

**THE INSTITUTE OF PAPER SCIENCE & TECHNOLOGY  
ATLANTA, GEORGIA**

**FOREST GENETICS PROJECT ADVISORY COMMITTEE**

**HANDOUTS**



**MARCH 28, 1990**

## AGENDA

## FOREST GENETICS PROJECT ADVISORY COMMITTEE

Institute of Paper Science and Technology  
 Radisson Conference Center  
 Atlanta, Georgia

Wednesday, March 28, 1990

8:00 a.m.	Registration, Coffee and Doughnuts	
8:30 a.m.	Welcome and Introductions Overview and PAC Recommendations	Dinus
9:00 a.m.	Somatic Embryo Maturation Update on Loblolly Pine	Uddin
9:25 a.m.	Embryo Classification and Germination	Webb
9:45 a.m.	Coffee Break	
10:00 a.m.	Zygotic Embryo Composition	Nagmani
10:40 a.m.	Student Presentation	Wood
11:00 a.m.	Hardwood Regeneration Leaf Section System Cell Suspensions	Uddin
11:30 a.m.	Summary and Near-Term Plans	Dinus/Malcolm
Noon	Lunch, IPST Conference Room	
1:30 p.m.	Laboratory and Greenhouse Tour  Laboratories Open and Personnel Available	Committee
5:00	Reception, IPST Conference Room	
6:00	Group Dinner, Dutch Treat	

## Agenda, Cont'd

Thursday, March 29, 1990

8:00 a.m.	Agenda for Morning	Leach/Dinus
8:15 a.m.	PAC Operations	Yeske/Malcolm
9:15 a.m.	Discussion/Deliberations	Committee
10:00 a.m.	Coffee Break	
10:20 a.m.	Discussion/Deliberations	Committee
11:00 a.m.	Closing Remarks	Leach, Dinus, and Malcolm
11:30 a.m.	Adjournment	

FOREST GENETICS

## Project Advisory Committee

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## 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)<sup>a</sup>

-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).

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<sup>a</sup>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

7  
 CONE SOURCES - 1988

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

<sup>a</sup>Cones obtained from progeny of the given clone.

8  
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 ( $P < 0.05$ ). The number of replications is indicated by N.

INTRODUCTORY REMARKS - RON DINUS

WELCOME

MEMBERS & GUESTS

OVERVIEW

STATUS OF FACILITIES

STUDENTS

PAC RECOMMENDATIONS

GROUND RULES & ANNOUNCEMENTS

THANKS, LET'S HEAR SOME RESULTS

**STUDENT PROJECTS****Lois Forde -****M.S., "Phenylalanine ammonia lyase and  
lignin biosynthesis." Advisors,  
T.E. Conners and R.J. Dinus.****Peasely Shorter -****M.S., "Promotion of additional auxin  
synthesis in Populus deltoides via  
transformation with Agrobacterium  
tumefaciens." Advisor, D.T. Webb.**

**STUDENT PROJECTS****Colleen Walker -****Ph.D., Tentative Dissertation Topic:  
"Comparison of biological and other  
methods for delignification of kraft  
pulps." Advisor, R.J. Dinus.****Michael Wood -****M.S., "Effect of cold shocking on cell  
cultures of Larix decidua." Advisor,  
R.J. Dinus.**

STUDENT INTERACTIONS

FALL ORIENTATION: BOWATERS SEED ORCHARD & NURSERY COMPLEX,  
PLANTATIONS, & HARVESTING OPERATION

CLASS FIELD TRIP: UNION CAMP TREE IMPROVEMENT CENTER  
PINE GENETICS  
HARDWOOD PROPAGATION  
SEED PROCESSING  
PRODUCTION NURSERY

## PAC RECOMMENDATIONS

ISSUE	ACTION OR PLAN
PROCESS DIAGRAMS	ADJUSTING AS SUGGESTED
PAC OPERATIONS	RON YESKE, TOMORROW
KEY GOALS	WORKING ON THEM BALANCED APPROACH EVALUATION: ACCOMPLISHMENTS + PROGNOSIS
PLANNING/PUBLICATION	WEEKLY & LAB MEETINGS PEER REVIEW COURSE WORK
EMBRYO CLASSIFICATION SYSTEM	USING & ENLARGING
MATURATION EXPERIEMENTS	NS: TEST & REFINE PROTOCOL MOVE ON KEY GOALS  LP: ISOLATE FACTORS PROBE INTERACTIONS
INITIATION, TARGET SPECIES	DF: SUMMER RESULTS NEW ZEALAND  ZYGOTIC EMBRYO COMPOSITION  DF & LP: NEW COLLECTIONS
RECRUITING/HIRING	ADDED 2 TEMPORARIES ADDED 2 TECHNICIANS INTERVIEWING FOR OTHERS
PATENT	ADDITIONAL SEARCH RECENT COMPETING PATENT PROCEEDING WITH APPLICATION
SHARING CULTURES	SEEKING COLLABORATION EXCEPTIONS



November 29, 1989

Dr. Ronald J. Dinus  
The Institute of Paper Science  
and Technology  
575 14th Street, N.W.  
Atlanta GA 30318

Dear Ron:

On behalf of the Project Advisory Committee on Forest Genetics, I want to thank you for the update on the projects underway in the biological sciences department, and the tour/orientation with the facility and organization in Atlanta during our PAC meeting of October 25, 1989. It was relieving to hear that the cultures and experiments in progress had made the move successfully, and that you are getting established in the new "temporary, temporary" laboratory. Despite the major disruption from the move, the team still managed to make progress on maturation and other issues; these efforts are appreciated.

The major points of discussion at the meeting and other topics brought to my attention by PAC members are summarized below.

REPORTS/MEETINGS

1. The report and presentations were good. The process diagrams clearly show the stage of progress with the different species, and are just what the PAC asked for. The only suggestions for change were to make sure that the units of any numbers not expressed as percentages (i.e. embryos per gram of callus) are clearly labelled. Also, the explanations as to the meanings of the different percentages might be put on a single, separate page.
2. There was fairly strong support from the PAC for continuing to have committee meetings twice per year. However, preparation for the meetings could be reduced by only distributing the handouts and eliminating the status reports. At least one of the meetings should extend over a two-day period to allow PAC time to assimilate the presentations and discussion before the deliberations and recommendations. The other meeting could be less formal, for one-day (similar to this meeting), and focus more on long-term goals and directions than short-term results. An annual meeting where embryogenesis is discussed in

general terms for the broader IPST membership seems unnecessary, and would require significant preparation to be understandable to non-PAC members.

3. The annual "wood grain" reports could be eliminated and only issued, as suggested, at significant points of development in the research.
4. There appears to be little need to change the makeup of the Forest Biology PAC. If the committee were restricted only to members involved in tissue culture, it would be very small and not representative of the IPST membership. Although PAC discussions on technical issues are certainly dominated by those involved with tissue culture, it does not appear the more "lay" members are hindering such discussion or require extensive, simplified presentations. Some of the less technical members also bring a valuable larger view of the research and its application, as well as providing useful input on organization, goals, and other issues.

Perhaps most importantly, the PAC members are able to provide accurate explanations of the current status and likely future prospects for tissue culture research to their respective member companies. Although the RAC evidently did not feel this was so, it is difficult to accept that the "newsy two-pager" periodic letter proposed would carry the same weight in influencing a member company's position on the project as the report of their own representative. A newsletter would certainly supplement communication of research results, but it could not substitute for someone who is very familiar with the project via the PAC.

5. The PAC had mixed views on the necessity of a lawyer at the meetings. Generally there was little comment, although at least one member thought a lawyer should be present and one did not think it was necessary. No member expressed the view that a lawyer definitely should not be present, just that it seemed unnecessary. I would comment that a lawyer is not present at any of the other meetings of forestry research cooperatives with which I am familiar. Perhaps IPST member companies' policies on this issue should be investigated, to see if any require it.

#### GOALS

1. Thanks to you and the sub-committee for developing the key goals. The PAC agreed these are some of keys to success of the project, and are expressed as requested at the March, 1989, PAC meeting. To re-emphasize a couple of points about the key goals: 1) they should not be pursued to the exclusion of everything else, and 2) if they are not accomplished, the PAC does not necessarily want to see

the project terminated or substantially changed; that would depend on the degree on accomplishment and the prognosis for further progress.

2. There was little discussion on the short-term goals presented. In regards to publication, there should be an emphasis on publication in refereed scientific journals. In regard to conifer initiation, there was support for exploring inflorescences and other tissues of mature trees in future experiments.

#### CONIFER CRITICAL ISSUES

1. Thank you for implementing the standard system of describing embryo development, as suggested at the last PAC meeting. This should help avoid confusion when discussing success in producing and maturing somatic embryos.
2. There was considerable discussion concerning conversion of mature embryos to plants:
  - Several comments were made on the improved quality seen in the Norway spruce embryos; compliments to the team for this success.
  - In experiments involving germination of NS zygotic embryos, culture of the entire megagametophyte might be tried. Also, different ways of "feeding" the somatic embryo, such as different placement in the medium, or putting it in the megagametophyte tissue in place of the zygotic embryo might be tried.
  - In the loblolly maturation experiments (p. 72-73 in handouts) there was considerable variation in number of embryos/clump between the two experiments, and it was difficult to separate the effects of the carbohydrate source from the ABA/IBA and the transfer process itself, since these differed between the two experiments. Proper controls (e.g. sucrose and -IBA) are needed to determine what effect changes made between experiments have on maturation.
  - Several pre-treatments of the SE's might be tried to enhance germination (e.g. dehydration, photoperiod, stratification, ABA, diurnal variation in water stress and/or temperature). Optimization of media for zygotic embryo germination might also help somatic embryos.
3. Although new initiation experiments were not begun since the last PAC meeting, there were some suggestions for future initiation experiments. Steve Wann questioned why do loblolly explants die so soon in culture, and what could be done to get them to live longer? There was also

a suggestion to pre-treat cones with hormones to encourage SE before obtaining explants.

4. Other suggestions made by PAC members:
  - A field or greenhouse trial using Norway spruce SE produced plants
  - Use of incomplete experimental designs like split plot which could increase the number of variables being examined without increasing experiment size. Significant main effects discovered this way could be fine tuned later.
  - Exploratory research at a low level in gene transfer in softwoods.

#### PERSONNEL/ADMINISTRATION

1. The PAC appreciates the leadership of David Webb in the project. His presentation and discussion show a positive influence in organizing and coordinating the research.
2. While the experiments which had been done since the last PAC meeting showed good progress, they also tended to be more empirical. The need for addition of a biochemist and plant physiologist is evident in order to get more fundamental understandings out of experiments. It will be difficult for IPST to maintain a scientifically competitive edge with other conifer embryogenesis groups without a fundamental and mechanistic approach.
3. In regard to patenting of the process which led to the development of pre-cotyledonary and cotyledonary embryos, Steve Wann felt the first literature search for the patent was inappropriate, and believed a second search should be completed.
4. There was general agreement in the PAC that cultures need not be given to anyone who asks for them. It is recommended cultures only be given to those with whom a formal collaboration has been established from which the IPST will benefit.

Mr. Ronald J. Dinus

18

November 29, 1989

Page 5

The PAC meeting was short but productive. Although the past six months were demanding, the tissue culture work is progressing well.

Sincerely,



Gregory N. Leach  
Research and Development Manager  
Western Florida Region

cc. PAC Committee Members



January 2, 1990

Mr. Greg Leach  
Research and Development Manager  
Western Florida Region  
Champion International Corporation  
117 Pace Parkway  
P. O. Box 875  
Cantonment, FL 32533

Dear Greg:

Thanks for your recent letter of response to the Fall meeting of the Forest Genetics Project Advisory Committee (PAC). We were pleased to demonstrate the success of our move and some progress on somatic embryo maturation. The tour of the Atlanta facility and familiarization with our new organization was productive for all. Please be reminded, however, that successfully moving cultures would not have been possible without help from several member companies, especially Westvaco and Union Camp.

#### REPORTS/MEETINGS

- 1) Your compliments on process diagrams are appreciated. The additional suggestions are worthwhile, and should not be difficult to implement.
  
- 2-5) Team members agree with your recommendations concerning meeting types and numbers, status and annual reports, PAC composition, PAC member reporting vs. newsletters, and involvement of lawyers. As discussed in our several telephone conversations, I conveyed these and earlier recommendations to Earl Malcolm and Ron Yeske. Our input is

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being blended with responses from other PAC meetings into a new Institute policy. Thanks again, for your thoroughness.

### GOALS

- 1) All concerned seem satisfied with the "Key Goals," and we are working to attain them. Team members genuinely appreciate your emphasis on a balanced approach, including some exploratory research, and willingness to judge project worth via likelihood of further progress as well as degree of accomplishment.
- 2) We recognize importance of publishing, especially in referred journals. Recent stiffening of weekly meetings and research plan reviews should improve research quality and publication rates. Initiation from tissues of more mature plants is an important thrust, and we plan to execute several experiments per year.

### CONIFER CRITICAL ISSUES

- 1) The standard system for describing embryo development is a valuable innovation from several standpoints, and we plan to increase utility with additional anatomical, histochemical, and biochemical data.
- 2) Compliments on improved quality of Norway spruce somatic embryos are appreciated. Nevertheless, much work remains, and we will utilize several of your suggestions to define, secure, and assure quality. In addition, we plan to test several new treatments and media in our continuing effort to improve germination. We agree that proper controls and a more systematic approach are needed to clarify factors responsible for improved loblolly pine somatic embryo maturation, and will execute two factorial experiments as quickly as culture rooms and required cultures become available. The first will test carbohydrate sources and levels as well as varying ABA concentrations. The second will build on the first by examining different amounts and timing of IBA additions against a background of preferred carbohydrate and ABA treatments. The pair should enable us to separate effects of the factors and probe their interactions.

3) Some of your suggestions concerning initiation in target species seem worthwhile. We will try several of them with explants stored from last summer, and new materials collected in Summer 1990. We decided not to attempt initiation with winter collections from the Southern Hemisphere. Facilities have been limiting, and sufficient cultures are available for continued work on maturation. This tack, hopefully, will also free some time for compiling and interpreting past work on zygotic embryo composition, thereby enabling tests of better media next summer.

4) Growth chamber, greenhouse, and field trials of Norway spruce "somatic seedlings" are part and parcel of our Key Goals. Changes made last summer in our research planning and review of process should facilitate our devising and implementing more exacting and economical experimental designs. Also, personnel lacking statistical expertise have been advised to take appropriate course work. For the present, exploratory research on softwood gene transfer will be pursued via collaboration with molecular biologists in other laboratories.

#### PERSONNEL/ADMINISTRATION

1) Dr. Webb is making major contributions on many fronts, and we are all grateful for his talent, energy, and commitment.

2) As indicated at our meeting, personnel recruiting and interviews are well underway. We are seeking to add outstanding individuals with skills that will build on our fine record and set us apart from others in the field.

3) Consultations and discussions on the patent front continue.

4) Requests for cultures are received routinely, and negotiations are underway with several prospective collaborators. We intend to provide cultures to those workers who are willing and able to collaborate and only after formal agreements have been secured. Exceptions may be made to accommodate workers who can use our materials to examine issues or solve problems that we are not able to address.

On other fronts, we expect to start 1990 in our regular "temporary" laboratories. Most of the residual electrical and plumbing work was completed before the holidays. Barring any setbacks or accidents, we should finally relocate by January 15. To help you start the new year, I am enclosing a copy of our "1989 Executive's Conference Proceedings." The views, confidence, and support expressed by David Luke of Westvaco (pages 35-38), are much appreciated.

Once again, many thanks, best wishes for 1990, and we look forward to sharing continued progress with you at our next meeting, March 28-29.

Sincerely,

*Ronald J. Dinus*

Ronald J. Dinus  
Forest Biology

dsh

Enc.

GROUND RULES

BREAK AT 9:45 AM, WILL ADJUST IF NEEDED

QUESTIONS & IDEAS, PLEASE FEEL FREE

PRESENTATIONS INCLUDE TALK + QUESTIONS,

EXPECT TO STAY ON SCHEDULE

LUNCH AT NOON

THE INSTITUTE OF PAPER SCIENCE AND TECHNOLOGY  
Atlanta, Georgia

Status Report  
to the  
FOREST GENETICS  
PROJECT ADVISORY COMMITTEE

March 28, 1990

## PROJECT SUMMARY FORM

DATE: March 28, 1990

PROJECT NO. 3223-00: MASS CLONAL PROPAGATION OF IMPROVED CONIFERS

PROJECT LEADERS: R.J. Dinus, N. Rangaswamy, M. R. Uddin, D. T. Webb

IPST GOAL: To develop and assure low-cost supplies of quality fiber

OBJECTIVE:

To develop reliable cell and tissue culture systems for mass clonal propagation of improved conifers.

CURRENT FISCAL YEAR BUDGET: \$500,000

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 performance and foster development of value-added or new products.

PRIOR RESULTS:

Past research on cell and tissue culture systems has brought somatic emryogenesis, one method of mass cloning, closer to commercialization. Embryogenesis in Norway spruce, our model system, is now reproducible and straightforward. Embryogenic

cultures can be obtained from immature and mature spruce seed and from tissues of newly germinated seedlings.

In accordance with earlier plans, Norway spruce work remains focused on somatic embryo development and maturation. The archive detailing culture origin, past treatment, and current composition is nearing completion. Action to date has streamlined our culture bank, reduced workloads associated with subculturing, and established bases for both future research on embryo maturation and seedling production. Research continues on gauging embryo maturity and documenting germination events. Collective results should yield improved procedures for maturation, germination, and acclimatization. Somatic seedlings derived from past research are alive and well.

Available loblolly pine embryogenic cultures have been and are being used to improve maturation frequencies. Efforts center on extending recent success with carbohydrates and abscisic acid. A factorial experiment has been installed to test several levels of three carbohydrates and varying concentrations of abscisic acid. Cultures are also being expanded for a second factorial involving best carbohydrate and abscisic acid treatments and differing levels and durations of exposure to Indolebutyric acid. Several exploratory trials are also planned.

In Douglas-fir, efforts to initiate new embryogenic cultures from summer cone collections gave mixed results. Relocation to temporary quarters and later to present facilities inhibited our processing cones as quickly and effectively as desired. In addition, a number of cultures appeared promising at the outset, but later deteriorated or became nonembryogenic. Even so, four new embryogenic lines were obtained. Growth rates, however, have been slow and numbers are insufficient for maturation research at this time. Hence, we are attempting to initiate yet new cultures from mature cones collected last summer in the US and this winter in New Zealand. Most cultures from past years remain viable, but generally slow growth rates are hampering all but exploratory work in maturation.

Difficulties associated with initiation and proliferation of Douglas-fir and loblolly pine prompted a survey of past Institute work on physical and chemical properties of zygotic embryos, gametophytes, and seed. Preliminary findings suggest that conventional culture media differ from environments afforded by developing seed. Importance of deviations will be evaluated as quickly as surveys of institute databases and available literature are completed. The outcome may justify adjustment of current media composition. In any event, new research concerning effects of anionic composition seems warranted, and a grant proposal has been submitted to the National Science Foundation.

**PLANNED ACTIVITY FOR THE PERIOD:**

Complete staffing plan. Complete renovation of laboratory, office, and greenhouse facilities. Raise initiation frequencies in and obtain additional embryogenic cultures of Douglas-fir and loblolly pine. Improve maturation frequencies and raise efficiency of conversion of seedlings; extend best treatments to target species. Document course of zygotic embryo development, maturation, and germination as well as early growth and development of zygotic seedlings so as to establish guideposts for manipulating somatic materials.

**POTENTIAL FUTURE ACTIVITIES:**

Renew research on suspension cultures and initiation from explants of trees mature enough to have been proven genetically superior. Adapt cell and tissue culture techniques to study xylogenesis in vitro; explore factors affecting fiber formation and lignification.

