOPMENT FROM LIQUID SUSPENSIONS
FACTORIAL EXPERIMENT DESIGN

FACTOR	LEVEL	VALUE OF LEVEL
MEDIUM	2	1 = DCR, 2 = ELM
PLANT GROWTH REGULATORS (PGR's)	2	1 = 2,4-D / BA 2 = ABA / BA / KIN

SOMATIC EMBRYO DEVELOPMENT
FROM LIQUID SUSPENSION CULTURES
OF LOBLOLLY PINE

TREATMENT		SOMATIC EMBRYO		
MEDIUM	PGR's	NUMBER	SIZE	SUSPENSION DRY WEIGHT
		(per ml)	(mm)	(mg/ml)
DCR	2,4-D/BA	32 a	0.15 bc	2.6 a
DCR	ABA/BA/KIN	70 a	0.20 ab	2.1 a
ELM	2,4-D/BA	34 a	0.12 c	3.0 a
ELM	ABA/BA/KIN	30 a	0.22 a	3.4 a

SUMMARY & CONCLUSIONS

1. The basal medium and the level of ABA significantly effected the number of somatic embryos which developed in embryogenic callus of loblolly pine, whereas light vs. dark did not.

2. The basal medium also significantly effected the number of "large" (stage 2) somatic embryos.

3. The highest number of large and total somatic embryos was on the MSG medium with 30 μm ABA.

4. These results demonstrate a potential for empirically improving somatic embryo development in loblolly pine by using factorial experiments which quantify the effect of several factors simultaneously.

5. Similarly, experiments with liquid embryogenic cultures of loblolly pine suggested that significant improvements in somatic embryo development can be obtained by altering media and growth regulator combination.

BIOCHEMISTRY OF DEVELOPMENT
STORAGE OF LIPIDS

RUSS FEIRER

THE BIOCHEMISTRY OF DEVELOPMENT

COMPARISON OF ZYGOTIC AND SOMATIC EMBRYO DEVELOPMENT

- **Ultrastructure** - choroplasts
  - lipid bodies
- **Biochemistry** - triglycerides
  - proteins

HYPOTHESIS: **Similarity in the ultrastructure of somatic and zygotic embryos is expected. Factors which promote development of somatic embryos (ie. ABA) should also affect the ultrastructure.**

CONCLUSION: **Chloroplast maturation or development in somatic embryos is stimulated by ABA.**

PURPOSE: **Lipid bodies are numerous in developing and mature zygotic embryos. These reserve lipids are important to seed development / germination.**

- **Are lipid bodies found in cultured tissues?**
- **Can formation of lipid bodies be stimulated in somatic embryos?**

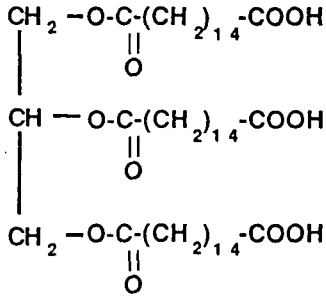
CONCLUSIONS:

- **Cultured conifer tissues contain few, if any, lipid bodies**
- **ABA leads to formation of lipid bodies**

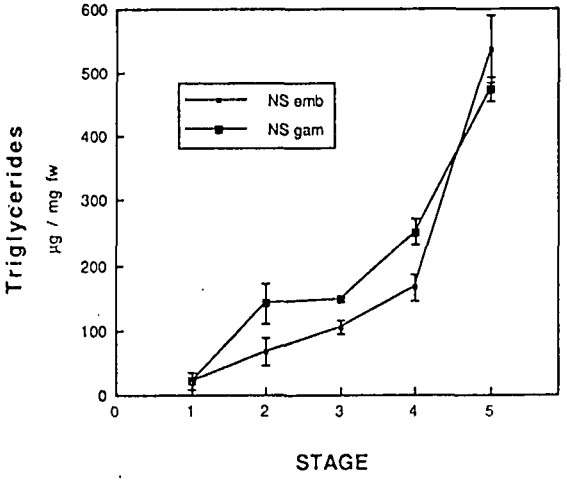
Experimental Approach to Storage Lipid Work

- **Descriptive study of zygotic embryogenesis**
- **Descriptive study of cultured tissues**
- **Manipulation of triglyceride levels in cultured tissues (somatic embryos)**

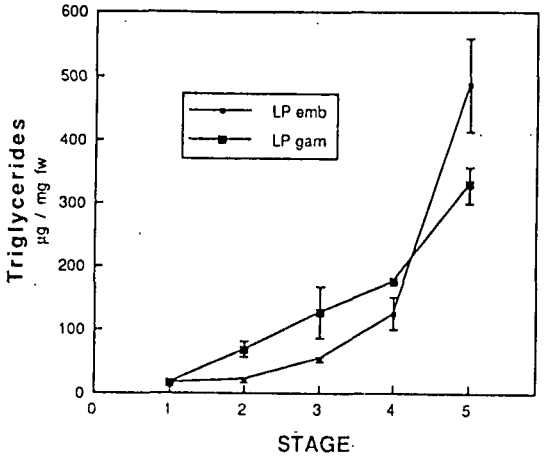
Hypothetical Triglyceride



RP674 Triglycerides in Developing NS Zygotic



RP673 Triglycerides in Developing Zygotic LP

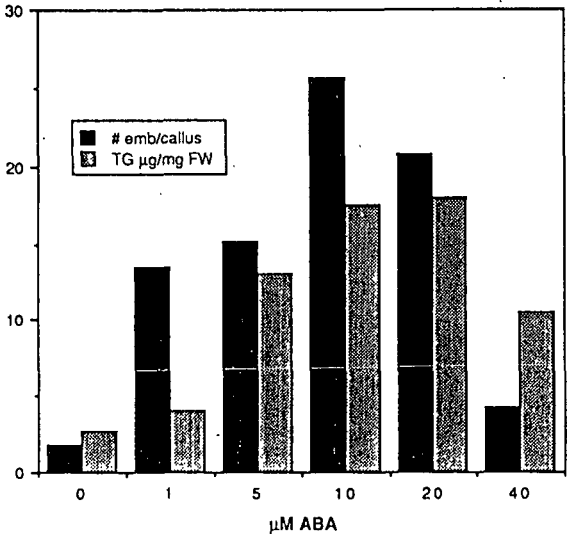


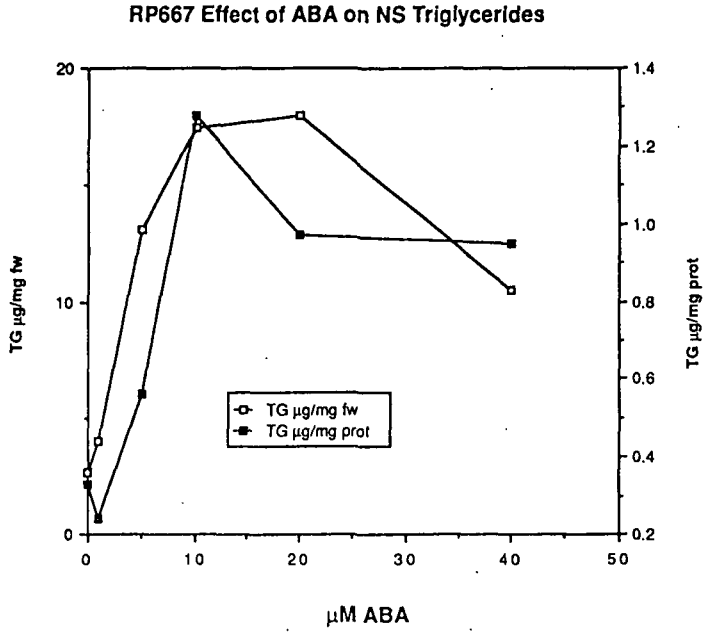
COMPARISON OF TRIGLYCERIDE LEVELS IN VARIOUS TISSUES

Tissue	Triglyceride (µg/mg fw)
NS embryogenic calli	0.5 - 4
NS somatic embryos	0.7 - 8
LP embryogenic callus	0.8
carrot embryogenic calli	2-18
carrot embryogenic susp.	3 - 23
LP mature seed	418

