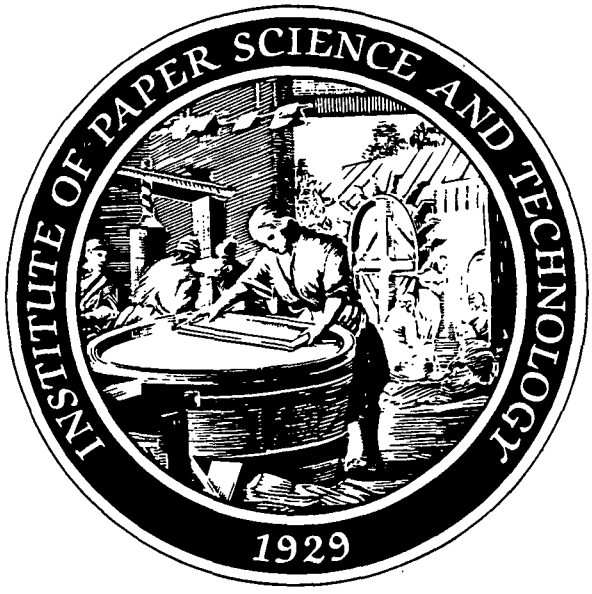


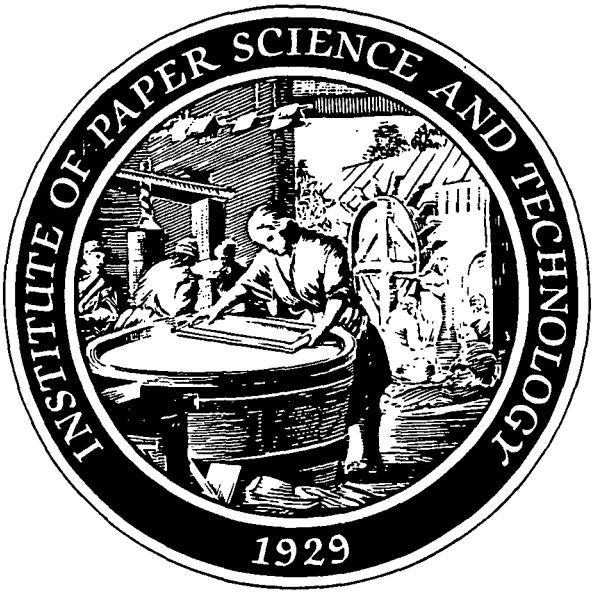
THE INSTITUTE OF PAPER SCIENCE AND TECHNOLOGY, ATLANTA, GEORGIA

STATUS REPORT AND HANDOUTS

TO THE

FOREST GENETICS PROJECT ADVISORY COMMITTEE

OCTOBER 25, 1989



THE INSTITUTE OF PAPER SCIENCE AND TECHNOLOGY, ATLANTA, GEORGIA

STATUS REPORT AND HANDOUTS

TO THE

FOREST GENETICS PROJECT ADVISORY COMMITTEE

OCTOBER 25, 1989

AGENDA

FOREST GENETICS PROJECT ADVISORY COMMITTEE

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

Wednesday, October 25, 1989

8:30 a.m.	Welcome and Introductions	Dinus
8:40	Relocation/Reorganization Update	Yeske
9:00	Overview and PAC Recommendations	Dinus
9:15	Status of Cultures	Ozturk
9:45	Germination: Events and Timing, Norway Spruce Zygotic Embryos	Webb
10:10	Coffee Break	
	Somatic Embryo Development/Maturation	
10:30	Norway Spruce	Nagmani
11:00	Loblolly Pine	Uddin
11:30	Summary and Near-Term Plans	Dinus
NOON	Lunch and Tour of IPST	
1:30 p.m.	Discussion/Deliberations	Committee
3:00	Coffee Break	
3:20	Discussion/Deliberations	Committee
4:00	Closing Remarks	Malcolm, Dinus, and Leach
4:30	Adjournment	

NEXT MEETING: Late March, 1990

FOREST GENETICS

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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)[a]

-1 = time of initiation or treatment identification

2 = line or genetic source, e.g., seedling No. 2

E = Immature embryo; explant type (only used if cell line derived from more than one explant within a research plan).

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

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

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

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

CONE SOURCES - 1988

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

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

STATISTICS

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

OVERVIEW

BACKGROUND

RELOCATION

- RAT
- EARLY MOVE
- STATUS OF LABS, OFFICES, & GREENHOUSE
- ON-CAMPUS BUILDING

COOPERATIVE INTERACTIONS

- CONTINUING
- NEW, CULTURES/COLLABORATION

STUDENTS, OLD & NEW

GROUNDRULES

COFFEE ANYTIME

BREAK AT 10:10; WILL ADJUST IF NEEDED

QUESTIONS AND IDEAS, PLEASE FEEL FREE

PRESENTATION TIMES INCLUDE TALK AND QUESTIONS

EXPECT TO STAY ON SCHEDULE

117 Pace Parkway
P.O. Box 875
Cantonment, Florida 32533
904 968-3010

Champion
Champion International Corporation

April 10, 1989

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

Dear Ron:

The Project Advisory Committee on Forest Genetics thanks you for the productive advisory committee meeting of March 29 and 30, 1989. Overall the committee was impressed with the progress that has been made on several fronts. The limited success with maturation of loblolly and Douglas-fir embryos was a very pleasant surprise; congratulations on a significant advancement of the project. 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 written report and the presentations were generally good in explaining the hypotheses, execution, and conclusions of experiments. While there was discussion on the design of certain experiments, the team has progressed well in clarifying their reporting of the work to the PAC.

2. Everyone agreed the process diagrams showing the success at different steps for different species were much improved. They make it much clearer to the PAC how the project is progressing. Some refinements suggested were to only count the percentage of "well-formed" embryos which mature, and to express maturation in terms of embryos/gram of callus instead of a percentage of all embryos. When such changes are made on future diagrams, please make a point of them to the PAC so direct comparisons won't be made to numbers on previous diagrams.

3. Thanks for having reprints of recent publications available as the PAC requested. The PAC encourages continued publication of the work in refereed journals. It is especially important in the case of scientists who will be retiring that their results be submitted for publication or well summarized before they leave the Institute.

KEY GOALS

1. The PAC appreciates your efforts in developing key goals; however, some changes to your list are necessary. Key goals were defined as significant objectives to be achieved in the near-term (two to three years). They are accomplishments that have to be made in that time if the project is to proceed at a reasonable pace. Conversely, they could be viewed as points at which the project might be stopped or changed significantly if they cannot be achieved in that time.

   Your suggestion to form a sub-committee to work on the key goals is a good one. Some needed characteristics of the key goals are:

   - relatively few (2-4) goals
   - should reasonably be expected to be achieved within a 2-3 year period
   - should be quantitatively expressed as much as possible
   - should be true "keys" to progress of the project, as defined above

   Key goals will change with time as (hopefully) the current goals are achieved and new ones are set. Your list of key goals is a good place to start; however, it is too ambitious for a 2-3 year period. You and the sub-committee should focus on just a few of these, and agree on some numbers representing levels of success to attach to them. Westvaco has volunteered Mike Becwar's knowledge in this area to help write or comment on the key goals.

2. The purpose of the key goals is to better help the PAC and the research team evaluate progress. The PAC does not intend to use them as threats or absolutes in terms of expectations of the project, and the failure to achieve them would not automatically mean a change in the project. If not achieved, the PAC would want to consider what has been attempted, and if additional effort appears warranted.

3. Certainly the PAC does not mean that the key goals should be focused on to the exclusion of everything else. Different steps of somatic embryogenesis will become key goals as current goals are accomplished, with work in progress before they become the new key goals. And as always, the PAC encourages limited pioneering and novel efforts related to the project but away from the main-line program.

CONIFER CRITICAL ISSUES

1. The discussion on the quality of work boiled down to two concerns:

   - lack of proper controls in some experiments (specifically in effects of light on germination and carbohydrate source/ABA on maturation)
   - experiments not being designed to get at more fundamental understandings

   There was considerable variation among the PAC members as to the seriousness of these problems. While some PAC members will disagree, I heard a general consensus that the work being done is of good quality and that the recent progress on maturation was especially positive, but that there is room for improvement in experimental design. Part of the problem may be poor or incomplete communication to the PAC on the basis for the design of experiments. The PAC recommends that:

   - design of experiments be critically reviewed among the project scientists before work begins
   - rationale for experimental treatments is made clear to the PAC

2. Continued factorial experiments to examine glucose/ABA levels for loblolly maturation beyond those tested are a natural extension of the work done to date. Also, try Norway spruce to how it responds. The specificity of glucose as the carbohydrate source should be tested on additional callus lines, with the inclusion of sucrose as a "control" carbohydrate.

3. Experiments should seek to answer why only certain levels of these factors appear effective. Also, why (or are) conditions different for maturation of loblolly pine and Douglas-fir?

4. Work with triglycerides remains interesting, especially the depressed levels seen in somatic as compared to zygotic embryos. This looks like a good topic of further biochemical investigations, and possibly to help assay cultures for effects that are not readily visible. Also, activated charcoal decreased the level of trigylcerides in loblolly pine somatic embryos, yet the activated charcoal treatment was apparently a critical step in the somatic embryo development protocol. Further work is needed to clarify this apparent contradiction.

5. Terminology related to embryo development was confusing; it was suggested the research team look at the terminology proposed by Hakman and von Arnold (1988) relating to embryo development stage. Most important is that the terminology be consistent throughout presentations and publications from the research team.

6. The old data for LM medium, which was developed from analyses of developing zygotic embryos, would be good to look at when considering media modifications.

7. The importance of pH in cultures has hopefully not been overlooked, and should be controlled or monitored as appropriate (or practical) for different experiments.

HARDWOOD CRITICAL ISSUES

1. The Ad Hoc committee and you are commended for narrowing the possibilities for species to work on and research topics. However, there was some confusion over exactly what was being proposed. The PAC decided emphasis should be on developing in-house expertise in genetic transformations. Although this might be focused initially on herbicide tolerance, the knowledge gained would be broadly applicable to other traits. The PAC realizes the first step may be the development of a somatic embryogenesis system for the species chosen. The research team should also realize the purpose of a somatic embryogenesis system would be to regenerate transformed cells into plants, not to perfect mass propagation.

2. Along with the possibility of government grants, IPC funds for hardwood transformation might be leveraged with special contributions from member companies, and/or from herbicide manufacturers. These should be explored.

3. Goals for this project should be set realistically in light of the relatively low funding level.

PERSONNEL/ADMINISTRATION

1. The PAC welcomes the arrival of David Webb and looks forward to his involvement with the project. The PAC was pleased that you are assessing your research needs in regard to the several personnel replacements that will be necessary with the move to Atlanta. A biochemist/physiologist would seem essential since we will be losing both Morris and Russ.

2. Everyone realizes the move and personnel turnover will be extremely disruptive during the next six months. The emphasis should be on keeping experiments/cultures viable during the transition.

3. The patenting of the process which led to the development of pre-cotyledonary and cotyledonary embryos was suggested by the PAC. Bob Lazar volunteered Union Camp's assistance in procuring a patent.

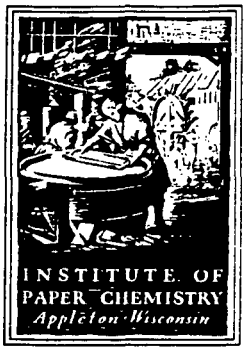
The PAC meeting was the last time many of us will visit Appleton. On behalf of the PAC, I wish to thank Morris for his excellent and amusing review of project 3223. We will miss him and the other members of the research team who will not be going to Atlanta. We wish them well, and look forward to continued association with the remaining researchers and the new ones who join the team in Georgia.

Sincerely,

Greg Leach

Gregory N. Leach
Research and Development Manager
Western Florida Region

cc. PAC Committee Members



THE INSTITUTE OF PAPER CHEMISTRY
Post Office Box 1039
Appleton, Wisconsin 54912
Phone: 414/734-9251
FAX: 414/738-3448
Telex: 469289

June 22, 1989

Mr. Gregory N. Leach
Research & Development Manager
Champion International Corporation
Southern Timberlands Division
P. O. Box 875
Cantonment, FL 32533

Dear Greg:

Thank you for the favorable response to our spring PAC meeting. We too were pleased with progress since the fall session, and were especially happy to share the exciting results on maturation of loblolly pine and Douglas-fir.

Most of our time and effort since spring has been devoted to maturation experiments, and especially to preparing for relocation. The potential for delays in construction prompted us to devise a set of alternative plans. To minimize disruption/downtime, we decided to design a temporary laboratory in another part of the building, and have prepared to move as early as possible. Thus, we will move essential equipment, supplies, and cultures during the last week of June or first week of July. Remaining items have been packed and will be shipped in late August or early September.

Effective July 10, we can be reached at:

Institute of Paper Science and Technology
575 14th Street NW
Atlanta, GA 30318

404-853-9500

REPORTS/MEETINGS

Your comments on reports and presentations are appreciated, and we are developing some new approaches to raise quality and clarity. We continue to emphasize publication, with efforts concentrated on publishing recent results as well as on publishing or documenting work performed by departing employees. Several of us will give papers at conferences this summer and fall, despite heavy involvement in relocation planning and implementation.

Concerning process diagrams, we all agree that numbers of "well-formed, mature embryos" per gram of callus is the best expression. We are working on a clear definition of "well-formed and mature," and are developing a standard key to ensure consistency among observers, presentations, and meetings.

KEY GOALS

Need for and importance of key goals is understood. We welcome and will exploit the opportunity to work with a sub-committee of selected Institute, company, and university members. Working together, we will construct two to four realistic goals that are critical to progress, can be expressed more or less quantitatively, are likely to be achieved in two or three years, and can be used to judge the value of continued work.

Please be reminded, however, that establishing state-of-the-art frequencies, and predicting likely improvements will be difficult. Developments in this area often occur in "fits and starts." We must all understand therefore that the goals should be flexible, that they should not be regarded as "absolutes," that midcourse corrections may prove necessary, and that some "exploratory, novel, or pioneering" efforts will be encouraged.

CONIFER CRITICAL ISSUES

We too have been and are concerned about quality of experimental design, interpretation, and explanation. Room for improvement clearly exists. As a result, a more strenuous and demanding system for planning and review has been developed and will be implemented by staff members moving to Atlanta.

Additional work on carbohydrate/ABA interactions is underway with target and model species. An effort is being made to include more callus lines, and to gather information on why certain sources and levels are more effective than others.

The issue of trigylcerides, embryo maturation, and activated charcoal is being addressed. Means must also be found to increase storage reserves well above levels noted to date. Indeed, we will seek, in the near future, to evaluate the entire protocol against those from other laboratories and verify the utility of individual steps. We suspect that considerable simplification and streamlining is possible.

As mentioned above, we are working on a standard key for embryo developmental stages. More consistent terminology will benefit all of us. In addition, Morris and others are re-examining older data from analyses of zygotic embryos and from experiments where pH was monitored/controlled.

HARDWOOD CRITICAL ISSUES

Despite some confusion, we all seem to agree that the Hardwood Project should concentrate on genetic transformation, and that an embryogenic system may be a convenient vehicle for regenerating transformed plants and/or somaclonal variants. Project efforts and hiring will therefore proceed along these lines. We will also seek additional funding from specific members and other sources.

PERSONNEL/ADMINISTRATION

Our newest employees are indeed doing well, and we look forward to working with them and others added in Atlanta. Position descriptions have been developed around our projected needs, and we have identified several prospective candidates. Advertising and actual interviews will begin in the near future.

Loss of personnel and moving undoubtedly will cause some slowing, if not disruption, of research. We are working to minimize downtime by moving early, and quickly re-establishing basic functions. In addition, we are forwarding cultures to member companies for insurance, and have divided our stock cultures into two separate lots. A caretaker and one lot will remain in Appleton until we are satisfied with our facilities and growth of the second lot.

The offer to help with patenting the maturation protocol was accepted. We assembled a Record of Invention and forwarded it plus supporting documentation to Union Camp attorneys. Preliminary readings were positive, but Counsel recommended and is seeking a second opinion from a more specialized law firm. We are anxiously awaiting the outcome, and will keep you apprised of developments.

All will miss those employees who have decided to leave, and the several PAC members who will rotate off the committee. We wish them well, and look forward to interaction with the remaining veterans as well as with future employees and members. Many thanks for your strong and continuing support.

Sincerely,

Ronald J. Dinus
Director
Forest Biology Division

RJD/jmm
Copies to: PAC Members

PAC RECOMMENDATIONS

ISSUE	ACTION OR PLAN
PROCESS DIAGRAMS	
MATURE EMBRYOS	EMBRYO STAGE KEY
# / GRAM	WILL USE, OR WILL NOTE OTHERWISE
KEY GOALS	WILL DISCUSS LATER
SOFTWOOD CRITICAL ISSUES	
DESIGN, RATIONALE, & INTERPRETATION	IMPLEMENTED REVIEW & APPROVAL PROCESS
CHO WORK, MATURATION	SOME WORK CONFIRMING & EXTENDING; MORE COMING
LIPIDS	COMPLETED/STOPPED, & SUMMARIZED
EMBRYO STAGES, TERMINOLOGY	ASSAY CULTURES, EMBRYO STAGE KEY
EMBRYO/MEDIA COMPOSITION	STARTED; INCOMPLETE
HARDWOOD CRITICAL ISSUES	
FOCUS	TRANSFORM & PROPAGATE
LEVERAGE	WILL SEEK FUNDS

PAC RECOMMENDATIONS, CONT'D

PERSONNEL/ADMINISTRATION

RECRUITING/HIRING	TEMP, REPLACE, & NEW
RELOCATION/DISRUPTION	EARLY MOVE & SLOWDOWN; INSURANCE CULTURES; PROBLEMS/PROGNOSIS
PATENT	SEARCH COMPLETED; HAVE APPROVAL, WILL PREPARE APPLN

THE INSTITUTE OF PAPER SCIENCE AND TECHNOLOGY

Atlanta, Georgia

Status Report

to the

FOREST GENETICS

PROJECT ADVISORY COMMITTEE

Project 3223-00

MASS CLONAL PROPAGATION OF IMPROVED SOFTWOODS

Project 3223-02

BIOCHEMISTRY OF CLONAL PROPAGATION

Project 3223-03

MASS CLONAL PROPAGATION OF GENETICALLY IMPROVED/ENGINEERED HARDWOODS

October 25, 1989

PROJECT SUMMARY FORM

DATE: October 2, 1989

PROJECT NO. 3223-00 - Mass Clonal Propagation of Improved Conifers

PROJECT STAFF: Dinus, Nagmani, Uddin, Webb

PROJECT OBJECTIVE/GOAL:

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

PROJECT RATIONALE:

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

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

CURRENT FISCAL YEAR BUDGET: $525,000

SUMMARY OF RESULTS SINCE LAST REPORT: (March 1989 - September 1989)

Past research on conifer cell and tissue culture systems has brought somatic embryogenesis, 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 seed and from tissues of newly germinated seedlings.