Project 3223-00

Status Report

## MASS CLONAL PROPAGATION OF IMPROVED CONIFERS

PROJECT 3223-00

## GENERAL SUMMARY OF RECENT ACTIVITIES

March 28, 1990

This project is designed to develop reliable cell and tissue culture systems for rapid, low-cost mass clonal propagation of genetically improved softwoods. All IPST member companies rely on US forests for raw material, and most operate intensive forest management programs on their own woodlands. The management programs typically include aggressive tree breeding efforts. Economic returns from tree breeding are sizeable, accruing from increased growth and shorter rotations as well as improved quality. Much progress has been made toward ensuring lower cost and stable supplies of raw material.

Such benefits could be increased even further by a cost-effective method of mass clonal propagation. Cloning would maximize genetic gain from existing breeding programs, permit tailoring of planting materials to specific sites and markets, and greatly decrease variability in raw material size and quality.

Somatic embryogenesis, one approach to cloning, has been the focus of this project since its start a little over 10 years ago. The process involves production of "artificial seeds" in sterile culture from a small amount of cells or tissue collected from a donor tree of value. The method is preferred over others, e.g., rooting cuttings, because of its multiplying power. Successfully deployed, the process can provide millions, not tens or thousands, of genetically identical seedlings for planting. Mass cloning only the very best genetically improved materials will thus enable member to maximize returns from their considerable investments in land, forest management, and tree breeding. In addition, such seedlings are expected to suffer minimally, if at all, from the lingering age and position effects commonly encountered in plants cloned by other means.

Reliable cell and tissue culture systems will also open the way for eventual development and application of several other technologies: "genetic engineering" and delivery of new genetic combinations having exceptional properties not otherwise obtainable; screening for and selecting useful variants in culture to lower costs of and accelerate the pace of conventional tree breeding; and investigation under controlled conditions of the factors regulating fiber length, diameter, flexibility, and other properties affecting pulping and papermaking properties.

## RESULTS TO DATE:

Initially, project activities focused on studies of natural conifer seed development and manipulation of cells and tissues in culture. An in-depth database and considerable expertise were developed. Other, non-conifer systems; e.g., carrot and coffee, were also investigated with the aim of extending somatic embryogenesis from them to conifers. The phenomenon, however, was not observed in conifers until 1985. At that time, Swedish workers obtained low levels of somatic embryogenesis in Norway spruce, and IPST scientists quickly adopted the species as a model system. The coupling of the in-depth IPST experience with a reasonably workable model engendered rapid process. IPST scientists have since optimized many process steps in the model system, and have extended the process across three related species to the target species, Douglas-fir and loblolly pine.

The process is best viewed as consisting of four major steps: Initiation, Maintenance, Maturation, and Conversion to Seedlings. In the first or Initiation Step, a few cells or small amount of tissue are collected and established in sterile culture. At present, embryos from developing or ripened natural seed are the usual starting material, but the process is gradually being extended to older materials; e.g., the first leaves or needles of newly germinated seedlings. Extension to older materials is an important part of ongoing and future research as commercialization depends on cloning trees old enough to have been certified as genetically superior. Once established in culture, cells or tissues are given a mix of growth regulators and nutrients to foster rapid cell division and growth. Within a few weeks, certain cells within the enlarging mass cease normal growth and differentiate into embryos. The initiation of this embryogenic state is straightforward and reproducible for the model system, Norway spruce. Frequencies of initiation now average 80 percent for cultures started from developing embryos, 25 percent for embryos from ripened seed, and 1 percent for the first needles from germinating seedlings. The target species are more difficult to manipulate, and success rates currently average less than one percent. A major portion of current project resources is thus devoted to optimizing conditions for Douglas-fir and loblolly pine.

In the Maintenance Step, embryogenic cultures are transferred to culture media designed to maintain the embryogenic state, foster culture growth, and promote proliferation of embryo numbers. The medium typically is similar to that used for initiation, but often contains a different mix or concentration of growth regulators. Maintenance of Norway spruce cultures has proven rather easy, and major effort has not been devoted to optimizing it. Culture numbers sufficient for research on subsequent steps generally have been obtained without difficulty. Thus, over 50 percent of embryogenic Norway spruce cultures can be maintained. In addition, growth rates are such that culture numbers double every two weeks, and developing embryos number 750 or more per gram of culture.

Somewhat similar success has been achieved with loblolly pine. Maintenance frequencies average 80 percent, and growth rates, though slower, are adequate. Loblolly pine cultures, however, contain far fewer embryos per gram, and much effort is being given to understanding and remedying the cause of this important difference.

Maintenance of Douglas-fir is more difficult, even though roughly 50 percent of the embryogenic cultures can be maintained for reasonable lengths of time. Growth rates are slow; doubling times average three or more weeks. In addition, perhaps 25 percent of the cultures deteriorate or revert to a nonembryogenic condition within a year or so. This is in direct contrast to Norway spruce and loblolly where cultures have remained embryogenic for five and three years, respectively. This problem has retarded research on subsequent steps, and is receiving and will continue to receive major attention.

Manipulations in the Maturation Step are structured to slow overall growth of the cultures, stop proliferation of new embryos, and stimulate growth, development, and maturation of individual embryos. The objective is to grow the many small embryos produced in cultures during the Initiation and Maintenance steps to a size and organizational state equal to that of natural embryos in ripened seed. Toward these ends, growth regulators used to foster growth and proliferation must be removed, and a new mix substituted in their place. Recent findings also indicate that the nature and level of both nitrogen and carbohydrate sources play significant roles in maturation.

Embryo maturation has proven a difficult step, regardless of species. Fully mature Norway spruce embryos were produced at IPST several years ago, but numbers were low and the results sporadic until recently. Increased reliability was secured only after a lengthy series of experiments testing various growth regulators and nitrogen sources. The most recent experiment, completed in 1989, raised mature embryo yields five-fold. On the average, each gram of culture now yields 130 embryos, mature and ready for germination. Best treatments yield roughly 350. Despite this improvement, however, conditions are far from optimal in that each gram contains 750 potential embryos. Further improvement to this key step is critical to realizing the tremendous multiplying power of somatic embryogenesis.

Considerable effort has also been devoted to extending such results to the target species. Small but significant numbers of mature embryos have been obtained in both loblolly pine and Douglas-fir. In loblolly pine, the very best treatments produce two or three mature embryos per gram. Results are reproducible, and microscopic assays indicate that the embryos are structurally identical to their natural counterparts. Reasons for the low yields are not yet clear, but several factors have been implicated. Firstly, loblolly pine embryogenic cultures contain fewer embryos in the Initiation and Maintenance Steps. Secondly, the few that are present apparently require stimuli different from those effective in Norway spruce. As an example, preliminary trials suggested that different carbohydrate sources and concentrations were needed. Thus, comprehensive tests of

the interaction between growth regulators, nitrogen regimes, and carbohydrates are underway. Prospects for overcoming the barrier posed by the Maturation Step clearly have improved in recent times. It nevertheless remains a serious obstacle, and major effort will be required to resolve it.

The last step, Conversion to Seedlings, entails harvesting mature embryos, germinating them, and moving the newly germinated embryos through a series of controlled environments that condition them for transfer to a greenhouse or nursery and eventually to field trials. Having developed under protected conditions, somatic embryos and seedlings are likely to be quite sensitive to natural levels of and changes in temperature, humidity, and moisture. The transition to natural conditions must therefore be gradual, with embryos germinated in sterile culture and planted in closed containers of soil. Acclimatization can then be accomplished by slowly reducing the frequency of watering and gradually opening the containers to ambient temperatures and humidities. Fertilizer and fungicide applications may also be necessary, but little is known about specific requirements.

Work on this step has to date involved only Norway spruce. Varying numbers of seedlings have been produced over the last four years, but they have been more the by-product of research on other steps than a goal in and of themselves. Thus, experiments on germination and acclimatization have been conducted on an ad hoc basis. Germination trials initially gave poor and highly variable results. Important factors were identified, and more recent trials have given more uniform results. Germination currently averages 19 percent, considerably higher than in most earlier trials but still far too low.

The first few seedlings obtained at IPST are now entering their fifth growing season. Throughout their lives, they have started and stopped growth in synchrony with their natural standards, and have remained similar in most other external appearances as well. The only obvious difference has been a tendency for the somatic seedlings to have fewer and finer branches. Summed over all years, roughly a hundred seedlings of various ages and in different stages of development have been produced. Regrettably, these represent only 10 to 25 percent of the embryos that germinated and were planted in soil. Difficulties with acclimatization were planted in soil. Difficulties with acclimatization were numerous and critical factors are not understood. Clearly, more reliable procedures for both germination and acclimatization are needed. Recent improvements to the Maturation Step should enable us to produce sufficient mature embryos for definitive trials. These will be designed to improve the overall conversion process, and establish replicated greenhouse and field trials.

#### NEAR-TERM PLANS:

Mass clonal propagation of genetically improved conifers has been moved closer to commercialization in recent years. As noted above, however,

significant obstacles remain. Over the near-term, project efforts and resources will be focused as follows:

- Model System - Document events in natural systems so as to develop guidelines for manipulating somatic materials  
Improve maturation frequencies  
Raise efficiency of conversion to seedlings  
Adapt best treatments and methods for application to target species  
Develop methods for initiation from older materials
- Target Species - Raise initiation frequencies  
Obtain sufficient embryogenic cultures for work on later steps  
Improve maturation frequencies  
Initiate research on conversion to seedlings

#### POSSIBLE FUTURE DIRECTIONS:

\* Establish replicated greenhouse and field trials of seedlings from the model system; use the results to accelerate production and testing of seedlings from the target species.

\* Utilize techniques developed in Project 3223-02 to evaluate genetic fidelity of somatic embryos and seedlings.

\* Evaluate liquid suspension cultures as a means for increasing embryo production

\* Investigate feasibility of and techniques for constructing "synthetic seeds", ie., encasing somatic embryos in a nutrient medium and polymer coating that will permit easy handling, storage, and nursery sowing.

\* Adapt cell and tissue culture methods for study of factors affecting lignification, fiber dimensions, and other aspects of fiber formation.

\* Devise methods for screening for and selection of novel variants in culture.

\* Initiate research on "genetic engineering"; genes of possible interest include those regulating lignin biosynthesis, production of growth regulators and other factors affecting fiber formation, and resistance to diseases and insects.

## PROJECT SUMMARY FORM

DATE: March 28, 1990

PROJECT NO. 3223-02: BIOCHEMISTRY OF CLONAL PROPAGATION

PROJECT LEADERS: Vacant (2)

IPST GOAL: To develop and assure low-cost supplies of quality fiber

## OBJECTIVE:

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.

CURRENT FISCAL YEAR BUDGET: \$150,000

## 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.

## PRIOR RESULTS:

Past Institute efforts have been made somatic embryogenesis in Norway spruce, our model system, straightforward and reproducible. Embryo numbers can be quantified, and seedlings have been recovered. Somatic embryogenesis has also been obtained in our target species, loblolly pine and Douglas-fir, but initiation frequencies remain low and seedlings have not been recovered.

Earlier work on the biochemistry of embryogenesis yielded useful data on differences between embryogenic and nonembryogenic cultures, and some knowledge of factors affecting the process. Such differences and associated markers are used to screen cultures for embryogenic potential, and monitor effects of modified or new

protocols. In addition, techniques for isolating, purifying, and characterizing proteins, lipids, enzymes, RNA, and DNA have been developed or refined. These are now available for use in increasing initiation and maturation frequencies, facilitating conversion to seedlings, and evaluating seedling performance and fidelity.

Investigation of biochemical and molecular properties of zygotic and somatic embryos slowed upon relocation as all involved personnel chose not to move. Efforts since then have focused on completing publications and internal documents. Much effort has also been given to readying facilities, unpacking equipment, and recruiting new employees. Once new personnel are hired, work on using biochemical and molecular methods to promote embryo maturation will be renewed and accelerated. Efforts to quantify and stimulate biosynthesis of proteins and lipids will receive particular attention. In anticipation of renewed research, numerous cultures from selected experiments have been and are being frozen and stored.

Work continues on quantifying levels of growth regulators, especially abscisic acid, in developing embryos. Cooperative arrangements for assay, using monoclonal antibodies, have been finalized with industrial and university scientists, in the Republic of South Africa. To ensure successful completion, similar arrangements are being negotiated with several US scientists. Growth regulators are major factors in maturation, and successfully documenting their ebb and flow should enable us to improve maturation frequencies.

Recruiting/hiring is underway and a number of likely candidates have been identified.

#### PLANNED ACTIVITIES FOR THE PERIOD:

Complete recruiting/hiring, and ready facilities for use. Renew work on similarities/differences of zygotic and somatic embryos, with emphasis on proteins and lipids as well as on using substrates and inhibitors to stimulate maturation. Execute cooperative efforts to quantify abscisic acid levels in developing embryos. Direct and facilitate student project on documenting onset and course of lignification.

**POTENTIAL FUTURE ACTIVITIES:**

Refine and apply methods for certifying genetic fidelity of seedlings derived from cloning of softwoods and hardwoods. Document differences between mature and immature tissues, compare characteristics of mature tissues to those of explants known to have embryogenic potential, and devise means for rendering mature tissues more easily manipulated in culture. Refine and develop promising new techniques. Explore procedures for early selection and testing.

Project 3223-02

Status Report

BIOCHEMISTRY OF CLONAL PROPAGATION  
GENERAL SUMMARY OF RECENT ACTIVITIES

March 28, 1990

This recently established project was originally part of Project 3223-02, Mass Clonal Propagation of Improved Conifers, and work specifically supported that project. Separation was effected to ensure more precise direction, encourage exploratory research in other areas, and provide support to newly chartered Project 3223-03, Mass Clonal Propagation of Genetically Improved/Engineered Hardwoods. Improved understanding of and ability to manipulate biochemical and molecular mechanisms affecting clonal propagation remain the primary goal. Results are expected to make research on cloning less empirical and more efficient, hasten commercialization, and facilitate extension to trees mature enough to have been proven genetically superior.

## RESULTS TO DATE:

Earlier work on the biochemistry of somatic embryogenesis yielded useful data on differences between embryogenic and nonembryogenic cultures and on factors affecting the process. Such differences and associated markers are being used to screen cultures for embryogenic potential and monitor effects of changes in culture media. Techniques for isolating, purifying, and characterizing proteins, fats, and enzymes, RNA, and DNA were also adapted or developed. Several are now available for use in increasing production of embryogenic cultures and fostering maturation of somatic embryos. Further work on others is essential before they can be applied routinely, especially in verifying genetic fidelity of somatic embryos and seedlings and in monitoring gene expression in genetically engineered hardwoods.

Efforts in recent years centered on stimulating biosynthesis of storage products, proteins and fats, in developing embryos. Types and quantities of such compounds were first followed across developmental stages in natural embryos. Analyses were then extended to somatic embryos. Comparisons showed that somatic embryos accumulated the same compounds but in smaller amounts and somewhat later. Attempts were then made to stimulate biosynthesis and thereby promote embryo maturation and readiness to germinate. Some improvement was obtained, but the research is on hold since involved personnel chose not to move with IPST. Such products provide the wherewithal for embryo germination and early seedling growth, and ability to control their biosynthesis is critical to success with somatic embryogenesis. The research will therefore be activated and

given high priority as new personnel are put in place. In anticipation of renewed effort, cultures and embryos of interest are being harvested, frozen, and stored for later analysis.

Work is proceeding apace on quantifying levels of growth regulators in developing somatic embryos. Cooperative arrangements for assay have been finalized with an internationally recognized team of scientists, and similar arrangements are being negotiated with others as insurance. Growth regulators are major factors in embryo maturation, and documenting their ebb and flow should enable us to improve several steps in the process of somatic embryogenesis.

#### NEAR-TERM PLANS:

- \*Renew development of molecular and biochemical techniques
- \*Reactivate analyses of protein and lipid biosyntheses
- \*Initiate research on use of inhibitors and substrates to stimulate embryo maturation
- \*Execute cooperative assay of growth regulator levels
- \*Initiate research on hardwood genetic engineering

#### POSSIBLE FUTURE DIRECTIONS:

- \*Refine molecular methods for verifying genetic fidelity and gene expression
- \*Devise means for rendering tissues from older trees more easily manipulated in culture
- \*Develop methods for early selection and testing
- \*Acquire or develop new techniques
- \*Explore opportunities for influencing fiber formation in culture

## PROJECT SUMMARY FORM

DATE: March 28, 1990

PROJECT NO. 3223-03: MASS CLONAL PROPAGATION OF GENETICALLY IMPROVED/ENGINEERED HARDWOODS

PROJECT LEADERS: R.J. Dinus, M.R. Uddin

IPST GOAL: To develop and assure low-cost supplies of quality fiber

## OBJECTIVE:

To develop reliable cell and tissue culture systems for mass clonal propagation of genetically improved and/or engineered hardwoods.

## 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.

## PRIOR RESULTS:

Considerable hardwood research has been done at the Institute in past years. This work resulted in production of plants from tissue culture, and successful application of polyploidy to forest tree breeding. Other exploratory work at the Institute suggested that tissue culture methods can be used to test for disease resistance. Results from these efforts and those of other organizations indicate that hardwood tissues, cells, and protoplasts can be

manipulated in culture with relative ease. In addition, the first demonstration of gene transfer and expression in forest trees was accomplished with a hardwood. Still other work infers that novel variants can be produced in culture, isolated, and used to introduce new traits into breeding and/or planting stock.

In accordance with earlier plans, this project seeks to develop technologies for transferring genes for herbicide tolerance into commercially important species, and for efficient mass propagation, testing and release of genetically modified plant materials. Herbicide tolerance may also be sought, more on an exploratory or insurance basis, via somaclonal variation and selection. Since project plans were finalized, most efforts have been devoted to selecting research approaches, collecting suitable plant materials, and establishing stable cultures.

Both diploid and haploid explants of cottonwood and aspen were obtained from IPST sources and cooperating organizations, and used to establish cultures for subsequent expansion and manipulation. Stable cultures and protocols for adaptation to our needs were also obtained from collaborators at the University of Nebraska and Tuskegee University. Cuttings of additional elite trees will be provided in the near future by a member company, Tuskegee University, and the University of Minnesota.

In cottonwood, several donor trees are represented by stable diploid and/or haploid cultures. A system suitable for transformation and easy regeneration has been developed with cultures from one of the aforementioned donor trees. Leaf sections were isolated from stabilized shoot cultures, surface sterilized, and plated on modified Woody Plant Medium containing Naphthaleneacetic acid and 6-Benzylaminopurine. Adventitious buds formed within several weeks, and shoot elongation occurred after transfer to the same basal medium with half-strength growth regulators. Excised shoots root readily on the basal medium supplemented with Indolebutyric acid. Significant numbers of rooted plants have been produced and are ready for acclimatization and transfer to the greenhouse. The system is also being used for a student project on genetic transformation.

Suspension cultures have also been established from leaf callus of two donor trees. Successful regeneration from suspensions will provide another avenue for attack on transformation and/or somaclonal variation/selection. In an effort to regenerate plants, samples of the suspensions were plated on solid medium and manipulated to form callus. Cultures from one of the donor trees yielded micro-calli, and these are being used to obtain regeneration with this system.

Aspen shoot cultures were stablized with relative ease, and diploid cultures of two elite aspens and one triploid hybrid are available. Also, cultures from past exploratory work on tetraploid aspen and native sweetgum are being maintained.

#### PLANNED ACTIVITIES FOR THE PERIOD:

Expand existing cultures, and initiate or secure and stablize additional cultures. Complete greenhouse construction, secure additional plant materials, and establish "clean" greenhouse populations. Refine technologies for mas propagation, and ensure that resultant systems are suitable for genetic transformation. Direct and facilitate student project on genetic transformation. Complete recruiting key personnel, and accelerate research on gene transfer and expression.

