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PROGRESS REPORT

NEW VARIETIES OF RICE FOR SALINE AND ACID SOIL THROUGH TISSUE CULTURE

AID/SCI PROPOSAL 2A-15

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U.S. International Development
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Bangkok

Department of Botany
Chulalongkorn University
Bangkok

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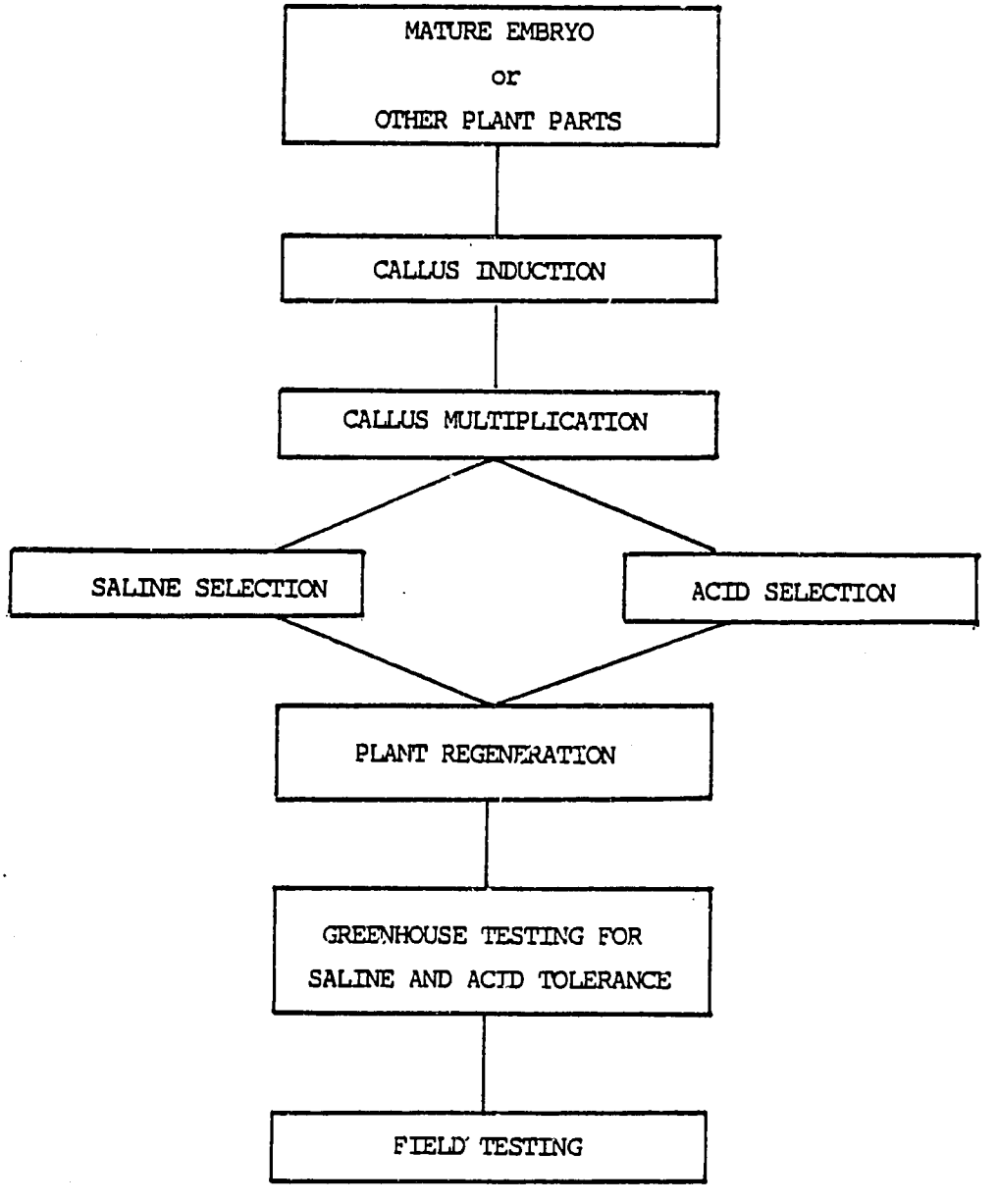
PROPOSED METHODS

FOR

PRODUCTION OF SALINE AND ACID TOLERANT RICE



THE RICE TISSUE CULTURE LABORATORY

The new tissue culture laboratory is on the second floor of Botany Building. It has total floor area of 100 square meters (1076 sq. ft.) which half of the area is a preparation room and another half divides into a transfer room and another half divides into a transfer room and a culture (incubation) room.

The preparation is, almost self sufficient, equipped with a number of standard equipment and facilities necessary for plant tissue culture; excepting the autoclaves which are in separate compound. Six transfer chambers are in a separate air condition room, three of which are of laminar flow type and three being simple plexiglass chambers. One of each are reserved for spares while the rest are being used most of the time. The culture room comprises mainly of seven culture stacks, three cabinets and two shakers. Maximum shelf area is 52.5 square meters (565 sq. ft.), divided into 64 % dark area which can accommodate 13,000 bottles and 36 % light area for 8,000 bottles. These areas, have been used near its maximum capacity most of the time since Febuary, 1984; which hold approximately 21,000 bottles at any time. Each week, two thousand bottles are replaced by the new culture bottles, the ones that are through with the experiments or are contaminated with micro-organisms are washed and reused immediately.

The abovementioned operation have been made possible by a number of persons and organizations which we take this opportunity to express our gratitute to the following names. The conversion of the rooms into laboratory was made possible by Phya Anukul Siamkit Upaniksit Siamrath Fund, amount of US $ 13,050.00, donated by Mrs. Sri Oonsiri one of his descendants. Many of the equipment and glassware, amount of US $ 21,740.00, used in this laboratory

PREAMBLE TO THE PROGRESS REPORT III

Most advancement in rice tissue culture have been made in Japan, China and India. However, in 1977, IRRI inthe Philippines begin a comprehensive program for rice improvement through tissue culture. These include tissue tissue culture of microspores and somatic isolates, screening and collection of germplasm that produce special characteristics for immediate and future use.

Many problems restrict the use of tissue culture in rice improvement program. Scientist working on rice tissue culture now a day are still faced with the problems of callus culture and plant regeneration, due partly to erratic responses to the treatments among rice varieties or due to some unknow biological factors characteristic of this particular group of plants.

In our laboratory, contamination of the culture was as high as 40 % during the wet season in the first year and was reduced sucessfully to 8-10 % by altering seed treatment before culture (see Progress Report I, and II). Callus induction is found to be easy provided that varietal difference is taken into account. Two different media have been selected, from the experiment, from callus production of eight rice varieties (see Progress Report II). Some varieties respond equally well to both media but some require specific treatments. Generally, immature seed give larger callus but does not seem to regenerate more plant than the mature seeds are use in most experiments.

Regeneration is the most difficult part in rice tissue culture. In screening of mutants with special characteristics, a very large number of plant regenerated from callus is needed. This project would not be materialized without having enough plants regenerated from calli. At present time, regeneration is still fairly difficult in rice, most calli develop to certain stages,

were donated by Khunying Chern Pisolyabutr. The research grant acquired from the USAID enable us to add more equipment, technical staff, and other expenses to the laboratory which facilitates our work greatly. Last and the most important of all, is the generosity of the Head Department of Botany and their staff for not only allocate the area for housing the new laboratory but also give full support to this project.

Recently, the university recognizes this laboratory as a unit cell for research and human resources development. To this end, the university will directly involve in the development of our laboratory by expansion of working area, providing more technical staff and additional budget necessary for good operation. This will enable us to improve our working condition, which inturn will improve and not only on work outputs but also increase efficiency of our laboratory in the future. At the same time, we are preparing to be amember of the International Plant Biotechnology Network now being formed. This project has been proposed by Professor M. W. Nabors at Colorado State University for cooperation among laboratories working in the same directions of plant tissue culture. Which the members of the network will work together in information exchange, technical assistance, personal exchange, training of scientists, and organizing conferences. This will further expand our international cooperation in the development of science for benifit of mankind.

turn brown and eventually die. Some treatments reported in the Progress II, induced more greenspot formation than the others which indicate early development of shoot apices. Development beyond that stage is still not satisfactory. However, much of the information obtained from the said experiments were used in planning of the experiments being report here.