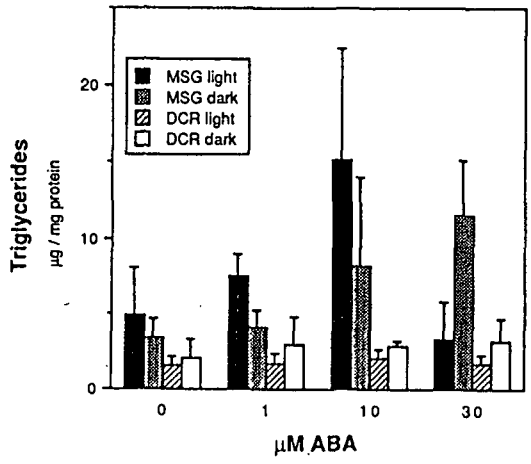
RP667

Effect of ABA on NS Triglycerides and Embryo#

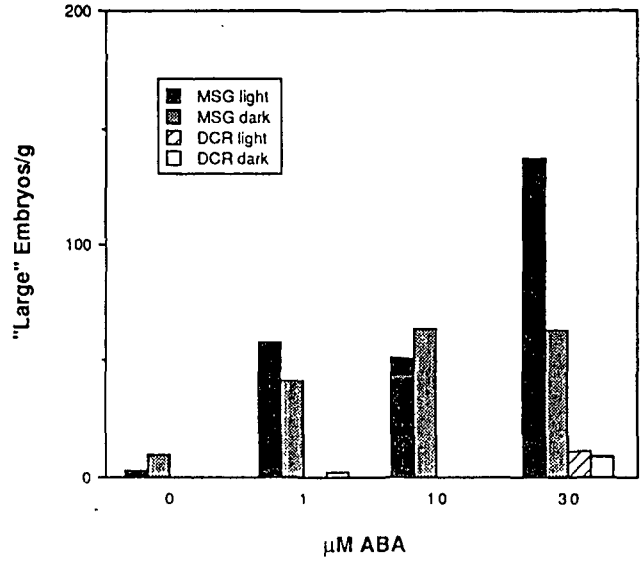



RP667 Effect of ABA on NS Triglycerides
TG µg/mg fw
TG µg/mg prot
µM ABA
TG µg/mg fw
TG µg/mg prot
0
10
20
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
40
50

RP659 Effect of ABA on LP Triglycerides



RP659 Effect of ABA on LP Embryo#



RP659 EFFECT OF MEDIA, LIGHT, ABA ON LP TRIGLYCERIDES

FACTORIAL ANOVA

SOURCE	DF	MS	F	Prob.	
Treatment	15	61.87	7.66	.0001	*
Media	1	401.40	49.67	.0001	*
Light	1	0.07	.009	0.927	ns
ABA	3	48.01	5.94	0.002	*
media x light	1	14.41	1.78	0.188	ns
media x ABA	3	36.92	4.57	0.007	*
light x ABA	3	45.88	5.68	0.002	*
med. x L x ABA	3	39.95	4.94	0.005	*
Error	48	8.081			
Total	63				

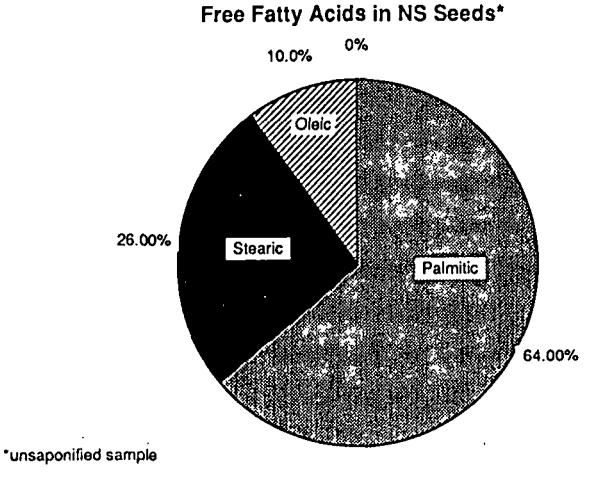
* = significant
ns = non-significant

CONCLUSIONS:

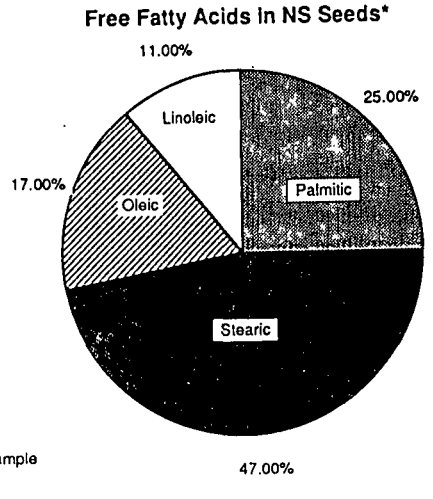
- **Triglycerides accumulate in developing zygotic embryos, to represent nearly 30% of embryo weight.**
- **Triglycerides are dramatically lower (deficient ?!) in cultured conifer tissues.**
- **ABA promotes triglyceride accumulation, as well as development, in somatic embryos.**

Experimental approach to triglyceride pertubation studies

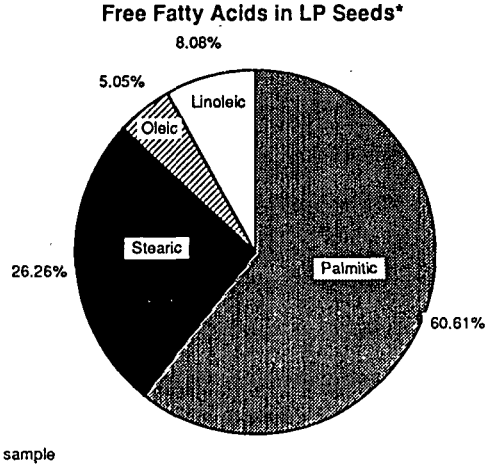
- **Continue to determine effect of media, ABA, etc. on triglyceride accumulation**
- **Add substrates of triglyceride biosynthesis to culture medium**



*unsaponified sample



*saponified sample



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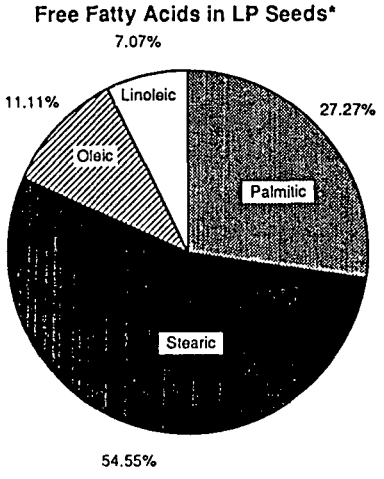


*saponified sample

BIOCHEMISTRY OF DEVELOPMENT
CHANGES IN POLAR LIPIDS AND ISOPEROXIDASE ACTIVITY DURING THE DEVELOPMENT OF CONIFER ZYGOTIC AND SOMATIC EMBRYOS

MORRIS JOHNSON

CHANGES IN POLAR LIPIDS AND ISOPEROXIDASE ACTIVITY DURING THE DEVELOPMENT OF CONIFER ZYGOTIC AND SOMATIC EMBRYOS

OVERALL OBJECTIVES

1. DETERMINE THE MANNER IN WHICH BIOCHEMICAL PARAMETERS CHANGE DURING ZYGOTIC EMBRYO DEVELOPMENT AND THE EXTENT TO WHICH SOMATIC EMBRYO DEVELOPMENT IN THE NORWAY SPRUCE MODEL SYSTEM PARALLELS THAT OF ZYGOTIC EMBRYO DEVELOPMENT IN TERMS OF THOSE PARAMETERS.

2. USE THESE FINDINGS TO DESIGN EXPERIMENTS TO IMPROVE SOMATIC EMBRYO DEVELOPMENT IN THE TARGET SPECIES.

CONE SOURCES ANALYZED

DEVELOPING ZYGOTIC EMBRYOS AND GAMETOPHYTES WERE OBTAINED FROM LOBLOLLY PINE SOURCE F AND NORWAY SPRUCE SOURCES NS-1-88, SKY PINE, SYRACUSE-1, SYRACUSE-9, SYRACUSE-16, AND SYRACUSE-18.