#### POTENTIAL FUTURE ACTIVITIES:

Explore novel methods for accelerating conventional tree improvement by early testing and selection in culture, generating haploid cultures and dihaploids, effecting protoplast fusion, and creating polyploid or hybrid individuals.

Project 3223-03

Status Report

MASS CLONAL PROPAGATION  
OF  
GENETICALLY IMPROVED/ENGINEERED HARDWOODS

GENERAL SUMMARY OF RECENT ACTIVITIES

March 28, 1990

Demand for high quality hardwoods has increased dramatically in recent years. Conversion of mills from manufacturer of commodity grade products to those having higher profit margins has been the principle driving force, especially in the Southeastern Coastal Plain. In the Pacific Northwest, escalating export demand has also played a significant role. In yet other regions, supplies are adequate but are located in wet or mountainous areas, economically harvestable volumes are scattered, or stands are situated at sizeable haul distances from mills. In addition, the products in question typically require fiber of consistently high quality, but wood deliveries are highly variable in terms of species, age, and quality. Thus, significant numbers of IPST member companies require more and better hardwood in a time of tightening supplies and rising costs. Many have become convinced that breeding, planting, and management of hardwoods is warranted.

Planting and management, however, can be costly. Sites suitable for prime species; e.g., cottonwood and aspen, are fertile and support a variety of competing vegetation. Commercially available herbicides cannot be used to control competition because managed species are as susceptible as competitors. As a result, competition control is either not done and volume productivity is reduced or control is effected by mechanical means and establishment costs rise to near prohibitive levels. Sensing an opportunity to help member companies, IPST recently chartered the present project which seeks to develop technologies for transferring genes for herbicide tolerance into commercially important hardwoods, and for efficient mass propagation, testing, and release of genetically transformed trees.

RESULTS TO DATE:

Considerable hardwood research has been done at IPST in past years. This work resulted in production of improved trees from tissue culture, and successfully demonstrated that tissue culture could benefit tree breeding. Other exploratory work at IPST suggested that tissue culture methods can be used to test for disease resistance. In addition, the first demonstration of genetic engineering in forest trees was accomplished with a hardwood. Still other work infers that novel variants can be produced in culture, and used to introduce new traits into breeding and/or planting stock. These collective results indicate that hardwood cells and tissues can be manipulated in culture and genetically engineered with relative ease.

Initially, most project efforts were devoted to selecting research approaches, collecting suitable plant material, and establishing stable cultures. Tissue samples of cottonwood and aspen were obtained from cooperating member companies and universities, and used to establish cultures for subsequent expansion and manipulation. Stable cultures and procedures were also obtained from collaborators at several universities.

More recently, work has focused on production of intact plants from culture, and a system suitable for genetic engineering and easy regeneration has been developed. Briefly, leaf sections are isolated from stabilized shoot cultures and placed on conventional culture medium supplemented with a unique mix of growth regulators. Numerous buds form within several weeks and these elongate into shoots after transfer to culture medium containing a different mix of growth regulators. Excised shoots can be rooted with ease, and significant numbers of intact plants have been produced for transfer to the greenhouse. The system is also being used for a student project on genetic engineering.

Liquid cells suspensions of cottonwood have also been established with relative ease. Addition of normally lethal doses of herbicides to such suspensions may result in isolation of one or a few cells with above average tolerance. Availability of rapidly growing cell suspension allows this approach to be tested, on an exploratory or insurance basis, as another route to herbicide tolerance.

Aspen cultures have also been stabilized with relative ease, and cultures of two elite and one hybrid aspen are available. Also, cultures from past exploratory work with a polyploid aspen tree and several sweetgum selections are being maintained for future use.

#### NEAR-TERM PLANS:

- \* Increase number and availability of existing cultures
- \* Initiate or secure additional cultures
- \* Secure additional plant materials for creation of a reserve greenhouse population
- \* Refine technologies for mass propagation
- \* Accelerate research on genetic engineering
- \* Regenerate genetically engineered trees

#### POSSIBLE FUTURE DIRECTIONS:

- \* Verify herbicide tolerance of genetically engineered trees
- \* Explore creation of novel variants and hybrids in culture
- \* Examine feasibility of studying factors affecting lignification, fiber dimensions, and other aspects of fiber formation in culture

- \* Explore opportunities for genetically engineering other traits; e.g.; lignification, growth regulator production, other fiber characteristics, and pest resistance

Project 3223-0

Status Report

## COOPERATIVE INTERACTIONS

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

SAPPI Forests Ltd. and Stellenbosch University - Joint assay with Drs. Barbour and Cutting, of respectively, of growth regulators in embryogenic and nonembryogenic cultures and in developing zygotic and somatic embryos.

University of Nebraska - Cottonwood cultures and protocols supplied by Dr. S. Ernst.

Tuskegee University - Cottonwood cultures, cuttings and protocols supplied by Dr. C. Prakash.

Joint research arrangements are also being sought or negotiated with Dr. J. Caruso, University of Cincinnati; Drs. J. Choi and J. Mathis, Georgia Tech; Dr. K.E. Eriksson, University of GA; Dr. D. Neale, US For. Serv., Berkeley, CA; and Dr. S. Strauss, Oregon State University.

## RELATED STUDENT RESEARCH:

Completed in 1989

- Lisa T. Dudek - M.S., "Encapsulation of zygotic and somatic embryos of conifer species." Advisor, N. Rangaswamy.
- Patricia Exarhos - M.S., "Electron microscopy study of ultra-structure on Picea abies plants obtained via somatic embryogenesis." Advisor, T. E. Conners.
- Frederick Lang - M.S., "Application of recombinant DNA technology in construction of a gene library." Advisor, R. J. Dinus.
- Lorrain Logsdan - M.S., "Patterns of gene expression in maturing and germinating tree seeds." Advisor, R.J. Dinus.
- Mary Kay Lynde-Maas - M.S., "Fructose utilization by embryogenic and nonembryogenic suspension cultures of Norway spruce." Advisor, M.A. Johnson.
- Colleen Walker - M.S., "Optimization and quantification of embryogenic cultures of several conifer species in bioreactors." Advisors, M.R. Becwar and R.J. Dinus.

In Progress

- Lois Forde - M.S., "Phenylalanine ammonia lyase and lignin biosynthesis." Advisors, T.E. Conners and R.J. Dinus.
- Peasely Shorter - M.S., "Promotion of additional auxin synthesis in Populus deltoides via transformation with Agrobacterium tumefaciens." Advisor, D.T. Webb.
- Colleen Walker - Ph.D., Tentative Dissertation Topic: "Comparison of biological and other methods for delignification of kraft pulps." Advisor, R.J. Dinus.

Michael Wood - M.S., "Effect of cold shocking on cell cultures of Larix decidua." Advisor, R.J. Dinus.

Others of Potential Interest

Ingegerd Uhlin - Ph.D., "Influence of hemicelluloses on structure of bacterial cellulose." Committee participation, R.J. Dinus.

James Bond - "A Raman microspectroscopic investigation of the patterns of molecular order in secondary walls of southern pine tracheids." Committee participation, R.J. Dinus.

**M. RAFIQUE UDDIN**

**DEVELOPMENT & MATURATION  
OF  
LOBLOLLY PINE SOMATIC EMBRYOS**

**PRELIMINARY RESULTS & NEAR TERM PLANS**

**OBJECTIVE: ENHANCE DEVELOPMENT AND MATURATION OF  
LOBLOLLY PINE SOMATIC EMBRYOS**

**EXPERIMENT 1: ONGOING - CONFIRM & EXPAND EARLIER  
FINDINGS CONCERNING UTILITY OF  
CARBOHYDRATES & ABA**

**HYPOTHESES**

- (1) GLUCOSE AND MALTOSE ENHANCE EMBRYO MATURATION**
- (2) ABA STIMULATES MATURATION WHEN APPLIED WITH  
CERTAIN CARBOHYDRATES**

**MATERIALS AND METHODS**

**CARBOHYDRATES: GLUCOSE, MALTOSE, & SUCROSE,  
AT 4 EQUIMOLAR LEVELS**

**ABA: = FIVE LEVELS 0, 10, 20, 30 and 40  $\mu$ M**

**REPLICATION: 5**

## MATERIALS AND METHODS

### CULTURES: ORIGIN AND COMPOSITION

CULTURES	EXPLANT TYPE	DEVELOPMENTAL STATUS*
(672 LP8F)1**	IMMATURE EMBRYOS	++
(672 LP7H)1	IMMATURE EMBRYOS	++++
(574 LP2G)1**	FERTILIZED OVULE	++++
(575 LP7F)1	IMMATURE EMBRYOS	++++

\* ++ = SMALL PROEMBRYOGENIC MASSES

++++ = LARGE PROEMBRYOGENIC MASSES

\*\* COMMON TO EARLIER WORK

**PRELIMINARY RESULTS - REPLICATION 1, WEEK 4**

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<b>HISTORY/TREATMENTS</b>	<b>DEVELOPMENTAL STAGE</b>
<b>MAINTENANCE MEDIUM</b>	<b>NO DISTINCTIVE EMBRYOS</b>
<b>TRANSITION MEDIUM</b>	<b>" " "</b>

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<b>MATURATION MEDIUM</b>	
<b>SUCROSE</b>	<b>NO DISTINCTIVE EMBRYOS</b>
<b>GLUCOSE</b>	
<b>LOWEST 3 LEVELS</b>	<b>STAGE 1 EMBRYOS</b>
	<b>(8 OF 15 TREATMENTS, HIGHER ABA LEVELS)</b>
<b>HIGHEST LEVELS</b>	<b>NO DISTINCTIVE EMBRYOS</b>
<b>MALTOSE</b>	<b>STAGE 1 &amp; 2 EMBRYOS</b>
	<b>(13 OF 20 TREATMENTS, ALL ABA LEVELS)</b>

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### **PRELIMINARY CONCLUSIONS**

- (1) RESULTS TO DATE CONFIRM EARLIER FINDINGS; GLUCOSE AND MALTOSE STIMULATE MATURATION**
- (2) MALTOSE APPEARS MORE EFFECTIVE THAN GLUCOSE**
- (3) ABA ALSO APPEAR ESSENTIAL**

**EXPERIMENT 2: PROPOSED - CLARIFY ROLE OF IBA RELATIVE  
TO CARBOHYDRATE & ABA**

**BACKGROUND AND RATIONALE**

**IN EARLIER EXPLORATORY WORK, MATURING EMBRYOS WERE  
OBSERVED, BUT EFFECTS OF CARBOHYDRATES, ABA, & IBA  
WERE CONFOUNDED.**

**ADDITIONAL EXPERIMENTS ARE NEEDED TO DOCUMENT  
IMPORTANCE OF EACH, GENERATE RESPONSE CURVE, &  
UNDERSTAND INTERACTIONS.**

**ALSO, APPLICABILITY TO OTHER GENOTYPES MUST BE VERIFIED.**

## **HYPOTHESES**

- (1) IBA PLAYS A CRITICAL ROLE IN EMBRYO MATURATION**
- (2) IBA IS MOST INFLUENTIAL IN LATER DEVELOPMENTAL STAGES**
- (3) SELECTED CARBOHYDRATES + ABA WILL STIMULATE  
MATURATION IN AN ARRAY OF GENOTYPES**

## MATERIALS & METHODS

### CULTURES: ORIGIN AND COMPOSITION

LINES	EXPLANT TYPE	DEVELOPMENTAL STATUS*
(672 LP 7D)	IMMATURE EMBRYO	++
(672 LP8F)1**	IMMATURE EMBRYO	++
(672 LP7H)1**	IMMATURE EMBRYO	++++
(666 LP 4H)	FERTILIZED OVULE	++
(574 LP2G)1**	FERTILIZED OVULE	++++
(575 LP7F)1**	IMMATURE EMBRYO	++

\* ++ = SMALL PEM'S

++++ = LARGE PEM'S

\*\* COMMON TO EARLIER WORK

## **MATERIALS & METHODS**

**CARBOHYDRATES: GLUCOSE, MALTOSE, & SUCROSE AT 4  
EQUIMOLAR LEVELS**

**IBA: 5 LEVELS = 0.0, 0.1, 0.5, 1.0, 5.0  $\mu$ M**

**IBA TIMING: EARLY (FIRST 6 WEEKS) AND LATE (LAST 6  
WEEKS) IBA TREATMENTS ARE ALSO INCLUDED FOR  
CONTRAST TO THE IBA 0.0 CONTROL AND CONTINUOUS  
EXPOSURE TO 0.5  $\mu$ M.**

**ABA: CONTINUOUS EXPOSURE TO 30  $\mu$ M**

**REPLICATION: 5**

**CARBOHYDRATE & ABA TREATMENTS WILL BE ADJUSTED IN  
ACCORDANCE WITH RESULTS FROM EXPERIMENT 1**

**NEAR TERM PLAN**

- (1) ESTABLISH REMAINING REPLICATIONS IN EXPERIMENT 1**
- (2) CONTINUE SUBSAMPLING, MEASUREMENT, & MICROSCOPY**
- (3) ANALYZE AND PUBLISH FINDINGS**
- (4) REFINE & INSTALL EXPERIMENT 2**

EMBRYO CLASSIFICATION & GERMINATION

DAVID T. WEBB

**EMBRYO CLASSIFICATION****BACKGROUND:**

- 1] VARIOUS CLASSIFICATION SYSTEMS ARE BEING USED BY  
SCIENTISTS WORKING IN THE AREA OF CONIFER SOMATIC  
EMBRYOGENESIS
  
- 2] THERE IS NO OVERALL AGREEMENT REGARDING A STANDARD WAY TO  
CLASSIFY CONIFER SOMATIC EMBRYOS
  
- 3] NONE OF THE PRESENT SYSTEMS IS BASED ON THE WELL KNOWN  
STAGES OF ZYGOTIC EMBRYOGENESIS IN THE PINE FAMILY  
WHICH INCLUDES PINUS, PICEA & PSEUDOTSUGA

**OBJECTIVES:**

- 1] PROVIDE A UNIFORM AND COHERENT BASIS FOR CLASSIFYING  
ZYGOTIC EMBRYOS FOR INITIATION EXPERIMENTS
  
- 2] PROVIDE A FRAMEWORK FOR ASSESSING THE "QUALITY" &  
MATURITY OF SOMATIC EMBRYOS BASED ON COMPARISONS OF
  - A] EXTERNAL MORPHOLOGY
  - B] MEASUREMENTS OF TOTAL LENGTH, COTYLEDON LENGTH &  
AXIS DIAMETER
  - C] FRESH & DRY WEIGHTS
  - D] ANATOMY & HISTOCHEMISTRY [LIPIDS, PROTEINS & STARCH]
  - E] BIOCHEMICAL QUANTIFICATION OF TOTAL LIPIDS &  
PROTEINS

- 1] SEVEN STAGES OF EMBRYO DEVELOPMENT HAVE BEEN DEFINED BY US AND ARE IN AGREEMENT WITH THE LITERATURE ON THE MID-LATE STAGES OF ZYGOTIC EMBRYOGENESIS.
- 2] THIS SCHEME NEEDS TO BE REFINED TO ACCOUNT FOR THE LARGE NUMBER OF EARLY-STAGE EMBRYOS WHICH FIT BETWEEN STAGES 1 - 2.
- 3] ANOTHER STAGE [PROEMBRYO] MAY ALSO NEED TO BE ADDED.

## EMBRYO DEVELOPMENTAL STAGES

### PRE-COTYLEDONARY EMBRYOS

STAGE 1: EMBRYOS WITH MICROSCOPIC EMBRYO PROPER ["HEAD"]

STAGE 2: EMBRYOS WITH A MACROSCOPIC, OPAQUE EMBRYO PROPER WITHOUT A CLEARLY DISCERNABLE SHOOT APICAL MERISTEM ["BULLET STAGE"]