As per earlier plans, work in Norway spruce focused on somatic embryo development and maturation. Several trials executed before relocation confirm that addition of glutamine in lieu of ammonium nitrate and higher levels of abscisic acid foster maturation. Maturity and quality were further characterized by tracking germination and early growth.

To better gauge somatic embryo maturity, tests were conducted to document events associated with germination of Norway spruce zygotic embryos on standard germination medium. Structures at various times during germination were also collected for subsequent evaluation of anatomy and storage compounds. Zygotic embryos germinated rapidly, much more rapidly than noted to date for somatic embryos. Within 12 days, over 85 percent showed signs of germination; roughly 60 percent had visible radicles and 25 percent or so were swollen at the radicle end. Results were essentially equal in light and dark environments. In some cases, swollen tissues turned brown and produced a colored exudate similar to that often found on germinating somatic embryos. Radicle growth appeared inhibited or abnormal when browning and exudate were prominent. Microscopic examination of swellings and exudate are underway. Extant and future results should lead to improved definitions of somatic embryo quality and better protocols for germination.

Large definitive tests of conversion to seedlings have been deferred until all facilities and equipment are ready for use in Atlanta. "Somatic seedlings" from past experiments have been moved to Atlanta and are being maintained in soil.

Some additional embryogenic cultures were obtained from cotyledons and hypocotyls of "somatic seedlings". Initiation frequencies were low, but maturation frequencies equaled those of cultures from which the seedlings originated.

Loblolly pine embryogenic cultures initiated in earlier years were used to evaluate additional maturation treatments. The aim was to extend earlier work with carbohydrates and abscisic acid, and thereby set the stage for more definitive work after relocation. Maturation frequencies were somewhat increased over those observed earlier, mainly in response to carbohydrate source and sequence. Cultures are now being expanded for a large factorial experiment retesting the best treatments noted to date. In addition, a search of "prior art" yielded favorable results and approval has been secured to apply for a maturation protocol patent.

Some exploratory work on maturation of Douglas-fir somatic embryos was executed before relocation. Results were encouraging, and best treatments will be re-examined in larger trials once relocation is complete. Efforts to initiate new cultures using immature embryos from the Southern Hemisphere, stored seed, and pre-meiotic tissues of developing cones were inconclusive. Another attempt using immature embryos is in progress.

Preparing for relocation provided opportunities to review and revamp laboratory organization. After completing comprehensive inventories, culture numbers, for example, were adjusted to levels realistic for relocation. Two sets of key cultures were sent to Westvaco and Union Camp for insurance and for later recovery. The remainder were later moved to Atlanta, again as two separate sets to minimize loss or damage. Databases derived from inventories enabled us to implement improved or new procedures in Atlanta.

A centralized stock culture system was established, a streamlined coding procedure was implemented, and all media, culture, and subculture records were computerized. Also nearing completion are an archive detailing origin, treatment, and composition of all cultures, and a standardized key to zygotic and somatic embryo stages. Such actions should improve research quality and efficiency.

SHORT TERM GOALS:

Goals for Remainder of FY 89-90

1) Replace personnel not relocating or leaving sincere location.

2) Transfer remaining equipment and supplies to Atlanta.

3) Complete laboratories, offices, and greenhouse; finish design work on new on-campus facility.

4) Raise initiation frequency in Douglas-fir; obtain additional embryogenic cultures.

5) Improve protocols for increasing maturation frequencies, and raising efficiency of conversion to seedlings; extend best treatments from model to target species.

6) Continue documenting 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.

7) Explore means for initiation with explants from more mature plant materials.

8) Provide for prompt presentation/publication of findings.

PROJECT SUMMARY FORM

DATE: October 2, 1989

PROJECT NO. 3223-02 - Biochemistry of Clonal Propagation

PROJECT STAFF: Vacant (2)

PROJECT OBJECTIVE/GOAL:

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

PROJECT RATIONALE:

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

CURRENT FISCAL YEAR BUDGET: $150,000

SUMMARY OF RESULTS SINCE LAST REPORT: (March 1989 - September 1989)

Past Institute efforts have 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 also yielded useful data on differences between embryogenic and nonembryogenic cultures, and some knowledge of factors affecting the process. Such differences and associated markers can be 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, facilitate conversion to seedlings, and evaluate seedling performance and fidelity.

Collection of baseline data on biochemical and molecular characteristics of embryogenesis in zygotic and somatic systems continued until preparations for relocation began in earnest. In keeping with earlier plans, work in progress mainly concerned promoting embryo development rather than merely tracking changes accompanying development. Results from such work on triglycerides, polar lipids, proteins, and peroxidase isozymes generally confirmed and complemented earlier findings. Much effort was, therefore, devoted to summarizing results from the most recent experiments and incorporating them along with earlier findings into publications and internal documents. Considerable effort was also devoted to inventorying equipment, supplies, and samples, packing them, and otherwise preparing for relocation. All involved personnel chose not to relocate.

Work continued on adapting and applying techniques for extracting, purifying, derivatizing, and quantifying abscisic acid from developing embryos. Assays are being done by a cooperating scientist at the University of Cincinnati. In addition, a similar arrangement, involving use of monoclonal antibodies, is being negotiated with a cooperator at the University of Natal, Republic of South Africa. These efforts, if successful, may yield techniques sufficiently sensitive to obtain accurate estimates of quantities and trends.

Recruiting/hiring is underway, and a number of likely candidates have been identified for each of the four vacancies occasioned by relocation to Atlanta.

SHORT TERM GOALS:

Goals for Remainder of FY 89-90

1) Complete recruiting and hiring.

2) Transfer remaining equipment and supplies to Atlanta.

3) Complete renovation of temporary laboratories, and design of on-campus facility.

4) Reactivate work on similarities/differences of zygotic and somatic embryos, with emphasis on using substrates and inhibitors to stimulate maturation.

5) Seek cooperators to execute selected aspects on needed work at least until new hires are in place.

6) Continue cooperative efforts to quantify abscisic acid levels in developing embryos.

PROJECT SUMMARY FORM

DATE: October 2, 1989

PROJECT NO. 3223-03 - Mass Clonal Propagation of Improved Hardwoods

PROJECT STAFF: Dinus, Uddin

PROJECT OBJECTIVE/GOAL:

Overall - Develop reliable, low-cost 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.

CURRENT FISCAL YEAR BUDGET: $100,000

SUMMARY OF RESULTS SINCE LAST REPORT: (March 1989 - September 1989)

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 new project is aimed at developing technologies for transferring genes for herbicide tolerance into commercially important species, and for efficient mass propagation, testing, and release of genetically transformed plant materials. Similar outcomes may be sought more on an exploratory basis, via somaclonal variation/selection. Efforts during the interim period were concentrated on selecting research approaches required to develop the technologies, collecting suitable plant material, and establishing stable cultures.

Both diploid and haploid explants of cottonwood and aspen were obtained from Institute sources and cooperating organizations and used to establish cultures for subsequent stabilization, expansion, and manipulation.

Cottonwood proved difficult to culture. Sterilizing explants presented problems, but even more difficulty was encountered in stabilization and expansion. The most serious problem was excessive production of tannins, followed by gradual deterioration and eventual death. Numerous remedies were tried, with only a few giving positive, repeatable results. Stable cultures, however, have been obtained, with much help from cooperators at the University of Kentucky, Tuskegee University, University of Nebraska, University of Iowa, the US Forest Service, and SAPPI, Republic of South Africa. Three elite donor trees are represented, one by diploid & haploid culture, one by diploid cultures, and one by haploid cultures. These should provide suitable bases for work on gene transfer, and perhaps somaclonal variation/selection. Even so, we will attempt

to initiate cultures from dormant explants of other elite donors this winter. Arrangements are also being made to secure additional cultures from the University of Nebraska, Tuskegee University, and the US Forest Service.

Aspen shoot cultures, in contrast, were established and stabilized with relative ease. Thus, cultures for further work are available from two elite aspens and one triploid hybrid. In addition, cultures from past exploratory work on tetraploid aspen and native sweetgum have been transferred to and are being maintained in our Atlanta facility.

SHORT TERM GOALS:

Goals for Remainder of FY 89-90

1) Recruit and hire new scientist.

2) Expand existing cultures, and initiate/secure additional cultures.

3) Secure plant material for establishment of "clean" greenhouse populations.

4) Initiate research on gene transfer and expression, and propagation of transformed or novel materials.

5) Explore methods for accelerating conventional tree improvement by early testing and selection in culture, production of useful variants via somaclonal variation/selection and protoplast fusion.

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.

University of Cincinnati - Joint assay with Dr. J. Caruso of endogenous abscisic acid levels in embryogenic and nonembryogenic cultures and in developing zygotic and somatic embryos.

Tuskegee University - Supply of cottonwood cultures by Dr. C. Prakash for hardwood research.

University of Nebraska - Supply of cottonwood cultures by Dr. S. Ernst for hardwood research.

Joint research arrangements are also being sought or negotiated with Dr. P. Hofmann, University of Natal, Republic of South Africa; Dr. K. Eriksson, University of Georgia; Dr. S. Strauss, Oregon State University; Dr. D. Neale, US For. Serv., Berkeley, CA; Drs. J. Choi and J. Mathis, Georgia Tech; and Dr. C. Michler, US For. Serv., Rhinelander, WI.

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 ultrastructure 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 Logsden - 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 somatice embryogenic cultures of several conifer species in bioreactors." Advisors, M. R. Becwar and R. J. Dinus. Now on leave of absence for industrial experience; will return in January to start Ph.D.

In Progress

Daniel Bunker - Ph.D., "An investigation of the role of drying strategy in the structure of pigment-adhesive films." Advisor, T. E. Conners.

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

Note: Several members of incoming class have expressed an interest in working on related research. Final decisions will be known by November.

STATUS OF CULTURES

SONJA OZTURK

TRANSFER OF STOCK CULTURES FROM IPC TO IPST AND ESTABLISHMENT OF THE TISSUE CULTURE LABORATORY IN ATLANTA

SONJA OZTURK, DAVID T. WEBB & RONALD J. DINUS

MOVE GOALS

1] SUCCESSFULLY TRANSFER OUR KEY EXPERIMENTAL CULTURES

2] SET UP TEMPORARY LAB UNTIL OUR LAB IN THE IRF IS READY

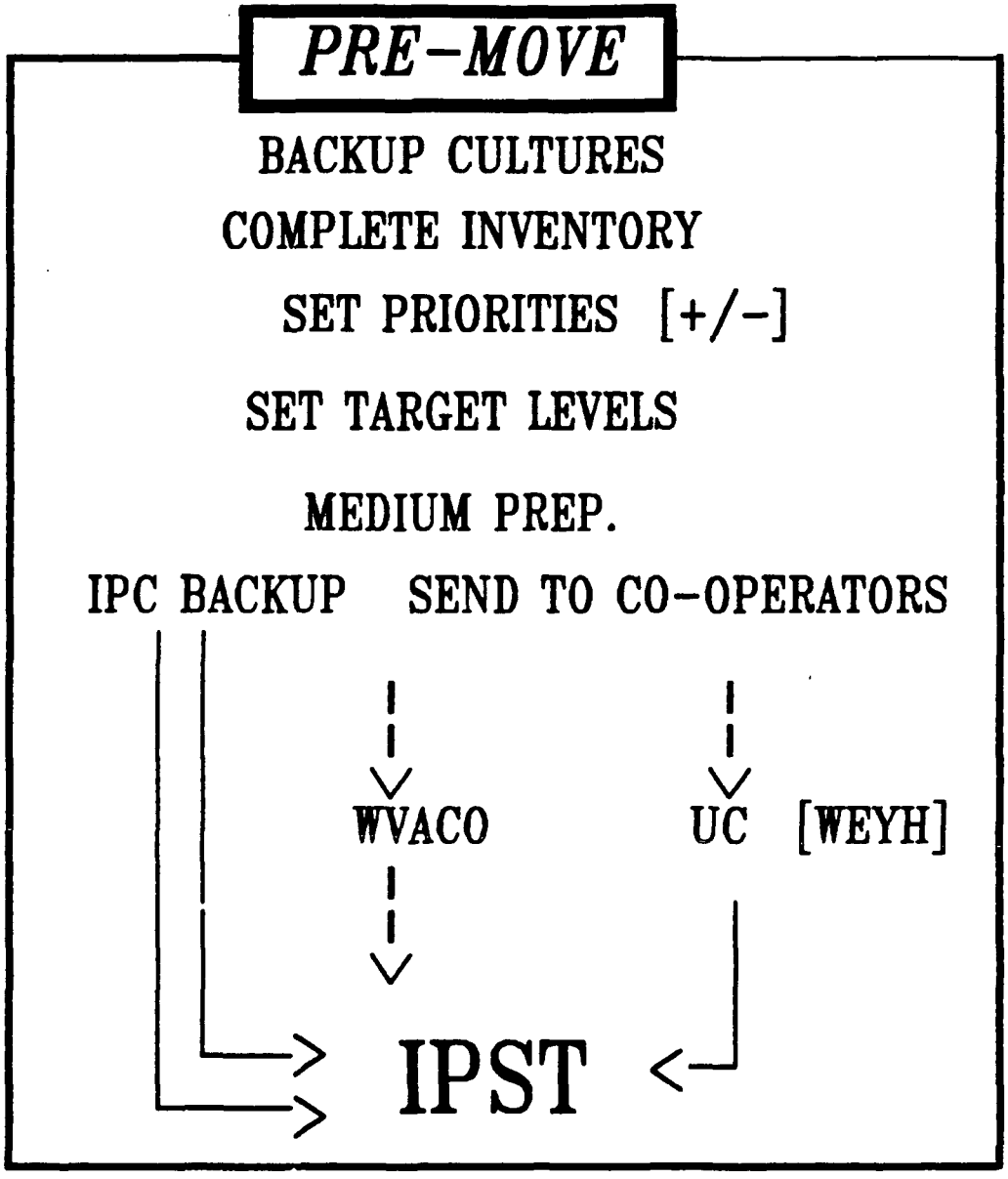
3] EVALUATE OLD LAB PROCEDURES AND IMPLEMENT NEW ONES WHERE IT APPEARED NECESSARY

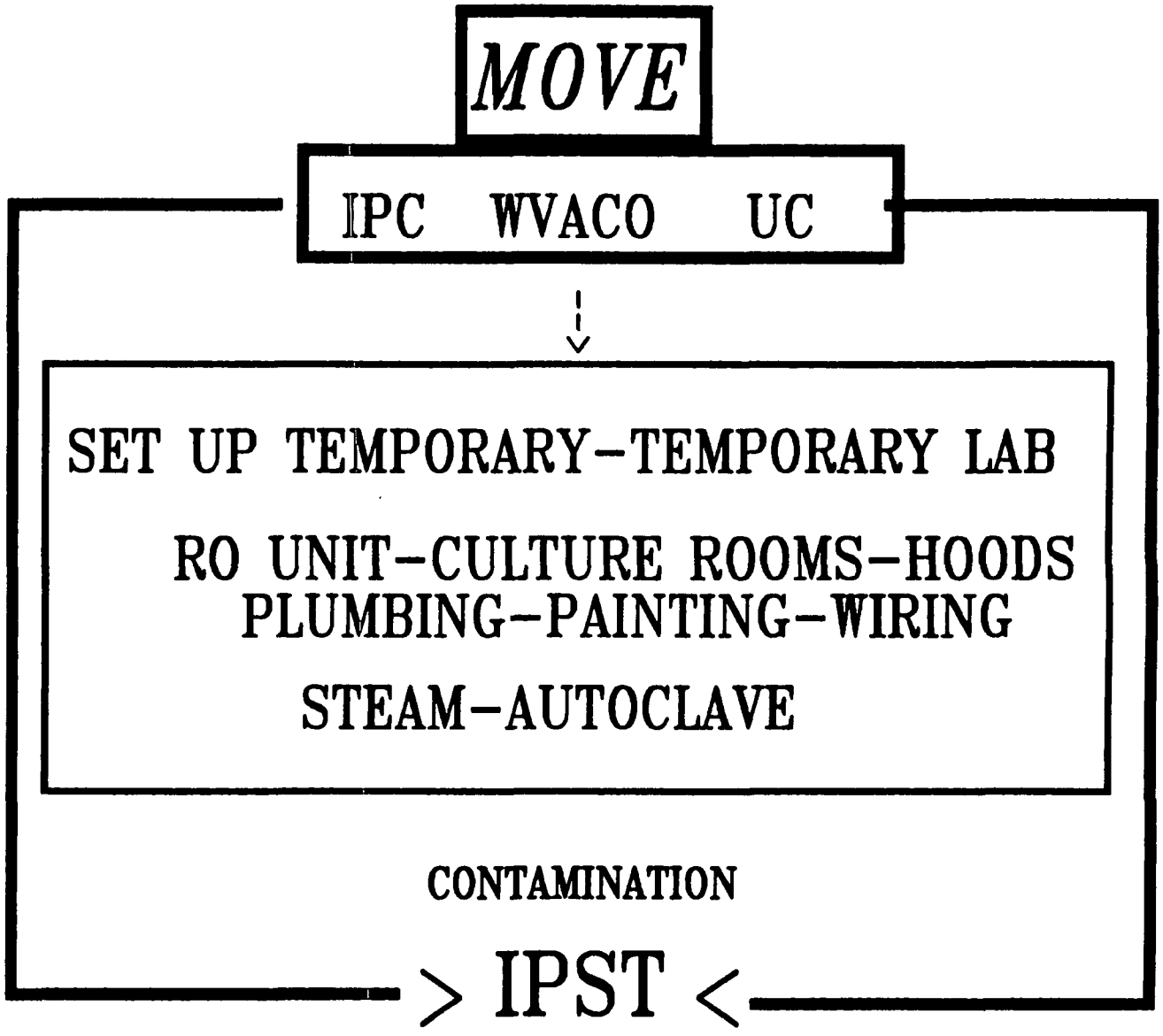
PHASES OF THE MOVE

PRE–MOVE

MOVE

POST–MOVE


PRE-MOVE
BACKUP CULTURES
COMPLETE INVENTORY
SET PRIORITIES [+/-]
SET TARGET LEVELS
MEDIUM PREP.
IPC BACKUP
SEND TO CO-OPERATORS
WVACO
UC [WEYH]
IPST