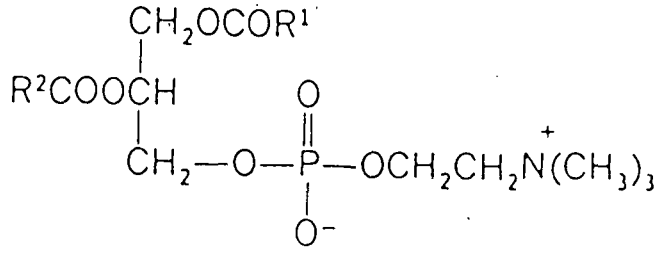
HYPOTHESIS I

1. CHANGES IN POLAR LIPIDS THAT ACCOMPANY ZYGOTIC EMBRYO DEVELOPMENT SHOULD ALSO OCCUR DURING SOMATIC EMBRYO DEVELOPMENT.

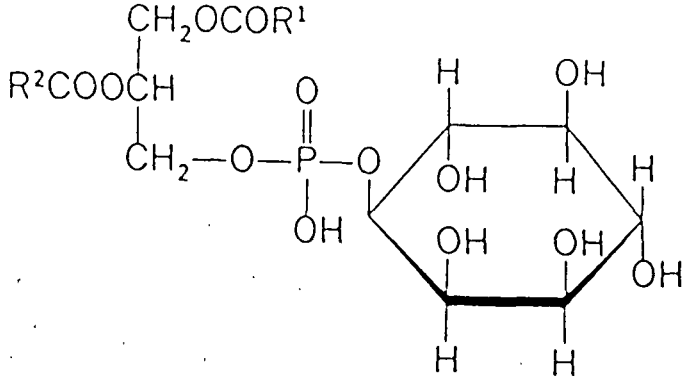
2. DETERMINE THE CHANGES IN THE POLAR LIPID COMPOSITION OF DEVELOPING ZYGOTIC EMBRYOS (AND GAMETOPHYTES) OF NORWAY SPRUCE AND LOBLOLLY PINE.

3. COMPARE THE RESULTS WITH ANALOGOUS DATA AVAILABLE SO FAR ON SOMATIC EMBRYO DEVELOPMENT.

3-*sn*-Phosphatidyl choline



3-*sn*-Phosphatidyl inositol



CONCLUSIONS I

1. POLAR LIPID COMPOSITION CHANGED DURING BOTH NORWAY SPRUCE AND LOBLOLLY PINE ZYGOTIC EMBRYO DEVELOPMENT.

2. LITTLE DIFFERENCE WAS NOTED BETWEEN SPECIES AND WITHIN A GIVEN SPECIES (NORWAY SPRUCE) LITTLE SOURCE VARIATION WAS OBSERVED.

3. NORWAY SPRUCE SOMATIC EMBRYO DEVELOPMENT SHOWED SIMILAR BUT NOT IDENTICAL CHANGES.

4. MANY POLAR LIPID COMPONENTS OF ZYGOTIC EMBRYOS ARE HELD IN COMMON WITH THE SURROUNDING GAMETOPHYTIC TISSUE WHICH CHANGED LESS DURING DEVELOPMENT.

HYPOTHESIS II

1. IT IS POSTULATED THAT PEROXIDASE ACTION IS PART OF A GENERAL CHANGE IN OXIDATION STATE THAT ACCOMPANIES PLANT DEVELOPMENT AND THAT BOTH TOTAL AND SPECIFIC INCREASES IN PEROXIDASE ACTIVITY SHOULD ACCOMPANY EMBRYO MATURATION.

2. DETERMINE THE CHANGES IN PEROXIDASE ACTIVITY THAT OCCUR IN DEVELOPING ZYGOTIC EMBRYOS AND GAMETOPHYTES OF NORWAY SPRUCE AND LOBLOLLY PINE.

3. COMPARE THE RESULTS WITH ANALOGOUS DATA AVAILABLE SO FAR ON SOMATIC EMBRYO DEVELOPMENT.

CONCLUSIONS II

1. ZYGOTIC EMBRYOS OF BOTH NORWAY SPRUCE AND LOBLOLLY PINE EXHIBITED INCREASED INTENSITY AND NUMBER OF ISOPEROXIDASE BANDS WITH DEVELOPMENT (MATURATION) WHEREAS THE NUTRITIVE GAMETOPHYTIC TISSUES DID NOT.

2. THE ISOZYME PATTERNS VARIED SOMEWHAT BOTH WITHIN AND BETWEEN SPECIES, BUT THE GENERAL TREND IN ALL CASES WAS THE SAME AS THE EMBRYOS MATURED.