STAGE 3: EMBRYOS WITH POINTED SHOOT APICAL MERISTEMS

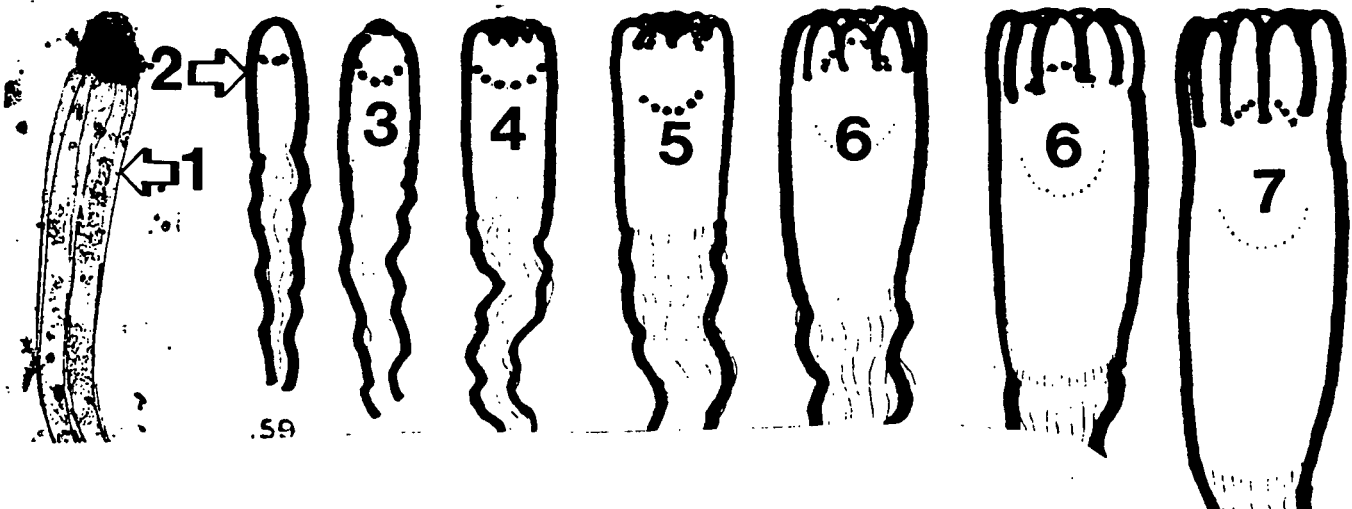
### COTYLEDONARY EMBRYOS

STAGE 4: SAME AS STAGE 3 BUT COTYLEDON PRIMORDIA BARELY VISIBLE

STAGE 5: EMBRYOS WITH ELONGATED COTYLEDONS WHICH DO NOT OVERTOP THE SHOOT APICAL MERISTEM

STAGE 6: ELONGATED COTYLEDONS OVERTOP THE SHOOT APEX BUT HAVE NOT CLOSED TO OBSCURE IT AS VIEWED FROM ABOVE

STAGE 7: ELONGATED COTYLEDON PRIMORDIA COMPLETELY OBSCURE THE SHOOT APEX

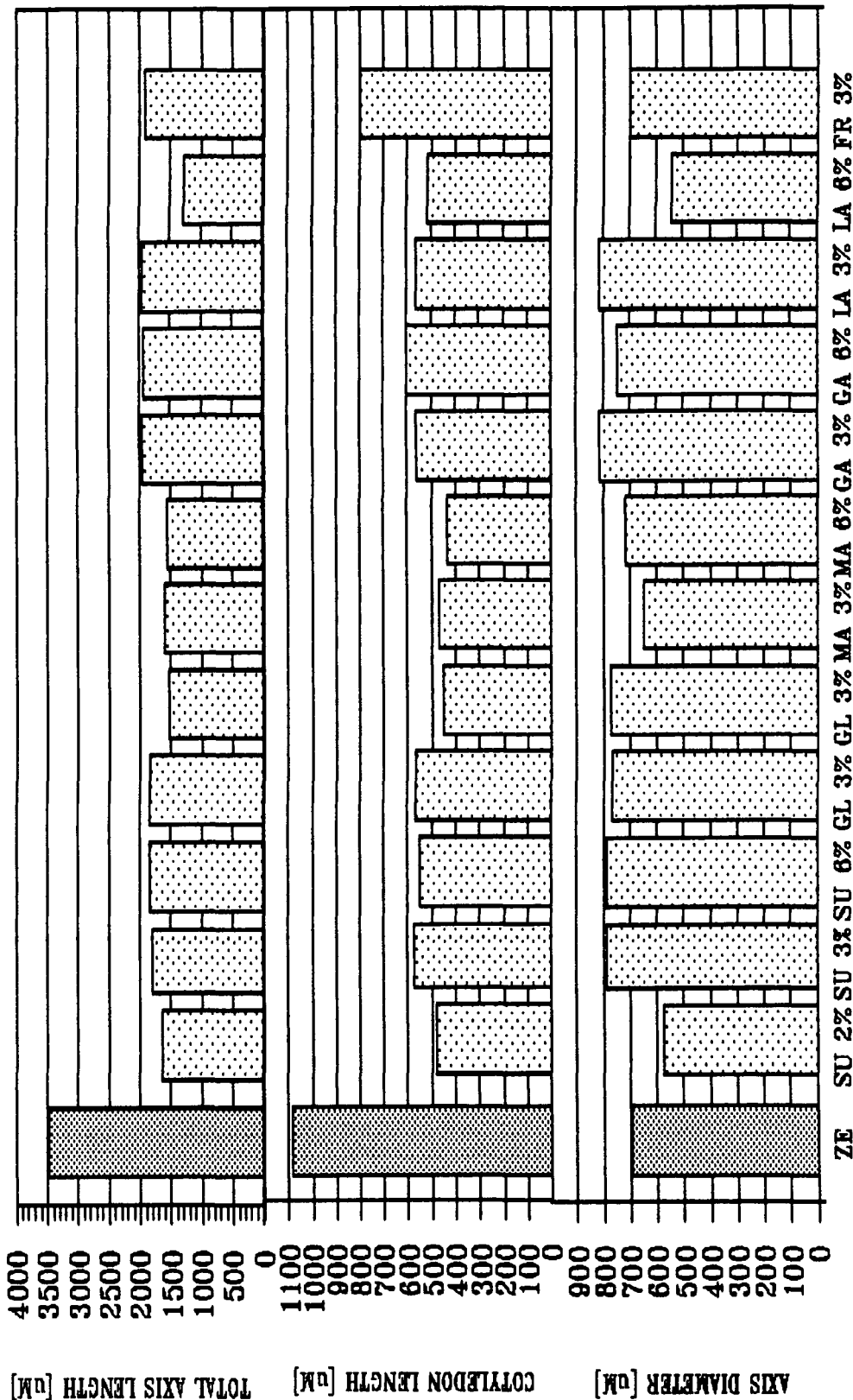


## MATERIALS &amp; METHODS

- 1] CALLUS LINE NS1(5) GROWN ON HM 2/1 WITH 3% SUCROSE
- 3] TRANSFERED TO ACTIVATED CHARCOAL MEDIUM (1 WK.) WITH 2%  
SUCROSE
- 4] SUBCULTURED TO BLG 2/1 CONTAINING DIFFERENT CARBOHYDRATES  
FOR 12 WEEKS
- 5] MATURE SOMATIC EMBRYOS HARVESTED AND MEASURED

- 1] ZYGOTIC EMBRYOS OF NORWAY SPRUCE DIFFERED FROM SOMATIC EMBRYOS PRODUCED WITH DIFFERENT CARBOHYDRATES IN TERMS OF TOTAL AXIAL LENGTH AND COTYLEDON LENGTH
  
- 2] ZYGOTIC & SOMATIC EMBRYOS HAD SIMILAR DIAMETERS AT THE BASE OF THE COTYLEDONS.

COMPARISON OF ZYGOTIC EMBRYOS TO SOMATIC EMBRYOS  
PRODUCED WITH DIFFERENT CARBOHYDRATES

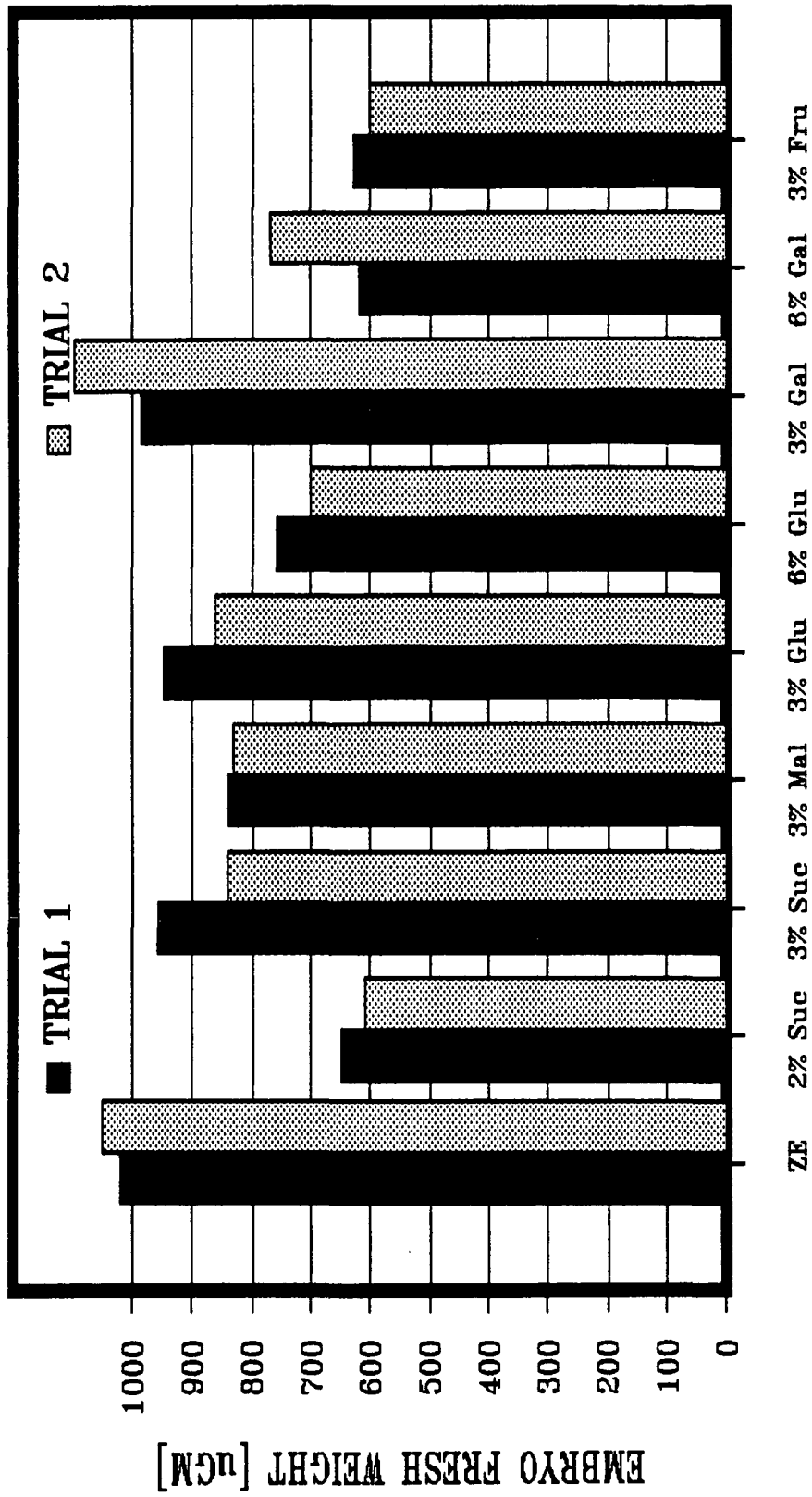


CARBOHYDRATE SOURCE

ZE SU 2% SU 3% SU 6% SU 3% GL 6% GL 3% MA 6% MA 3% GA 6% GA 3% LA 6% LA 3% FR 6% FR

- 1] SOMATIC EMBRYOS PRODUCED WITH DIFFERENT CARBOHYDRATES  
TENDED TO HAVE SMALLER FRESH WEIGHTS COMPARED TO  
HYDRATED ZYGOTIC EMBRYOS.
  
- 2] IN SEVERAL CASES, THERE WAS LITTLE FRESH WEIGHT  
DIFFERENCE BETWEEN SOMATIC AND ZYGOTIC EMBRYOS.

# COMPARISON OF ZYGOTIC EMBRYOS TO SOMATIC EMBRYOS PRODUCED WITH DIFFERENT CARBOHYDRATES



## EMBRYO SOURCE

## ONGOING WORK

SOMATIC & ZYGOTIC EMBRYOS OF NORWAY SPRUCE ARE BEING  
COMPARED MICROSCOPICALLY FOR

## 1] HISTOCHEMICAL DIFFERENCES IN STORAGE PRODUCTS

- A] LIPIDS
- B] PROTEINS
- C] STARCH

## 2] ANATOMY &amp; CYTOLOGY OF

- A] STORAGE CELLS
- B] APICAL MERISTEMS
- C] VASCULAR SYSTEM

**FUTURE WORK**

- 1] REFINE THE PRESENT SYSTEM BY IDENTIFYING PROEMBRYO STAGES WHICH OCCUR PRIOR TO THE PRESENT STAGE 1
- 2] DEFINE STAGES WHICH ARE INTERMEDIATE BETWEEN THE PRESENT STAGES 1 & 2
- 3] COMBINE ACCURATE MEASUREMENTS OF EMBRYOS WITH STAGE CLASSIFICATION
- 4] DEVELOP A BATTERY OF EMBRYO QUALITY TESTS TO BE PERFORMED AS PART OF ALL DEVELOPMENT AND MATURATION EXPERIMENTS. THIS SHOULD INCLUDE:
  - 1] ACCURATE STAGING
  - 2] MEASUREMENTS OF AXIAL & COTYLEDON LENGTH
  - 3] FRESH & DRY WEIGHT DETERMINATIONS
  - 4] DETERMINATIONS OF TOTAL
    - A] LIPIDS
    - B] PROTEINS
    - C] CARBOHYDRATES
  - 5] ANATOMICAL ANALYSIS OF KEY ORGANS & MERISTEMATIC AREAS

**EMBRYO GERMINATION****BACKGROUND:**

- 1] THE GERMINATION OF NORWAY SPRUCE SOMATIC EMBRYOS IS  
VARIABLE AND AVERAGES LESS THAN 50%
- 2] GERMINATION OF SOMATIC EMBRYOS IS SLOW
- 3] LIGHT MAY INHIBIT GERMINATION
- 4] LIGHT MAY INFLUENCE ROOT, HYPOCOTYL & COTYLEDON  
ELONGATION
- 4] SOMATIC EMBRYOS APPEAR TO GERMINATE PRECOCIOUSLY IN THAT  
HYPOCOTYL & COTYLEDON GROWTH PRECEDE ROOT GROWTH
- 5] THE PRESENCE OF AN ACTIVE & WELL-ORGANIZED ROOT APICAL  
MERISTEM HAS NOT BEEN DOCUMENTED IN SOMATIC EMBRYOS
- 6] A BROWN-BLACK MASS ["SKIRT"] DEVELOPS AROUND THE RADICLE  
OF SOMATIC EMBRYOS AND MAY IMPEDE OR INHIBIT ROOT  
GROWTH
- 7] THE ANATOMICAL & HISTOCHEMICAL EVENTS OCCURRING DURING  
THE GERMINATION OF ZYGOTIC & SOMATIC EMBRYOS ARE  
UNKNOWN

## OBJECTIVES:

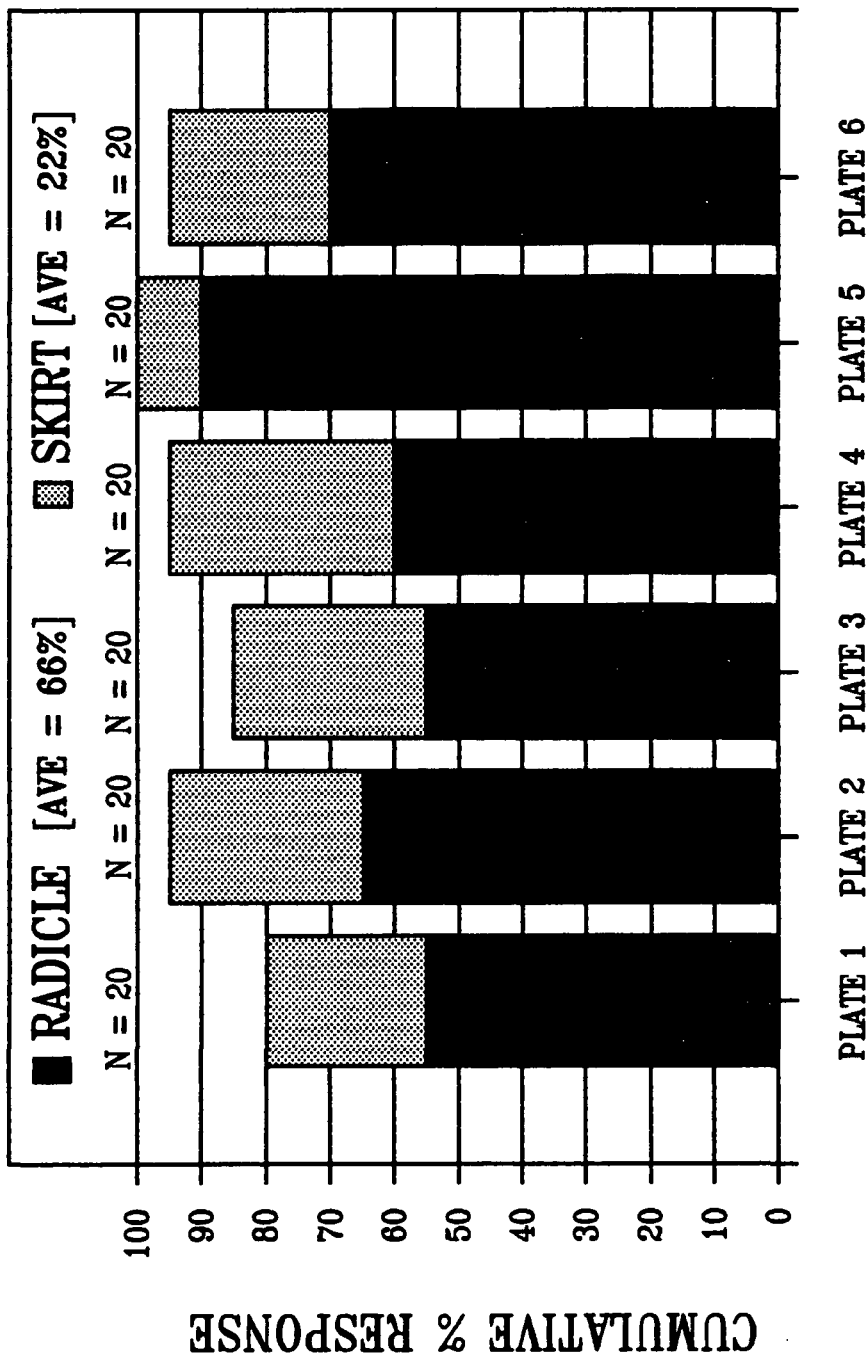
- 1] DETERMINE THE FREQUENCY & RATE OF ZYGOTIC EMBRYO  
GERMINATION ON MEDIUM USED FOR GERMINATING SOMATIC  
EMBRYOS OF NORWAY SPRUCE
- 2] COMPARE GERMINATION OF ZYGOTIC EMBRYOS IN LIGHT &  
DARKNESS
- 3] DOCUMENT THE RELATIVE GROWTH RATES OF THE ROOT, HYPOCOTYL  
AND COTYLEDONS DURING THE GERMINATION OF ZYGOTIC  
EMBRYOS
- 4] DETERMINE WHETHER OR NOT THE "SKIRT" IS PRESENT WITH  
ZYGOTIC EMBRYOS GERMINATING IN VITRO
- 5] UNDERSTAND THE ANATOMICAL & HISTOCHEMICAL CHANGES IN AND  
AROUND THE RADICLE TO ACCOUNT FOR THE APPEARANCE OF THE  
"SKIRT" WHICH DEVELOPS AT THE OUTSET OF SOMATIC EMBRYO  
GERMINATION
- 6] COMPARE THE ANATOMY AND HISTOCHEMISTRY OF GERMINATION  
EVENTS IN THE RADICLE REGION

## MATERIALS &amp; METHODS

- 1] SEEDS OF NORWAY SPRUCE [LOT #53] WERE HYDRATED AT 5 °C  
FOR 48 HRS
- 2] EXCISED EMBRYOS WERE CULTURED HORIZONTALLY IN PETRI  
PLATES CONTAINING 1/2 DCR 25/25 MEDIUM IN LIGHT OR  
DARKNESS
- 3] EACH PLATE CONTAINED 20 EMBRYOS & 4-6 PLATES WERE SCORED  
AT EACH SAMPLING DATE [3, 5, 10 & 12 DAYS]
- 4] THE PERCENTAGES OF EMBRYOS PRODUCING A RADICLE OR "SKIRT"  
WERE RECORDED
- 5] THE RADICLES, HYPOCOTYLS AND COTYLEDONS OF GERMINATING  
EMBRYOS WERE MEASURED
- 6] SAMPLES WERE FIXED AND EMBEDDED FOR MICROSCOPICAL  
ANALYSIS

- 1] APPROXIMATELY 90% OF ZYGOTIC EMBRYOS SHOWED SIGNS OF  
GERMINATION
- 2] A RADICLE WAS PRESENT WITH 66% OF DARK CULTURED EMBRYOS
- 3] A "SKIRT" WAS PRESENT WITH 22% OF DARK CULTURED EMBRYOS