Due to some disagreement among the results reported by several workers and ours, which may be either the variation in responses of rice varieties or physical environment in the culture rooms besides the composition of media. This makes the problem even more complex. This report is base on the results obtained from the experiments which compare the results of other workers and our previous results. The experiments were then designed accordingly to cover several aspects broadly, so the more systematic approach and experiments in depth will be found in the next report.

Some problems has arisen in the course of the experiments, some of which are beyond our control. Failure to control the temperature occurred several time in the summer, because of the excessive heat from out side of the culture room raised room temperature up to 30 C for several consecutive days. It was observed that high temperature hastened calli to turn brown and died before shoots were formed, or it might have direct effect on inhibition of shoot apex differentiation. At present, two out of three air conditioners in the culture room can be used at one time, otherwise the main electric line would not be able to bear the load. The main line would be replaced by a larger one in the near future but it may not be possible in this coming summer. However alternation of lighting system was done by moving ballasts of the fluorescent system up on top of the stacks, so the heat now dissipates to the upper layer of air instead of heating up the shelves where the bottles are. It is hope that this will help keeping the temperature around culture bottles down,

which would benefit a great deal in the summer.

Even the rate of plant regeneration is still not satisfactory, a fair number of plant regenerated from calli are obtained. Plants from different varieties are being grown in sand culture, some have already produced seeds. Now, such seeds are under further investigation for plant regeneration in tissue culture and also for the evaluation for other characteristics. Some of the calli were treated with 0.5, 0.8, 1.0, 2.0, 3.0 and 4.0 % NaCl; few survived through the treatments. Among these, some complete plants have been obtained. These plants, of cause, are to be tested again for salt tolerance in pot later.

ABBREVIATIONS USED IN THIS REPORT

RICE VARIETIES

RD 23	Rice Department No.23
RD 25	Rice Department No.25
KDML	Kao-dok-mali-105
LPT	Lueng-pa-tiew
NSPT	Neo-sun-pa-tong

MACRO- and MICRONUTRIENT FORMULATIONS

LS	Linsmaier and Skoog (1965)
MS	Murashige and Skoog (1962)
SH	Schenk and Hildebrandt (1972)
W	White (1954)

GROWTH REGULATORS

IAA	Indole-3-acetic acid
2,4-D	2,4-Dichlorophenoxy acetic acid
K	Kinetin

ORGANIC ADDITIVES

CW	Coconut water (liquid endosperm)

TERMINOLOGIES

A — Green area coverage $= \frac{\text{Area covered with greenspots}}{\text{Total callus surface area}} \times 1$

GS — Greenspot $= \frac{\text{No. of calli with greenspots}}{\text{No of total calli}} \times 100$

Sh — Shoot $= \frac{\text{No. of calli with shoots}}{\text{No. of total calli}} \times 100$

Figure A. Preparation room where 2000 culture bottles are prepared every week.

B. Miss Poonsapaya is checking pH of the agar medium before autoclaving.

M

Figure C. Calli are being transfered in a laminar flow cabinet.

D. Liquid culture on the shaker.

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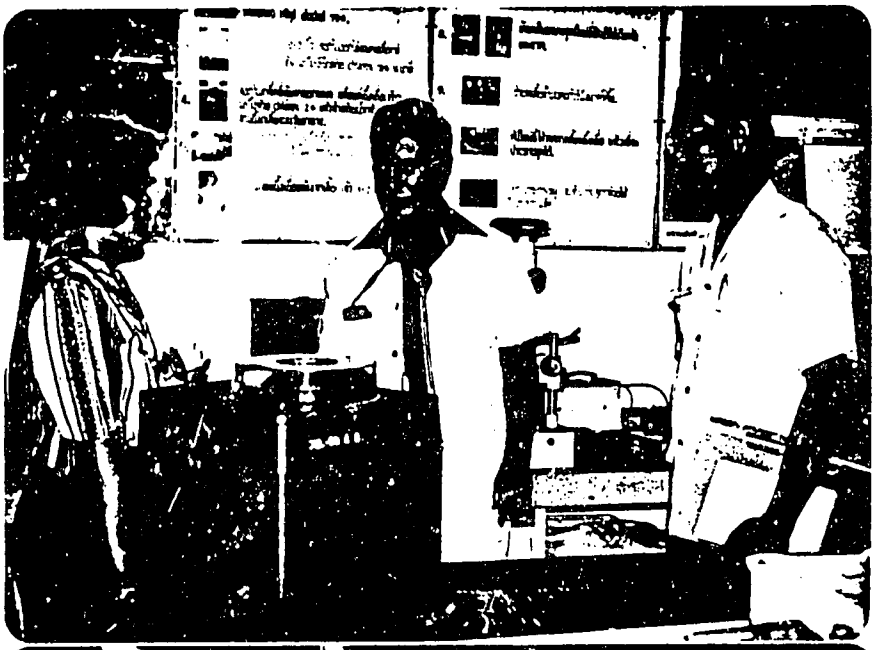

Figure E. Dr. James L. Walker, research coordinator of USAID (right) is inspecting the culture room.

F. Dr. Walker (middle), Dr. Kamnualta (right) and principal investigator are discussing on the management problems.

Figure G. Dr. D.A. Evans of the DNA Plant Technology Corp. as the USAID expert visits our laboratory;

H. Professor Y. Yamada (sitting) of Kyoto University with our staff and graduate student.

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Figure I. Professor M.W. Nabors (middle). the cooperative investigator with Associate Professor M. Vajrabhaya (right). principal investigator and Professor T. Vajrabhaya (dark shirt). investigator;

J. Both are listening to the talk on plant regeneration by one of the project staff.

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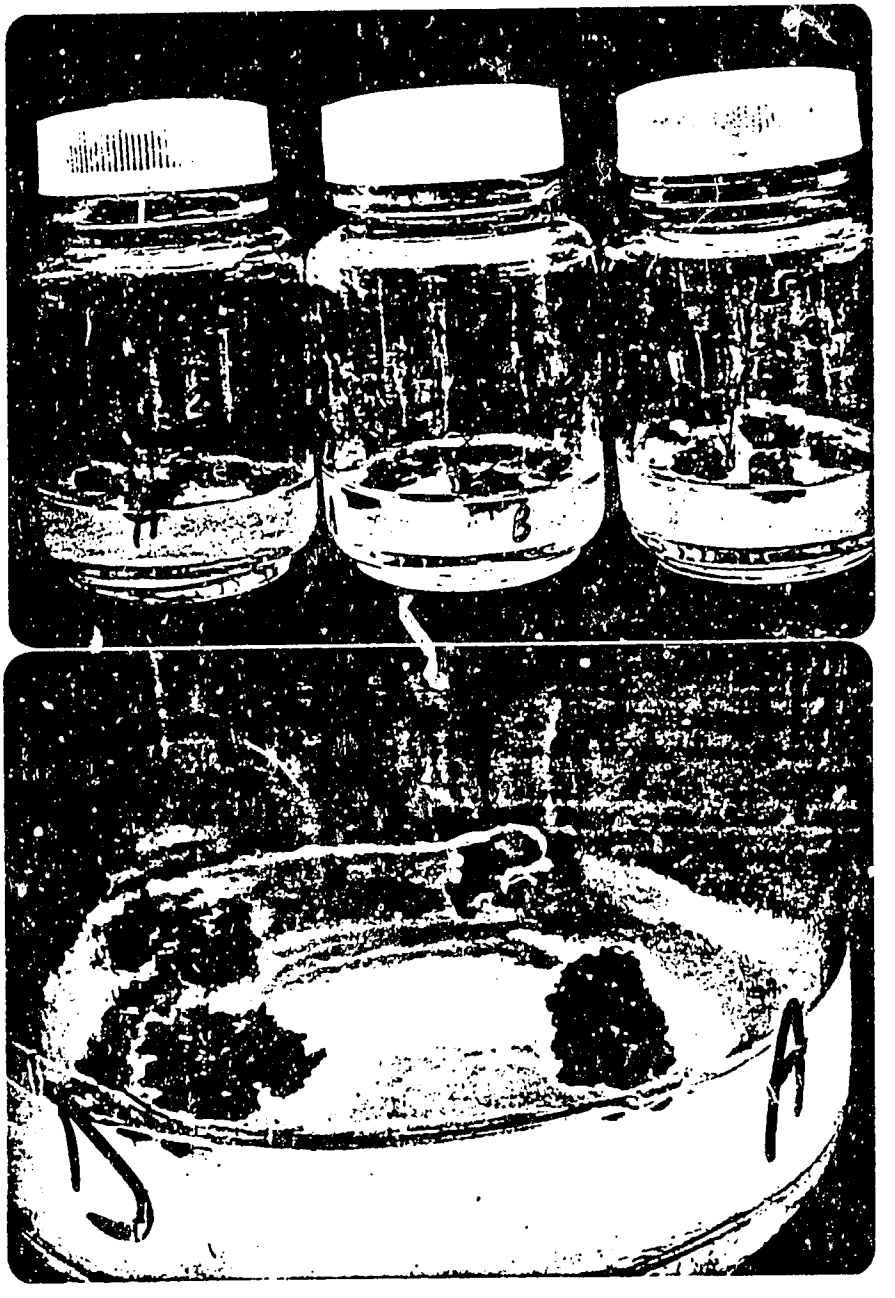