3. NORWAY SPRUCE MODEL SYSTEM SOMATIC EMBRYOS DISPLAYED THE SAME TREND ALTHOUGH IT WAS MORE GRADUAL.

4. AT A GIVEN STAGE OF ZYGOTIC EMBRYO DEVELOPMENT, LOBLOLLY PINE SEEMED TO BE LESS OXIDATIVE THAN NORWAY SPRUCE.

SUMMARY AND DISCUSSION

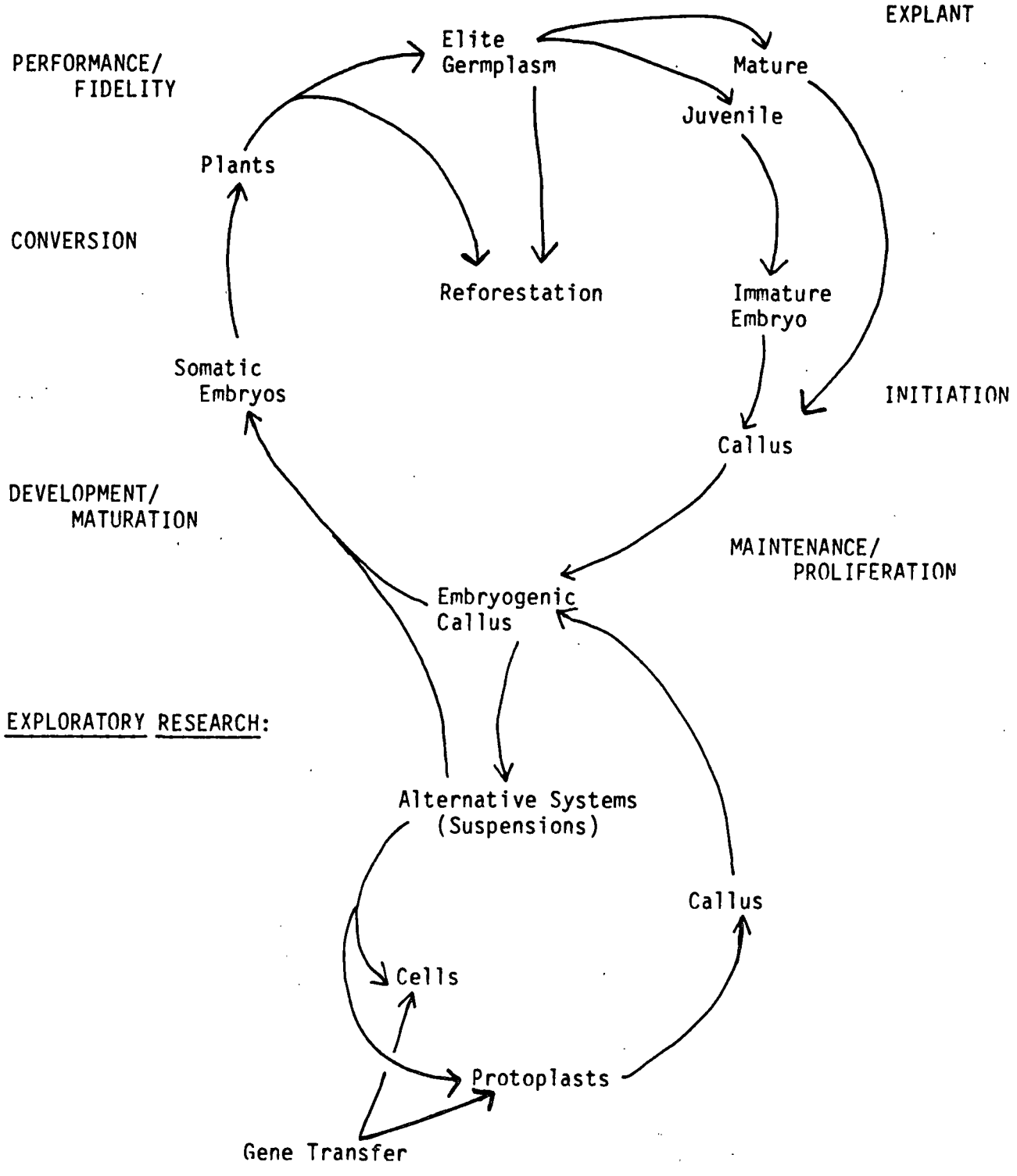
RON DINUS

PROGRESS: PAC TO PAC

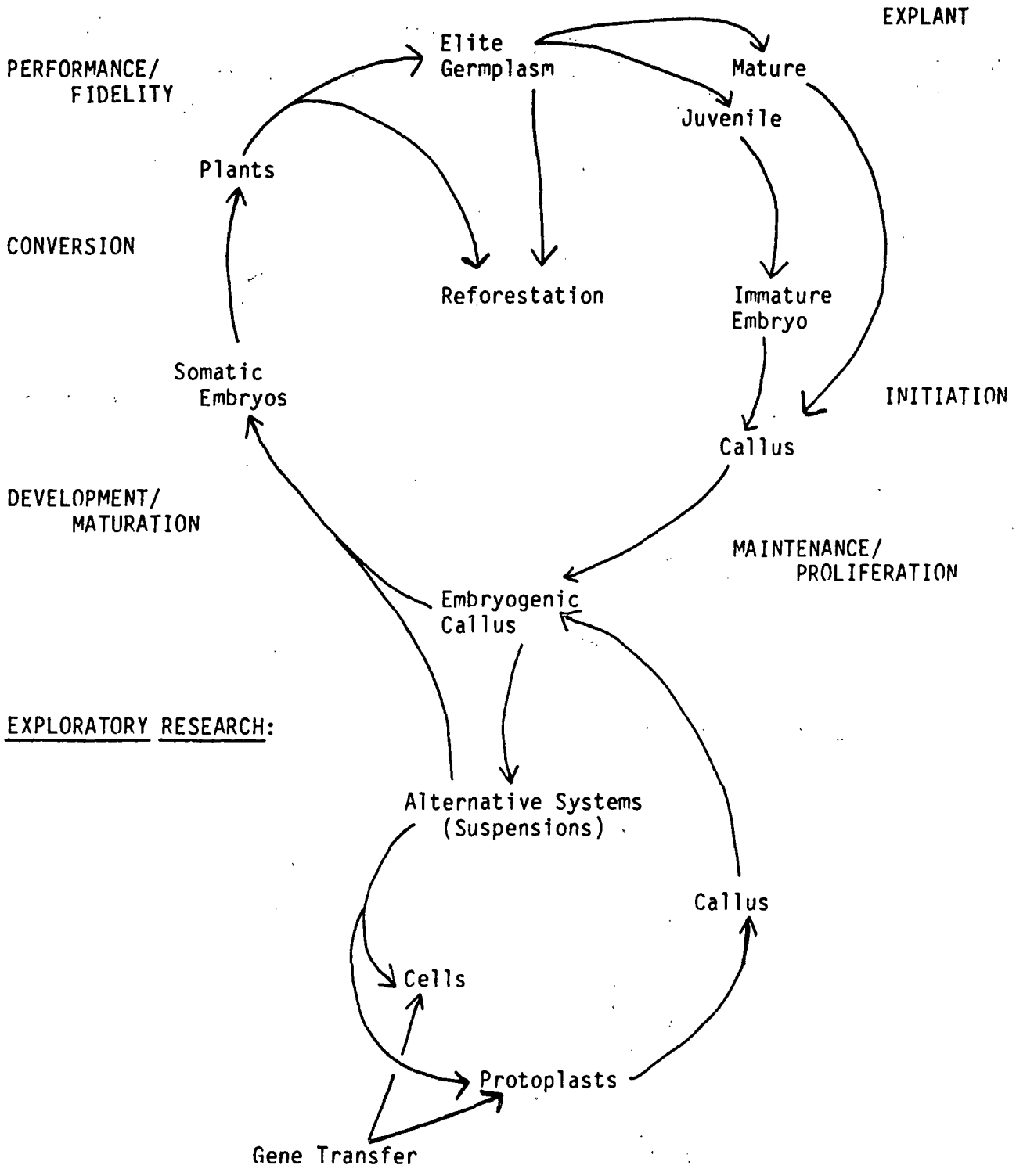
SHORT TERM GOALS	ACCOMPLISHMENTS/ISSUES
RAISE INITIATION FREQUENCIES, TARGET SPECIES	LOBLOLLY - STILL LOW, VERIFIED BEST MEDIA, & CLONAL DIFFERENCES
	DOUGLAS-FIR - WE SCORED! MUST PUSH IN WINTER
BASELINE DATA, BIOCHEMICAL & MOLECULAR	SECURED MATERIALS; DOCUMENTED LIPID, PROTEIN, & ISOZYME PATTERNS; NEED MORE Z/S COMPARISONS
USE BIOCHEMISTRY TO IMPROVE DEVELOPMENT/MATURATION	STARTED WORK WITH PRECURSORS, INHIBITORS, & ABA
INCREASE FREQUENCIES FOR DEVELOPMENT/MATURATION & CONVERSION	N SPRUCE - MAJOR THRUST; MORE EMBRYOS TO COTYLEDONARY STAGE, HIGHER GERMINATION, & 500 PLANTS
	LOBLOLLY - HAVE GOOD LINES + MORE AND LARGER EMBRYOS, BUT NO PLANTS; CONTINUE TO PUSH
IMPROVE ALTERNATIVE CULTURE SYSTEMS	TESTED NEW PROTOCOLS, VERIFIED ABA EFFECT, CONTINUING
INITIATION FROM MORE MATURE EXPLANTS	SPRUCE COTYLEDONS = SAME AS LAST SPRING; ALSO TESTING SOMATIC VS ZYGOTIC
EXECUTE EXPLORATORY RESEARCH	NOT MUCH NEW, BUT CONTINUING; MUCH STUDENT INVOLVEMENT

RECRUIT & HIRE NEW EMPLOYEES	POST-DOC WORKING REPLACED 3 TECHS INTERVIEWING SCIENTISTS MUST COMPLETE & PREPARE FOR NEXT ROUND
DEVELOP HARDWOOD WHITE PAPER	COMPILED DRAFT FOR DISCUSSION
SECURE GRANTS AND CONTRACTS	SUBMITTED 2ND DOE PROPOSAL EXPLORING CONTRACTS; FY '90 PROSPECTS
PUBLISH/PRESENT PROMPTLY	ABOUT THE SAME: CONTINUED EMPHASIS