MOVE
IPC WVACO UC
SET UP TEMPORARY-TEMPORARY LAB
RO UNIT-CULTURE ROOMS-HOODS
PLUMBING-PAINTING-WIRING
STEAM-AUTOCLAVE
CONTAMINATION
> IPST <

POST MOVE

LOGISTICS–CONSTRUCTION–CONTAMINATION

REVISED & NEW PROCEDURES

SIMPLIFIED CULTURE & MEDIA CODES

INTERNAL STOCK CULTURE BANK

INVENTORY–SUB.RECORD & COMPUTER BACK UP

CULTURE ARCHIVE

WEEKLY MEETINGS

RESEARCH PLANS

PLAN EXPERIMENTS

REVISE CULTURE TARGET LEVELS

SUMMARY

GOALS ACHIEVED

REVISED & NEW PROCEDURES INITIATED

GERMINATION: EVENTS & TIMING, NORWAY SPRUCE ZYGOTIC EMBRYOS

DAVID WEBB

DEFINING STAGES OF ZYGOTIC AND SOMATIC EMBRYOS

DAVID T. WEBB AND NAGMANI RANGASWAMY

STAGES OF ZYGOTIC EMBRYOGENESIS

1] PROVIDE A COHERENT BASIS FOR THE CLASSSIFICATION OF ZYGOTIC EMBRYOS FOR INITIATION EXPERIMENTS

2] PROVIDE AN IMPROVED THE FRAMEWORK FOR DETERMINING THE "QUALITY" OF SOMATIC EMBRYOS BASED ON A COMPARISON OF

A] EXTERNAL MORPHOLOGY

B] MEASUREMENTS OF TOTAL LENGT, COTYLEDON LENGTH & AXIS DIAMETER

C] FRESH & DRY WEIGHTS

D] ANATOMICAL & HISTOCHEMICAL [LIPID, PROTEIN & STARCH] TRAITS

E] TOTAL LIPIDS & PROTEINS

EMBRYO DEVELOPMENTAL STAGES

PRE-COTYLEDONARY EMBRYOS

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

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

STAGE 3: EMBRYOS WITH POINTED SHOOT APICAL MERISTEMS

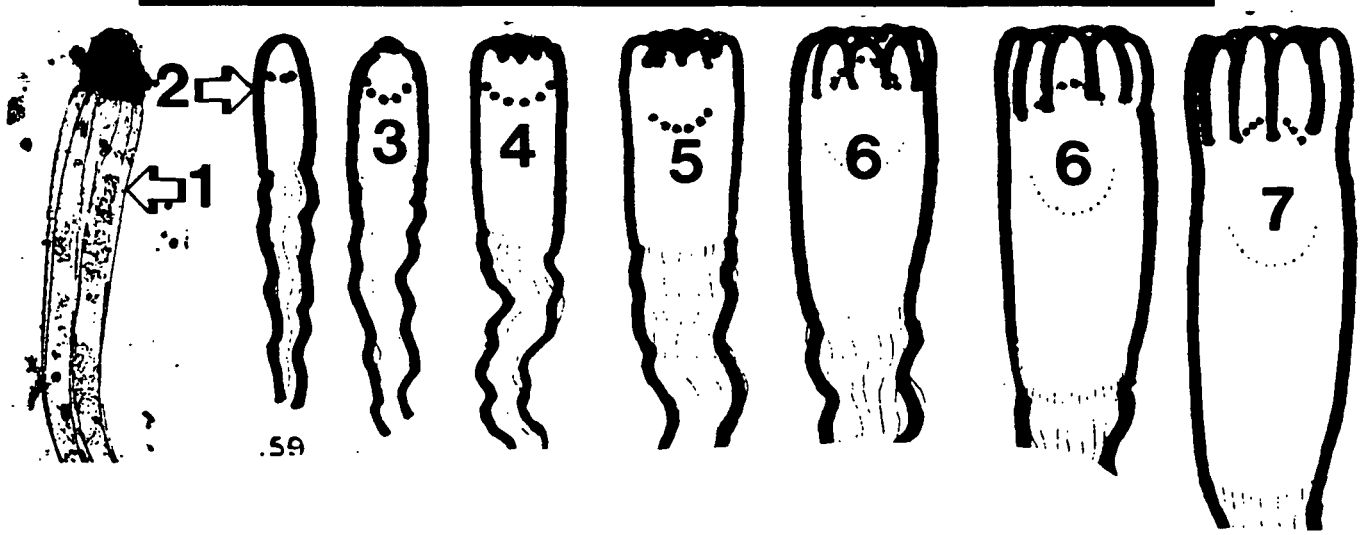
COTYLEDONARY EMBRYOS

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

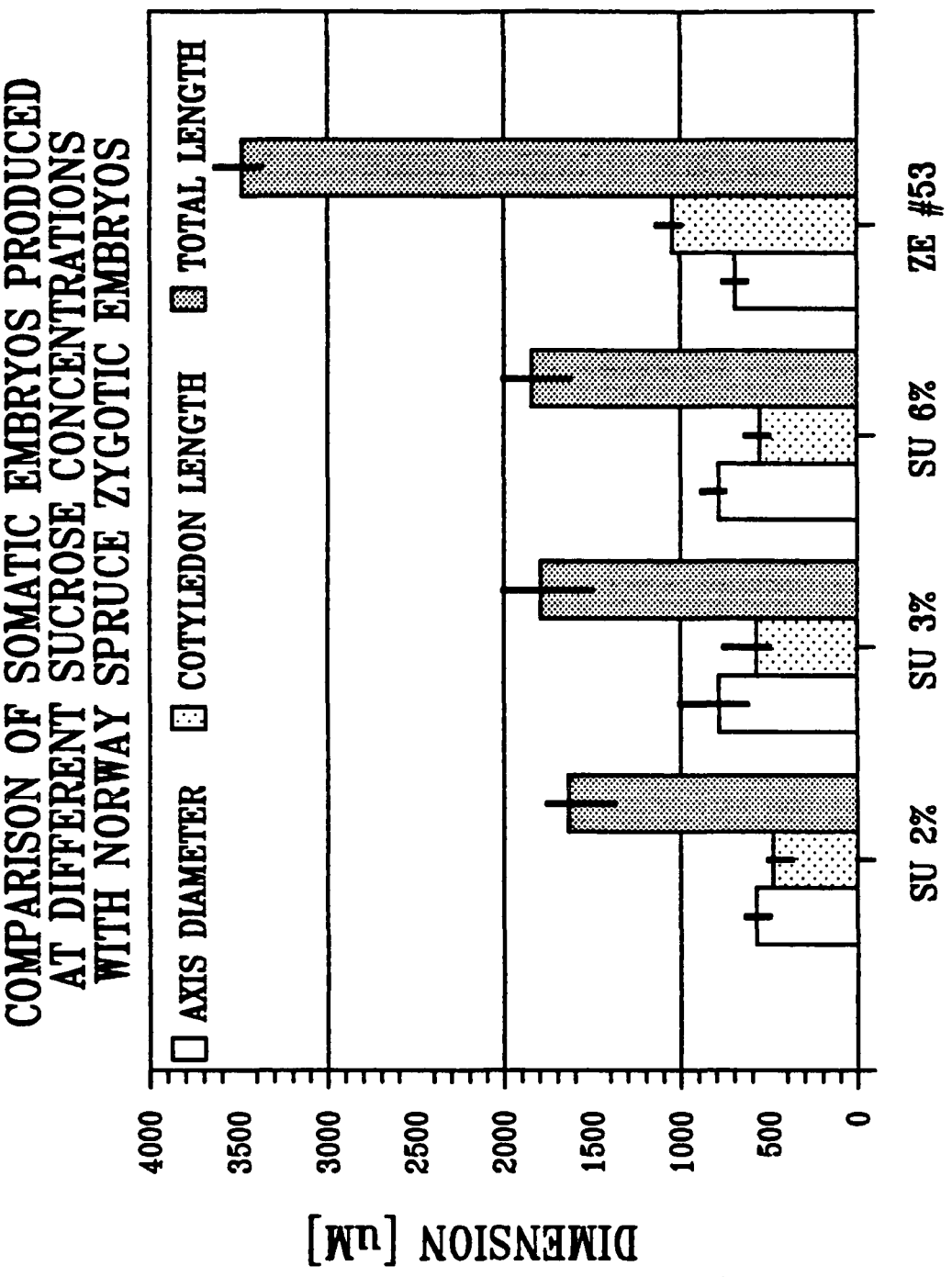
STAGE 6: ELONGATED COTYLEDONS OVERTOP THE SHOOT APEX BUT HAVE NOT CLOSED TO OBSCURE IT AS VIEWED FROM ABOVE

STAGE 7: ELONGATED COTYLEDON PRIMORDIA COMPLETELY OBSCURE THE SHOOT APEX



COMPARISON OF ZYGOTIC EMBRYOS OF NORWAY SPRUCE WITH SOMATIC EMBRYOS PRODUCED WITH DIFFERENT SUCROSE CONCENTRATIONS

DAVID T. WEBB AND RAFIQUE UDDIN


COMPARISON OF SOMATIC EMBRYOS PRODUCED
AT DIFFERENT SUCROSE CONCENTRATIONS
WITH NORWAY SPRUCE ZYGOTIC EMBRYOS
AXIS DIAMETER
COTYLEDON LENGTH
TOTAL LENGTH
DIMENSION [uM]
4000
3500
3000
2500
2000
1500
1000
500
0
SU 2%
SU 3%
SU 6%
ZE #53
CARBOHYDRATE SOURCE

GERNMINATION OF NORWAY SPRUCE ZYGOTIC EMBRYOS

DAVID T. WEBB

RATIONALE

1] GERMINATION OF SOMATIC EMBRYOS IS VARIABLE IN FREQUENCY & RANGES FROM 45 - 100%.

2] GERMINATION OF SOMATIC EMBRYOS IS SLOW AND PEAKS AT 38 DAYS.

3] LIGHT MAY INHIBIT GERMINATION DURING THE FIRST 10 DAYS OF CULTURE.

4] A BLACK EXUDATE OFTEN FORMS AT THE RADICLE END OF SOMATIC EMBRYOS & MAY INTERFERE WITH RADICLE PROTRUSION.

5] SOMATIC EMBRYOS APPEAR TO GERMINATE PRECOCIOUSLY IN THAT HYPOCOTYL AND COTYLEDON GROWTH OCCURRS BEFORE RADICLE EMERGENCE.

6] THE PRESENCE OF AN ORGANIZED & ACTIVE ROOT APICAL MERISTEM HAS NOT BEEN VERIFIED FOR "MATURE" GERMINATING SOMATIC EMBRYOS.

7] THE ANATOMICAL & HISTOCHEMICAL EVENTS OCCURRING DURING GERMINATION OF SOMATIC & ZYGOTIC EMBRYOS IS UNKNOWN.

8] STUDYING THE GERMINATION RESPONSE OF ZYGOTIC EMBRYOS WILL HELP TO SEPARATE PROBLEMS ARISING FROM THE CULTURE CONDITIONS VS THE DEVELOPMENTAL STATUS OF SOMATIC EMBRYOS.

GOALS

1] DETERMINE THE FREQUENCY & RATE OF GERMINATION FOR ZYGOTIC EMBRYOS ON MEDIUM USED FOR THE GERMINATION OF SOMATIC EMBRYOS

2] COMPARE GERMINATION BY ZYGOTIC EMBRYOS IN LIGHT & DARKNESS

3] UNDERSTAND THE ANATOMICAL & HISTOCHEMICAL CHANGES OCCURRING IN THE RADICLE REGION DURING GERMINATION

4] COMPARE THE ANATOMY AND HISTOCHEMISTRY OF ZYGOTIC & "MATURE" SOMATIC EMBRYOS

MATERIALS & METHODS

1] SEEDS OF NORWAY SPRUCE [LOT # 53] WERE HYDRATED AT 5 °C FOR 48 Hrs.

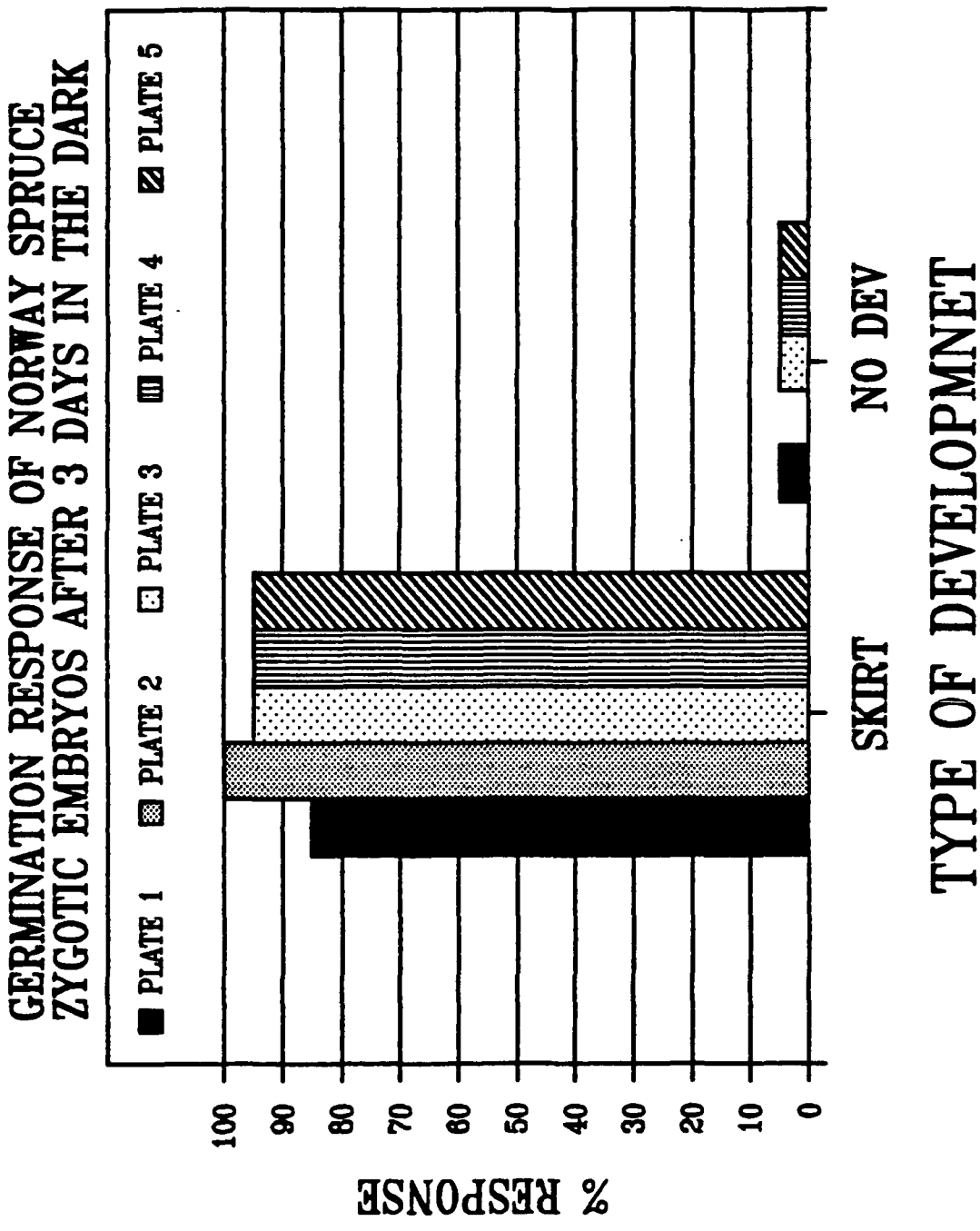
2] EMBRYOS WERE EXCISED AND CULTURED HORIZONTALLY IN PETRI PLATES ON 1/2 DCR 25/25 MEDIUM IN THE DARK OR LIGHT.

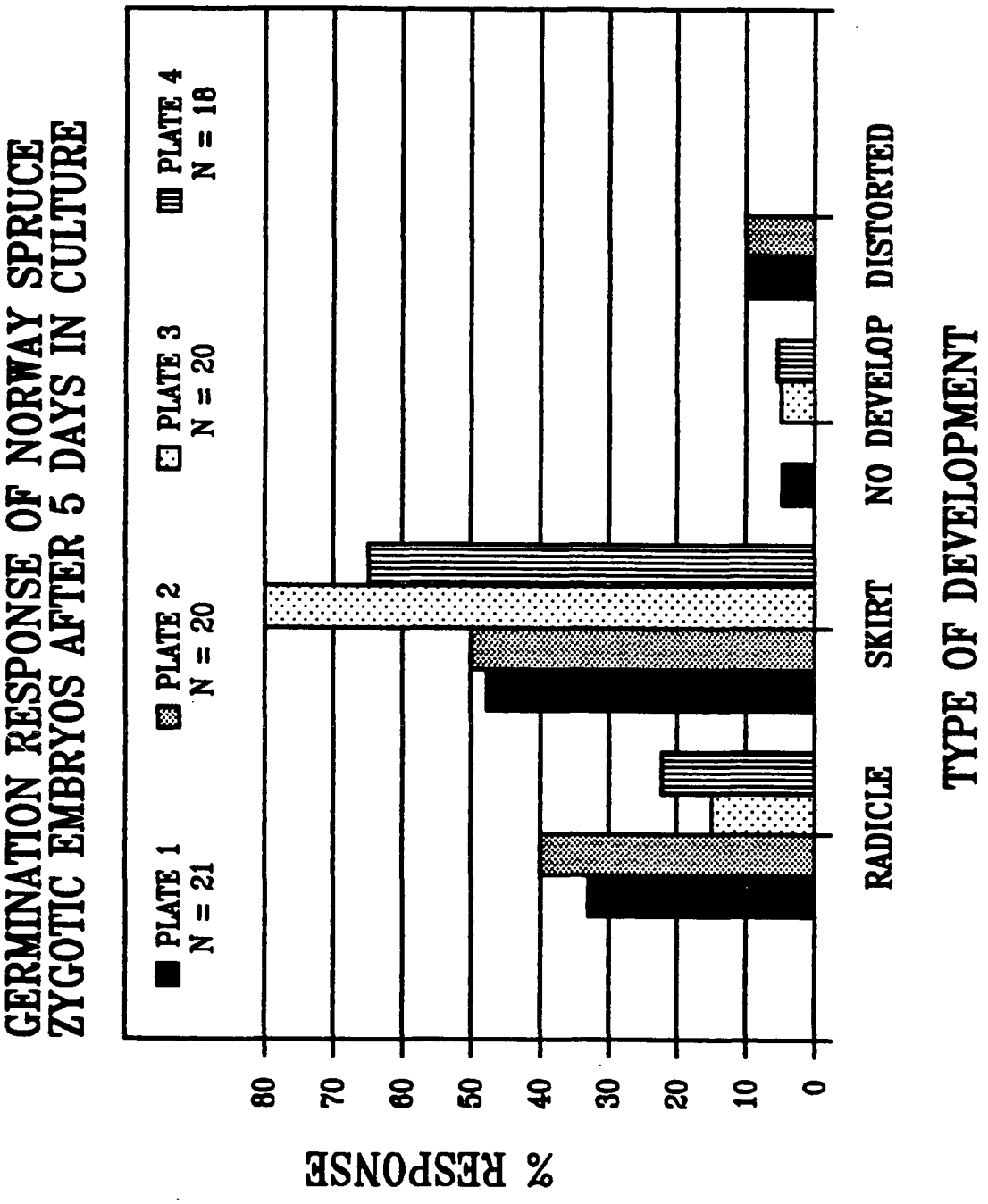
3] EACH PLATE CONTAINED 20 EMBRYOS & 4-6 PLATES WERE ANALYZED AT EACH SAMPLING DATE [3, 5, 10 & 12 DAYS].