Figure K. Calli with greenspots are forming shoots in a modified White's medium.

L. A close-up photograph showing the type of callus that will regenerate into plantlet.

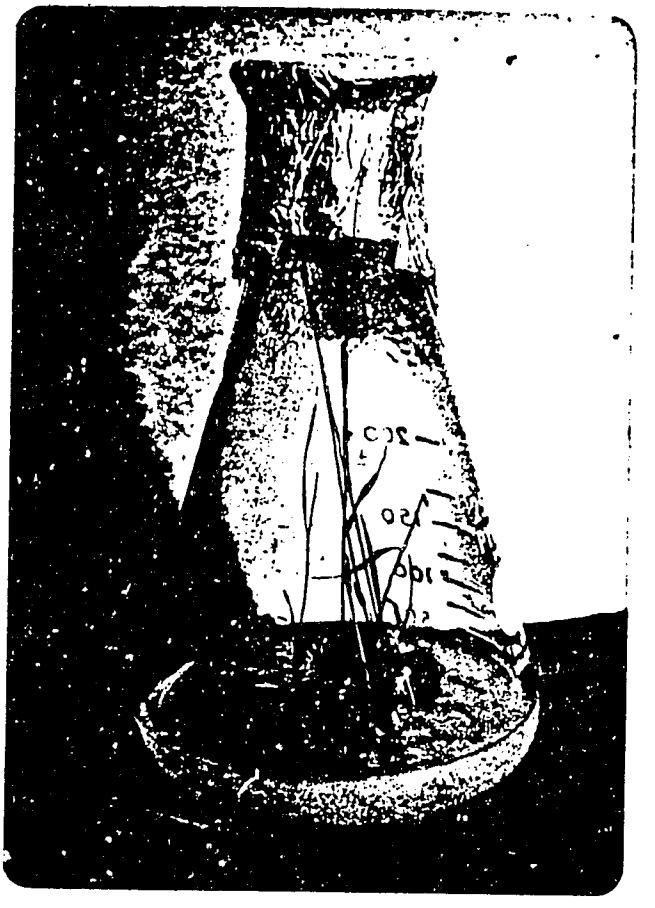

Figure M, Rice plants regenerated from calli in a modified LS medium, with 6 % sucrose and 10 % coconut water added, are ready to be planted in natural condition.

WORK DONE DURING JULY– DECEMBER 1984

OBJECTIVES

To establish suitable media for regeneration of plant from callus by the adjustment of inorganic and organic compositions of the media, and by altering physical environment both within and outside media.

BACKGROUND

All of the experiments performed in this period are the continuation of the preliminary work during January and June 1984 as reported in The Progress Report II. As reported earlier that the regeneration of rice plant from callus is still a problem which partly due to varietal differences. The response of Japonica type to the treatments reported by many workers in the United States and Japan have not been reported successfully with the Indica type, whereas the references on regeneration from somatic cell callus are scarce and not very encouraging. Much of the time spent during this period was to find basic causes of failure found in the first part of the year, and the results obtained were satisfactory.

Two major factors that cause failure in regeneration have been discov ered in the cause of experiments. First, in all experiments performed in the past indicated that the present of sucrose in media supplemented with coconut water reduced greenspot formation from 4.4 to 6 times. In the absent of coconut water, greenspot formation was very low in both with or without sucrose. This finding contradics with other reports on rice tissue culture, but surprisingly it is in agreement with the works on monopodial orchid tissues. This may be due to differences between the tissues of low land and high land rice varieties or possible other unknown factors. Second, the high salt media (L.S.) consistently give much better result in the initial stages of callus induction and growth but on the contrary the give distinctly poor result in

greenspot formation if compared to the low salt media (White's). The differences are likely to be the concentration of some ions which interfere with metabolism of the tissue. Other finding in the previous experiments are also taken into consideration in planing further experiments.

There are many different media used by tissue culturists around the world at the present time. Some have been used widely for long time while some are formulated quite recently as a result of some experiments and accumulation of information. Most of this recommendations are from the workers that have worked out the method for a specific group of plants but can also be applied to others as well White;1943; 1954; Murashige and Skoog, 1962; and Linsmaier and Skoog,1965). Some derive a formula from a series of test in both monocotyledonous and dicotlyledonous tissues (Schenk and Hildebrandt, 1972). In addition to these, the number of recommended organic compounds (and the combination of these) used in media are very numerous. That renders a systematic testing of the effects of these compounds extremely difficult. Therefore, we selected only some and tried to our own work.

The following report on the experiments are based largely on the the two major factors stated above yet other factors are taken into account. These are the organic additives, ratios of cytokinin and auxin, concentration of NH_4^+ , NO_3^-, K^+ ions, physical factors both in the media and outside, and also some combinations of these.

MATERIALS AND METHODS

All calli used in the experiments were initiated from mature embryos, some of which had been stored for long time before used. Bacterial and fungal contamination are fairly common due to high humidity during storage. Some microorganisms are seed born, such as Pythium, and some only attach to the hull surface. Such contaminations occur twice as much in the rainy season,

a surface sterilization method is describe in the Progress Report I and its improved method in the Progress Report II.

Calli were obtained by culturing embryo in the dark using Linsmaier and Skoog (1965) basic salts and vitamins, 3 % sucrose, 0.8 % agar, 1-2 ppm 2,4-D, and either 0.3 ppm kinetin or 10 % v/v coconut water. this callus induction medium has been thoroughly tested and standardized for the initiation of callus to be used in any experiment. The detailed procedures are described in the Progress I and II.

After two weeks of culture in the dark, the shoot and the remnant of seed are cut off. Unless otherwise specified, calli are then transfered to a greenspot induction medium which composes of a modified White's (1954) medium (W) by using only its major element composition and substitutes minor elements (including iron compound) by a half strength of Linsmaier and Skoog's (1965) compound(LS). Ten percent of distilled water is substituted by coconut water (CW) from green fruit. Sucrose is ommitted. Culture vessels are kept under a regime of 14/10 or 16/8 hours light/dark photoperiods, 2000 lux light intensity provided by Philips, Tl 40 W/33, fluorescent bulbs. Temperature is maintained between 24 and 26 ^{o} C by using window typed air conditions.