GERMINATION RESPONSE OF NORWAY SPRUCE  
ZYGOTIC EMBRYOS AFTER 12 DAYS IN THE DARK



TRIAL

PLATE 1 PLATE 2 PLATE 3 PLATE 4 PLATE 5 PLATE 6

THE FREQUENCIES OF RADICLE AND "SKIRT" DEVELOPMENT IN THE  
LIGHT WERE SIMILAR TO THOSE OBTAINED IN THE DARK

GERMINATION RESPONSE OF NORWAY SPRUCE  
ZYGOTIC EMBRYOS AFTER 12 DAYS IN LIGHT

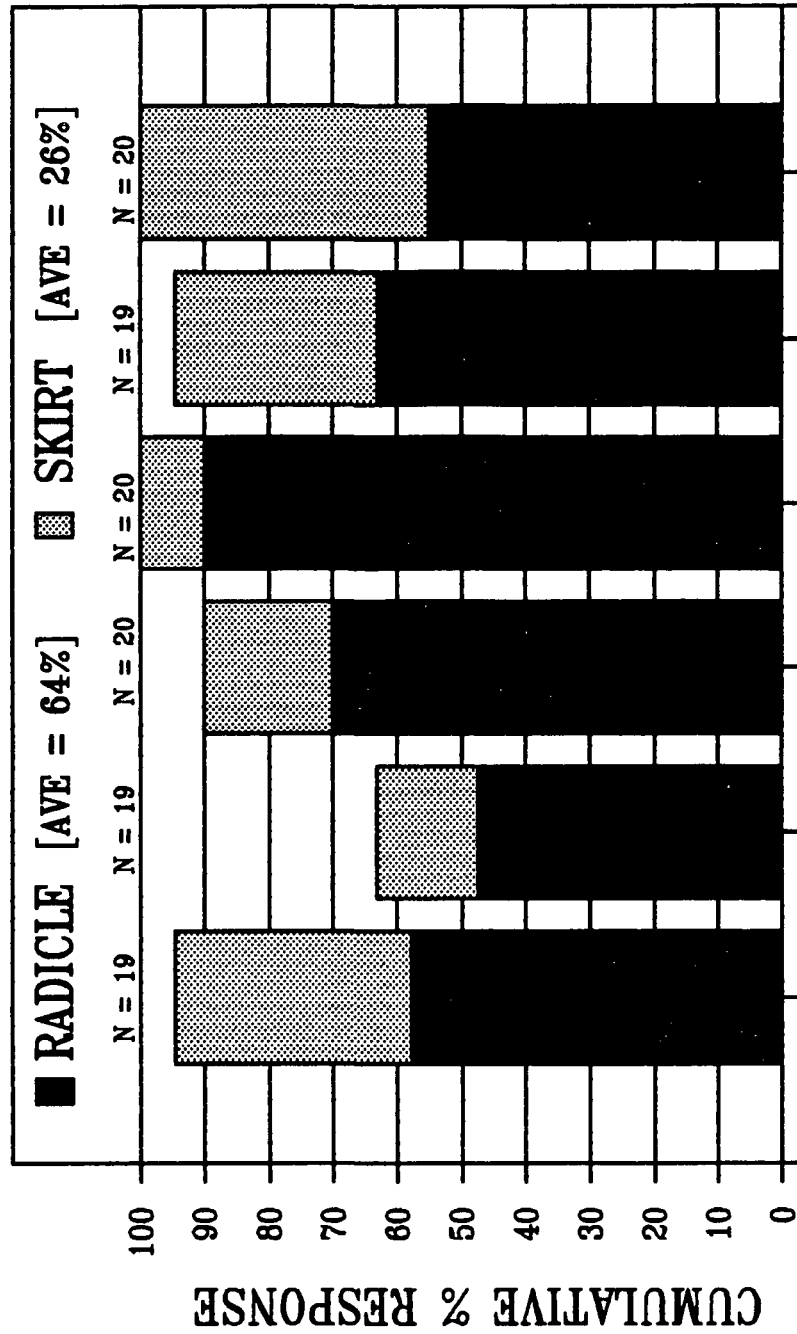


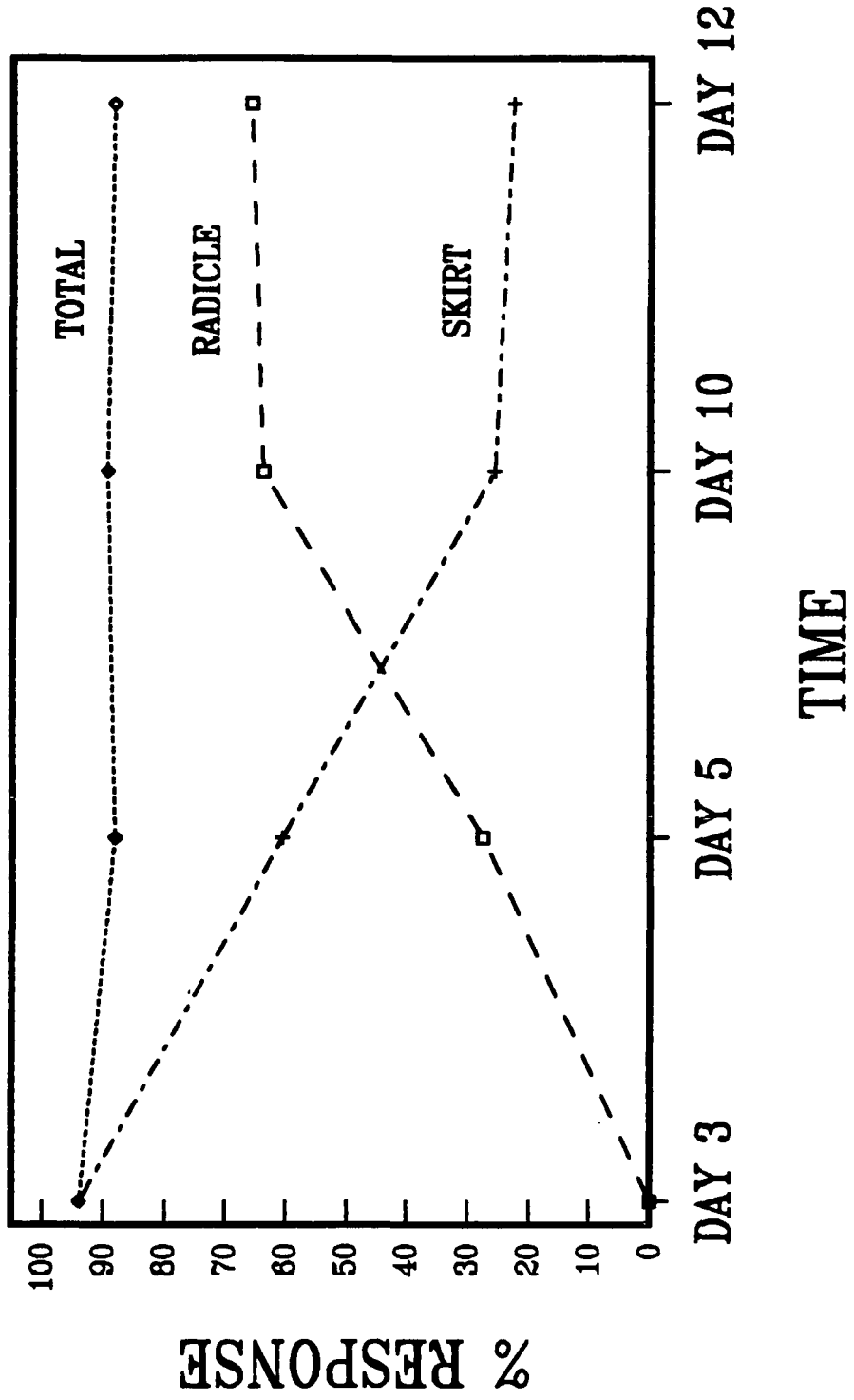
PLATE 1 PLATE 2 PLATE 3 PLATE 4 PLATE 5 PLATE 6

TRIAL

1] SIGNS OF ACTIVITY AT THE RADICLE POLE WERE EVIDENT AFTER  
3 DAYS

2] GERMINATION OF CULTURED ZYGOTIC EMBRYOS PEAKED BY DAY 10

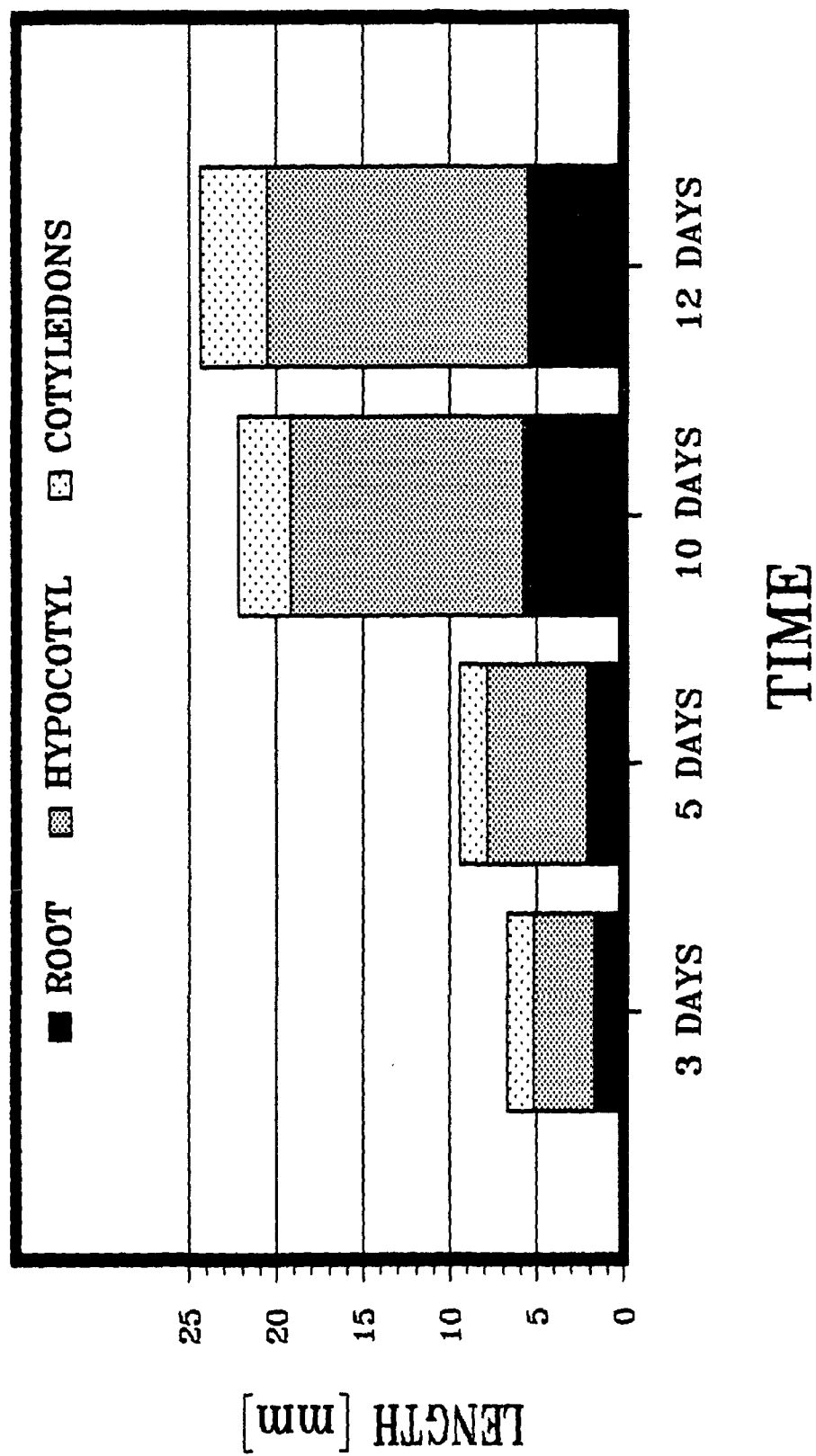
GERMINATION RESPONSE OF NORWAY SPRUCE  
ZYGOTIC EMBRYOS CULTURED IN THE DARK



1] THE HYPOCOTYL ELONGATED RAPIDLY

2] BOTH THE RADICLE AND THE COTYLEDONS EXHIBITED DELAYED  
ELONGATION AND GREW SLOWLY COMPARED TO THE HYPOCOTYL

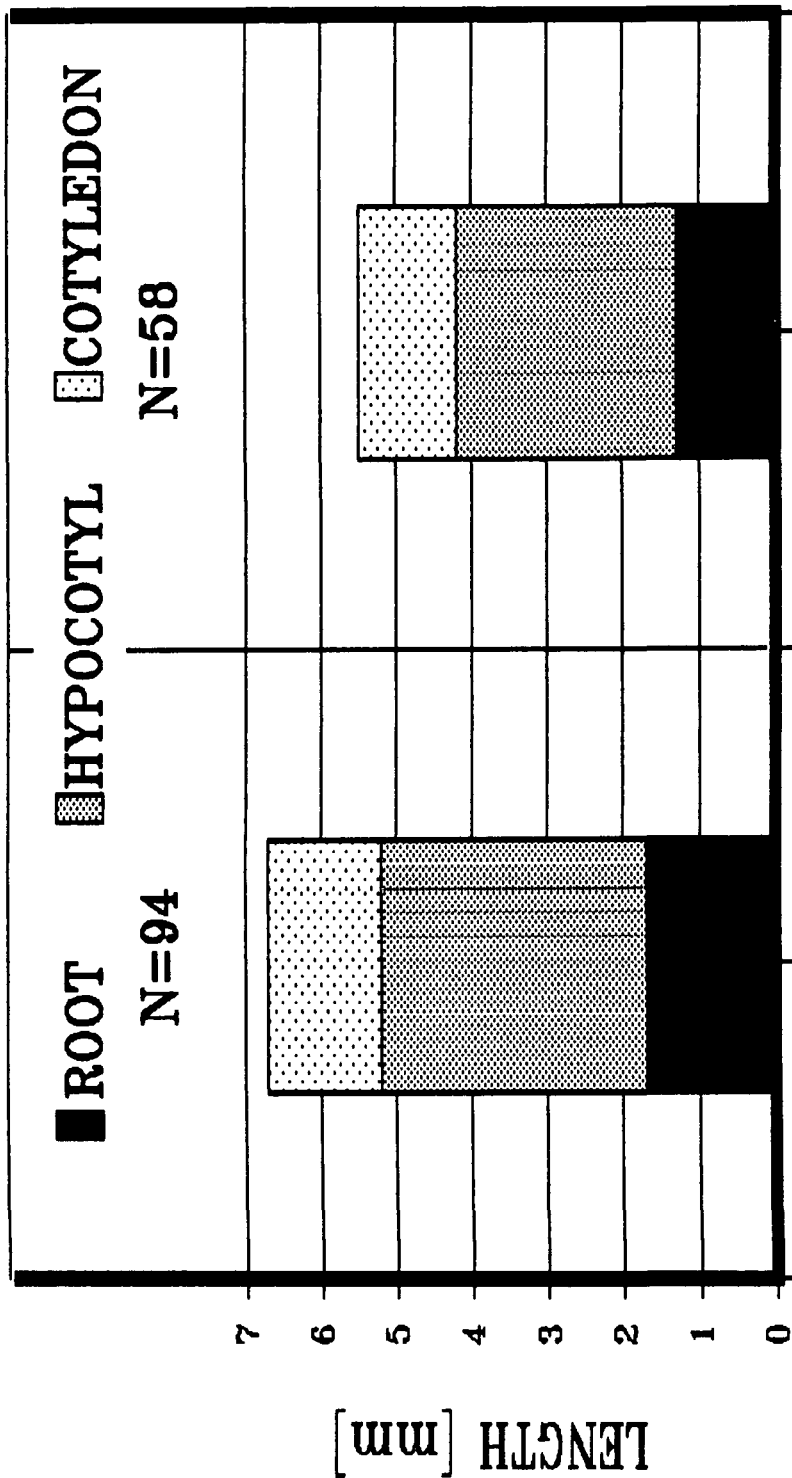
COMPARISON OF ZYGOTIC EMBRYOS GERMINATED  
FOR DIFFERENT PERIODS IN THE DARK



1] DIFFERENCES IN GROWTH BETWEEN LIGHT & DARK-CULTURED  
EMBRYOS WERE APPARENT AFTER 3 DAYS

2] THE ELONGATION OF ALL THREE EMBRYONIC ORGANS WAS  
INHIBITED BY LIGHT

# COMPARISON OF ZYGOTIC EMBRYOS CULTURED IN LIGHT OR DARKNESS



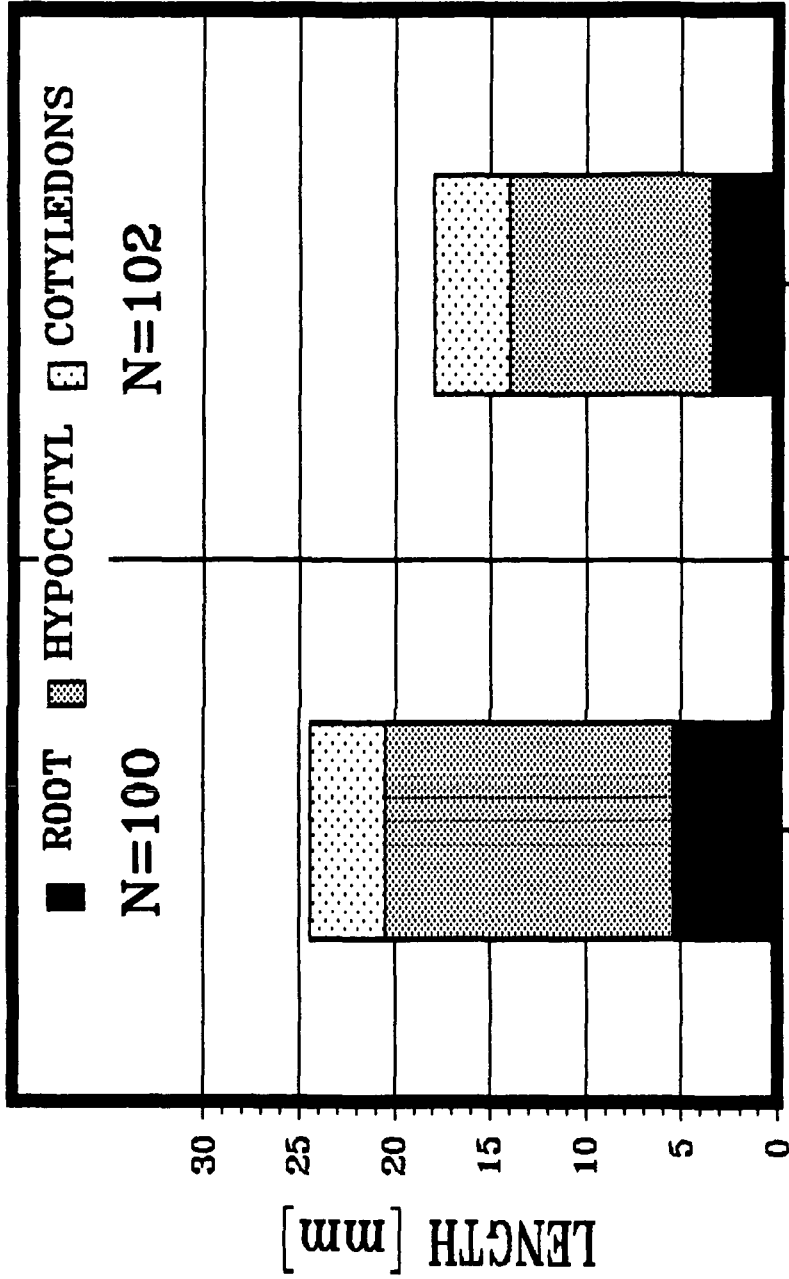
3 DAYS LT.