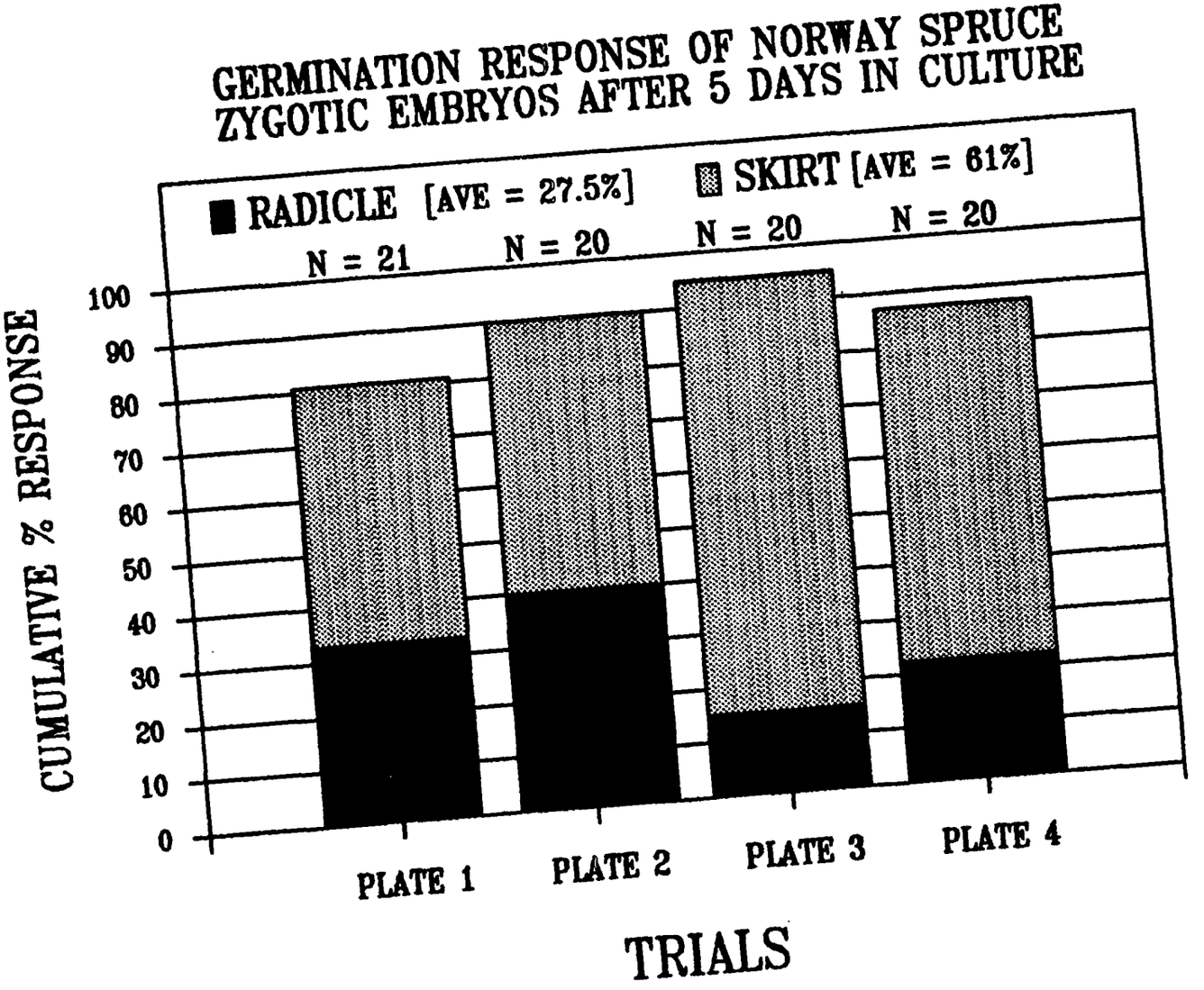
4] THE % OF EMBRYOS PRODUCING A RADICLE OR "SKIRT" WAS RECORDED AS WELL AS THE % OF HIGHLY DISTORTED EMBRYOS AND THOSE WHICH SHOWED NO EVIDENCE OF DEVELOPMENT.

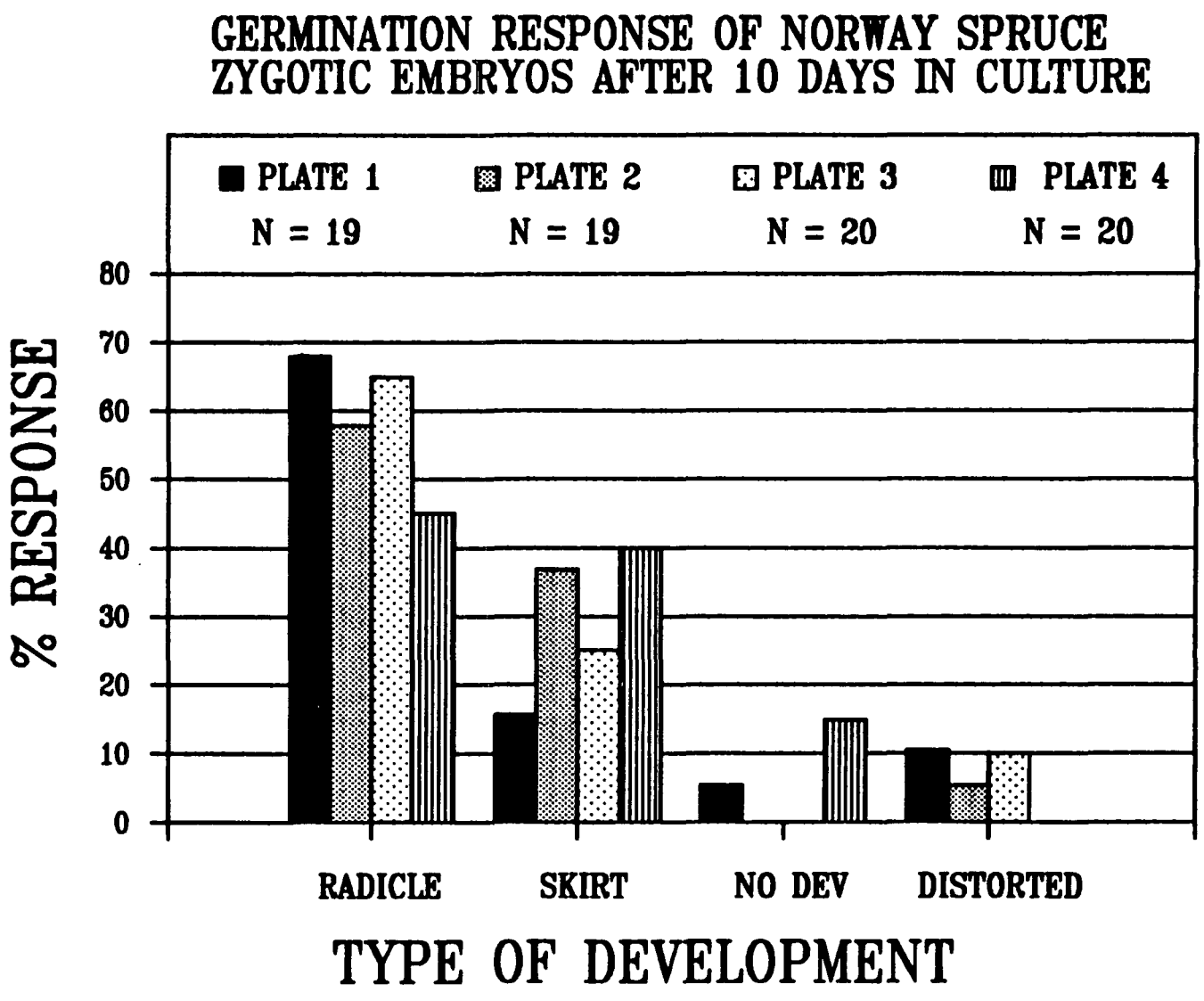
5] SAMPLES WERE FIXED & EMBEDDED IN RESIN OR PARAFIN FOR LATER ANATOMICAL & HISTOCHEMICAL ANALYSIS OF THE RADICLE & ROOT-SHOOT JUNCTION.

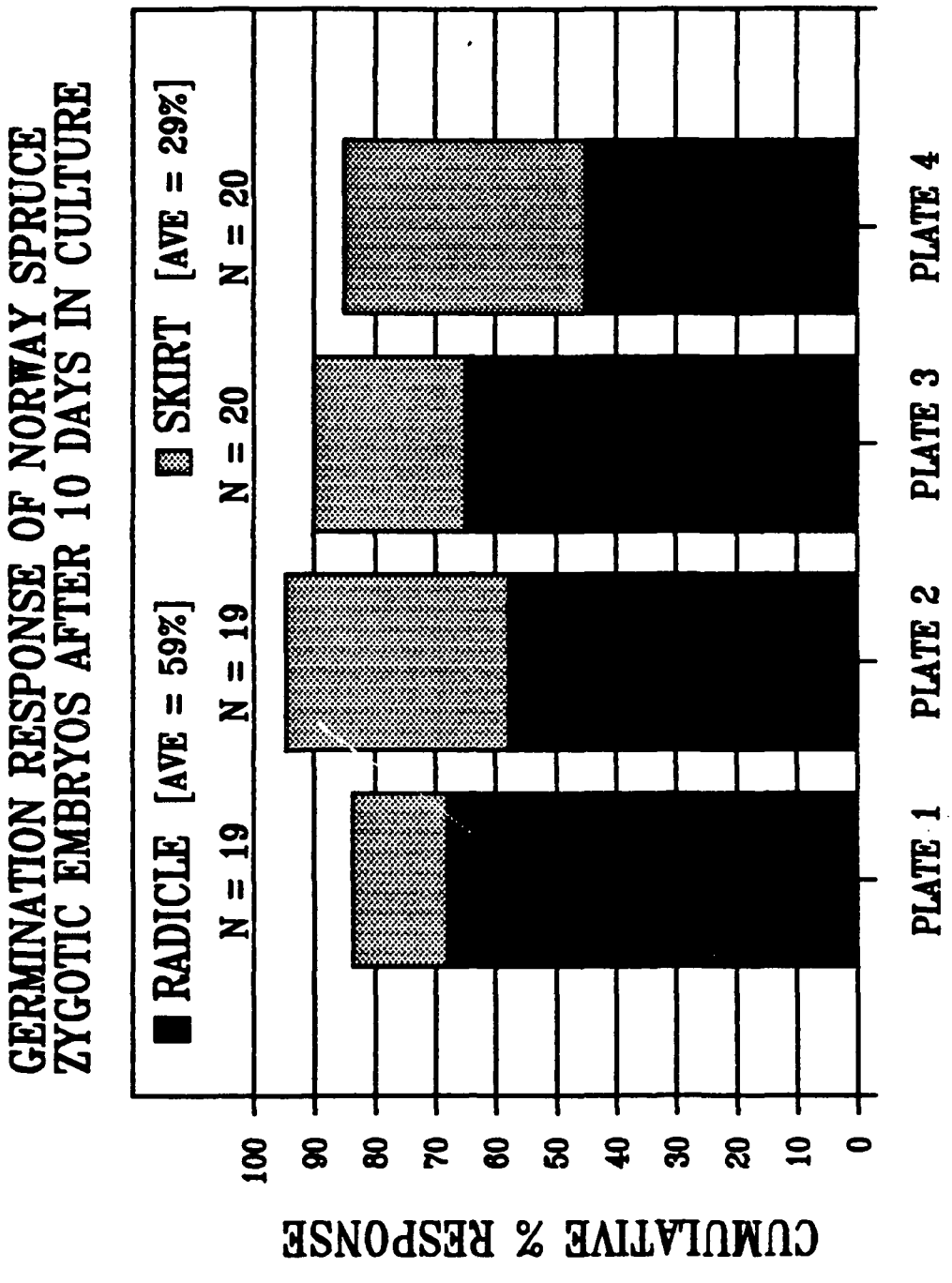
6] SAMPLES WERE ALSO TAKEN FOR TOTAL LIPID & PROTEIN ANALYSIS.

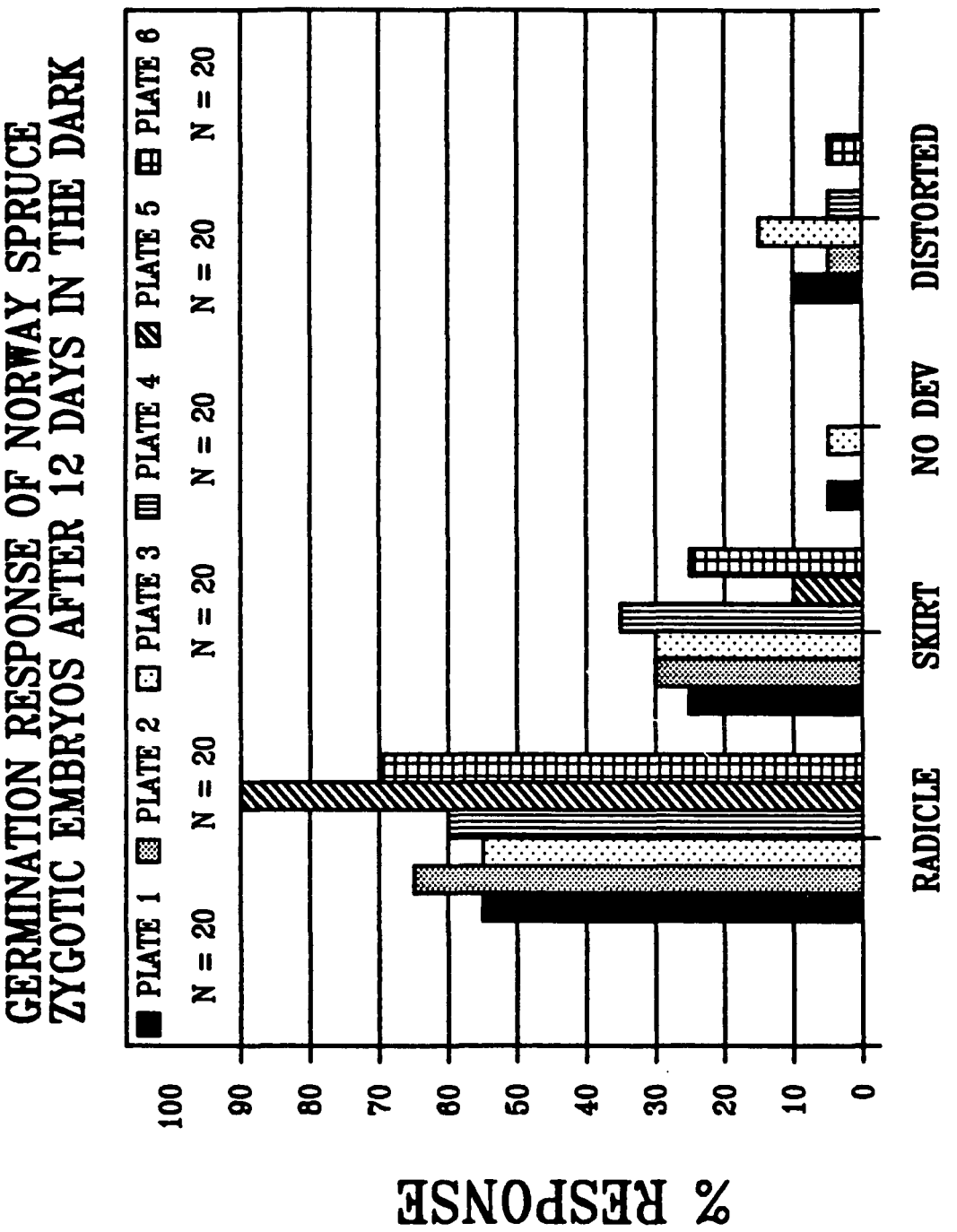

GERMINATION RESPONSE OF NORWAY SPRUCE
ZYGOTIC EMBRYOS AFTER 3 DAYS IN THE DARK
PLATE 1
PLATE 2
PLATE 3
PLATE 4
PLATE 5
100
90
80
70
60
50
40
30
20
10
0
% RESPONSE
SKIRT
NO DEV
TYPE OF DEVELOPMNET