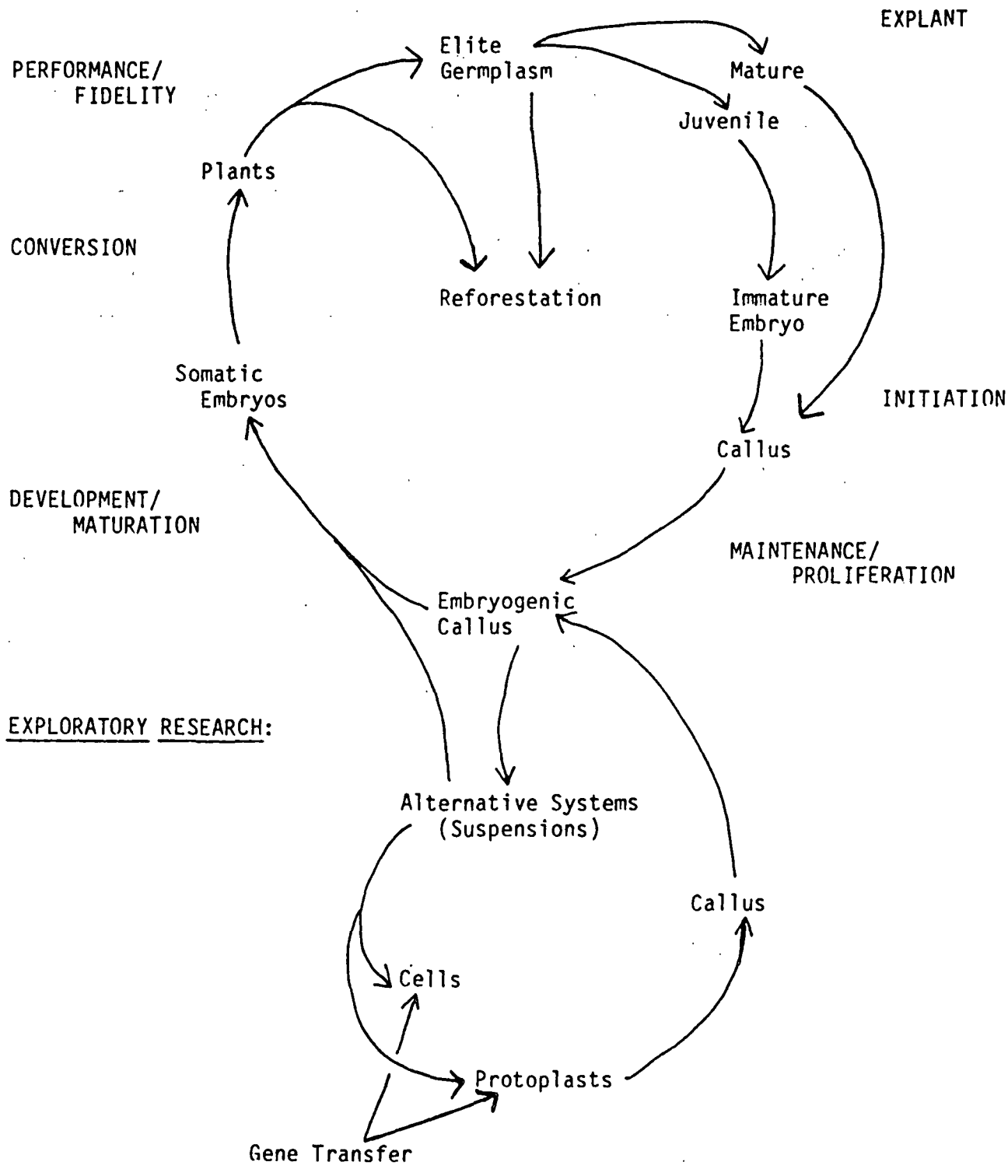
MASS PROPAGATION OF IMPROVED CONIFERS



MASS PROPAGATION OF IMPROVED CONIFERS



MASS PROPAGATION OF IMPROVED CONIFERS



RELATIVE LEVELS OF EFFORT AS INDICATED BY:

RESEARCH PLANS DEVELOPED AND IMPLEMENTED SINCE LAST PAC MEETING FOR EACH RESEARCH AREA AS PERCENTAGE OF TOTAL.

RESEARCH AREA	PLANT MATERIAL		TOTALS
	MODEL SPECIES	TARGET SPECIES	
INITIATION	12	24	36
DEVELOPMENT/ MATURATION	33	25	58
CONVERSION	2	2	4
PERFORMANCE/ FIDELITY	-	-	-
EXPLORATORY	-	2	2
TOTALS	47	53	100

TIME SURVEYS = SIMILAR PATTERN

SHORT TERM GOALS/CRITICAL ISSUES

CLONING SOFTWOODS

- INCREASE INITIATION FREQUENCIES FOR TARGET SPECIES
- RAISE DEVELOPMENT/MATURATION FREQUENCIES, INCREASE CONVERSION EFFICIENCY, AND GENERATE MATERIAL FOR REPLICATED TRIALS
- IMPROVE ALTERNATIVE CULTURE SYSTEMS
- EXTEND TO MORE MATURE EXPLANTS, NORWAY SPRUCE AND LOBLOLLY
- SECURE EXPLANT SOURCES FROM SOUTHERN HEMISPHERE
- TRACK MORPHOLOGICAL/ANATOMICAL CHANGES DURING DEVELOPMENT, ESPECIALLY AS CONCERNS ABNORMALITIES
- EXECUTE EXPLORATORY RESEARCH
- RECRUIT AND HIRE NEW EMPLOYEES
- SECURE GRANTS AND CONTRACTS
- PUBLISH/PRESENT PROMPTLY

BIOCHEMISTY

PROMOTE EMBRYO DEVELOPMENT VIA ADDITION OF PRECURSORS AND INHIBITORS -

- LIPID SYNTHESIS/ACCUMULATION
- ETHYLENE DEPLETION
- PEROXIDASE ISOZYME TIMING AND PATTERNS
- GLUTATHIONE SYNTHESIS/DEGRADATION

QUANTIFY ABSCISSIC ACID LEVELS IN DEVELOPING EMBRYOS, AND MONITOR FATE OF ADDED ABSCISSIC ACID

SECURE DEVELOPING EMBRYOS, IF NEEDED, FROM SOUTHERN HEMISPHERE

BEGIN COMPARING BIOCHEMISTRY OF MORE MATURE EXPLANTS TO TISSUES HAVING EMBRYOGENIC COMPETENCE

EXECUTE EXPLORATORY RESEARCH

PUBLISH/PRESENT PROMPTLY

CLONING HARDWOODS

FINALIZE PROJECT GOALS, OBJECTIVES, AND DIRECTIONS

CHOOSE APPROACHES AND DEVELOP SPECIFIC PLANS

IDENTIFY AND SECURE PLANT MATERIAL

ESTABLISH GREENHOUSE AND/OR IN VITRO POPULATIONS

EVALUATE CLONING METHODS FOR DESIGNATED SPECIES

EXPLORE METHODS FOR PRODUCING/SCREENING USEFUL VARIANTS

GLOSSARY

Adventitious - Roots, shoots, embryos, or other organs or tissues developing in an abnormal position.

Agar - Polysaccharide complex extracted from algae. Used as gelling agent in tissue culture medium.

Agarose - A gelling agent derived from agar: the neutral (charge) fraction of agar.

Agrobacterium tumefaciens - Bacterial plant pathogen responsible for crown gall in plants. Harbors a tumor inducing (Ti) plasmid which can be used to transport a foreign gene into a plant cell.

Antibiotic resistance gene - A gene that codes for a protein, which imparts resistance to an antibiotic that allows cells to live in the presence of the drug that would normally kill them.

Archegonium - The flask-shaped container of the ovum (egg cell) of some gymnosperms. The swollen base (venter) contains the egg cell and is surrounded by the neck, with neck canal cells.

Aseptic culture - Surface sterilization of parental explants, free from pathogens, but not necessarily free of internal symbionts.

Asexual reproduction - Reproduction without fertilization. New individuals may develop from vegetative parts such as tubers, bulbs, or rooted stems, or from sexual parts such as unfertilized eggs or other cells in the ovule.

Auxins - A class of plant growth hormones of diverse makeup which cause cell enlargement, apical dominance, and root initiation.

Bacillus thuringiensis - Bacterium which produces a protein having a strong insecticidal activity. Depending upon the strain of the bacteria, the toxin may exhibit specificity toward Lepidopteran, Dipteran or Coleopteran insect groups.

Bacteriophage - A virus that attacks bacteria; also called a phage.

Base (nucleic acid) - A flat, ring compound that forms part of one of the nucleotide links of a nucleic acid chain. The bases are adenine, thymine, guanine, cytosine and uracil (commonly abbreviated A, T, G, C, U).

Base pair - Two bases, one in each strand of a double stranded DNA molecule, which are attracted to each other by weak chemical interactions. Only certain combinations of bases will pair: A-T, G-C and A-U.

Callus culture - Proliferation from a parental explant of many cells in protoplasmic continuity, but having no equivalence with any normal tissue. Same as tissue culture.

Cell differentiation - Internal chemical or ultrastructural changes preceding or accompanying specialization of function.

Cell suspension - Culture of single cells in moving liquid medium, often used to describe suspension cultures of cells and cell aggregates.

Chloroplast - A membrane-enclosed subcellular organelle containing chlorophyll. Chloroplasts are the sites of photosynthesis. They contain DNA and ribosomes and can replicate.

Clonal propagation - Propagation of a group of plants derived from a single individual (ortet) by asexual reproduction. All members (ramets) of a clone have the same genotype and consequently tend to be uniform.

Clone - 1. (verb) to undergo the process of creating a group of identical DNA molecules or genes derived from a single source. 2. (noun) a group of genetically identical cells (plants), all derived from a single ancestor.