Five rice varieties were used in these experiments these are among the recomended varieties of Rice Division, Department of Agriculture. They are RD 23, RD 25, KDML, LPT and NSPT, the last varity being sticky rice.

Experiment 1

OBJECTIVES: To study the effects of organic additives on regeneration of plant from callus; to study the relationship between organic additives and varied concentrations of auxin; and to determine varietal differences in response to the treatments.

BASIC CULTURE MEDIUM: Linsmaier and Skoog's (1965) with 3 ppm kinetin and 3 % sucrose. (Table 1)

RICE VARIETIES: RD25 KDML and NSPT.

CALLUS AGES: Two, four and six weeks.

LIGHT REGIME: 14/10 light/dark.

NUMBER OF TREATMENTS: 15 different media; 3 rice varieties.

NUMBER OF REPLICATIONS: 20-40 calli for each treatment.

CONCLUSION: Overall results in this experiment is rather poor, however, media supplemented with 0.3 casein hydrolysate gave best result in both greenspot formation and plant regeneration. Two weeks old calli responded to the treatment better than the older ones in any variety. Calli of NSPT variety differentiated into greenspot better than other varieties tested.

RESULTS AND DISCUSSION: The result of plant regeneration in this experiment is rather poor, but it gives us better perspective in designing further experiments. Calli in all treatments began to turn brown after two weeks in culture. However, all treatments gave rise to root initiation and some calli produce shoots and plantlets at later stages of development. Even the number of plants regenerated from the treatments was rather low, nevertheless the medium supplemented with 0.3 % casein hydrolysate gave the best result either without or with low concentration (0.1, 0.5 ppm) of IAA. (see Table 2, 3, 4 and 8). This result is in agreement with that of Inoue and Maeda (1980). The same medium stated above also induced more greenspot formation than other media. Therefore, it is rather safe to assume that greenspot is an early step in shoot apex initiation prior to shoot development. However, one should be aware that the requirement of initiation and development of shoot apex may not be the same.

Table 1 Fifteen modified LS media used in the experiment, all are supplemented with 3 ppm kinetin.

Medium	IAA ppm	Yeast extract (%)	Casine hydrolysate (%)
A_1	-	-	-
A_2	0.1	-	-
A_3	0.5	-	-
A_4	1.0	-	-
B_1	-	0.30	-
B_2	0.1	0.30	-
B_3	0.5	0.30	-
B_4	1.0	0.30	-
C_1	-	-	0.30
C_2	0.1	-	0.30
C_3	0.5	-	0.30
C_4	1.0	-	0.30
D_1	-	0.15	0.15
D_2	0.1	0.15	0.15
D_3	-	0.30	0.30

Table 2 Two week old calli in 15 modified media.

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		SH		N	C	GS		R		SH		N	C	GS		R		SH	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
A_1	2	40	3.25	0	0	2	5	0	0	32	3.47	0	0	0	0	0	0	28	3.86	0	0	0	0	0	0
LS + 3 ppm K + 3 % S	4	40	2.13	0	0	13	33	0	0	32	2.44	3	9.4	2	6.3	1	3.1	28	2.14	5	17.9	5	17.9	0	0
	6	40	2.03	0	0	20	50	0	0	32	2.06	0	0	7	21.9	1	3.1	28	1.96	4	14.3	4	14.3	0	0
A_2	2	40	3.33	0	0	8	20	0	0	40	3.73	0	0	1	2.5	0	0	28	3.68	0	0	0	0	0	0
LS + 3 ppm K + 0.1 ppm	4	40	2.15	1	2.5	19	48	1	2.5	40	2.80	11	27.5	7	17.5	0	0	28	2.11	5	17.9	6	21.4	0	0
IAA + 3 % S	6	40	1.93	4	10.0	27	68	1	2.5	40	2.15	2	5.0	10	25.0	0	0	28	1.96	1	3.6	6	21.4	0	0
A_3	2	40	3.25	0	0	2	5	0	0	40	3.73	0	0	4	10.0	0	0	28	3.68	0	0	0	0	0	0
LS + 3 ppm K + 0.5 ppm	4	40	2.28	2	5	26	65	0	0	40	2.80	11	27.5	11	27.5	0	0	28	2.18	11	39.3	8	28.6	0	0
IAA + 3 % S	6	40	2.03	1	2.5	27	68	0	0	40	2.25	7	17.5	10	25.0	0	0	28	1.96	3	10.7	9	32.1	0	0

Table 2 Two week old calli in 15 modified media. (continue)

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sb		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
A_4	2	40	3.43	0	0	2	5.0	0	0	40	3.68	0	0	4	10.0	0	0	24	3.83	0	0	0	0	0	0
LS + 3 ppm K + 1 ppmIAA	4	40	2.50	2	5.0	21	52.5	1	2.5	40	2.63	4	10.0	9	22.5	0	0	24	2.25	9	37.5	7	29.2	0	0
+ 3 % S	6	40	2.10	1	2.5	28	70.0	1	2.5	40	2.20	3	7.5	5	12.5	0	0	24	1.88	4	16.6	8	33.3	0	0
B_1	2	40	3.05	0	0	2	5.0	0	0	40	3.40	0	0	0	0	0	0	24	3.83	0	0	0	0	0	0
LS + 3 ppm K + 0.3 % Y	4	40	2.25	3	7.5	19	47.5	0	0	40	2.30	0	0	11	27.5	0	0	24	2.00	4	16.7	7	29.2	0	0
+ 3 % S	6	40	2.05	0	0	26	65.0	1	2.5	40	2.00	0	0	17	42.5	0	0	24	2.00	0	0	5	20.8	0	0
B_2	2	40	3.23	0	0	0	0	0	0	40	3.48	0	0	1	2.5	0	0	20	3.65	0	0	0	0	0	0
LS + 3 ppm K + 0.3 % Y	4	40	2.05	2	5	20	50.0	0	0	40	2.40	4	10.0	8	20.0	0	0	20	2.20	6	30.0	2	10.0	0	0
+ 0.1 ppm IAA + 3 % S	6	40	1.95	0	0	21	52.5	0	0	40	2.05	1	2.5	12	30.0	1	2.5	20	1.95	0	0	4	20.0	0	0
B_3	2	32	3.56	0	0	0	0	0	0	40	3.18	0	0	3	7.5	0	0	28	3.64	0	0	2	7.1	0	0
LS + 3 ppm K + 0.3 % Y	4	32	2.16	1	3.1	11	34.4	0	0	40	2.55	5	12.5	7	17.5	0	0	28	2.07	18	64.3	16	57.1	0	0
+ 0.5 ppm IAA + 3 % S	6	32	2.00	0	0	21	65.6	0	0	40	1.95	0	0	12	30.0	0	0	28	2.00	6	21.4	20	71.4	0	0

Table 2 Two week old calli in 15 modified media. (continue)