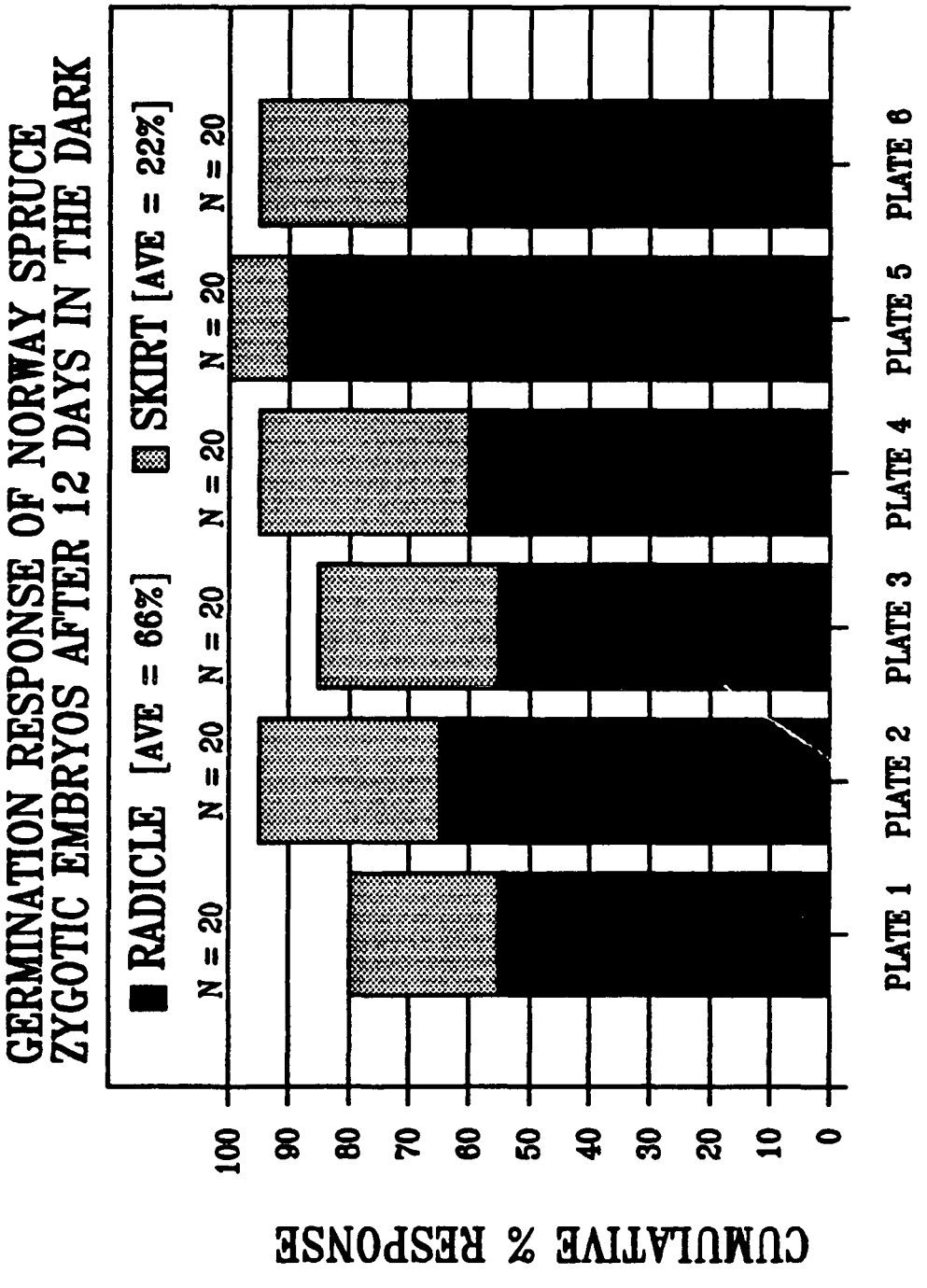

GERMINATION RESPONSE OF NORWAY SPRUCE
ZYGOTIC EMBRYOS AFTER 5 DAYS IN CULTURE
PLATE 1
N = 21
PLATE 2
N = 20
PLATE 3
N = 20
PLATE 4
N = 18
% RESPONSE
80
70
60
50
40
30
20
10
0
RADICLE
SKIRT
NO DEVELOP
DISTORTED
TYPE OF DEVELOPMENT

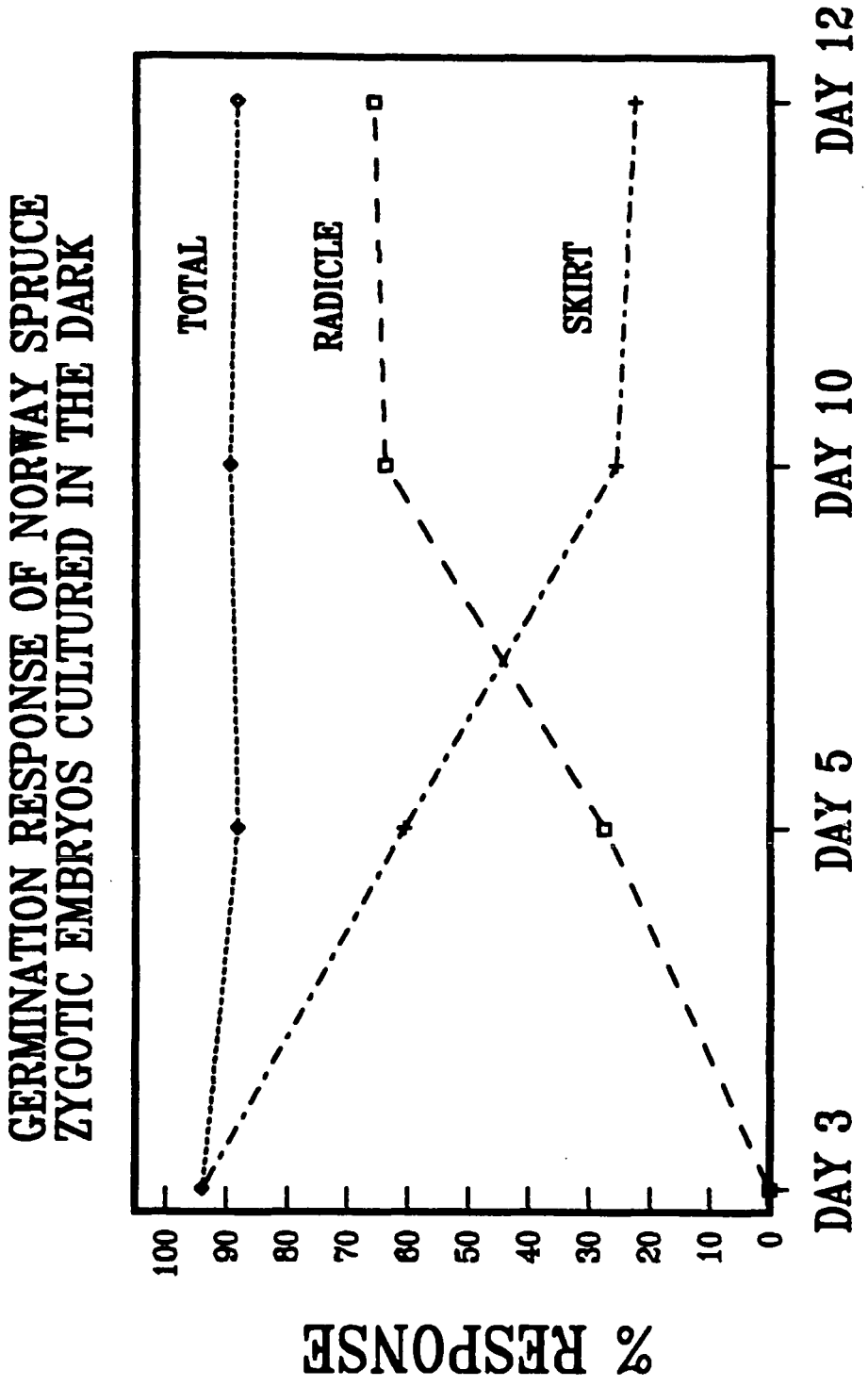

GERMINATION RESPONSE OF NORWAY SPRUCE
ZYGOTIC EMBRYOS AFTER 5 DAYS IN CULTURE
RADICLE [AVE = 27.5%]
SKIRT [AVE = 61%]
N = 21
N = 20
N = 20
N = 20
CUMULATIVE % RESPONSE
100
90
80
70
60
50
40
30
20
10
0
PLATE 1
PLATE 2
PLATE 3
PLATE 4
TRIALS

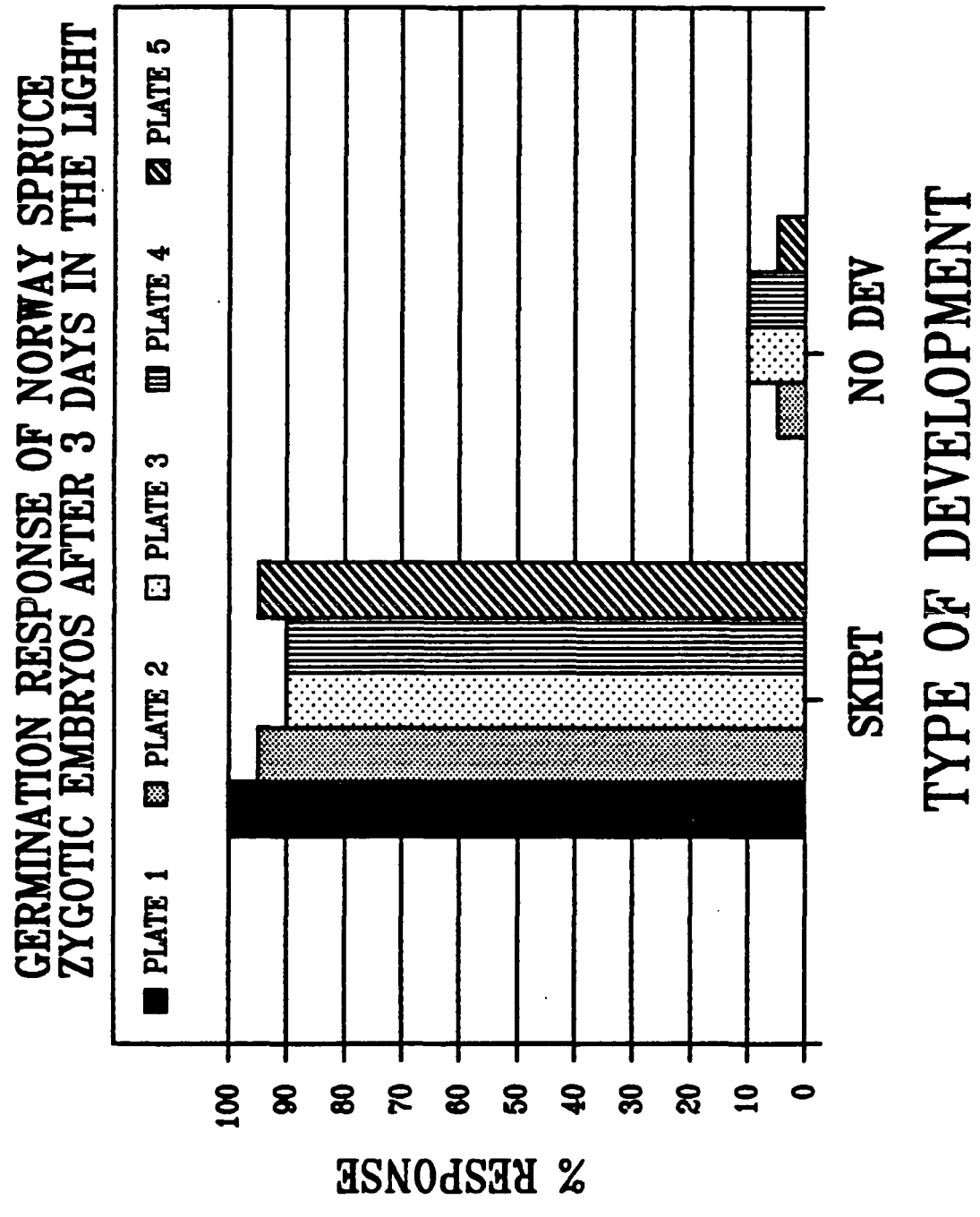

GERMINATION RESPONSE OF NORWAY SPRUCE
ZYGOTIC EMBRYOS AFTER 10 DAYS IN CULTURE
PLATE 1
N = 19
PLATE 2
N = 19
PLATE 3
N = 20
PLATE 4
N = 20
% RESPONSE
80
70
60
50
40
30
20
10
0
RADICLE
SKIRT
NO DEV
DISTORTED
TYPE OF DEVELOPMENT

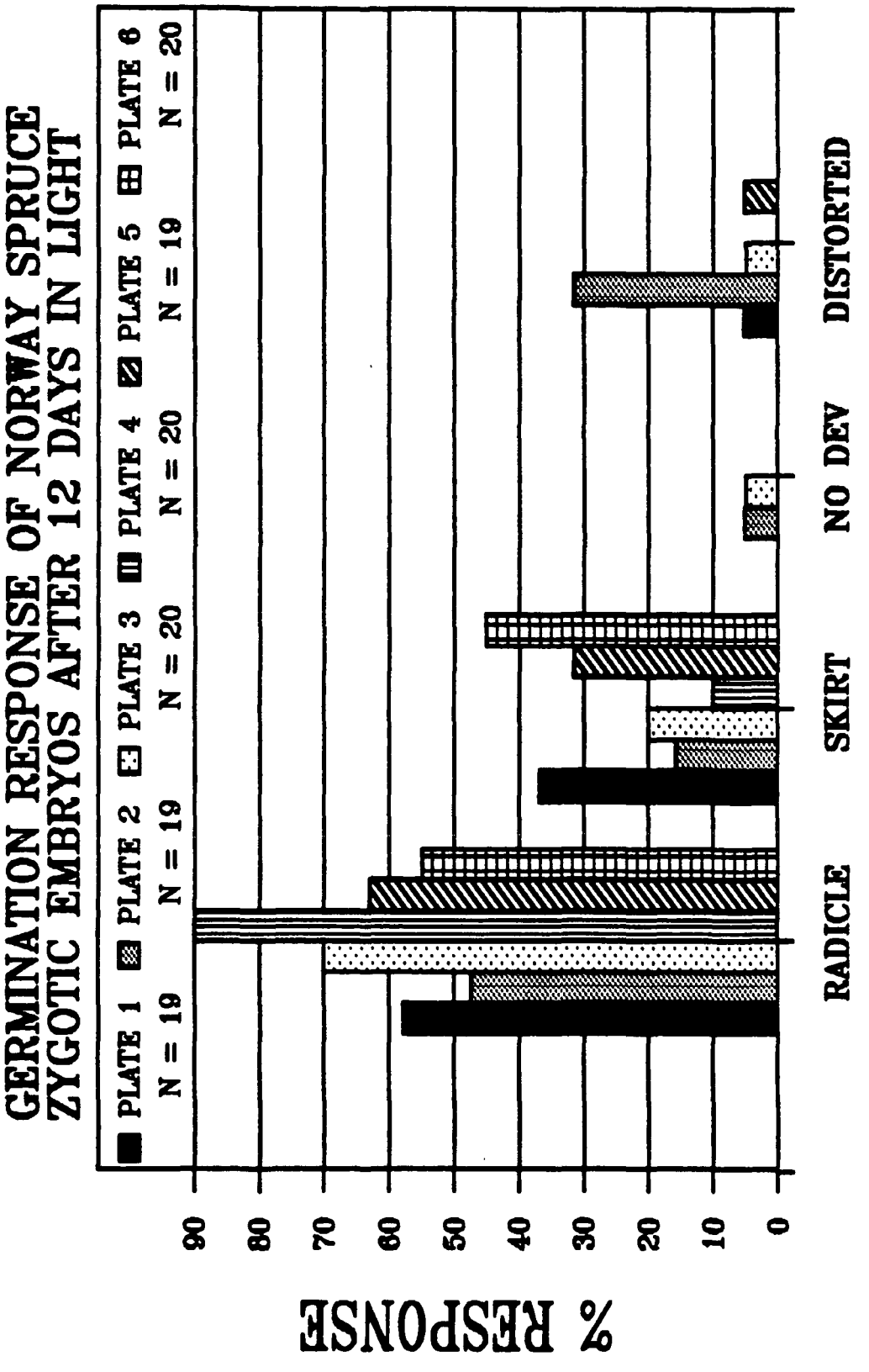

GERMINATION RESPONSE OF NORWAY SPRUCE
ZYGOTIC EMBRYOS AFTER 10 DAYS IN CULTURE
RADICLE [AVE = 59%]
SKIRT [AVE = 29%]
N = 19
N = 19
N = 20
N = 20
CUMULATIVE % RESPONSE
100
90
80
70
60
50
40
30
20
10
0
PLATE 1
PLATE 2
PLATE 3
PLATE 4
TRIALS