Cloning vector - Small plasmid, phage or virus DNA molecules used to transfer a DNA fragment or gene from a test tube to a living cell. Some vectors are capable of multiplying inside living cells (bacteria) to result in the multiplication or cloning of the transferred DNA or gene.

Codon - A group of three nucleotides coding for an amino acid.

Conversion or Transfer to Soil - Survival and continued growth of an <u>in vitro</u> derived plantlet (germinant) in soil (nonaxenic conditions).

Coumarins - A class of phenylpropanoid phenolic compounds of which coumarin itself typifies the structures.

Cotyledon - The leaf formed directly from the embryo of an angiosperm or gymnosperm. There may be one (in monocotyledons), two (in dicotyledons), or several (in gymnosperms). They act as storage organs in nonendospermous seeds and as the first photosynthetic organs in endospermous seeds.

Cytokinins - A class of plant growth hormones associated with cell division, assisting with the transmission of the genetic information from the genes to the proteins.

cDNA (complementary DNA) - DNA synthesized from an RNA template in test tubes using the enzyme reverse transcriptase. The DNA sequence is thus complementary to that of the RNA. cDNA is usually made with radioactive nucleotides and is used as a hybridization probe to detect specific RNA or DNA molecules (genes).

Denature - In reference to DNA, denaturation means conversion of double stranded to single stranded DNA.

Development - Any or all of the steps subsequent to the first asymmetric cell division that result in the formation of a complete plant.

2D TLC - Two-dimensional thin-layer chromatography.

Diploid - Having two sets of chromosomes in the nucleus. One-half of the chromosomes are contributed by one parent, one-half by the other parent. Many higher organisms are diploid except for their sex cells and associated tissue.

Electroinjection - Method of transporting naked DNA into a plant cell having a cell wall using a short duration DC electrical pulse (see electroporation).

Electroporation - Method of transporting naked DNA (gene) into a protoplast using a short duration DC electrical pulse.

E. coli (Escherichia coli) - A bacterium commonly found in the digestive tracts of many mammals, including humans.

EM - Electron microscope.

Embryo - The young plant developing in the megagametophyte from the fertilization of an egg cell, or without fertilization. In aseptic cultures, adventitious embryos show polarization followed by the growth of a shoot from one end and a root from the other end.

Embryogenesis - Initiation of embryoids or embryos from cultured cells.

Embryoid - A cell group approximating an embryo, but having a more random cell arrangement.

Enzyme - A protein molecule that catalyzes a specific chemical reaction.

ER - Endoplasmic reticulum. A system of membranes (originating from the external membrane of the nuclear envelope) that permeates the cytoplasm and that may or may not be covered with ribosomes.

Erosion zone - Zone in the gametophytic tissue below the archegonium that is degraded by the developing embryo.

Eucaryotic cells - Cells with true nuclei bounded by nuclear membranes and which undergo meiosis.

Excise - To cut or isolate callus tissue from its parental explant or to remove adventitious shoots from callus tissue for rooting.

Explant - A plant part excised and prepared for aseptic culture by surface sterilization followed by the exposure of live cells to a nutrient medium.

Fertilization - The normal union of two gametes during sexual reproduction.

Fidelity - Preservation of the original genotype and phenotype.

Flavonoids - A class of phenolic compounds usually consisting of two hydroxylated aromatic rings joined by a three-carbon chain.

Gametophytic tissue - Haploid tissue of the seed that surrounds the developing embryo during the latter stages of embryogenesis.

Gel electrophoresis - A method for separating molecules based on their size and/or electrical charge. Molecules are forced to run through a gel (e.g., agarose or polyacrylamide) by placing them in an electric field. The speed at which they move depends on their size and/or charge.

Gene - One of the units of inherited material carried on a chromosome; arranged in a linear fashion and indivisible.

Gene cloning - A way to use microorganisms to produce millions of identical copies of a specific region of DNA or gene.

Gene pool - Reservoir of genetic variability available for use in genetic improvement of tree species.

Genetic engineering - The formation of new combinations of heritable material by the insertion of nucleic acid molecules into a vector system so as to allow their stable incorporation into a host organism in which they do not naturally occur.

Genetic gains - Average improvement in progeny over the mean of the parents.

Genetic variability - The variation existing in a given population (species, for example) with respect to particular genes or arrangement of genes.

Genome - May refer to the full genetic complement in the haploid set of chromosomes of a species, but one may speak of nuclear, chloroplastid and mitochondrial genomes.

Genotype - The genetic makeup of an individual; carried in the chromosomes.

Germination - Production of a germinant (plantlet with primary root) from a mature embryo.

Glycolipid - A lipid molecule containing carbohydrate.

Grana - Association of thylakoids in a stack.

Groundplasm - Homogeneous plasma (matrix) remaining after cell organelles and particles have been excluded.

Haploid - Having the reduced chromosome number, i.e., having one set of chromosomes in the nucleus. This is normal in sex cells, which have only half the number of sets occurring in diploid vegetative cells.

Homologous - Describing regions of DNA molecules that have the same nucleotide sequence. Complementary base pairing can occur between homologous regions in two different DNA molecules.

Hormone - Any growth substance which is generally transported to the site of action and can stimulate growth or cell enlargement (auxins), cell division (cytokinins), stem elongation (gibberellins), or can retard growth as in the abscission of leaves (ethylene).

Hybrid vigor - The increase in vigor, size and fertility of a hybrid as compared with its parents, resulting from the union of genetically different gametes and assumed to be due to special recombinations of dominant and recessive genes (heterosis).

Hybridization - The production of offspring of genetically different parents.

Hypocotyl - The part of a seedling axis between the radicle and the cotyledon(s).

Induction - To cause initiation of a plant structure, organ or process.

Initiation - The formation of callus from an explant.

Inoculation density - "ID" is the volume of cells per unit of medium, i.e.,)L/mL.

Inoculum - A small piece of tissue cut from callus, or a small amount of cell material from a suspension culture placed in contact with fresh medium for continued growth of the culture. Inocula (plural).

Interspecific hybrid - The progeny from matings between species.

Intraspecific hybrid - The progeny from matings within species.

Intron - A noncoding section of a gene that is spliced out of mRNA before translation into proteins.

In vitro - Outside the living organism.

In vivo - Within the living organism.

Isozymes - Multiple forms of a single enzyme.

Kanamycin - Antibiotic that disrupts protein synthesis in some bacteria and plants.

Lamda - The name of a particular bacteriophage (virus) used extensively in gene cloning.

Launch - (Induction), to cause the initiation of a process that will result in the development of a plant structure (shoots, roots, or embryos); sometimes used to describe the log phase of the growth cycle.

Lipids - Any of a group of biochemicals which are variably soluble in organic solvents and barely soluble in water.

Maintenance - The perpetuation of callus by subculture.

Maturation - Development of proembryo to cotyledonary (mature) embryo.

Milieu - The whole chemical and physical environment of a culture.

Meristem - A localized group of cells, actively dividing and undifferentiated but ultimately giving rise to permanent tissue such as shoots, roots, wood or bark.

Meristemoid - A localized group of cells in callus tissue, characterized by an accumulation of starch, RNA and protein, and giving rise to adventitious shoots or roots.

Mitochondria - Small bodies in spaces of the cytoplasm. They are spheres or rods, and are the sites of many important aerobic enzymatic processes. The inner layer of the wall is infolded into fingerlike processes.

Morphogenesis - Initiation of organized tissue in callus or suspension cultures.

mRNA (messenger RNA) - RNA that is used by the ribosome to synthesize proteins.

Nick translation - A procedure for radiolabelling DNA in vitro. Used to make a radioactive probe.

Nuclease - A general term for an enzyme that cuts DNA or RNA.

Nucleic acid - DNA or RNA.

Nucleotide - One of the building blocks of nucleic acids. A nucleotide consists of three parts: a base, a sugar and a phosphate.

Nutrient medium - A solid or liquid combination of major and minor salts, an energy source (sucrose), vitamins, hormones, and occasionally other defined or undefined supplements. Usually made up from previously prepared stock solution, then sterilized by autoclaving or filtering through a micropore filter. Media (plural).

Organized tissue - Tissue composed of regularly differentiated cells.

Organelle - A complex cytoplasmic structure of characteristic morphology and function, such as a mitochondrion or plastid.