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
B_4	2	40	3.40	0	0	1	2.5	0	0	40	3.43	0	0	2	5.0	0	0	28	3.68	0	0	0	0	0	0
LS + 3 ppm K + 0.3 %Y	4	40	2.15	2	5.0	16	40.0	0	0	40	2.43	6	15.0	7	17.5	0	0	28	2.00	6	21.4	3	10.7	0	0
+ 1 ppm IAA + 3 % S	6	40	2.00	3	7.5	20	50.0	0	0	40	2.08	0	0	14	35.0	0	0	28	1.89	0	0	9	32.1	0	0
C_1	2	40	3.35	0	0	4	10.0	0	0	40	3.40	0	0	1	2.5	0	0	28	3.61	0	0	0	0	0	0
LS + 3 ppm K + 0.3 % C	4	40	2.08	3	7.5	26	65.0	0	0	40	2.53	8	20.0	4	10.0	1	2.5	28	2.07	10	35.7	8	28.6	0	0
+ 3 % S	6	40	1.93	0	0	21	52.5	0	0	40	2.10	0	0	5	12.5	1	2.5	28	2.00	7	25.0	13	46.4	0	0
C_2	2	40	3.58	0	0	2	5.0	0	0	36	3.50	0	0	5	13.9	0	0	28	3.75	0	0	0	0	0	0
LS + 3 ppm K + 0.3 % C	4	40	2.20	2	5.0	20	50.0	0	0	36	2.53	4	11.1	3	8.3	0	0	28	2.07	14	50.0	10	35.7	0	0
+ 0.1 ppm IAA + 3 % S	6	40	2.03	1	2.5	21	52.5	2	5	36	1.86	2	5.6	7	19.4	0	0	28	2.00	5	17.9	16	57.1	2	7.14
C_3	2	40	3.38	0	0	4	10.0	0	0	40	3.30	0	0	2	5.0	0	0	28	3.64	0	0	0	0	0	0
LS + 3 ppm K + 0.3 % C	4	40	2.10	8	20.0	22	55.0	0	0	40	2.53	6	15.0	7	14.5	0	0	28	2.11	10	35.7	3	10.7	0	0
+ 0.5 ppm IAA + 3 % S	6	40	1.93	1	2.5	29	72.5	0	0	40	1.93	3	7.5	4	10.0	1	2.5	28	2.00	2	7.1	11	39.3	0	0

Table 2 Two week old calli in 15 modified media. (continue)

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
C_4	2	40	2.95	0	0	2	5.0	0	0	40	3.28	0	0	2	5.0	0	0	28	3.64	0	0	0	0	0	0
LS + 3 ppm K + 0.3 % C	4	40	2.05	2	5.0	24	60.0	0	0	40	2.18	4	10	5	12.5	0	0	28	2.25	14	50.0	8	28.6	0	0
+ 1.0 ppm IAA + 3 % S	6	40	1.95	2	5.0	31	77.5	0	0	40	2.00	0	0	6	15.0	0	0	28	1.93	4	14.3	12	42.9	0	0
D_1	2	40	3.40	0	0	2	5.0	0	0	40	3.33	0	0	2	5.0	0	0	28	3.71	0	0	0	0	0	0
LS + 3 ppm K + 0.15 % Y	4	40	2.18	0	0	27	67.5	0	0	40	2.15	0	0	8	20.0	0	0	28	2.00	7	25.0	7	25.0	0	0
+ 0.15 % C + 3 % S	6	40	2.00	0	0	30	75.0	0	0	40	1.98	0	0	6	15.0	0	0	28	2.00	0	0	8	28.6	0	0
D_2	2	40	3.18	0	0	2	5.0	0	0	40	3.33	0	0	3	7.5	0	0	28	3.57	0	0	0	0	0	0
LS + 3 ppm K + 0.15 % Y	4	40	2.20	0	0	12	30.0	0	0	40	2.08	2	5	4	10.0	0	0	28	2.04	8	28.6	4	14.3	0	0
+ 0.15 % C + 0.1 ppm IAA + 3 % S	6	40	2.08	3	7.5	21	52.5	0	0	40	1.98	0	0	5	12.5	0	0	28	2.00	0	0	8	28.6	0	0
D_3	2	40	3.20	0	0	1	2.5	0	0	40	3.23	0	0	1	2.5	0	0	28	3.32	0	0	0	0	0	0
LS + 3 ppm K + 0.3 % Y	4	40	2.15	0	0	26	65.0	0	0	40	2.00	1	2.5	7	17.5	1	2.5	28	2.11	8	28.6	8	28.6	0	0
+ 0.3 % C + 3 % S	6	40	2.10	1	5	32	80.0	0	0	40	1.98	0	0	6	15.0	1	2.5	28	1.89	0	0	9	32.1	0	0

Table 3 Four week old calli in 15 modified media.

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
A_1	4	32	2.81	0	0	0	0	0	0	40	1.93	0	0	5	12.5	0	0	36	2.72	0	0	6	16.7	0	0
	6	32	2.09	1	3.1	15	46.9	0	0	40	1.43	1	2.5	6	15.0	0	0	36	2.00	3	8.3	7	19.4	0	0
	8	32	2.03	1	3.1	21	65.6	0	0	40	1.33	0	0	9	22.5	0	0	36	2.00	0	0	7	19.4	0	0
A_2	4	32	2.91	0	0	4	12.5	0	0	40	3.15	0	0	5	12.5	0	0	40	2.93	0	0	2	5.0	0	0
	6	32	2.13	0	0	28	87.5	0	0	40	2.53	1	2.5	6	15.0	0	0	40	2.00	3	7.5	5	12.5	0	0
	8	32	2.09	0	0	28	87.5	0	0	40	2.45	0	0	6	15.0	0	0	40	2.00	1	2.5	9	22.5	0	0
A_3	4	20	3.20	0	0	1	5.0	0	0	40	2.05	0	0	6	15.0	0	0	40	2.75	0	0	3	7.5	0	0
	6	20	2.25	1	5.0	20	100	0	0	40	1.68	1	2.5	7	17.5	0	0	40	2.00	4	10	9	22.5	0	0
	8	20	2.15	1	5.0	20	100	0	0	40	1.45	3	7.5	8	20.0	0	0	40	1.98	4	10	9	22.5	0	0
A_4	4	20	3.00	0	0	4	20.0	0	0	40	3.28	0	0	5	12.5	0	0	36	2.92	0	0	2	5.6	0	0
	6	20	2.10	0	0	13	65.0	0	0	40	2.60	1	2.5	11	27.5	0	0	36	2.03	6	16.7	7	19.4	0	0
	8	20	2.10	0	0	11	55.0	0	0	40	2.33	1	2.5	10	25.0	0	0	36	2.00	1	2.8	16	44.4	0	0

ble 3 Four week old calli in 15 modified media. (continue)

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
B_1	4	32	2.97	0	0	7	21.9	0	0	40	2.73	0	0	6	15.0	0	0	32	2.47	0	0	1	3.1	0	0
	6	32	2.06	0	0	22	68.8	0	0	40	2.20	0	0	5	12.5	0	0	32	1.94	0	0	6	18.8	0	0
	8	32	2.03	0	0	20	62.5	0	0	40	2.13	0	0	6	15.5	0	0	32	1.94	1	3.1	13	40.6	0	0
B_2	4	32	2.50	0	0	6	18.8	0	0	40	2.83	0	0	2	5.0	0	0	36	2.66	0	0	3	8.3	0	0
	6	32	2.06	0	0	21	65.6	0	0	40	2.38	0	0	5	12.5	0	0	36	2.03	1	2.8	4	11.1	0	0
	8	32	2.06	0	0	21	65.6	0	0	40	2.18	0	0	7	17.5	0	0	36	2.00	2	5.6	6	16.7	0	0
B_3	4	32	3.06	0	0	1	3.1	0	0	40	2.88	0	0	8	20.0	0	0	36	2.47	0	0	4	11.1	0	0
	6	32	2.16	0	0	23	71.9	0	0	40	2.23	0	0	8	20.0	0	0	36	2.03	2	5.6	4	11.1	0	0
	8	32	2.06	0	0	23	71.9	0	0	40	2.03	0	0	11	27.5	0	0	36	1.97	2	5.6	12	33.3	0	0
B_4	4	32	3.09	0	0	11	34.4	0	0	40	2.70	0	0	2	5.0	0	0	40	2.38	0	0	3	7.5	0	0
	6	32	2.00	0	0	22	68.8	0	0	40	2.25	0	0	6	15.0	0	0	40	1.98	2	5.0	5	12.5	0	0
	8	32	2.00	0	0	22	68.8	0	0	40	2.20	0	0	10	25.0	0	0	40	1.98	1	2.5	6	15.0	0	0

Table 3 Four week old calli in 15 modified media. (continue)