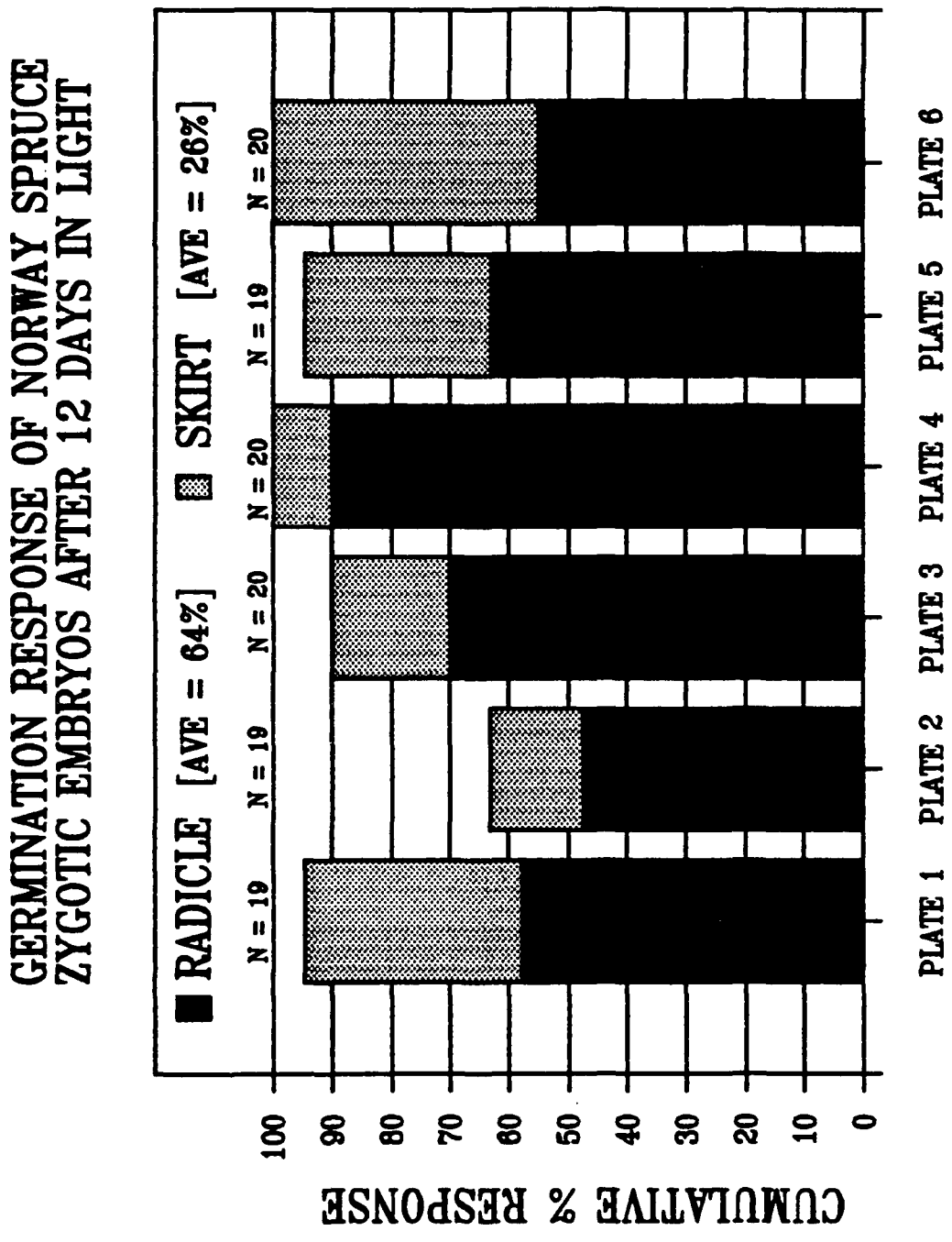

GERMINATION RESPONSE OF NORWAY SPRUCE
ZYGOTIC EMBRYOS AFTER 12 DAYS IN THE DARK
PLATE 1
PLATE 2
PLATE 3
PLATE 4
PLATE 5
PLATE 6
N = 20
N = 20
N = 20
N = 20
N = 20
N = 20
% RESPONSE
100
90
80
70
60
50
40
30
20
10
0
RADICLE
SKIRT
NO DEV
DISTORTED
TYPE OF DEVELOPMENT


GERMINATION RESPONSE OF NORWAY SPRUCE
ZYGOTIC EMBRYOS AFTER 12 DAYS IN THE DARK
RADICLE [AVE = 66%]
SKIRT [AVE = 22%]
N = 20
N = 20
N = 20
N = 20
N = 20
N = 20
100
90
80
70
60
50
40
30
20
10
0
CUMULATIVE % RESPONSE
PLATE 1
PLATE 2
PLATE 3
PLATE 4
PLATE 5
PLATE 6
TRIAL


GERMINATION RESPONSE OF NORWAY SPRUCE
ZYGOTIC EMBRYOS CULTURED IN THE DARK
TOTAL
RADICLE
SKIRT
100
90
80
70
60
50
40
30
20
10
0
% RESPONSE
DAY 3
DAY 5
DAY 10
DAY 12
TIME


GERMINATION RESPONSE OF NORWAY SPRUCE
ZYGOTIC EMBRYOS AFTER 3 DAYS IN THE LIGHT
PLATE 1
PLATE 2
PLATE 3
PLATE 4
PLATE 5
% RESPONSE
100
90
80
70
60
50
40
30
20
10
0
SKIRT
NO DEV
TYPE OF DEVELOPMENT


GERMINATION RESPONSE OF NORWAY SPRUCE
ZYGOTIC EMBRYOS AFTER 12 DAYS IN LIGHT
PLATE 1 N = 19
PLATE 2 N = 19
PLATE 3 N = 20
PLATE 4 N = 20
PLATE 5 N = 19
PLATE 6 N = 20
% RESPONSE
90
80
70
60
50
40
30
20
10
0
RADICLE
SKIRT
NO DEV
DISTORTED
TYPE OF DEVELOPMNET


GERMINATION RESPONSE OF NORWAY SPRUCE
ZYGOTIC EMBRYOS AFTER 12 DAYS IN LIGHT
RADICLE [AVE = 64%]
SKIRT [AVE = 26%]
N = 19
N = 19
N = 20
N = 20
N = 19
N = 20
CUMULATIVE % RESPONSE
100
90
80
70
60
50
40
30
20
10
0
PLATE 1
PLATE 2
PLATE 3
PLATE 4
PLATE 5
PLATE 6
TRIAL

CONCLUSIONS

1] ZYGOTIC EMBRYOS GERMINATE RAPIDLY & REACH A PEAK BY DAY 10.

2] 90% OF THE EMBRYOS SHOW SIGNS OF GERMINATING BUT ONLY 60% PRODUCE A RADICLE BY DAY 12.

3] 30% OF THE EMBRYOS DEVELOP A BROWN COLLAR "SKIRT" AT THE RADICLE & THIS APPEARS TO BE SIMILAR TO THE DARK BROWN AREA OBSERVED WITH SOMATIC EMBRYOS.

4] THERE IS NO MARKED DIFFERENCE IN THE FREQUENCY OF GERMINATION BY EMBRYOS CULTURED FOR 12 DAYS IN LIGHT OR DARKNESS.

5] THE MEDIUM USED IS SUITABLE FOR GERMINATING ZYGOTIC EMBRYOS BUT IS NOT IDEAL.

SOMATIC EMBRYO DEVELOPMENT & MATURATION IN LOBLOLLY PINE

M. RAFIQUE UDDIN

OBJECTIVE:

ENHANCE DEVELOPMENT AND MATURATION OF LOBLOLLY PINE SOMATIC EMBRYOS

SUB-OBJECTIVE

1) CONFIRM EARLIER CARBOHYDRATE RESULTS

2) EXPLORE ADDITIONAL TREATMENTS

HYPOTHESES:

1) GLUCOSE AND MALTOSE ENHANCE EMBRYO DEVELOPMENT AND MATURATION

2) SEQUENTIAL APPLICATION OF CARBOHYDRATES WILL FURTHER ENHANCE DEVELOPMENT AND MATURATION

MATERIALS AND METHODS:

CALLUS LINE: LP 2G1

CULTURE MEDIUM: MSG

CARBOHYDRATE SOURCES (6%): SUCROSE, GLUCOSE AND MALTOSE

GROWTH REGULATORS: ABA, IBA

DARK/LIGHT = LIGHT

CULTURE PERIOD: 12 WEEKS

MICROSCOPY:

1) HISTOCHEMICAL STUDY - SAMPLES FIXED AND STORED

2) ANATOMY - SAMPLES FIXED, STAINED, SECTIONED AND DOCUMENTED

BACKGROUND:

1) EARLIER RESULTS: GLUCOSE AND MALTOSE STIMULATED SOMATIC EMBRYO DEVELOPMENT.

Low frequency cotyledonary embryo formation was achieved with 6% glucose and 20 μM ABA on MSG medium (Table 1).

The frequency of precotyledonary embryos was higher with 6% maltose and 20 μM ABA on MSG medium (Table 2).

2) IN CACAO SWITCHING CARBOHYDRATE SOURCES WAS NECESSARY FOR MATURATION OF SOMATIC EMBRYOS (KONONOWICZ & JANICK, 1984) .

Table 1. Number of cotyledonary embryos on MSG medium with 6% glucose and 20 μM ABA, Line G1.

REPS #	# of cotyledonary embryos/clump
1	0.50
2	0.50
3	0
4	0
Mean	0.25

Table 2. Mean number of stage 2 embryos (per 100 mg) on MSG medium with various carbohydrates (6%) and 20 uM ABA.

Callus line	Sucrose	Glucose	Maltose
G1	58.5c	45.0b	69.4c
F1	23.0a	19.0a	26.0a

Mean value of four observations. Means followed by unlike letters were significantly different as determined by an analysis of variance with Duncan's New Multiple Range tests ($p < 0.05$).

BASED ON EARLIER RESULTS:

GLUCOSE & MALTOSE >> SUCROSE

30 ABA > 20 ABA

IBA ENHANCES MATURATION

AND,

WORK ON CACAO EMBRYOGENESIS.

BASED ON THE ABOVE AN EXPLORATORY STUDY WAS DESIGNED TO TEST THE EFFECTS OF SUCROSE, GLUCOSE AND MALTOSE INDIVIDUALLY OR IN SEQUENCE WITH AND WITHOUT ABA.

Table 3. Mean number of cotyledonary somatic embryos /clump as influenced by carbohydrate sources and ABA.

ABA (μM)	CARBOHYDRATE SOURCE (6%)		
	Sucrose	Glucose	Maltose
0	0.0	0.0	0.0
30	0.0	0.08 ± 0.10	0.12 ± 0.09
Earlier Results:		0.25	

Table 4. Formation of cotyledonary stage somatic embryo as influenced by alternating carbohydrate source (6%)[1].

CHO SOURCE SEQUENCE & DURATION	# OF COT. STAGE EMBRYO/CLUMP (n)
GLUCOSE ----> MALTOSE 4 WKS 4-6 WKS	1.28 ± 0.23 (25)
MALTOSE ----> MALTOSE 4 WKS 4-6 WKS	1.36 ± 0.51 (25)
MALTOSE ----> GLUCOSE 4 WKS 4-6 WKS	0.45 ± 0.30 (20)
GLUCOSE ----> GLUCOSE 4 WKS 4-6 WKS	0.40 ± 0.16 (20)

PAST RESULTS GLUCOSE

[1]In all cases callus was exposed to 20 μM ABA for the first 4 weeks. Then they were transferred to media containing 30 μM ABA and 0.5 μM IBA.

SUMMARY & CONCLUSION:

PART I

1) CARBOHYDRATES WITHOUT ABA WERE NOT EFFECTIVE IN PRODUCING COTYLEDONARY EMBRYOS.

2) CONTINUOUS TREATMENT WITH MALTOSE AND GLUCOSE PLUS ABA YIELDED COTYLEDONARY EMBRYOS AT A LOW FREQUENCY

PART II

1) CONTINUOUS INCUBATION WITH MALTOSE OR SUBSTITUTION OF MALTOSE FOR GLUCOSE DURING THE LAST SIX WEEKS GAVE THE BEST RESULTS WITH RESPECT TO THE NUMBER OF COTYLEDONARY STAGE SOMATIC EMBRYOS.

2) PRESENCE OF MALTOSE DURING THE LATER PART OF EMBRYO DEVELOPMENT APPEARS TO BE IMPORTANT IN DETERMINING THE NUMBER OF SOMATIC EMBRYOS DEVELOPING TO COTYLEDON STAGE.

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

OBJECTIVES

INCREASE DEVELOPMENT AND MATURATION FREQUENCIES BY

1. TWO TEST MEDIA
2. MODIFY THE TWO TEST MEDIA TO ALTER REDUCED NITROGEN SOURCES
3. INCREASE ABA LEVELS

HYPOTHESES

1. REDUCED ORGANIC NITROGEN IN THE FORM OF GLUTAMINE IN CULTURE MEDIUM REPLACING AMMONIUM NITRATE MIGHT STIMULATE SOMATIC EMBRYO DEVELOPMENT
2. ABA PROMOTES SOMATIC EMBRYO DEVELOPMENT& ABA AT HIGHER LEVELS ENHANCES SOMATIC EMBRYO DEVELOPMENT(BASED ON RESULTS IN ANGIOSPERMS;AMMIRATO, WILLIAMSON,ET.AL; 1987) AND IN NORWAY SPRUCE(BECWAR ET.AL,L987; VON ARNOLD,1988).

MATERIALS AND METHODS

MATERIAL: EC LINE 86(NS-1)5

DESIGN : 2 CLUMPS PER PLATE
(EACH CLUMP WEIGHS 1 gm)

2 PLATES/TREATMENT
(REPLICATES)

TREATMENTS: TEST MEDIA: HM & BLG
REDUCED NITROGEN SOURCES:
NH_4NO_3 AND/OR GLUTAMINE
VARYING ABA LEVELS:
(0-40 μM ABA)

TEST MEDIA SELECTED : HM & BLG

REDUCED NITROGEN: COMPARISON

COMPONENTS mg/L	HM	mHM	BLG	mBLG	mmBLG
NH_4NO_3	1200	-	- --	1200	1200
GLN	____	1500	1500	1500	____

EXPERIMENTAL PROTOCOL

MAINTENANCE MEDIUM

↓

(TEST MEDIA + 1 % ACTIVATED CHARCOAL)

(1 WEEK)

↓

TEST MEDIA + 1 μM IBA + VARIED ABA LEVELS

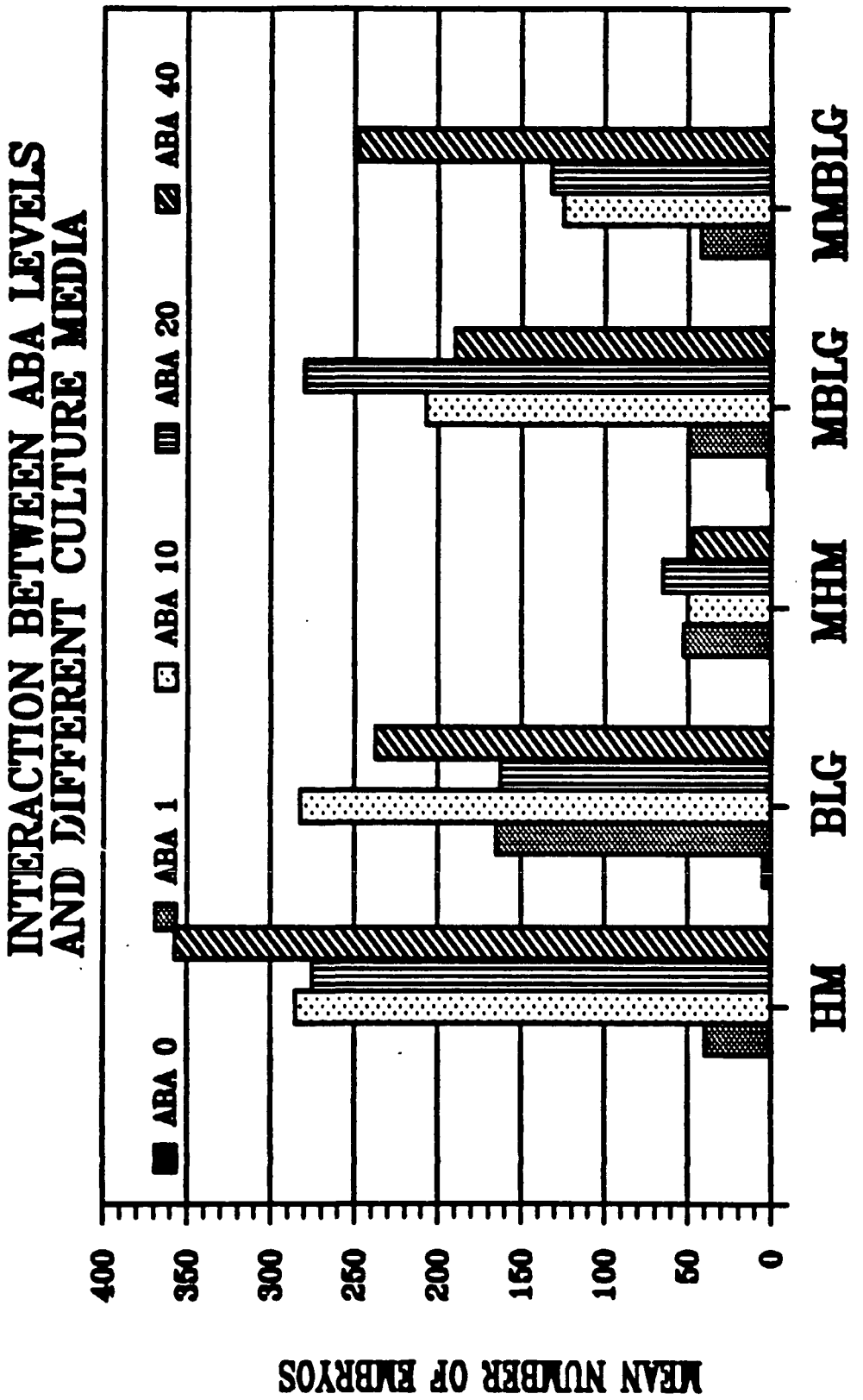
(2-4 WEEKS)