3 DAYS DK.

CULTURE TREATMENT

- 1] THE DIFFERENCES IN ELONGATION BETWEEN EMBRYOS CULTURED IN LIGHT OR DARKNESS WERE MORE EVIDENT AFTER 12 DAYS
- 2] HYPOCOTYL ELONGATION WAS MOST AFFECTED BY LIGHT & RADICLE ELONGATION WAS ALSO INHIBITED
- 3] COTYLEDON ELONGATION WAS SIMILAR IN LIGHT OR DARKNESS

# COMPARISON OF ZYGOTIC EMBRYOS CULTURED IN LIGHT OR DARKNESS



12 DAY DK.                      12 DAY LT.

## CULTURE TREATMENTS

## CONCLUSIONS

- 1] ZYGOTIC EMBRYOS GERMINATE RAPIDLY IN VITRO
- 2] APPROXIMATELY 90% OF THE EMBRYOS SHOW SIGNS OF  
GERMINATION BY DAY 3
- 3] PRODUCTION OF A "SKIRT" PRECEDES RADICLE EMERGENCE
- 4] AFTER 12 DAYS APPROXIMATELY 25% OF THE EMBRYOS PRODUCE A  
SKIRT WITH NO VISIBLE RADICLE
- 5] AFTER 12 DAYS APPROXIMATELY 65% OF THE EMBRYOS PRODUCE A  
RADICLE WHICH EMERGES FROM THE "SKIRT"
- 6] HYPOCOTYL ELONGATION IS RAPID AND COMPRISES THE MAJOR  
PORTION OF EMBRYO GROWTH DURING THE FIRST 12 DAYS
- 7] LIGHT DOES NOT INHIBIT THE FREQUENCY OF GERMINATION BUT  
DOES RETARD HYPOCOTYL ELONGATION AND ROOT ELONGATION
- 7] THE OVERALL PATTERN OF ZYGOTIC EMBRYO GERMINATION IN  
VITRO IS SIMILAR TO THAT OF SOMATIC EMBRYOS GERMINATED  
ON BLG 25/25
- 8] EMBRYO GERMINATION IS PRECOCIOUS IN BOTH CASES
- 9] PRESENT\_\_\_IN VITRO\_\_\_GERMINATION CONDITIONS ARE NOT OPTIMAL  
FOR ZYGOTIC EMBRYOS AND CONSEQUENTLY THEY ARE PROBABLY  
NOT OPTIMAL FOR SOMATIC EMBRYOS

## FUTURE WORK

- 1] MAKE SIDE BY SIDE COMPARISONS OF GERMINATION USING
  - A] WHOLE SEED
  - B] EXCISED ZYGOTIC EMBRYOS
  - C] SOMATIC EMBRYOS
- 2] EXPAND EXPERIMENTS TO INCLUDE REPRESENTATIVE SEED LOTS AND CALLUS LINES
- 3] TEST DIFFERENT MEDIA PAYING SPECIAL ATTENTION TO NITROGEN FORMS AND LEVELS
- 4] TEST DIFFERENT ORIENTATIONS OF THE EMBRYOS IN THE CULTURE MEDIUM & DIFFERENT MECHANISMS FOR DELIVERING CULTURE MEDIA
- 5] DEVELOP AN ARTIFICIAL MEGAGAMETOPHYTE BASED ON ANALYSIS OF CONIFER MEGAGAMETOPHYTES & OTHER MODEL SYSTEMS LIKE CASTOR BEAN
- 6] EXAMINE THE EFFECTS OF DIFFERENT LIGHT QUALITIES [BLUE, GREEN, RED, FAR-RED] & INTENSITIES ON RADICLE, HYPOCOTYL & COTYLEDON GROWTH
- 7] CLARIFY THE ANATOMICAL & HISTOCHEMICAL EVENTS ASSOCIATED WITH GERMINATION PAYING SPECIAL ATTENTION TO THE ORIGIN AND COMPOSITION OF THE "SKIRT"

## **IMMATURE SEEDS OF CONIFERS: PHYSICAL AND CHEMICAL CHARACTERISTICS**

### **RATIONALE**

**Understanding the physical and chemical characteristics of zygotic systems would help in designing the media composition for initiation and development of somatic embryo.**

### **OBJECTIVES**

**Review past IPST research**

**Identify other useful sources**

**Summarize pertinent information**

**Compare with the existing synthetic  
culture media**

**Compare data with somatic embryo  
composition**

**Recommend new research and/or  
protocol changes**

## PHYSICAL PARAMETERS

- pH of different zones in developing seeds
- moisture content
- osmolarity

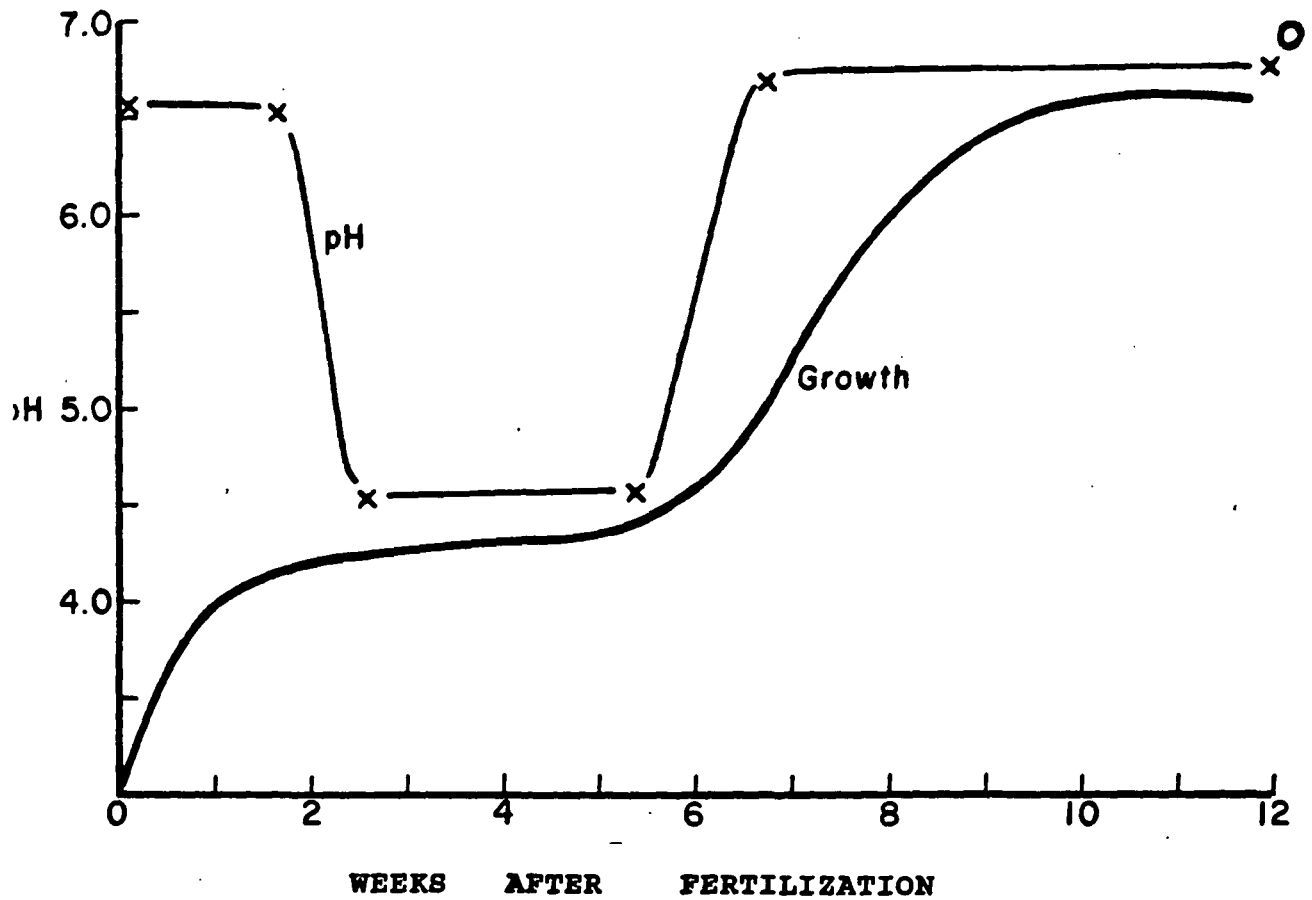
## CHEMICAL PARAMETERS

- carbohydrates
- amino acids
- lipids
- proteins
- elements

**ABBREVIATION**

- A** = **Archegonium or Egg Cell**
- A<sub>1</sub>A<sub>2</sub>** = **Archegonium (Duplicate Samples)**
- E=C** = **Corrosion Cavity**
- E<sub>1</sub>E<sub>2</sub>=C<sub>1</sub>C<sub>2</sub>** = **Corrosion Cavities (Duplicate Samples)**
- EM** = **Mature Embryo**
- IG** = **Immature Gametophyte**
- IG<sub>1</sub>IG<sub>2</sub>** = **Immature Gametophytes (Duplicate Samples)**
- G** = **Mature Gametophyte**
- G<sub>1</sub>G<sub>2</sub>** = **Mature Gametophytes (Duplicate Samples)**





CHANGING pH ENCOUNTERED BY LOBLOLLY PINE EMBRYOS DURING DEVELOPMENT

o INDEPENDENT VALUE FOR SEED EXTRACT

REFERENCE : The mass production of conifer hybrids;  
Progress Report 9 ( 1982 )

**WEIGHT AND MOISTURE CONTENT OF <sup>1</sup>  
DEVELOPING LOBLOLLY PINE SEED**

Parameter	Weeks After Fertilization					
	0	2	4	6	8	10
Fresh Wt. mg/seed	3.30	6.23	6.05	7.50	10.40	12.46
Dry Wt. mg/seed	0.505	0.625	0.890	2.92	5.18	7.72
Wt. Water mg/seed	2.80	5.61	5.19	4.58	5.22	4.74
M.C.%	85	90	85	65	50	38

M.C. = Moisture Content

<sup>1</sup> = Reference: The mass production of conifer hybrids: Project 3223; Progress Report 9 (1982)

CARBOHYDRATES IN DOUGLAS-FIR OVULE<sup>1</sup>  
BEFORE OR AT FERTILIZATION

Carbohydrates	% of Sample, Dry Wt.		
	A	C	IG
Rhamnan	-	0.3	0.2
Ribian	0.2	0.3	0.2
Araban	3.8	6.0	2.3
Xylan	1.4	1.7	0.5
Mannan	0.8	2.0	1.8
Galactan	2.2	3.2	2.5
Glucan	9.4	15.5	9.4

<sup>1</sup> = Reference: Data sheet from Analytical Chemistry Laboratory, Institute of Paper Chemistry (1980)

CARBOHYDRATES IN MATURE EMBRYO AND <sup>2</sup>  
GAMETOPHYTES, DOUGLAS-FIR

Carbohydrates	% of Sample, Dry Wt.		
	EM	G <sub>1</sub> <sup>↓</sup>	G <sub>2</sub> <sup>↓</sup>
Rhamnan	0.07	0.03	0.03
Ribian	0.2	0.03	0.03
Araban	1.0	0.6	0.6
Xylan	0.2	0.1	0.2
Mannan	0.6	0.1	0.2
Galactan	1.6	0.5	0.6
Glucan	6.8	2.0	1.9

↓ Duplicate Samples

<sup>2</sup> = Reference: Data sheet from Analytical Chemistry Laboratory, Institute of Paper Chemistry (1980)

CARBOHYDRATES IN WATER EXTRACTS OF DOUGLAS-FIR SEED<sup>1</sup>

Carbohydrates	% of Sample, Dry Wt.	
	Frozen	Autoclaved
Rhamnan	0.2	0.07
Araban	0.3	0.4
Xylan	0.2	0.06
Mannan	1.4	1.2
Galactan	4.1	4.8
Glucan	8.1	18.4

1 = Reference: Data sheet from Analytical Chemistry Laboratory, Institute of Paper Chemistry (1978)

AMINO ACID COMPOSITION OF UNSTRATIFIED DOUGLAS-FIR SEED<sup>1</sup>

Amino Acids	EMBRYO		GAMETOPHYTE	
	Free	Protein	Free	Protein
Asn	150	**	122	**
Asp	2,358	19,500	796	57,950
Thr	96	9,100	14	13,730
Lys	135	14,110	58	12,990
Gln	93	**	41	**
Glu	1,087	49,330	375	115,480
Pro	1,045	8,860	128	32,680
γ-ABA	187	**	28	**

\*\*Indicates that the amino acid is not present in proteins or that it was degraded during the hydrolysis procedure.

<sup>1</sup> = Reference: Data sheet from Russ Feirer, Institute of Paper Chemistry (1982)

AMINO ACID COMPOSITION OF UNSTRATIFIED DOUGLAS-FIR<sup>1</sup>  
SEED (Cont'd)

µg/gram of Fresh Wt.

Amino Acids	EMBRYO		GAMETOPHYTE	
	Free	Protein	Free	Protein
Ala	221	12,510	97	24,750
Leu	79	17,330	30	36,790
Ile	71	9,700	25	20,700
Val	100	11,030	22	27,590
Ser	358	13,280	27	36,130
Gly	173	13,000	16	24,790
Met	48	3,590	16	9,500
Tyr	198	7,580	168	17,430
Phe	47	9,210	4	19,940
His	149	7,500	89	12,920
Trp	0	**	228	**
Orn	141	**	16	**
Arg	10,118	31,620	2,109	111,460

\*\*Indicates that the amino acid is not present in proteins or that it was degraded during the hydrolysis procedure.

1 = Reference: Data sheet from Russ Feirer, Institute of Paper Chemistry (1982)

AMINO ACID COMPOSITION OF UNSTRATIFIED DOUGLAS-FIR SEED<sup>1</sup>

µg/gram of Dry Wt.

Amino Acids	EMBRYO		GAMETOPHYTE	
	Free	Protein	Free	Protein
Asn	163	**	129	**
Asp	2,557	21,150	846	61,580
Thr	104	9,870	15	14,590
Lys	146	15,300	63	13,800
Gln	101	**	44	**
Glu	1,179	53,500	399	122,720
Pro	1,133	9,610	136	34,730
γ-ABA	203	**	30	**

\*\*Indicates that the amino acid is not present in proteins or that it was degraded during the hydrolysis procedure.

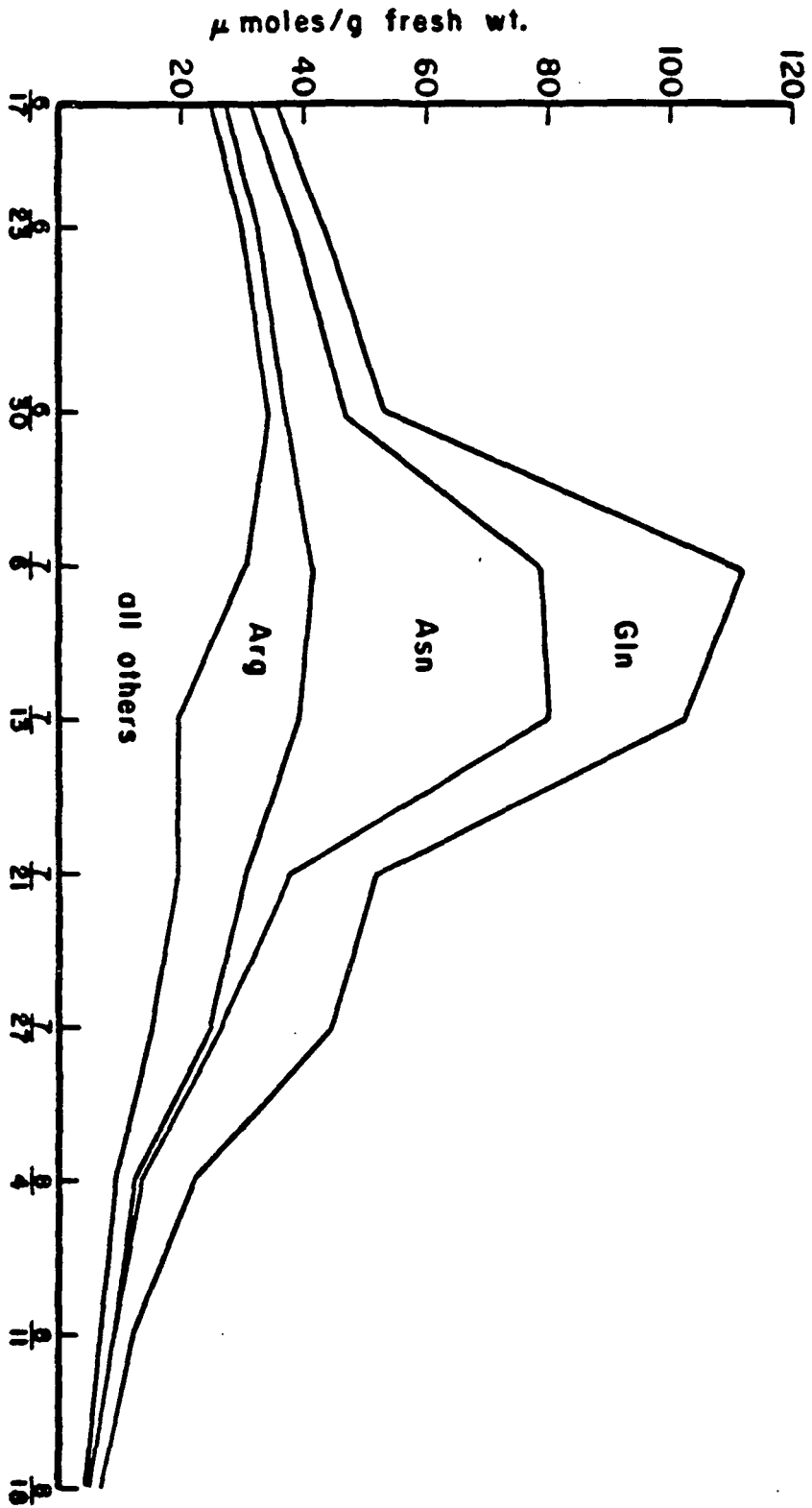
<sup>1</sup> = Reference: Data sheet from Russ Feirer, Institute of Paper Chemistry (1982)

AMINO ACID COMPOSITION OF UNSTRATIFIED DOUGLAS-FIR  
SEED<sup>1</sup> (Cont'd)

Amino Acids	μg/gram of Dry Wt.		
	Free	EMBRYO Protein	GAMETOPHYTE Free Protein
Ala	240	13,570	103
Leu	86	18,800	32
Ile	77	10,520	27
Val	108	11,960	23
Ser	388	14,400	29
Gly	188	14,100	17
Met	52	3,890	17
Tyr	215	8,320	179
Phe	51	9,990	4
His	162	8,130	95
Trp	0	**	242
Orn	153	**	17
Arg	10,974	39,300	2,241
			118,450

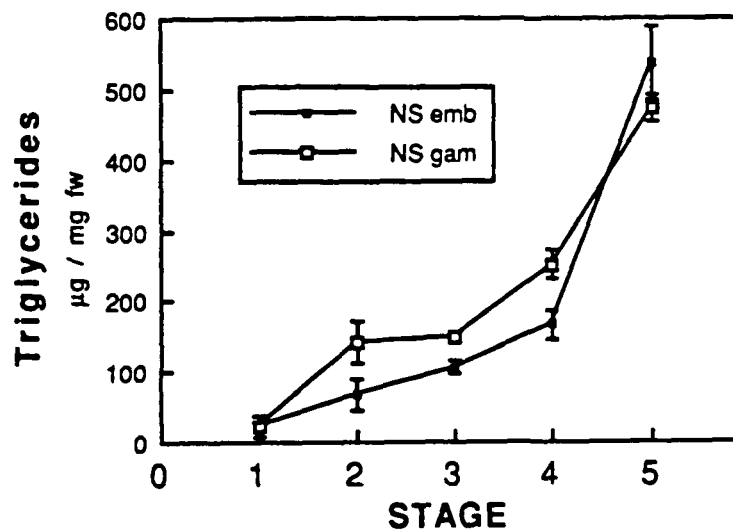
\*\*Indicates that the amino acid is not present in proteins or that it was degraded during the hydrolysis procedure.

1 = Reference: Data sheet from Russ Feirer, Institute of Paper Chemistry (1982)

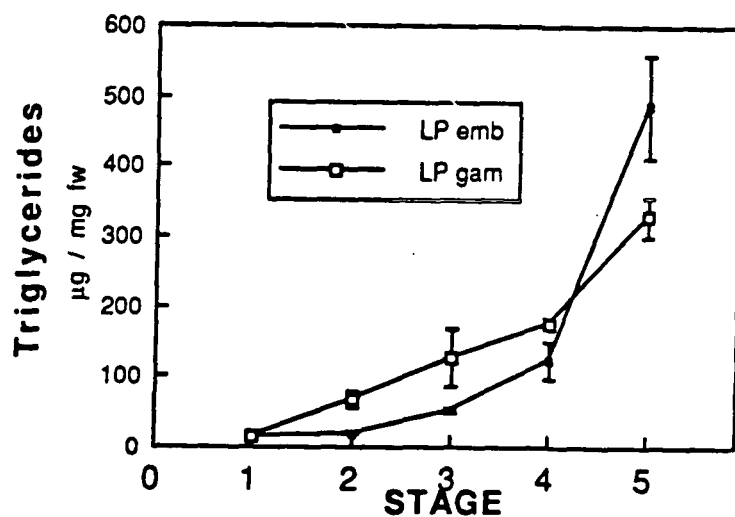


FREE AMINO ACID CONTENT OF DEVELOPING WHITE PINE OVULES  
( 1982 COLLECTION )

REFERENCE : The mass production of conifer hybrids;  
Progress Report 10 (1983).