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
C_1	4	20	3.35	0	0	4	20.0	0	0	40	2.95	0	0	4	10.0	0	0	36	2.94	0	0	3	8.3	0	0
	6	20	2.05	0	0	15	75.0	0	0	40	2.40	0	0	5	12.5	0	0	36	2.00	1	2.8	7	19.4	0	0
	8	20	2.00	0	0	15	75.0	0	0	40	2.05	1	2.5	10	25.0	1	2.5	36	2.00	3	8.3	9	25	0	0
C_2	4	32	3.03	0	0	8	25.0	0	0	40	2.83	0	0	12	30.0	0	0	32	3.19	0	0	0	0	0	0
	6	32	2.22	0	0	23	71.9	0	0	40	2.30	0	0	9	22.5	0	0	32	1.97	3	9.4	2	6.3	0	0
	8	32	2.06	2	6.3	17	53.1	0	0	40	2.13	0	0	13	32.5	0	0	32	1.97	0	0	5	15.5	0	0
C_3	4	32	1.94	0	0	5	15.6	0	0	40	3.35	0	0	3	7.5	0	0	32	3.22	0	0	2	6.3	0	0
	6	32	1.31	1	3.1	16	50.0	0	0	40	2.55	0	0	2	5.0	0	0	32	2.00	6	18.8	10	31.3	0	0
	8	32	1.31	0	0	15	46.9	0	0	40	2.28	0	0	5	12.5	0	0	32	1.97	0	0	13	40.6	0	0
C_4	4	32	2.28	0	0	6	18.8	0	0	40	2.90	0	0	5	12.5	0	0	40	3.20	0	0	0	0	0	0
	6	32	2.09	0	0	23	71.9	0	0	40	2.40	1	2.5	6	15.0	0	0	40	2.05	5	12.5	3	7.5	0	0
	8	32	2.00	0	0	26	81.3	0	0	40	2.20	0	0	9	22.5	0	0	40	2.00	0	0	4	10.0	0	0

Table 3 Four week old calli in 15 modified media. (continue)

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
D_1	4	32	3.03	0	0	3	9.4	0	0	40	2.68	0	0	6	15.5	0	0	36	2.48	0	0	2	5.0	0	0
	6	32	2.03	0	0	25	78.1	0	0	40	2.13	0	0	5	12.5	0	0	36	1.80	6	15.0	5	12.5	0	0
	8	32	2.03	0	0	25	78.1	0	0	40	2.03	0	0	5	12.5	0	0	36	1.8	1	2.5	6	15.0	0	0
D_2	4	32	3.03	0	0	4	12.5	0	0	40	1.90	0	0	1	2.5	0	0	36	3.00	0	0	1	2.8	0	0
	6	32	2.09	0	0	26	81.3	0	0	40	1.30	0	0	1	2.5	0	0	36	2.00	1	2.8	2	5.6	0	0
	8	32	2.03	0	0	24	75.0	0	0	40	1.25	0	0	8	20.0	0	0	36	2.00	0	0	2	5.6	0	0
D_3	4	32	2.91	0	0	6	18.8	0	0	40	2.80	0	0	4	10.0	0	0	32	2.91	0	0	2	6.3	0	0
	6	32	2.00	0	0	19	59.4	0	0	40	2.30	0	0	4	10.0	0	0	32	2.00	2	6.3	6	18.8	0	0
	8	32	2.00	0	0	21	65.6	0	0	40	2.00	0	0	11	27.5	0	0	32	2.00	0	0	8	25.0	0	0

Table 4 : Six- week old calli in 15 modified media.

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
A_1	6	32	2.06	0	0	1	3.1	0	0	40	3.38	0	0	0	0	0	0	36	2.61	0	0	6	16.7	0	0
	8	32	2.09	0	0	9	28.1	0	0	40	2.38	1	2.5	3	7.5	0	0	36	2.06	1	2.8	8	22.2	1	2.8
	10	32	2.06	0	0	23	71.9	0	0	40	2.08	0	0	4	10.0	0	0	36	2.00	1	2.8	14	38.9	1	2.8
A_2	6	28	2.46	0	0	0	0	0	0	40	3.48	0	0	0	0	0	0	40	2.90	0	0	2	5.0	0	0
	8	28	2.04	0	0	12	42.9	0	0	40	2.40	0	0	4	10.0	0	0	40	2.00	1	2.5	5	12.5	0	0
	10	28	2.00	0	0	19	67.9	0	0	40	2.28	0	0	8	20.0	0	0	40	2.00	1	2.5	15	37.5	0	0
A_3	6	24	2.58	0	0	0	0	0	0	40	3.38	0	0	1	2.5	0	0	40	3.03	0	0	5	12.5	0	0
	8	24	2.29	0	0	8	33.3	0	0	40	2.40	2	5.0	3	7.5	0	0	40	2.03	10	25.0	11	27.5	0	0
	10	24	2.21	0	0	15	62.5	0	0	40	2.15	0	0	9	22.5	0	0	40	2.00	4	10.0	14	35.0	0	0
A_4	6	28	2.43	0	0	1	3.6	0	0	40	3.45	0	0	0	0	0	0	40	2.90	0	0	3	7.5	0	0
	8	28	2.25	0	0	18	64.3	0	0	40	2.45	0	0	2	5.0	0	0	40	2.33	9	22.5	14	35.0	1	2.5
	10	28	2.07	0	0	22	78.6	0	0	40	2.05	0	0	3	7.5	0	0	40	1.90	1	2.5	17	42.5	1	2.5

Table 4 Six- week old calli in 15 modified media. (continue)

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
B_1	6	28	2.46	0	0	0	0	0	0	40	3.10	0	0	1	2.5	0	0	40	2.0	0	0	7	17.5	0	0
	8	28	2.11	0	0	7	25.0	0	0	40	2.13	0	0	1	2.5	0	0	40	2.03	4	10.0	9	22.5	0	0
	10	28	2.11	0	0	13	46.4	0	0	40	2.00	0	0	4	10.0	0	0	40	1.98	2	5.0	16	40.0	0	0
B_2	6	32	2.19	0	0	0	0	0	0	40	3.38	0	0	1	2.5	0	0	40	2.80	0	0	3	7.5	0	0
	8	32	2.00	0	0	10	31.3	0	0	40	2.18	0	0	4	10.0	0	0	40	2.10	2	5.0	8	20.0	0	0
	10	32	1.81	0	0	27	84.4	0	0	40	2.08	0	0	5	12.5	0	0	40	2.05	0	0	13	32.5	0	0
B_3	6	32	2.44	0	0	4	12.5	0	0	40	3.45	0	0	2	5.0	0	0	40	2.60	0	0	2	5.0	0	0
	8	32	2.13	0	0	22	68.8	0	0	40	2.10	1	2.5	7	17.5	0	0	40	2.00	1	2.5	7	17.5	0	0
	10	32	1.94	0	0	29	90.6	0	0	40	2.00	1	2.5	11	27.5	0	0	40	2.00	2	5.0	12	30.0	0	0
B_4	6	32	2.19	0	0	0	0	0	0	40	3.35	0	0	0	0	0	0	40	2.75	0	0	6	15.0	0	0
	8	32	2.03	0	0	13	40.6	0	0	40	2.18	0	0	5	12.5	0	0	40	2.03	3	7.5	12	30.0	0	0
	10	32	2.00	0	0	20	62.5	0	0	40	2.20	0	0	7	17.5	0	0	40	2.00	0	0	11	27.5	0	0

Table 4 Six- week old calli in 15 modified media. (continue)