Organogenesis - Initiation of roots or shoots from callus meristemoids.

Packed cell volume - "pcv" is the volume of cells determined by centrifugation.

Parasexual hybridization - Hybridization resulting from asexual fusion of cells, either diploid or haploid.

Passage - The duration of growth of callus or cell material from one subculture to another.

Performance - Response of the regenerated somatic plant to the environment relative to the original plant or suitable control plants.

Phospholipid - A lipid molecule containing phosphate, usually consisting of glycerol or sphingosine, phosphate, two esterified fatty acids, and an additional moiety such as choline, inositol, etc.

Photoperiod - Length of daily light cycle.

Plasmalemma - The semipermeable unit membrane surrounding and containing the cell cytoplasm. In plant cells, it is pressed up against the inner surface of the cell wall.

Plasmid - A small circular DNA molecule found inside bacterial cells. Plasmids reproduce every time the bacterial cell reproduces. Once infected, the bacteria will always contain a plasmid. Some plasmids continue to replicate in a bacterial cell so that a single cell may contain 200 plasmids. Plasmids are thus used to clone a gene.

Polyploidy - Having three or more times the haploid number of chromosomes.

Procaryotic cells - Single-celled organisms and reproducing entities that lack a membrane-bound nucleus; they do not undergo meiosis; these include the viruses, bacteria, and blue-green algae.

Probe - A radioactive DNA or RNA molecule used to detect the presence of its complementary strand on an electrophoretic "gel" by hybridization and autoradiography.

Proembryo - The very earliest stage of embryo development before suspensor cell elongation occurs.

Proliferation - Increase in mass of callus, cells, somatic proembryos, etc., involving an increase in numbers.

Prolamellar body - Semicrystalline structure from which thylakoid membranes arise during chloroplast development in dark grown seedlings.

Promotor - A short nucleotide sequence on DNA recognized by RNA polymerase to initiate transcription (synthesis of mRNA).

Proplastids - A group of plastids which are progenitors of chloroplasts.

Protoplast - Spherical cell protoplasm (cytoplasm + nucleus) bounded by a membrane but no cell wall.

Protoplast fusion - Union of two protoplasts into one cell.

Recombinant DNA (rDNA) - Chimeric DNA molecule formed by cutting and splicing of DNA (genes).

Recovery - The overall process of development starting with the proembryo. Recovery frequency = maturation frequency x germination frequency x conversion frequency.

Restriction endonucleases - (Restriction enzymes) enzymes that cut DNA at specific nucleotide sequences yielding fragments of various sizes. These enzymes are isolated from a variety of bacteria, and are identified by a three letter abbreviation consisting of the first letter of the genus and the first two letters of the bacterial species name, followed by the strain number (e.g., a particular enzyme isolated from an E. coli strain is designated Eco R1).

RFLPs (restriction fragment length polymorphisms) - DNA molecules from the same gene in two different individuals may differ slightly, and fragments of different length are formed when the gene is digested with a restriction enzyme. Since unequal-sized fragments travel at different speeds in an electrophoresis gel, the two fragments visualized by a radioactively-labeled homologous probe would appear as different bands on the gel. This is a RFLP.

Reverse transcriptase - An enzyme purified from tumor viruses that synthesizes DNA complementary to an RNA template.

Ribosomes - Organelles containing protein and RNA. They are seen as dense particles in electron micrographs. They are found in all types of cells in which protein is being synthesized.

RNA - Ribonucleic acid. RNA is usually single stranded.

RNA polymerase - The enzyme responsible for making RNA complementary to a DNA template. RNA polymerase binds at specific nucleotide sequences (promotors) in front of genes in DNA. It then moves through a gene and makes an RNA molecule that contains the information contained in the gene.

SEM - Scanning electron microscope.

Sequence - The order of the nucleotides in the DNA or RNA chain.

Somatic - Diploid body cells of an organism; those cells other than germ cells.

Somatic cell hybrid - The plant resulting from fusion of protoplasts from somatic cells of genetically different sources.

Splicing - Removal of introns from the "immature" form of eukaryotic mRNA. Carried out in the nucleus of the cell.

Subculture - Dividing agar grown callus or liquid cell suspensions for transfer to fresh medium.

Suspension culture - Cells or cell aggregates dispersed and growing in moving liquid medium.

Suspensor - Elongated, vacuolated cells subtending the embryonal cells in a developing zygotic embryo.

Tannins - A class of complex phenolic compounds known for their astringency and ability to tan the proteins of animal skins. There are two major types of tannins, the hydrolyzable and the condensed tannins.

TEM - Transmission electron microscope.

Template - A pattern of nucleotide sequences in DNA or RNA used by polymerases to specify the sequence in a new polymer by complementarity.

Tetracycline - An antibiotic that kills bacteria by blocking protein synthesis.

Thylakoids - Complex system of flattened membranes within a chloroplast; are often found in stacks to form grana.

Ti plasmid - The plasmid carried by the bacterium Agrobacter tumefaciens which is used to carry foreign genes into a plant cell.

Tissue culture - General term for callus and cell cultures.

Totipotency - A cell characteristic in which the cell retains the potential of forming all the cell types of the adult organism.

Transcription - The process of converting information in DNA into information in RNA. The copying of a gene into RNA. RNA polymerase is the enzyme that executes this conversion of information.

Transformation - The process whereby a cell takes up free DNA such that the free DNA (gene) becomes a permanent part of the cell's genome.

Translation - The process of converting the information in mRNA into protein. Also called protein synthesis.

Transposon - A short section of DNA capable of "jumping" to another region of a chromosome or to a different chromosome.

Transposon tagging - Method of using a transposon to locate a gene. When a transposon inserts into a chromosome, it causes a knockout mutation leading to a distinct mutant phenotype. A radioactive probe made from this transposon can then be used to identify the DNA sequence (gene) into which it had been inserted. The gene can then be localized on a gel and perhaps on a particular chromosome from the mutant plant. In short, the mutated gene is tagged or made identifiable by the transposon.

Triglyceride - A lipid molecule consisting of glycerol plus three esterified fatty acids; also, mono- and diglycerides which have only one or two fatty acids, respectively.

Ultrastructural - Sublight microscopic, intracellular structure.

Vacuole - A fluid-filled space in a cell. A single vacuole, taking up most of the volume of the cell, present in many plant cells, and containing a cell sap which is isotonic with the protoplasm.

Vegetative cells - Nonreproductive cells such as haploid cells from female gametophytes of conifers or diploid somatic cells.

Vesicle - Small membrane-bound body in the cytoplasm.

Zygote - Fusion product of male and female sex cells or fusion product of protoplasts.

AMINO ACIDS ABBREVIATIONS

ala	alanine
arg	arginine
asn	asparagine
asp	aspartic acid
cit	citrulline
cys	cysteine
q-aba	aminobutyric acid
gln	glutamine
glu	glutamic acid
gly	glycine
his	histidine
hyp	hydroxyproline
ile	isoleucine
leu	leucine
lys	lysine
met	methionine
orn	ornithine
phe	phenylalanine
pro	proline
ser	serine
thr	threonine
trp	tryptophan
tyr	tyrosine
val	valine

CUMULATIVE LIST OF ABBREVIATIONS

AA	Ascorbic acid
2,4-D	2,4-Dichlorophenoxyacetic acid
ABA	Abscisic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
ADC	Arginine decarboxylase
ADP	5'-Adenosine diphosphate
AMP	5'-Adenosine monophosphate
ANOVA	Analysis of variance
AOA	Aminooxyacetic acid
AOAA	Aminooxyacetic acid
AOPP	a-Aminooxy-b-phenylpropionic acid
ATP	Adenosine triphosphate
AVG	Aminoethoxyvinylglycine
BA	Benzylaminopurine = benzyl adenine
BAP	Benzylaminopurine = benzyl adenine
BLG	Brown and Lawrence medium + gln
BSA	Bovine serum albumin
BSO	Buthionine sulfoximine
cAMP	3',5'-Cyclic adenosine monophosphate
CBM	Bornman medium
C/N	Carbon/nitrogen
D	Dark
DCR	Durzan sugar pine medium
DF	Douglas-fir
DFMA	a-difluoromethylarginine
DFMO	a-difluoromethylornithine
DCHA	Dicyclohexylammonium sulfate
DHA	Dehydroascorbic acid
dSAM	Decarboxylated SAM
DW	Dry weight
E	Embryogenic
EC or ec	Embryogenic callus
EDTA	Ethylenediaminotetraacetic acid
E_i	Embryonal initial
FAA	Free amino acid(s)
FTIR	Fourier transform infrared
FW or fr.wt.	Fresh weight
G-1-P	Glucose-1-phosphate
GA	Gibberellic acid (gibberellin)
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
GD	Gresshof and Doy medium
GSH	Glutathione (reduced)
GSSG	Glutathione (oxidized)
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HFBI	Heptafluorobutyrylimidazole
HFSE	High frequency somatic embryogenesis
HM	Hakman medium
HPLC	High performance liquid chromatography
IAA	Indoleacetic acid