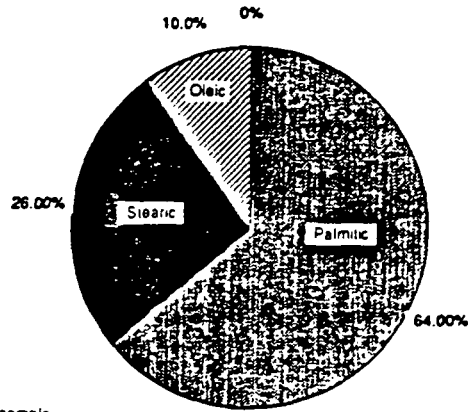
TRIGLYCERIDE LEVELS IN DEVELOPING NORWAY SPRUCE EMBRYOS AND GAMETOPHYTES



TRIGLYCERIDE LEVELS IN DEVELOPING LOBLOLLY PINE EMBRYOS AND GAMETOPHYTES

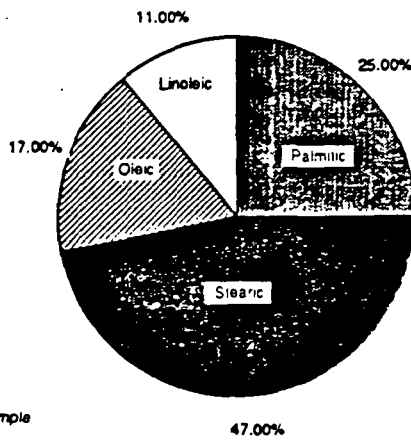
REFERENCE : Feirer, R.P.; Conkey, J.H.; Verghagen, S.A.  
 Triglycerides in embryonic conifer calli:  
 A comparison with zygotic embryos. Plant Cell  
 Reports 8 : 207-209.

Free Fatty Acids in NS Seeds\*



\*unsaponified sample

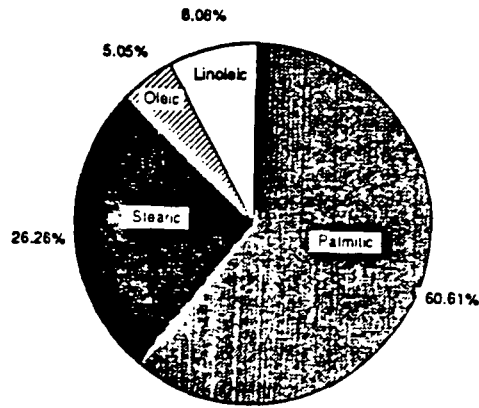
Free Fatty Acids in NS Seeds\*



\*saponified sample

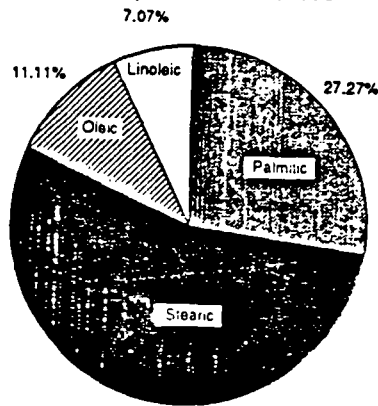
REFERENCE : PAC HANDOUT ; OCTOBER, 1988.

Free Fatty Acids in LP Seeds\*



\*unsaponified sample

Free Fatty Acids in LP Seeds\*



\*saponified sample

REFERENCE : PAC HANDOUT ; OCTOBER, 1988.

ELEMENTAL COMPOSITION OF DOUGLAS-FIR SEED ZONES<sup>1</sup>

µg/gram of oven dried samples Dry Wt.

Macro-elements	A <sup>1</sup>	C <sup>1</sup>	C <sup>2</sup>	IG <sup>3</sup>	G <sup>4</sup>
Calcium	740	690	50	375	305
Magnesium	3,050	2,800	2,400	2,300	2,250
Phosphorous	7,700	8,600	5,550	7,600	7,700
Potassium	41,100	55,000	2,400	64,000	68,300
Sodium	5,750	6,200	150	1,300	2,950

- A<sup>1</sup> = Average of 2 samples, Archegonia  
 C<sup>1</sup> = Single estimation of Corrosion Cavity  
 C<sup>2</sup> = Average of 2 samples, Corrosion Cavity  
 IG<sup>3</sup> = Average of 2 samples, Immature Gametophytes  
 G<sup>4</sup> = Average of 2 samples, Mature Gametophytes

<sup>1</sup> = Reference: Data sheet from Trace Elements Inc., 460 South Northwest Highway Park Ridge, Illinois 60068 (1980)

**ELEMENTAL COMPOSITION OF DOUGLAS-FIR SEED ZONES<sup>1</sup>**  
(Cont'd)

μg/gram of oven dried samples Dry Wt.

Micro-elements	A <sup>1</sup>	C <sup>1</sup>	C <sup>2</sup>	IG <sup>3</sup>	G <sup>4</sup>
Boron	155	760	<50	<50	112.5
Manganese	60	50	100	75	25
Zinc	2,140	1,500	<50	<50	390
Molybdenum	<50	<50	<50	<50	<50
Copper	<50	130	<50	<50	<50
Cobalt	<50	<50	<50	<50	<50
Nickel	<50	<50	<50	<50	<50
Iron	1,060	2,000	100	500	615

A<sup>1</sup> = Average of 2 samples, Archegonia  
 C<sup>1</sup> = Single estimation of Corrosion Cavity  
 C<sup>2</sup> = Average of 2 samples, Corrosion Cavity  
 IG<sup>3</sup> = Average of 2 samples, Immature Gametophytes  
 G<sup>4</sup> = Average of 2 samples, Mature Gametophytes

<sup>1</sup> = Reference: Data sheet from Trace Elements Inc., 460 South Northwest Highway Park Ridge, Illinois 60068 (1980)

ELEMENTAL COMPOSITION OF DOUGLAS-FIR SEED ZONES<sup>1</sup> (Cont'd)

µg/gram of oven dried samples Dry Wt.

Micro-elements	A <sup>1</sup>	C <sup>1</sup>	C <sup>2</sup>	IG <sup>3</sup>	G <sup>4</sup>
Aluminum	75	120	<50	25	95
Silicon	480	50	25	<50	25
Titanium	30	<50	<50	50	<50

107

- A<sup>1</sup> = Average of 2 samples, Archegonia  
 C<sup>1</sup> = Single estimation of Corrosion Cavity  
 C<sup>2</sup> = Average of 2 samples, Corrosion Cavity  
 IG<sup>3</sup> = Average of 2 samples, Immature Gametophytes  
 G<sup>4</sup> = Average of 2 samples, Mature Gametophytes

<sup>1</sup> = Reference: Data sheet from Trace Elements, Inc., 460, South Northwest Highway Park Ridge, Illinois 60068 (1980)

ELEMENTAL COMPOSITION OF MATURE EMBRYOS<sup>1</sup>  
AND GAMETOPHYTES, LOBLOLLY PINE

µg/gram of oven dried samples, Dry Wt.

Macro-elements      Embryos      Gametophytes

Calcium              180              400

Magnesium          5,800            5,000

Phosphorous        19,000           18,000

Potassium           37,000           29,000

Sodium              50                130

1 = Reference: Data sheet from Trace Elements, Inc., 460 South  
Northwest Highway Park Ridge, Illinois 60068 (1980)

ELEMENTAL COMPOSITION OF MATURE EMBRYOS<sup>1</sup>  
AND GAMETOPHYTES, LOBLOLLY PINE (Cont'd)

$\mu\text{g}/\text{gram}$  of oven dried samples, Dry Wt.

Micro-elements	Embryos	Gametophytes
Boron	<50	<50
Manganese	160	470
Zinc	110	150
Copper	<50	<50
Nickel	<50	<50
Aluminum	<50	80
Silicon	<50	<50
Titanium	<50	<50
Iron	200	100

1 = Reference: Data sheet from Trace Elements Inc., 460 South Northwest Highway Park Ridge, Illinois 60068 (1980)

ELEMENTAL COMPOSITION OF DOUGLAS-FIR SEEDS  
(BEFORE FERTILIZATION) AND MS MEDIUM -  
A COMPARISON

	<u>MS Medium</u>		<u>Seed Composition</u>	
Macro-elements	Metal elements	mg/1000g of water	mg/1000g of Fresh wt.	
NH <sub>4</sub> NO <sub>3</sub>	**	**	**	**
KNO <sub>3</sub>	Potassium	726.0	8,209.0	
KH <sub>2</sub> PO <sub>4</sub>	Potassium	49.1	1,224.6	
	Phosphorous	37.7	415.38	
MgSO <sub>4</sub> ·7H <sub>2</sub> O	Magnesium	59.2	92.30	

\*\* Reduced Nitrogen

ELEMENTAL COMPOSITION OF DOUGLAS-FIR SEEDS  
 (BEFORE FERTILIZATION) AND MS MEDIUM -  
 A COMPARISON (Cont'd)

	<u>MS Medium</u>	<u>Seed Composition</u>
Micro-elements	Metal elements mg/1000g of water	mg/1000g of Fresh wt.
$H_3BO_3$	Boron 1.08	46.9
$MnSO_4 \cdot 7H_2O$	Manganese 3.34	9.48
$ZnSO_4 \cdot 7H_2O$	Zinc 1.95	186.15
$Na_2MoO_4 \cdot 2H_2O$	Sodium 0.02	678.46
	Molybdenum 0.109	<50
$CuSO_4 \cdot 5H_2O$	Copper 0.006	6.6
$CoCl_2 \cdot 6H_2O$	Cobalt 0.006	<50
KI	Potassium 0.195	
$FeSO_4 \cdot 7H_2O$	Iron 5.58	182.5

## CONCLUSIONS

pH determinations of seed zones lacks precision.

Data on carbohydrates based on single determinations only and refer to monosachharides only.

Quantitative data on polar lipids and soluble proteins lacking.

Data on Elemental composition incomplete; anion composition not known; also, elemental composition of embryos lacking.

Data on embryo uptake of nutrients lacking.

**RECOMMENDED CHANGES IN PROTOCOL**

Improve methods for determination of pH of seed zones.

Use  $C^{13}$ NMR studies to determine carbohydrates.

Quantify data on soluble proteins and polar lipids.

Estimate metal elements by inductively coupled plasma spectrophotometry, and anions by ion chromatography with conductometric detection.

**M. RAFIQUE UDDIN**

**RECENT RESEARCH IN HARDWOOD TISSUE CULTURE**

**GENERAL OBJECTIVE**

**DEVELOP A REGENERATION SYSTEM SUITABLE FOR**

**(1) COCULTIVATION AND TRANSFORMATION  
EXPERIMENTS WITH AGROBACTERIUM TUMEFACIENS  
AND PRODUCTION OF TRANSGENIC HERBICIDE  
TOLERANT COTTONWOOD**

**(2) HERBICIDE SCREENING AND SELECTION OF  
SOMACLONAL VARIANTS FOR INCREASED TOLERANCE**

**PART I: A LEAF SECTION SYSTEM FOR REGENERATION OF  
COTTONWOOD**

**EXPERIMENT 1. SHOOT FORMATION**

**HYPOTHESIS**

**MANIPULATION OF GROWTH REGULATORS AND PHYSICAL  
ENVIRONMENT WILL STIMULATE ADVENTITIOUS SHOOT  
PRODUCTION FROM CUT EDGES OF IN VITRO GROWN LEAF  
SECTIONS**

## MATERIALS AND METHODS

**CLONE: C175 (ERNST, NEBRASKA)**

**PHYSICAL ENVIRONMENTS:(1) CONTINUOUS LIGHT (16 HR.)  
(2) THREE WEEKS DARK FOLLOWED BY LIGHT**

**BASAL MEDIUM: MODIFIED WPM (PRAKESH & THIELGES;  
1989) WITH 2% GLUCOSE.**

**GROWTH REGULATORS:**

**MAIN THRUST - BA = 0, 1.0, 2.0, 4.0, 8.0, AND 16  $\mu$ M  
NAA = 0, 1.0 AND 2.0  $\mu$ M**

**EXPLORATORY - THIDIAZURON = 1.0, 0.5 AND 0.1  $\mu$ M  
(PRAKESH & THIELGES, 1989)  
ZEATIN = 1.1  $\mu$ M  
(COLEMAN & ERNST, 1989)**

## RESULTS

EFFECTS OF GROWTH REGULATORS WITH WOODY PLANT MEDIUM  
ON NUMBER OF ADVENTITIOUS SHOOTS/EXPLANT

	<u>NAA</u> ( $\mu\text{M}$ )		
	0.0	1.0	2.0
0.0	0	0	0
1.0	0	$3.2 \pm 1.9$	$0.5 \pm 0.5$
2.0	0	$1.1 \pm 0.9$	$3.0 \pm 1.4$
4.0	0	$0.3 \pm 0.6$	$1.7 \pm 0.7$
8.0	0	0	$0.8 \pm 0.9$
16.0	0	0	0

BA ( $\mu\text{M}$ )

**EFFECTS OF GROWTH REGULATORS WITH WOODY PLANT MEDIUM  
ON NUMBER OF ADVENTITIOUS SHOOTS**

**EXPLORATORY**

<b>TREATMENT</b>	<b>LEVELS</b>	<b># OF SHOOTS/EXPLANT</b>
<b>THIDIAZURON</b>	<b>1.0 <math>\mu</math>M</b>	<b>0</b>
	<b>0.5 <math>\mu</math>M</b>	<b>0.4 <math>\pm</math> 0.6</b>
	<b>0.1 <math>\mu</math>M</b>	<b>2.3 <math>\pm</math> 1.1</b>
<b>ZEATIN</b>	<b>1.1 <math>\mu</math>M</b>	<b>0</b>

**EXPERIMENT II.EFFECTS OF GROWTH REGULATORS AND DKW-C  
MEDIUM ON ADVENTITIOUS SHOOT FORMATION**

**HYPOTHESIS**

**BEST MEDIA TRIED HERE AND ELSEWHERE WILL IMPROVE  
SHOOT FORMATION**

## MATERIALS & METHODS

**TEST MEDIA: DKW-C (MCGRANAHAN, 1987),  
SUPPLEMENTED**

**WITH BA + NAA; 1  $\mu$ M EACH AND 2.0  $\mu$ M EACH;  
THIDIAZURON 0.1  $\mu$ M, AND 1.1  $\mu$ M ZEATIN**

**CONTROL: WPM MEDIUM SUPPLEMENTED WITH 1  $\mu$ M  
EACH OF BA & NAA**

**PHYSICAL ENVIRONMENT: THREE WEEKS DARK  
FOLLOWED BY LIGHT INCUBATION**

**RESULTS****EFFECTS OF GROWTH REGULATORS AND BASAL  
MEDIUM ON NUMBER OF ADVENTITIOUS SHOOTS**

<b>BASAL MEDIUM</b>	<b>TREATMENTS</b>	<b># OF SHOOTS/EXPLANT</b>
DKW-C	Thidiazuron 0.1 $\mu$ M	2.3 $\pm$ 1.2
	1 $\mu$ M BA + 1 $\mu$ M NAA	3.9 $\pm$ 1.4
	2 $\mu$ M BA + 2 $\mu$ M NAA	2.2 $\pm$ 1.1
-----		
WPM	1 $\mu$ M BA + 1 $\mu$ M NAA	2.4 $\pm$ 1.1

## **ROOTING OF ADVENTITIOUS SHOOTS**

### **HYPOTHESES**

- (1) A SUITABLE MEDIUM SUCH AS WPM (UDDIN ET AL., 1988) SUPPLEMENTED WITH LOW LEVELS OF IBA WILL PROMOTE ROOTING**
- (2) 0.25 AND 0.5  $\mu$ M IBA APPEAR ATTRACTIVE BASED ON THE LITERATURE AND PAST EXPERIENCE**

**MATERIALS & METHODS**

**BASAL MEDIUM- WPM**

**GROWTH REGULATORS; IBA (0.25 AND .50 $\mu$ M)**

**SHOOT COLLECTED FROM EXPERIMENT 2, WPM & DKW**

**RESULTS****EFFECTS OF IBA ON FREQUENCY OF ROOTING OF  
ADVENTITIOUS SHOOTS**

SHOOTS FROM	IBA CONC. ( $\mu$ M)		
	0.0	0.25	0.5
WPM	0	92.9 $\pm$ 9.9	91.9 $\pm$ 11.3
DKW	0	95.4 $\pm$ 8.6	93.6 $\pm$ 11.1

## CONCLUSIONS

- (1) DKW MEDIUM WITH 1  $\mu\text{M}$  EACH OF BA & NAA GAVE THE BEST SHOOT PRODUCTION; 4 SHOOTS/EXPLANT
- (2) 0.25 OR 0.50  $\mu\text{M}$  IBA EQUALLY PROMOTED ROOTING; 92%
- (3) IT TOOK ONLY 10 WEEKS FROM SHOOT INDUCTION TO ROOTING TO DEVELOP COMPLETE PLANTS

**PART II. CELL SUSPENSION-EXPLORATORY**

**EXPERIMENT 1: PRODUCTION OF CALLUS FROM LEAF EXPLANT**

**HYPOTHESIS:**

**COMBINATION OF 2,4-D + BA OR 2,4-D + KINETIN WILL  
INDUCE FORMATION OF FRIABLE CALLUS.**

## **MATERIALS AND METHODS**

**BASAL MEDIUM : MS**

**CLONE: LEAF EXPLANT OF C175; 15 X 10 mm PIECES**

**GROWTH REGULATORS:**

**TEST OF 2,4-D & BA**

**5 LEVELS OF 2,4-D (0, 1.0, 2.0, 3.0 AND  
4.0 MG/L)  
4 LEVELS OF BA (0, 0.05, 0.1, AND 1.0  
MG/L)**

**TEST OF 2,4-D & KINETIN**

**3 LEVELS OF 2,4-D (0, 1.0, 2.0 MG/L)  
3 LEVELS OF KINETIN (0, 1.0, AND 2.0  
MG/L)**

**RESULTS**

- (A) CALLUS FORMED AT ALL TREATMENTS CONTAINING BA AND 2,4-D.**
- (B) CALLUS FORMED ONLY ON TREATMENT CONTAINING 2.0 MG/L 2,4-D AND 1.0 MG/L KINETIN.**

**EXPERIMENT II: DEVELOP SUSPENSION CULTURE FROM LEAF CALLUS**

**HYPOTHESIS:**

**RAPIDLY GROWING CELL SUSPENSION CULTURE CAN BE ESTABLISHED AND MAINTAINED USING SIMILAR RANGE OF 2,4-D & BA.**

## **MATERIALS AND METHODS**

**CLONE: C175; CALLUS DERIVED FROM EXPERIMENT 1**

**BASAL MEDIUM: MS**

**GROWTH REGULATORS:**

**3 LEVELS OF 2,4-D (1.0, 2.0 AND 4.0 MG/L) IN  
COMBINATION WITH 3 LEVELS OF BA (0.01, 0.1  
AND 1.0 MG/L) .**

**RESULTS**

**RAPIDLY GROWING FINE SUSPENSION CULTURE  
(ISODIAMETRIC CELLS) WAS DEVELOPED.**

**ONLY ONE COMBINATION OF GROWTH REGULATORS, 1.0  
MG/L 2,4-D AND 0.1 MG/L BA PROVED USEABLE.**

**EXPERIMENT III. PLATING OF SUSPENSION CULTURE TO  
PRODUCE MICROCALLUS**

**HYPOTHESIS**

**COMBINATION OF 2,4-D + BA SIMILAR TO THOSE USED  
TO INDUCE LEAF CALLUS CAN FORM CALLUS FROM  
PLATED SUSPENSION**

**MATERIALS & METHODS**

**CLONE: CELL SUSPENSION OF C175**

**BASAL MEDIA: MS**

**CULTURE CONDITION: DARK**

**GROWTH REGULATORS:**

**3 LEVEL OF 2,4-D (0, 1.0 AND 2.0 MG/L)**

**3 LEVELS OF BA (0, 0.05 AND 0.1 MG/L BA)**

**RESULTS**

**MICROCALLUS WAS FORMED AFTER 3-4 WEEKS & IS BEING MAINTAINED ON MEDIA CONTAINING 1.0 OR 2.0 MG/L 2,4-D PLUS 0.05 MG/L BA; BUT OTHER LEVELS DID NOT PRODUCE ANY CALLUS.**

## CONCLUSIONS

- (1) A RAPIDLY GROWING REGENERABLE SUSPENSION CULTURE WAS DEVELOPED FROM Populus deltoides LEAF CULTURE.
- (2) THE ONLY COMBINATION OF GROWTH REGULATORS SUITABLE FOR PRODUCING SUSPENSION CULTURE WAS 2,4-D AT 1.0 MG/L + BA AT 0.1 MG/L.
- (3) THE ONLY CALLUS THAT GAVE THIS SUSPENSION CULTURE WAS INITIATED WITH 2.0 MG/L 2,4-D IN COMBINATION WITH 1.0 MG/L KINETIN.
- (4) MAINTAINABLE RAPIDLY GROWING MICROCALLUS WERE OBTAINED ON MEDIA CONTAINING 1.0 OR 2.0 MG/L 2,4-D + 0.05 MG/L BA.