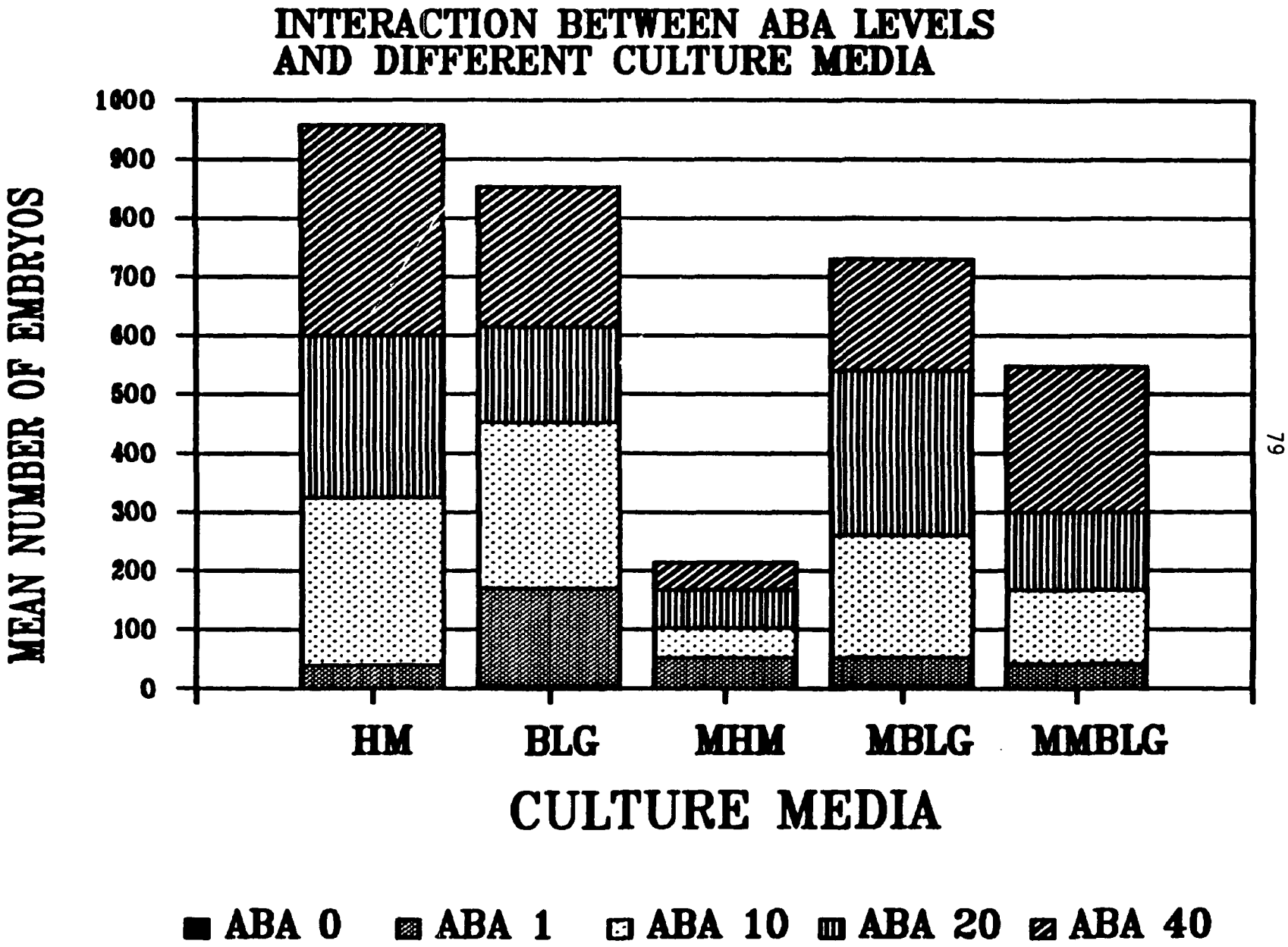
↓

HARVEST MATURE SOMATIC EMBRYOS AT STAGE 6

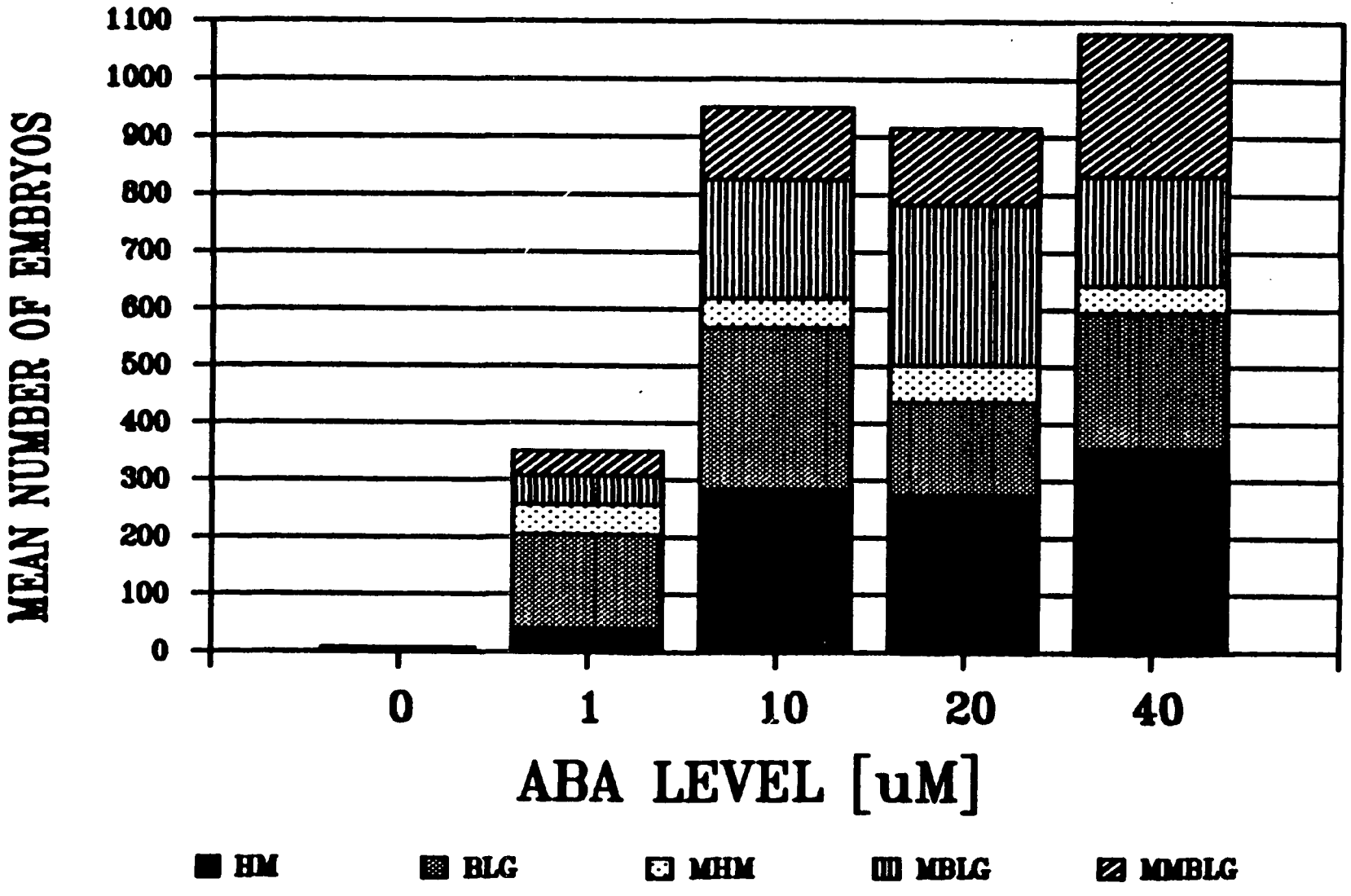
↓

GERMINATION ON ½ MS MEDIUM

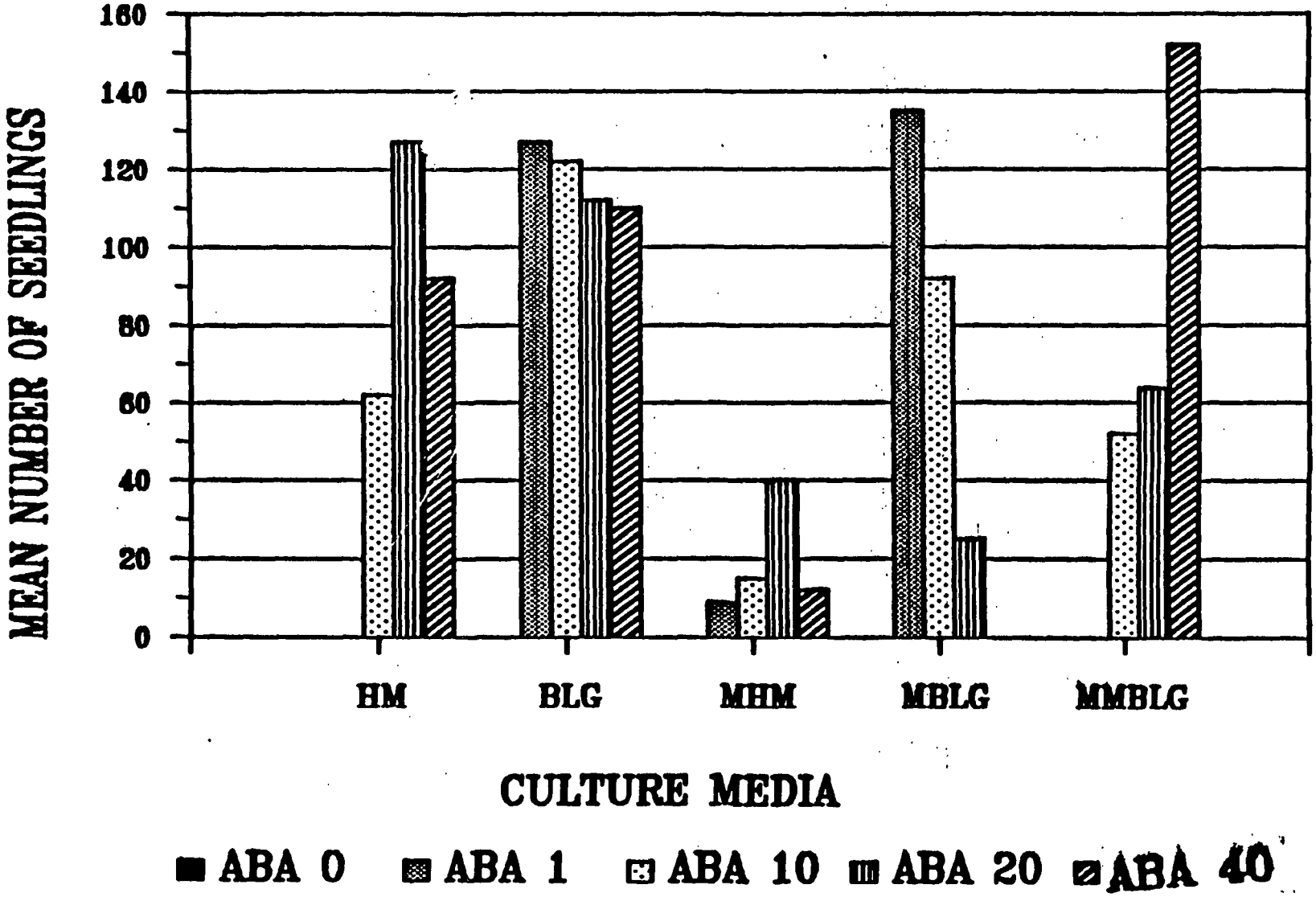

INTERACTION BETWEEN ABA LEVELS
AND DIFFERENT CULTURE MEDIA
ABA 0
ABA 1
ABA 10
ABA 20
ABA 40
400
350
300
250
200
150
100
50
0
MEAN NUMBER OF EMBRYOS
HM
BLG
MHM
MBLG
MMBLG
CULTURE MEDIA


INTERACTION BETWEEN ABA LEVELS
AND DIFFERENT CULTURE MEDIA
MEAN NUMBER OF EMBRYOS
1600
900
800
700
600
500
400
300
200
100
0
HM
BLG
MHM
MBLG
MMBLG
CULTURE MEDIA
ABA 0
ABA 1
ABA 10
ABA 20
ABA 40

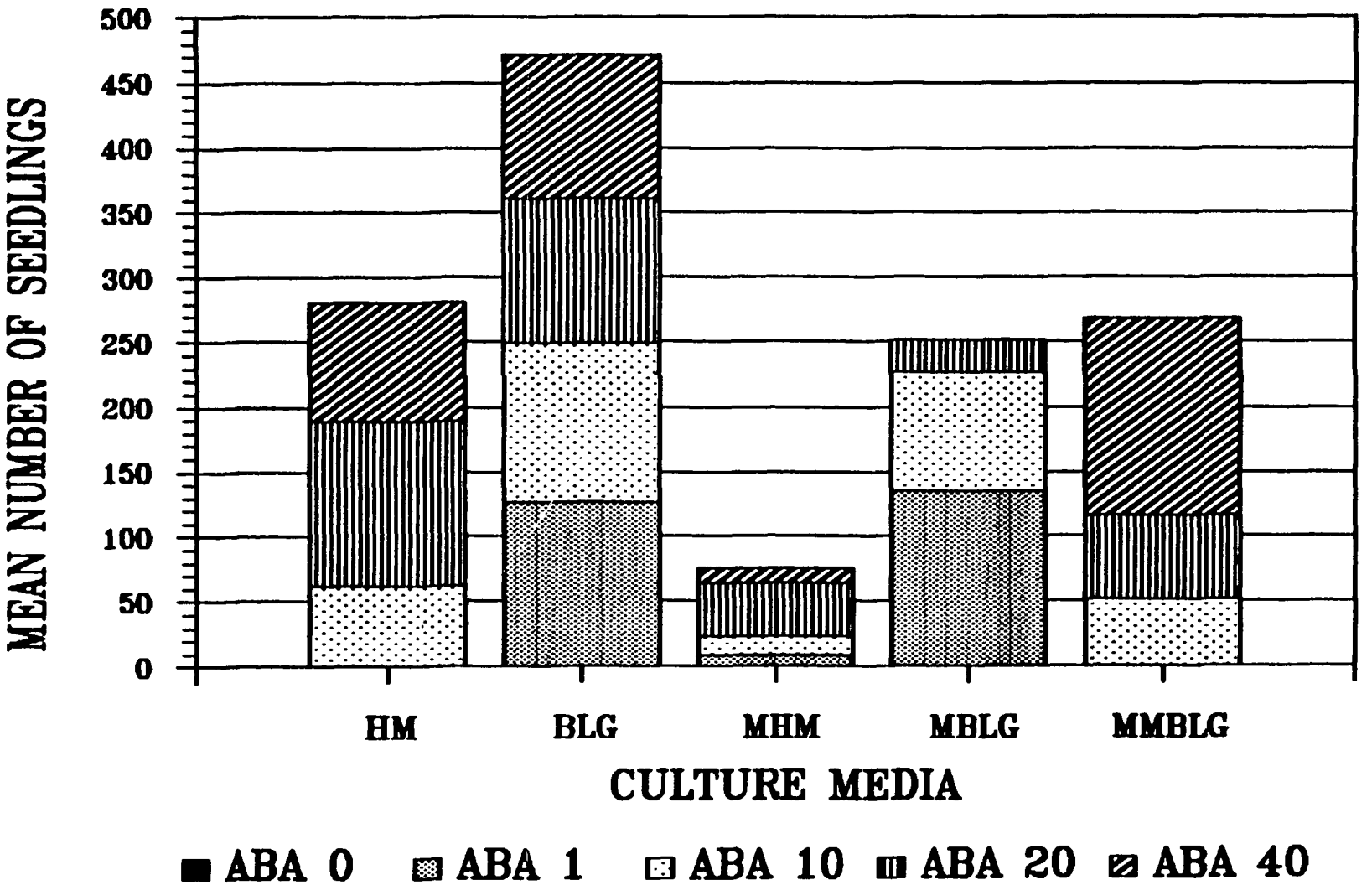
INTERACTION OF ABA LEVELS AND CULTURE MEDIA ON THE PRODUCTION OF MATURE NORWAY SPRUCE SOMATIC EMBRYOS

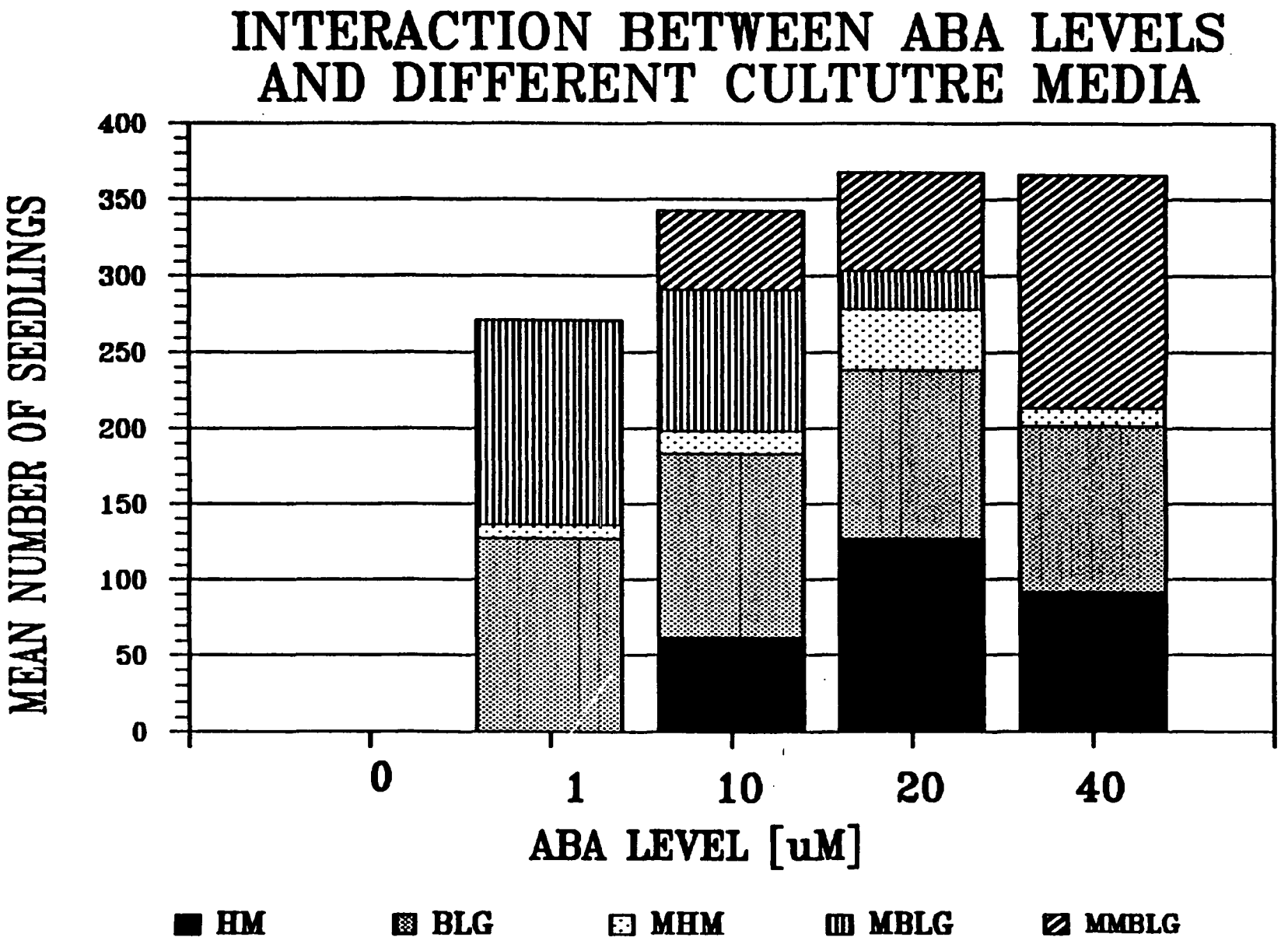


INTERACTION BETWEEN ABA LEVELS AND DIFFERENT CULTUTRE MEDIA



INTERACTION BETWEEN ABA LEVELS AND DIFFERENT CULTUTRE MEDIA




INTERACTION BETWEEN ABA LEVELS
AND DIFFERENT CULTUTRE MEDIA
MEAN NUMBER OF SEEDLINGS
400
350
300
250
200
150
100
50
0
0
1
10
20
40
ABA LEVEL [uM]
HM
BLG
MHM
MBLG
MMBLG

SUMMARY AND CONCLUSIONS

1. INCREASED NUMBER OF SOMATIC EMBRYOS ON HM MEDIUM AND INCREASED NUMBER OF SEEDLINGS ON BLG MEDIUM IRRESPECTIVE OF ABA LEVELS.