IBA	Indolebutyric acid
IEF	Isoelectric focusing
IPA	Isopentenylaminopurine = 2iP
L	Larch, light or liter
LFSE	Low frequency somatic embryogenesis
LM	Litvay medium
LP	Loblolly pine
lx	Lux
MEOI	Methyleneoxindole
MES	Morpholinoethane sulfonic acid
MOI	Methyloxindole
MOPS	Morpholinopropane sulfonic acid
MGBG	Methylglyoxal bis-guanyl hydrazone
MS	Murashige and Skoog medium
NAA	Naphthalene acetic acid
NAD^+	Nicotinamide adenine dinucleotide (oxidized)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidized)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NE	Nonembryogenic
NBT	Nitrobluetetrazolium
NOAA	Naphthoxyacetic acid
NS	Norway spruce
OBHA	o-benzylhydroxylamine
ODC	Ornithine decarboxylase
P	Putrescine or phosphate
PAL	Phenylalanine ammonia lyase
pcv	Packed cell volume
PEG	Polyethylene glycol
PEM or pem	Preembryonal mass
PO	Pond pine
PP	Pitch pine
PPi	Pyrophosphate
ProA	Proanthocyanidin
RP	Red pine or research plan
S	Suspensor
SAM	S-adenosylmethionine
Sd	Spermidine
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SE or se	Somatic embryo
S_i	Suspensor initial
SIM	Selective ion monitoring
Sp	Spermine
TLC	Thin-layer chromatography
TrpAM	Tryptamine
2iP	Isopentenylaminopurine
UDP	Uridine diphosphate
UDPG	Uridine diphosphate glucose
UTP	Uridine triphosphate
WC	Wild carrot
WCM	Wild carrot medium
WH	White's medium
WP	White pine
WS	White spruce

STATUS OF PUBLICATIONS AS OF 10/88

PUBLISHED OR IN PRESS:

1. Dinus, R. J. Integration of biotechnology and classical genetics. IUFRO Conference on Genetics and molecular biology of interactions between harmful agents and trees. Iowa State Univ., Ames, IA. October 16-21, 1988. (In press)

2. Becwar, M. R.; Wann, S. R.; Johnson, M. A.; Verhagen, S. A.; Feirer, R. P.; Nagmani, R. Development and characterization of in vitro embryogenic systems in conifers. In Somatic Cell Genetics of Woody Plants, M.R. Ahuja(ed.) Kluwer Academic Publishers, Norwell, MA, pp. 1-18 (1988).

3. Feirer, R. P. Plant growth: The role of polyamines. Invited contribution to McGraw-Hill Encyclopedia of Science and Technology. (In press)

4. Feirer, R. P. Chloroplast ultrastructure and gene expression in embryogenic conifer callus. In Molecular Genetics of Forest Trees, Cheliak, W. M. and Yapa, A. C. eds., Petawawa National Foresry Institute, Chalk River, Ontario, pp 89-95 (1988). (Proceedings of International Union of Forest Research Organizations-Molecular Genetics Working Group Conference., June 16-18. Chalk River, Ontario)

5. Slocum, R.; Bitonti, A.; McCann, P.; Feirer, R. P. DFMA metabolism in tobacco and mammalian cells: inhibition of ornithine decarboxylase activity following arginase-mediated hydrolysis of DFMA to DFMO. Biochem. J. (In press)

6. Verhagen, S.; Wann, S. R. Norway spruce somatic embryogenesis: high frequency initiation from eight cultured mature embryos. Plant Cell, Tissue and Organ Culture. (In press)

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1. Becwar, M. R.; Noland, T.; Wyckoff, J. Maturation, germination, and conversion of Norway spruce somatic embryos to plants. In Vitro Cell & Develop. Biol.

2. Feirer, R. P.; Becwar, M. R. Plastid ultrastructure in embryogenic conifer callus. In Vitro Cell & Develop. Biol.

3. Johnson, M. A.; Wann, S. R.; Becwar, M. R.; Nagmani, R.; Feirer, R. P.; Biochemical differences between embryogenic and nonembryogenic calli of conifers. Physiol. Plantarum.

4. Johnson, M. A.; Nealey, L. T.; Thompson, N. S. Properties of a xyloglucan isolated from the spent medium of suspension cultured loblolly pine (Pinus taeda) cells. Plant Physiol.

5. Nagmani, R.; Becwar, M. R.; Wann, S. R. A survey of in vitro induction frequency of embryogenic tissue from immature embryos of ten clones of loblolly pine (Pinus taeda). Can. J. For. Res.

6. Nagmani, R.; Becwar, M. R.; Wann, S. R. Factors regulating somatic embryo development in loblolly pine (Pinus taeda L.). Can. J. For. Res.

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1. Conners, T. E.; McLain, T. E. Modeling Moisture Gradient Effects on Bending Properties. Wood and Fiber Science 20(2):198-216(1988).

2. Conners, T. E. Segmented models for stress-strain diagrams. Accepted for publication in Wood Science and Technology (4/88)

3. MacGregor, M. A; Conners, T. E. Image Analysis of an LWC Paper Reveals Wire Mark in the Print Density Variations. Tappi J. 70(9):95-100 (September 1987).

PRESENTATIONS AND PARTICIPATION:

1. Becwar, M. R.; Wann, S. R.; Nagmani, R. A survey of *in vitro* induction frequency of embryogenic tissue from immature embryos of ten clones of loblolly pine (*Pinus Taeda*). International Conifer Tissue Culture Work Group; Saskatoon, Canada, August 8-13, 1988.

2. Becwar, M. R. "Conifer somatic embryogenesis and clonal forestry. Invited seminar presented at Rutgers Univ. May 2, 1988.

3. Becwar, M. R.; Wann, S. R.; Nagmani, R. Differences in initiation of somatic embryogenesis from immature embryos of *Pinus taeda* (loblolly pine) and *Picea glauca* (white sruce). TCA Meeting, Las Vegas, June 1988.

4. Dinus, R. J. Convened and moderated special contributed paper session on Advances in tree cell and tissue culture at the 39th Annual Meeting of the Tissue Culture Association, Las Vegas, NV. June 12-16, 1988.

5. Dinus, R. J. Moderated session on vegetative propagation at the 10th North American Forest Biology Workshop, Univ. of B.C., Vancouver, BC. July 20-22, 1988.

6. Johnson, M. A.; Carlson, J. A. Growth regulator effects on redox parameters in conifer embryo development. The 15th annual meeting of the Plant Growth Regulator Society of America. San Antonio, TX, July 31-August 4, 1988.

7. Johnson, M. A.; Becwar, M. R.; Dinus, R. J. Early performance and fidelity of plantlets derived from somatic embryos of Norway spruce. The 10th North American Forest Biology Workshop. Vancouver, British Columbia, Canada, July 20-22, 1988.

8. Johnson, M. A.; Carlson, J. A. Glutathione and related parameters in conifer embryo development. The 13th International Conference on Plant Growth Substances. Calgary, Alberta, Canada, July 17-22, 1988.

9. Nagmani, R.; Becwar, M. R.; Wann, S. R. Factors regulating somatic embryo development in loblolly pine. International Conifer Tissue Culture Work Group; Saskatoon, Canada, August 8-13, 1988.

10. Verhagen, S.; Hanson, D.; Becwar, M. R. Quantification of Embryogenic Suspension Cultures Derived from Norway Spruce Mature Embryo Callus. Poster: TCA Meeting, Las Vegas, June 1988.

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