**NEAR TERM PLAN**

- (1) EXTEND THE REPORTED ADVANCE TO OTHER CLONES**
- (2) ASSIST STUDENT WITH GENE TRANSFER PROJECT**
- (3) PROVIDE CULTURES FOR STUDENT PROJECT ON LIGNIFICATION**
- (4) REGENERATE INTACT PLANTS FROM SUSPENSION CULTURE**
- (5) INITIATE WORK ON SELECTION FOR HERBICIDE TOLERANCE**

SUMMARY & DISCUSSION

RON DINUS

## SOFTWOOD PROGRESS: PAC TO PAC

SHORT TERM GOALS

MOVE EQUIPMENT &amp; SUPPLIES

READY FACILITIES

DOUGLAS-FIR INITIATION

IMPROVE MATURATION &amp; CONVERSION

DOCUMENT ZYGOTIC EMBRYO  
DEVELOPMENT, GUIDEPOSTSEXPLORE INITIATION FROM MORE  
MATURE MATERIALS

COMPLETE STAFFING PLAN

PRESENT/PUBLISH PROMPTLY

ACCOMPLISHMENTSHAVE ADDED SOME ITEMS  
DEAL WITH OTHER SLATERMOSTLY OPERATIONAL  
CONTAMINATION  
OASIS FOR OTHER GROUPSMIXED RESULTS, 4 LINES  
MATURE CONE TRIALS

LOBLOLLY PINE PROTOCOL

REASSESSED PAST WORK  
ON ZYGOTIC EMBRYO  
PROPERTIESLP: NEW EXPERIMENTS,  
FIRST RESULTS  
CONFIRM PAST WORK  
ON CHO & ABA  
RESPONSE CURVESNS: COMPLETED ARCHIVE  
STREAMLINE BANK  
SET STAGE FOR NEW  
WORK ON PROTOCOL  
AND KEY GOALSDF: EXPLORATORY WORK,  
NO NEW RESULTSUSING & ENLARGING THE  
CLASSIFICATION SYSTEMDF: MATURE CONES,  
MORE TO COMEADDED 2 TEMPORARIES  
ADDED 2 TECHNICIANS  
INTERVIEWINGCOMPLETED 2 MAJOR PAPERS,  
SEVERAL IN PREPARATION  
SUMMER MEETINGS = 5

## BIOCHEMISTRY PROGRESS: PAC TO PAC

SHORT TERM GOALS

MOVE EQUIPMENT &amp; SUPPLIES

READY FACILITIES

RENEW WORK ON ZYGOTIC/SOMATIC  
COMPARISONS, LIPIDS, ETC.

SEEK INTERIM COOPERATORS

CONTINUE ABA ASSAYS

COMPLETE STAFFING PLAN

ACCOMPLISHMENTSNO ACTION, DEAL WITH  
THIS AS NEEDEDMOSTLY OPERATIONAL HEAVY  
USE BY OTHERSONLY ACTION = SELECTING  
& FREEZING SAMPLESEXPLORING OPTIONS AT  
GT & UGAFINALIZED AGREEMENT WITH  
GROUP 1; PRODUCING SAMPLES  
FOR ASSAYWORKING ON AGREEMENT  
WITH ANOTHER

EXPLORING FOR OTHERS

INTERVIEWING

## HARDWOOD PROGRESS: PAC TO PAC

SHORT TERM GOALS

EXPAND EXISTING CULTURES

INITIATE/SECURE ADDITIONAL  
CULTURESESTABLISH "CLEAN" GREENHOUSE  
POPULATION

START GENE TRANSFER WORK

READY METHOD FOR PROPAGATION

EXECUTE EXPLORATORY RESEARCH

COMPLETE STAFFING PLAN

ACCOMPLISHMENTSNUMBERS SUFFICIENT,  
SHORT ON VARIETYVARIETY INCREASING,  
UNIVCO OPERATORS INITIATE  
FROM GREENHOUSERECEIVING FROM UNIVERSITY  
COOPERATORS & MEMBER COMPANYSTUDENT PROJECT, INTERVIEWING  
FOR NEW HIRELEAF SECTION SYSTEM WORKS!  
WILL TEST TRANSFORMATION;  
MUST EXTEND TO OTHER MATERIALSESTABLISH SUSPENSIONS, OBTAINED  
CALLI, WORKING ON SHOOT  
INDUCTION

INTERVIEWING

\*EXAMPLE\*

SOMATIC EMBRYOGENESIS: PATHWAY, PROCESS, & PROGRESS

NORWAY SPRUCE, 3/89

PATHWAY	PROCESS	MEAN	SUCCESS RATES RANGE	COMMENTS
EXPLANT (IMMATURE EMBRYOS) ----- % -----				
	INITIATION	= 80	--	No New Data
	MAINTENANCE	= 50	--	No New Data
↓				
EMBRYOGENIC CALLI				
	DEVELOPMENT & MATURATION	130	0-358	Fall, 1989
↓				
SOMATIC EMBRYOS ----- #1 gram -----				
	CONVERSION	= 7	0- 7	Fall, 1987
	GERMINATION	= 19	0-20 (21-97)	Fall, 1987 1988
		24	(0-69)	Fall, 1989
↓				
SEEDLINGS				

CUMULATIVE RATES

EXPLANT TO EMBRYOGENIC CALLI = 40%

EMBRYOGENIC CALLI TO SEEDLINGS = <1 Seedling/Gram Culture

**SOMATIC EMBRYOGENESIS: PATHWAY, PROCESS, & PROGRESS**

**DEFINITIONS OF SUCCESS RATES**

**\* INITIATION (I) & MAINTENANCE (M)**

**EXPRESSED IN PERCENTS:**

**I = # EC LINES / # EXPLANTS**

**M = # EC LINES MAINTAINED / # INITIATED**

**CUMULATIVE = (I) (M), REFLECTS # EC LINES AVAILABLE  
FOR RESEARCH OR USE & EASE OF GETTING THEM**

**\* DEVELOPMENT/MATURATION (D/M) &**

**CONVERSION TO SEEDLINGS,**

**EXPRESSED AS NUMBERS:**

**D/M = # MATURE EMBRYOS / GRAM CULTURE**

**C = # SEEDLINGS SURVIVING @ 5 MONTHS/# MATURE EMBRYOS**

**G = # GERMINATED EMBRYOS / # MATURE EMBRYOS**

**CUMULATIVE = (D/M) (C) = # SEEDLINGS SURVIVING  
/ GRAM CULTURES, REFLECTS YIELD & UTILITY  
OF PROCESS**

## SOME ADDITIONAL ACTIVITIES

## GRANT PROPOSALS, SUBMITTED OR IN PREPARATION

## \* USDA PLANT GROWTH &amp; DEVELOPMENT PROGRAM

LIGNIN BIOSYNTHESIS: PI = ERIKSSON, UGA

IPST SUBCONTRACT - DINUS WEBB, CONNERS, WOODWARD,  
& FORDE (STUDENT)

## \* NSF BIOCHEMISTRY PROGRAM

NOVEL ENZYMES IN LIGNIN BIOSYNTHESIS: PI = ERIKSSON, UGA

IPST SUBCONTRACT - DINUS

## \* NSF BIOCHEMISTRY PROGRAM (IN REVISION)

LIGNIFICATION IN CELL & TISSUE CULTURES, SOMATIC EMBRYOS  
& SEEDLINGS, & THEIR ZYGOTIC COUNTERPARTS

PI'S = DINUS, WEBB, CONNERS, &amp; WOODWARD + ERIKSSON, UGA

## \* USDA FOREST BIOLOGY PROGRAM

LIGNIN DEPOSITION IN CELL CULTURES & DEVELOPING EMBRYOS  
& SEEDLINGS

PI'S = DINUS &amp; WEBB

SUBCONTRACT - ERIKSSON, UGA  
COLLABORATORS - CONNERS & WOODWARD, IPST  
ATALLA, FOREST PRODS. LAB  
SEAGULL, USDA-ARS

## \* NSF WOMEN'S CAREER ADVANCEMENT PROGRAM

CHEMICAL ANALYSES OF DEVELOPING CONIFER SEED

PI = NAGMANI, IPST COLLABORATOR = BANERJEE

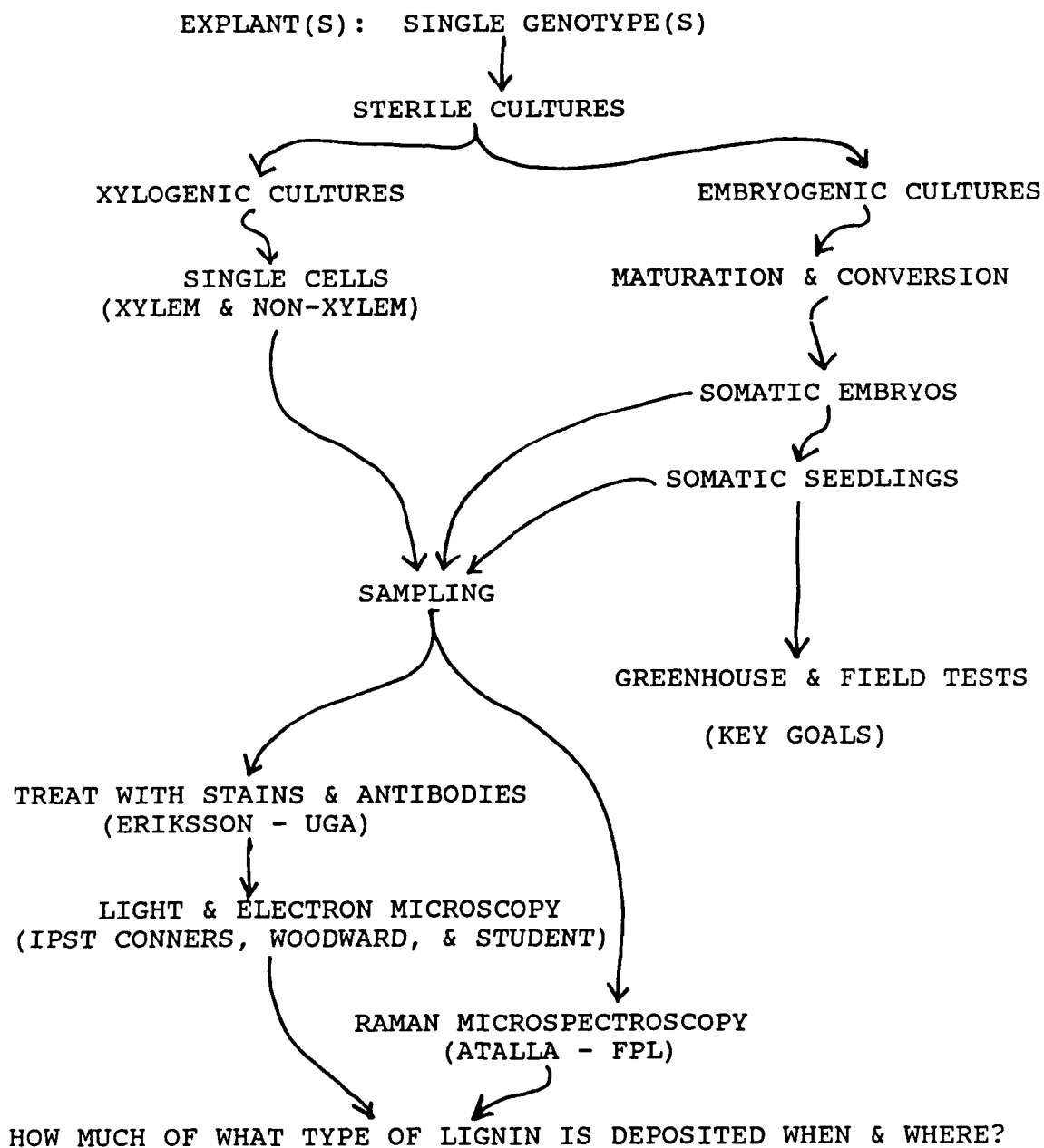
## \* USDOE BASIC ENERGY SCIENCES PROGRAM

LIGNIN SYNTHESIS &amp; DISTRIBUTION IN PLANT CELL WALLS

PI'S = DINUS &amp; ERIKSSON, UGA

PRE-APPLICATION, DECISION ON APPLICATION PENDING

GRANT PROPOSALS: RELATIONSHIP TO ONGOING WORK



STATUS OF NORWAY SPRUCE CULTURES  
BASIS FOR ATTACK ON KEY GOALS

TRANSFER OF CULTURES TO ATLANTA

INSURANCE CULTURES

APPLETON CULTURES

ATLANTA CULTURES

ATLANTA CULTURE BANK

RECOVERY

HISTORICAL ARCHIVE

CONSOLIDATION

SAMPLES FOR BIOCHEMICAL ASSAY

BASIS FOR FUTURE WORK

GENETIC BACKGROUND

HISTORY

COMPOSITION

POTENTIAL FOR MATURATION

LINES AVAILABLE & NEEDED

## STATUS OF NORWAY SPRUCE CULTURES

## BASIS FOR ATTACK ON KEY GOALS

GENTIC BACKGROUND	EXPLANT TYPE	CULTURE COMPOSITION	MATURATION POTENTIAL
APPLETON TREE # 2	IMMATURE EMBRYO	STAGE 1 & 2	1 = HIGH, TESTED 1 = GOOD, UNTESTED
"	MATURE EMBRYO	"	2 = POOR, TESTED
"	SOMATIC SEEDLING	STAGE 1	5 = GOOD, TESTED 4 = GOOD, UNTESTED
BULK SEEDLOT (53)	MATURE EMBRYO	STAGE 1	1 = HIGH, TESTED 5 = GOOD, UNTESTED 1 = POOR, TESTED
"	ZYGOTIC SEEDLING	"	7 = GOOD, UNTESTED
BULK SEEDLOT (55)	MATURE EMBRYO	"	2 = POOR, TESTED

STATUS OF NORWAY SPRUCE CULTURES

BASIS FOR ATTACK ON KEY GOALS

SUMMARY & TENTATIVE PLANS:

ONLY ONE KNOWN MOTHER TREE

7 TESTED GOOD OR BETTER, INCLUDING IMMATURE EMBRYO  
& SOMATIC SEEDLING EXPLANTS

INITIATE 2 OR MORE FROM MATURE EMBRYO EXPLANTS

SECURE ANOTHER 2 TO 4 KNOWN MOTHER TREES

INITIATE 2 OR MORE FROM IMMATURE & MATURE EMBRYO EXPLANTS

BULK SEEDLOTS (ASSUME DIFFERENT & NONRELATED GENOTYPES)

1 TESTED GOOD OR BETTER FROM MATURE EMBRYO EXPLANT

UNTESTED & GOOD, 5 FROM MATURE EMBRYO EXPLANTS  
+ 7 FROM ZYGOTIC SEEDLING EXPLANTS

\*UTILIZE A REPRESENTATIVE SAMPLE OF AVAILABLE CULTURES, INCLUDING  
(NS1) 5 AS A CONTROL, TO REFINE PROTOCOL, RAISE MATURATION  
FREQUENCIES TO 25%, & ENSURE RELIABILITY ACROSS GENOTYPES  
& EXPLANT TYPES

\*SECURE SUFFICIENT NUMBERS FOR PRODUCTION OF SEEDLINGS  
REPRESENTING 1 OR MORE EXPLANTS FROM EACH OF SEVERAL  
KNOWN MOTHER TREES

\*PRODUCE SEEDLINGS FROM BULK SEEDLOT EXPLANTS IF NEEDED

SHORT TERM GOALS - CRITICAL ISSUES

SOFTWOODS

COMPLETE STAFFING PLAN

COMPLETE RENOVATION OF FACILITIES & EQUIPMENT

IMPROVE INITIATION FREQUENCIES, TARGET SPECIES

OBTAIN NEEDED CULTURES, ALL SPECIES

IMPROVE MATURATION FREQUENCIES

DOCUMENT ZYGOTIC EMBRYO DEVELOPMENT, GUIDEPOSTS

EXPLORE INITIATION FROM MORE MATURE MATERIALS

SHORT TERM GOALS - CRITICAL ISSUES

BIOCHEMISTRY

COMPLETE STAFFING PLAN

READY FACILITIES & EQUIPMENT FOR USE

RENEW RESEARCH ON ZYGOTIC/SOMATIC COMPARISONS

EXECUTE ABA ASSAYS

FACILITATE STUDENT PROJECT ON LIGNIFICATION

SHORT TERM GOALS - CRITICAL ISSUES

HARDWOODS

COMPLETE STAFFING PLAN

COMPLETE EQUIPMENT OF GREENHOUSE

ESTABLISH "CLEAN" GREENHOUSE POPULATIONS

EXTEND EFFECTIVE PROPAGATION METHODS TO DIVERSE GENOTYPES

FACILITATE STUDENT PROJECT ON GENE TRANSFER

ACCELERATE GENE TRANSFER RESEARCH

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 in vitro 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.
- 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.,  $\mu\text{L}/\text{mL}$ .

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.

**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 RI).

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 (promoters) 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 *Agrobacterium 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.

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
$\gamma$ -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	l-Aminocyclopropane-l-carboxylic acid
ADC	Arginine decarboxylase
ADP	5'-Adenosine diphosphate
AMP	5'-Adenosine monophosphate
ANOVA	Analysis of variance
AOA	Aminoxyacetic acid
AOAA	Aminoxyacetic acid
AOPP	$\alpha$ -Aminoxy- $\beta$ -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	$\alpha$ -difluoromethylarginine
DFMO	$\alpha$ -difluoromethylornithine
DCHA	Dicyclohexylammonium sulfate
DHA	Dehydroascorbic acid
dSAM	Decarboxylated SAM
DW	Dry weight
E	Embryogenic
EC or ec	Embryogenic callus
EDTA	Ethylenediaminetetraacetic acid
E <sub>1</sub>	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	Heptafluorobutrylimidazole
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
MGBC	Methylglyoxal bis-guanyl hydrazone
MS	Murashige and Skoog medium
NAA	Naphthalene acetic acid
NAD <sup>+</sup>	Nicotinamide adenine dinucleotide (oxidized)
NADP <sup>+</sup>	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 <sub>i</sub>	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 RECENT PUBLICATIONS  
( NOVEMBER, 89- MARCH, 90 )

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