2. INCREASED NUMBER OF SOMATIC EMBRYOS WITH A CORRESPONDING INCREASE IN ABA LEVELS ON MEDIA WITH NO ORGANIC NITROGEN IN THE FORM OF GLUTAMINE(HM, mmBLG).

3. INCREASED NUMBER OF SOMATIC EMBRYOS AT LOW LEVELS OF ABA (0 & 1 μM) AND IN THE PRESCENCE OF GLUTAMINE (BLG).

4. THE YIELD OF MATURE SOMATIC EMBRYOS ON HM MEDIUM WITH 1μM ABA WAS VERY LOW COMPARED TO BLG MEDIUM WITH SAME LEVEL OF ABA. THESE RESULTS ARE CONSISTENT WITH THOSE PRESENTED LAST YEAR.

5. INCREASE IN THE YIELD OF MATURE SOMATIC EMBRYOS DOES NOT ALWAYS RESULT IN THE INCREASED YIELD OF SEEDLINGS.FOR EXAMPLE, ALTHOUGH ON HM MEDIUM MAXIMUM NUMBER OF SOMATIC EMBRYOS WERE PRODUCED THERE WAS NO CORRESPONDING INCREASE IN THE NUMBER OF SEEDLINGS.WHEREAS ON BLG MEDIUM, THE NUMBER OF SEEDLINGS PRODUCED PARALLED WITH THE NUMBER OF EMBRYOS PRODUCED.

6. ABA AT 10,20 AND 40μM PROMOTES DEVELOPMENT AND MATURATION OF SOMATIC EMBRYOS. HOWEVER, THE OPTIMAL ABA LEVEL VARIES FROM 10μM (BLG), 20μM (mBLG) TO 40 μM (HM, mmBLG).

FUTURE RESEARCH PLANS

1. QUANTIFY THE TOTAL NITROGEN CONTENT OF THE TWO TEST MEDIA (HM & BLG)AND DETERMINE THE OPTIMAL RATIO OF NO_3/NH_4 BY TESTING VARIOUS LEVELS;ALSO TO DETERMINE THE OPTIMAL RATIO OF INORGANIC AND ORGANIC REDUCED NITROGEN, WITHOUT ALTERING THE TOTAL NITROGEN CONTENT.

2. TEST HIGHER LEVELS OF ABA AGAINST DIFFERING REDUCED NITROGEN BACKGROUND.

SOFTWOOD PROGRESS: PAC TO PAC

SHORT TERM GOALS	STATUS
CONTINUE WORK ON:	
MATURATION/DEVELOPMENT	MAIN ENTERPRISE; TESTS IN NS, DF, &LP, POSITIVE RESULTS; MORE TO COME
CONVERSION/ACCLIMATIZATION	LIMITED EFFORT, LITTLE OR NO GAIN; ADD PLANT PHYSIOL
REPLICATED SEEDLING TRIAL	INCOMPLETE
DOCUMENT DEVELOPMENTAL PATTERNS	EMBRYO STAGE KEY; ALSO TRACKING EVENTS IN GERMINATION; ASSAY IN CULTURES
COMPLETE SUSPENSION CULTURES	COMPLETED, DOCUMENTED INTERNALLY; TIME DIVERTED TO RELOC
INITIATION, TARGET SPECIES	UNDERWAY IN DF, MODEST EFFORT
INITIATION, MORE MATURE EXPLANTS	NS= A FEW EMBRYOGENIC CULTURES FROM SOMATIC C & H; DF & SPRUCE+ RESULTS WERE INCONCLUSIVE

SOFTWOOD PROGRESS: PAC TO PAC
(CONTINUED)

SHORT TERM GOALS	STATUS
PRESENTATION/PUBLICATION	REASONABLE, BUT SLOWER THAN DESIRED
BLDG DESIGNS & RELOCATION	COMPLETED MOVE; IRF RENOVATION = FINAL STAGES; ON-CAMPUS= MUCH MORE WORK
PERSONNEL NEEDS & ORGANIZATION	HIRING PLAN APPROVED; INTERVIEWING

BIOCHEMISTRY PROGRESS: PAC TO PAC

SHORT TERM GOALS	STATUS
COMPARE ZYGOTIC & SOMATIC EMBRYOS	COMPLETED & DOCUMENTED WORK IN PROGRESS; RESULTS CONFIRMED OR COMPLEMENTED EARLIER ONES
	PACKED, ETC. FOR MOVE
PRECURSORS/SUBSTRATES, LEVERAGE MATURATION	SAME
INHIBITORS, LEVERAGE MATURATION	SAME
ASSAY ABA LEVELS	UNDERWAY VIA COOP ARRANGEMENTS
EXPLORATORY RESEARCH	DEFERRED
PRESENTATION/PUBLICATION	COMPLETED MAJOR ITEMS

HARDWOOD PROGRESS: PAC TO PAC

SHORT TERM GOALS	STATUS
SOLIDIFY OBJECTIVES & APPROACHES	FOCUS=TRANSFORMATION & EFFICIENT PROPAGATION
SECURE ADDITIONAL PLANT MATERIAL	CULTURES FROM NEB. & TUSKEGEE
EXPAND GREENHOUSE & IN VITRO POPULATIONS	GREENHOUSE = NO IN VITRO = SUFFICIENT FOR RESEARCH, BUT CONTINUING
PERSONNEL NEEDS & ORGANIZATION	HIRING PLAN APPROVED; INTERVIEWING
EXPLORATORY RESEARCH	NOTHING STARTLING

RELATIVE LEVELS OF EFFORT

ALL TIME NOT EXPENDED ON RELOCATION WAS DEVOTED TO:

CULTURE MAINTENANCE

DEVELOPMENT/MATURATION

HARDWOOD CULTURE ACQUISITION & STABILIZATION

CURRENT EFFORTS INVOLVE:

COMPLETION OF THE MOVE & LAB SETUP

CULTURE MAINTENANCE

DEVELOPMENT/MATURATION

NORWAY SPRUCE

LOBLOLLY PINE

DOUGLAS-FIR

DOUGLAS-FIR INITIATION

HARDWOODS, SAME AS EARLIER

SHORT TERM GOALS - CRITICAL ISSUES

CLONING SOFTWOODS

RECRUIT AND HIRE NEW TECHNICIANS

MOVE EQUIPMENT AND SUPPLIES

COMPLETE LABORATORIES, OFFICES, AND GREENHOUSE

DOUGLAS-FIR INITIATION AND CULTURES

RAISE MATURATION AND CONVERSION FREQUENCIES

DOCUMENT ZYGOTIC EMBRYO DEVELOPMENT, GUIDEPOSTS

EXPLORE INITIATION FROM MORE MATURE MATERIALS

PRESENT/PUBLISH PROMPTLY

SHORT TERM GOALS - CRITICAL ISSUES

BIOCHEMISTRY

RECRUIT AND HIRE SCIENTISTS AND TECHNICIANS

MOVE EQUIPMENT AND SUPPLIES

COMPLETE LABORATORY RENOVATION

REACTIVATE RESEARCH ON ZYGOTIC/SOMATIC COMPARISONS

SEEK INTERIM COOPERATORS

CONTINUE ABSCISIC ACID ASSAYS

SHORT TERM GOALS - CRITICAL ISSUES

CLONING HARDWOODS

RECRUIT AND HIRE NEW SCIENTIST

EXPAND EXISTING CULTURES

INITIATE AND/OR SECURE ADDITIONAL CULTURES

ESTABLISH "CLEAN" GREENHOUSE POPULATIONS

INITIATE RESEARCH ON GENE TRANSFER AND EXPRESSION

GET PROPAGATION METHOD READY FOR TRANSFORMATION

EXECUTE EXPLORATORY RESEARCH

SOMATIC EMBRYOGENESIS: PATHWAY, PROCESS, & PROGRESS

NORWAY SPRUCE, 3/89

PATHWAY	PROCESS	SUCCESS RATES (%) MEAN	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				
↓	CONVERSION =	7	0- 7	Fall 1987
	GERMINATION =	19	0-20	Fall 1987
			(21-97)	1988
SEEDLINGS		24	(0-69)	Fall, 1989

CUMULATIVE RATES (%):

EXPLANT TO EMBRYOGENIC CALLI = 40

EMBRYOGENIC CALLI TO SEEDLINGS = <1

SUCCESS RATES (%)

I = # EC LINES / # EXPLANTS
M = # EC LINES MAINTAINED / # INITIATED

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

D/M = # MATURE EMBRYOS / GRAM CALLUS
C = # SEEDLINGS SURVIVING @ 5 MONTHS / # MATURE EMBRYOS
G = # GERMINATED EMBRYOS / # MATURE EMBRYOS

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

SOMATIC EMBRYOGENESIS: PATHWAY, PROCESS, & PROGRESS

DOUGLAS FIR, 3/89

PATHWAY	PROCESS	SUCCESS RATES (%) MEAN	RANGE	COMMENTS
EXPLANT (IMMATURE EMBRYOS)				
↓	INITIATION =	<1	--	
↓	MAINTENANCE =	80	--	
EMBRYOGENIC CALLI				
↓	DEVELOPMENT & MATURATION =	√	--	
SOMATIC EMBRYOS				
↓	CONVERSION =	--	--	
	GERMINATION =			
SEEDLINGS				

CUMULATIVE RATES (%):

EXPLANT TO EMBRYOGENIC CALLI = <1

EMBRYOGENIC CALLI TO SEEDLINGS = --

SUCCESS RATES (%)

I = # EC LINES / # EXPLANTS
M = # EC LINES MAINTAINED / # INITIATED

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

D/M = # MATURE EMBRYOS / GRAM CALLUS
C = # SEEDLINGS SURVIVING @ 5 MONTHS / # MATURE EMBRYOS
G = # GERMINATED EMBRYOS / # MATURE EMBRYOS

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

SOMATIC EMBRYOGENESIS: PATHWAY, PROCESS, & PROGRESS

LOBLOLLY PINE, 3/89

PATHWAY	PROCESS	SUCCESS RATES (%) MEAN	RANGE	COMMENTS
EXPLANT (IMMATURE EMBRYOS)				
↓	INITIATION	= <1	0.0-0.7	
↓	MAINTENANCE	= 80,100	0-100	
EMBRYOGENIC CALLI				
↓	DEVELOPMENT & MATURATION	= ++	--	
SOMATIC EMBRYOS				
↓	CONVERSION	= --	--	
↓	GERMINATION	= --	--	
SEEDLINGS				

CUMULATIVE RATES (%):

EXPLANT TO EMBRYOGENIC CALLI = 0.2

EMBRYOGENIC CALLI TO SEEDLINGS = --

SUCCESS RATES (%)

I = # EC LINES / # EXPLANTS
M = # EC LINES MAINTAINED / # INITIATED

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

D/M = # MATURE EMBRYOS / GRAM CALLUS
C = # SEEDLINGS SURVIVING @ 5 MONTHS / # MATURE EMBRYOS
G = # GERMINATED EMBRYOS / # MATURE EMBRYOS

CUMULATIVE = (D/M) (C) = # SEEDLINGS SURVIVING / # EMBRYOS OR / GRAM CALLUS, REFLECTS YIELD & UTILITY OF PROCESS

***** KEY GOALS *****

SUNSET (SUNRISE) PROVISION REVISITED

ESTABLISH KEY GOALS

PERIODIC & OBJECTIVE EVALUATION EVERY 2 - 3 YRS

DECIDE TO CONTINUE, TURN, OR STOP

IN ADDITION TO BUT COINCIDENT WITH

MID-RANGE PLAN & PAC TO PAC PLANS

BASES FOR EVALUATION:

PROCESS STEP IMPROVEMENTS

ENHANCED UNDERSTANDING & NEW KNOWLEDGE

NEW OPPORTUNITIES

***** KEY GOALS *****

1) INCREASE DEVELOPMENT/MATURATION FREQUENCY IN MODEL SPECIES TO 25% ON A REPEATABLE BASIS, AND EXTEND TO TARGET SPECIES.

IE., MOVE 250 OR MORE OF THE 500 - 1000 POTENTIAL EMBRYOS PRESENT PER GRAM OF EMBRYOGENIC CALLUS TO MATURITY.

REQUIRES IMPROVED UNDERSTANDING OF ZYGOTIC SYSTEM.

2) IMPROVE CONVERSION PROTOCOLS FOR MODEL SPECIES & PRODUCE A POPULATION OF SUITABLE SIZE & VARIABILITY FOR REPLICATED GREENHOUSE & FIELD TRIALS.

IE., ACCUMULATE 375 USEABLE SEEDLINGS FROM EACH OF ONE OR MORE CALLUS LINES REPRESENTING EACH OF SEVERAL DONOR TREES.

REQUIRES IMPROVED UNDERSTANDING OF ZYGOTIC SYSTEM, REFINED GERMINATION METHODS, & IMPROVED ACCLIMATIZATION PROCEDURES.

3) RAISE INITIATION FREQUENCIES IN TARGET SPECIES TO 10% ON A REPEATABLE BASIS ACROSS EXPERIMENTS, SEASONS, AND GENOTYPES.

MAIN INTENT: IMPROVE RELIABILITY OF PROCESS STEP, AND GAIN ABILITY TO OBTAIN EMBRYOGENIC CALLUS AT WILL & WITH EASE FOR RESEARCH ON OTHER CRITICAL STEPS.

HONORABLE MENTION: OBTAIN EMBRYOGENIC CALLUS OF AT LEAST ONE SOFTWOOD, FROM EXPLANTS OF TREES OLD ENOUGH TO HAVE BEEN PROVEN GENETICALLY SUPERIOR.

GLOSSARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

EM - Electron microscope.

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

Embryogenesis - Initiation of embryoids or embryos from cultured cells.

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

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

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

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

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

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

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

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

Fidelity - Preservation of the original genotype and phenotype.

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

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

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

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

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

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

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

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

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

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

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

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

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., µL/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 R1).

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

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

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

RNA - Ribonucleic acid. RNA is usually single stranded.

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

SEM - Scanning electron microscope.

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

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

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

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

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

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

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

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

TEM - Transmission electron microscope.

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

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

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

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

Tissue culture - General term for callus and cell cultures.

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

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

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

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

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

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

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
γ-aba	aminobutyric acid
gln	glutamine
glu	glutamic acid
gly	glycine
his	histidine
hyp	hydroxyproline
ile	isoleucine
leu	leucine
lys	lysine
met	methionine
orn	ornithine
phe	phenylalanine
pro	proline
ser	serine
thr	threonine
trp	tryptophan
tyr	tyrosine
val	valine

CUMULATIVE LIST OF ABBREVIATIONS

AA	Ascorbic acid
2,4-D	2,4-Dichlorophenoxyacetic acid
ABA	Abscisic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
ADC	Arginine decarboxylase
ADP	5'-Adenosine diphosphate
AMP	5'-Adenosine monophosphate
ANOVA	Analysis of variance
AOA	Aminooxyacetic acid
AOAA	Aminooxyacetic acid
AOPP	α-Aminooxy-β-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	α-difluoromethylarginine
DFMO	α-difluoromethylornithine
DCHA	Dicyclohexylammonium sulfate
DHA	Dehydroascorbic acid
dSAM	Decarboxylated SAM
DW	Dry weight
E	Embryogenic
EC or ec	Embryogenic callus
EDTA	Ethylenediaminotetraacetic acid
E_I	Embryonal initial
FAA	Free amino acid(s)
FTIR	Fourier transform infrared
FW or fr.wt.	Fresh weight
G-1-P	Glucose-1-phosphate
GA	Gibberellic acid (gibberellin)
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
GD	Gresshof and Doy medium
GSH	Glutathione (reduced)
GSSG	Glutathione (oxidized)
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HFBI	Heptafluorobutyrylimidazole
HFSE	High frequency somatic embryogenesis
HM	Hakman medium
HPLC	High performance liquid chromatography
IAA	Indoleacetic acid

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

STATUS OF PUBLICATIONS AS OF 10/89

PUBLISHED OR IN PRESS:

1. Becwar, M.R; Noland, T.L; Wyckoff, J.L. Maturation, germination, and conversion of Norway spruce (Picea abies L.) somatic embryos to plants. In Vitro Cell. Dev. Biol. 25:575-580

2. Becwar, M.R; Feirer, R.P. Factors regulating loblolly pine (Pinus taeda L.) somatic embryo development. In: Proceedings of Southern Forest Tree Improvement Confeference 42:178-185 (1989).

3. Feirer, R.P; Conkey, J.L; Verhagen, S.A. Triglycerides in embryogenic conifer calli: a comparison with zygotic embryos. Plant Cell Reports 8:207-209 (1989).

4. Feirer, R.P; Wann, S.R. Preliminary evidence of transformation and foreign gene expression in sweet gum (Liquidamber styraciflua L.) and loblolly pine (Pinus taeda L.) In: Proceedings of Southern Forest Tree Improvement Conference 42:381-388 (1989).

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6. Wann, S.R; Becwar, M.R; Nagmani, R; Feirer, R.P; Johnson, M.A. Biochemical differences between embryogenic and nonembryogenic calli of conifers. Trees (In press;1989).

SUBMITTED OR IN REVISION:

1. Becwar, M.R; Nagmani, R; Wann, S.R. Initiation of embryogenic callus and somatic embryo development in loblolly pine (Pinus taeda L.) Can. J. For. Res.

OTHERS FOR INFORMATION:

1. Conners, T.E. Segmented models for stress-strain diagrams. Wood Science and Technology 23:65-73(1989)

2. Macgregor, M.A; Conners, T.E. MD microstriations in paper. Tappi Journal 72:177-181 (1989).