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	CS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
C_1	6	16	2.31	0	0	1	6.3	0	0	40	3.20	0	0	1	2.5	0	0	40	2.45	0	0	9	22.5	0	0
	8	16	2.25	0	0	11	68.8	0	0	40	2.23	1	2.5	4	10.0	0	0	40	2.15	0	0	12	30.0	0	0
	10	16	2.13	0	0	16	100	0	0	40	2.05	0	0	7	17.5	0	0	40	2.05	0	0	23	57.5	0	0
C_2	6	32	2.34	0	0	0	0	0	0	40	3.28	0	0	1	2.5	0	0	40	3.00	0	0	5	12.5	0	0
	8	32	2.00	0	0	11	34.4	0	0	40	2.10	2	5.0	4	10.0	0	0	40	2.15	8	20.0	15	37.5	0	0
	10	32	1.97	0	0	26	81.3	0	0	40	2.00	0	0	13	32.5	0	0	40	2.05	2	5.0	17	42.5	0	0
C_3	6	32	2.44	0	0	0	0	0	0	36	3.64	0	0	0	0	0	0	40	2.68	0	0	2	5.0	0	0
	8	32	2.03	0	0	15	46.9	0	0	36	2.19	2	6	3	8.3	0	0	40	2.00	1	2.5	8	20.0	0	0
	10	32	2.03	0	0	25	78.1	0	0	36	2.03	0	0	7	19.4	0	0	40	2.00	1	2.5	20	58.0	0	0
C_4	6	28	2.32	0	0	0	0	0	0	40	3.28	0	0	0	0	0	0	40	2.58	0	0	4	10.0	0	0
	8	28	2.00	0	0	12	42.9	0	0	40	2.28	3	8.3	2	5.0	0	0	40	2.10	5	12.5	13	32.5	0	0
	10	28	2.00	0	0	22	78.6	0	0	40	2.18	1	2.5	8	20.0	0	0	40	2.00	1	2.5	15	37.5	0	0

Table 4 Six- week old calli in 15 modified media. (continue)

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
D_1	6	32	2.34	0	0	0	0	0	0	40	3.28	0	0	1	2.5	0	0	40	2.65	0	0	4	10.0	0	0
	8	32	2.00	0	0	10	31.3	0	0	40	2.23	1	2.5	6	15.0	0	0	40	2.05	2	5.0	10	25.0	0	0
	10	32	1.25	0	0	27	84.4	0	0	40	2.10	0	0	10	25.0	0	0	40	2.00	0	0	12	30.0	0	0
D_2	6	28	2.43	0	0	0	0	0	0	40	3.28	0	0	1	2.5	0	0	40	2.70	0	0	5	12.5	0	0
	8	28	2.04	0	0	15	53.6	0	0	40	2.20	0	0	2	5.0	1	2.5	40	2.03	7	17.5	11	27.5	0	0
	10	28	2.00	0	0	19	67.9	0	0	40	2.00	0	0	3	7.5	1	2.5	40	1.95	0	0	11	27.5	1	2.5
D_3	6	32	2.31	0	0	0	0	0	0	32	3.34	0	0	0	0	0	0	36	2.72	0	0	1	2.8	0	0
	8	32	2.00	0	0	6	18.8	0	0	32	2.19	0	0	2	6.3	0	0	36	2.00	0	0	4	11.1	0	0
	10	32	2.00	0	0	18	56.3	0	0	32	2.00	0	0	5	15.6	0	0	36	2.00	0	0	10	27.8	0	0

Table 5 Greenspot induction in three different ages of RD 25 calli in 15 modified media

Callus age (week)	Weeks after culture	MEDIUM														
		A_1	A_2	A_3	A_4	B_1	B_2	B_3	B_4	C_1	C_2	C_3	C_4	D_1	D_2	D_3
2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	+	+	+	+	+	+	+	+	+	+ +	+	-	-	-
	4	-	+	+	+	-	-	-	+	-	+	+	+	-	+	+
4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-
	4	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-
6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- no greenspot, + 1-10% greenspot, ++ 11-20% greenspot.

Table 6 Greenspot induction in three different ages of KDML calli in 15 modified media

Callus age (week)	Weeks after culture	MEDIUM														
		A_1	A_2	A_3	A_4	B_1	B_2	B_3	B_4	C_1	C_2	C_3	C_4	D_1	D_2	D_3
2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	+	+++	+++	+	-	+	++	++	++	++	++	+	-	+	+
	4	-	+	++	+	-	+	-	-	-	+	+	-	-	-	-
4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-
	4	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	+	-	+	-	-	-	+	-	+	+	+	+	+	-	-
	4	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-

- no greenspot, + 1-10% greenspot, ++ 11-20% greenspot, +++ 21-30% greenspot.

Table 7 Greenspot induction in three different ages of NSPT calli in 15 modified media

Callus age (week)	Weeks after culture	MEDIUM														
		A_1	A_2	A_3	A_4	B_1	B_2	B_3	B_4	C_1	C_2	C_3	C_4	D_1	D_2	D_3
2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	++	++	++++	++++	++	+++	+++++	+++	++++	+++++	++++	+++++	+++	+++	+++
	4	++	+	++	++	-	-	+++	-	+++	++	+	++	-	-	-
4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	+	+	+	++	-	+	+	+	+	+	++	++	++	+	+
	4	-	+	+	+	+	+	+	+	+	-	-	-	+	-	-
6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	+	+	+++	+++	+	+	+	+	-	++	+	++	+	++	-
	4	+	+	+	+	+	-	+	-	-	+	+	+	-	-	-

- no greenspot, + 1-10% greenspot, ++ 11-20% greenspot, +++ 21-30% greenspot, ++++ 31-40% greenspot
+++++ 41-50% greenspot, ++++++ 51-60% greenspot, +++++++ 61-70% greenspot.

30

Table 8 Shoot initiation from three different callus ages of RD 25, KDML and NSPT, at the beginning of the experiment, culture in fifteen different media four weeks.

VARIETY	Callus age at the beginning of experiment	MEDIUM														
		A_1	A_2	A_3	A_4	B_1	B_2	B_3	B_4	C_1	C_2	C_3	C_4	D_1	D_2	D_3
RD 25	2		2.5%		2.5%	2.5%					5.0%					
	4															
	6															
KDML	2	3.1%					2.5%			2.5%		2.5%				2.5%
	4									2.5%						
	6														2.5%	
NSPT	2									7.14%						
	4															
	6	2.8%			2.5%										2.5%	

It in interesting to note that the result of this experiment is not in agreement with those of Davoyan and Smetanin (1979), Oono (1981); and Yatazawa et al (1967) that they obtained good results by using yeast extract or the combination of yeast extract and casein hydrolysate as organic addtives in the media. In our experiment, the present of yeast extract in the media either as an undefined organic additive alone or in combination with casein hydrosate gave poor result in all cases.

In studying the ability of regeneration of callus at different ages, it was found that two week old calli produce more greenspots and shoot than the four or six week old ones. This result shows that calli might loss their totipotency with time of culturing.

Varietal differences were demonstrated by the NSPT calli produced much larger number of greenspots in all treatments (see table 2 to 7). It is interesting to note that, media supplemented with yeast extract gave poor overall result in plant regeneration but gave best result in inducing greenspot formation in the NSPT variety. However, no further development of shoot was found, this gives us an impression that shoot development may require different treatments from greenspot formation.

Experiment 2

OBJECTIVES: To examine the deleterious effect of sucrose on growth and differentiation of callus reported in Progress Report II; to reexamine the beneficial effect of coconut water; to test the requirement of myo-inosital for regeneration.

BASIC CULTURE MEDIA: Modified White's (1954) supplemented with 10 % coconut water (CW) ; Linsmaier and Skoog's (1965) Schenk and Hildebrandt's (1972).

RICE VARIETIES: RD 23, RD 25, KDML and NSPT.

CALLUS AGES: Four, six and eight weeks.

LIGHT REGIME: 16/8 light/dark.

NUMBER OF TREATMENTS: 19 different media; 4 rice varieties.

NUMBER OF REPLICATIONS: 20-40 calli for each treatment.

CONCLUSION: Greenspot and shoot formation were reduced drastically as the concentration of sucrose rose from 0 to 3 and 6 % . This result indicates that the toxic effect of sucrose on cell occurs during shoot apex initiation. Coconut water could be used as substitutes for kinetin, myo-inosital and probably other organic additives. Furthermore, coconut water had effect on prolonging callus life and inducing greenspot formation especially in media without sucrose.

RESULTS AND DISCUSSION: This experiment is the reexamination of the results reported in the Progrees Report II; which showed that sucrose at the recommened concentration (3 to 6 %) had deleterious effect on callus, either by interfering with greenspot formation or by direct toxicity on callus cell at shoot initiation stage; and to test whether some organic additives have beneficial effects on plant regeneration.

This present result confirms our finding reported earlier that the presence of sucrose lowered the chance of obtaining greenspots. Calli of all varieties responded wellin modified Whites's media without sucrose (treatment W_1, W_4, W_7; fig. 3), and some calli also initiated many greenspots in the LS media without sucrose (treatments L_1, L_4; fig. 1). Calli became brown very quickly when transfered to media containing sucrose either in modified White's, LS's or SH's, which resulted in reduction in number of greenspots at later stages. The effect was even more pronounce when the concentration of sucrose was increased

to 6 % (treatment L_3, L_6, W_3, W_6, W_9; fig.1, 3). This result is not in agreement with those of Brar et al. (1974); Davoyan and Smetanin (1979); Ling et al. (1984); Nishi et al (1983); Nitsch (1981); Oono (1981); Siriwardana and Nabors (1983); Yan and Zhao (1982), who reported that 2 to 6 % sucrose in the media was optimum for plant regeneration from somatic callus.

It is surprising that ommision of sugar from the media entirely still give good results, this may due to sufficient energy sources are carried over from the induction stage (treatment L_1, L_4; fig.1). However, addition of coconut water into the media seems to support better growth of callus and induces more greenspot to form (treatments W_1, W_4, and W_7) because of the incresed amount of cytokins, sugars, amino acids, vitamins, and inorganic salts (Steward et al., 1969). Such media have been previously used sucessfully for callus culture and regeneration of monopodial orchids (Kunisaki et al., 1972; Teo et al.; 1973; and Vajrabhaya and Vajrabhaya, 1976). In other plant tissues, Cheng and Voqui (1977) found that excess sucrose reduced vitality of the shoot of Douglas fir and if the concentration of sucrose was increased further the plantlet formation was inhibited entirely. Rice callus behaves in similar fashion as some problem orchids and firs but the rate of regeneration at the present time is still not satisfactory. Further investigation on carbon sources incorporated in the media is urgently needed.

Myo-inositol is widely used in plant tissue culture as an organic additive in varying concentrations. In this experiment, 100 and 1000 mg/l were added to the LS basic medium (treatments L_1 to L_6) and found no beneficial effect on higher concentration. The attention should readily be given to the media containing no sucrose since they produced more greenspots, and even found that the greenspot formation was higher in the 100 mg/l treatment

Table 9 Nineteen different media used in the experiment.

MEDIUM	Modified basal medium	Myo-inositol ppm	Sucrose %
L_1	LS	100	0
L_2	LS	100	3
L_3	LS	100	6
L_4	LS	1000	0
L_5	LS	1000	3
L_6	LS	1000	6
SH_1	SH ½	0	3
SH_2	SH ½	0	6
SH_3	SH	0	3
SH_4	SH	0	6
W_1	White	0	0
W_2	White	0	3
W_3	White	0	6
W_4	White	100	0
W_5	White	100	3
W_6	White	100	6
W_7	White	1000	0
W_8	White	1000	3
W_9	White	1000	6

Table 10 Effects of myo-inositol and sucrose on greenspot and root formation in different basal medid.

MEDIUM	Age	RD 23								RD 25								KDML								NSPT							
		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
L_1	4	36	15.5	11	30.6	12	33.3	5	13.9	40	10.0	2	5.0	16	40.0	1	2.5	40	10.0	7	17.5	2	5.0	1	2.5	40	17.3	26	65.0	6	15.0	4	10.0
	6	36	22.6	19	52.8	14	38.9	10	27.8	40	0	0	0	9	22.5	0	0	40	12.9	14	35.0	4	10.0	4	10.0	40	11.0	10	25.0	10	25.0	2	5.0
	8	36	33.3	6	16.7	17	47.2	4	11.1	40	0	0	0	20	50.0	0	0	40	20.0	2	5.0	5	12.5	0	0	40	15.0	2	5.0	12	20.0	1	2.5
L_2	4	36	21.1	9	25.0	14	38.9	8	22.2	40	13.3	6	15.0	20	50.0	6	15.0	40	10.0	3	7.5	5	12.5	3	7.5	40	29.7	31	77.5	14	35.0	13	32.5
	6	36	17.1	7	19.4	27	75.0	7	19.4	40	15.6	9	22.5	33	82.5	9	22.5	40	10.0	6	15.0	10	25.0	3	7.5	40	12.5	4	10.0	22	55.0	3	7.5
	8	36	15.0	2	5.5	26	72.2	2	5.6	40	34.0	5	12.5	35	87.5	5	12.5	40	10.0	1	2.5	12	30.0	0	0	40	0	0	0	25	62.5	0	0
L_3	4	35	33.3	6	17.1	12	34.3	4	11.4	40	10.0	4	10.0	24	60.0	4	10.0	40	10.0	1	2.5	4	10.0	1	2.5	40	17.1	21	52.5	6	15.0	5	12.5
	6	35	50.0	2	5.7	22	62.9	2	5.7	40	0	0	0	28	70.0	0	0	40	0	0	0	9	22.5	0	0	40	10.0	3	7.5	12	30.0	3	7.5
	8	35	0	0	0	22	62.9	0	0	40	0	0	0	31	77.5	0	0	40	0	0	0	10	25.0	0	0	40	20.0	1	2.5	12	30.0	1	2.5
L_4	4	36	13.8	13	36.1	11	30.6	5	13.9	40	15.0	4	10.0	22	55.0	4	10.0	40	10.0	4	10.0	3	7.5	0	0	40	23.7	19	47.5	5	12.5	4	10.0
	6	36	24.7	15	41.7	13	36.1	9	25.0	40	10.0	1	2.5	9	22.5	1	2.5	40	10.0	1	2.5	6	15.0	1	2.5	40	20.0	6	15.0	12	30.0	4	10.0
	8	36	20.0	2	5.6	16	44.4	2	5.6	40	0	0	0	17	42.5	0	0	40	0	0	0	4	10.0	0	0	40	20.0	3	7.5	12	30.0	3	7.5
L_5	4	36	12.6	19	52.6	9	25.0	5	13.9	40	10.0	3	7.5	12	30.0	3	7.5	40	10.0	3	7.5	3	7.5	1	2.5	40	24.8	27	67.5	1	2.5	1	2.5
	6	36	11.3	8	22.2	26	72.2	6	16.7	40	10.0	3	7.5	20	50.0	3	7.5	40	10.0	2	5.0	4	10.0	1	2.5	40	0	0	0	10	25.0	0	0
	8	36	10.0	3	8.3	23	63.9	3	8.3	40	0	0	0	30	75.0	0	0	40	0	0	0	5	12.5	0	0	40	0	0	0	16	40.0	0	0

Table 10 Effects of myo-inositol and sucrose on greenspot and root formation in different basal media (continued)

MEDIUM	Age	RD 23								RD 25								KDML								NSPT							
		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
L_6	4	36	11.4	14	38.9	15	41.7	8	22.2	40	0	0	0	19	47.5	0	0	40	10.0	2	5.0	4	10.0	2	5.0	40	20.0	22	55.4	9	22.5	7	17.5
	6	36	0	0	0	26	72.2	0	0	40	0	0	0	29	72.5	0	0	40	0	0	0	7	17.5	0	0	40	0	0	0	11	27.5	0	0
	8	36	0	0	0	28	77.8	0	0	40	0	0	0	34	85.0	0	0	40	0	0	0	2	5.0	0	0	40	0	0	0	13	32.5	0	0
SH_1	4	32	17.0	13	40.6	17	53.1	10	31.2	40	13.3	6	15.0	15	37.5	5	12.5	40	10.0	4	10.0	3	7.5	0	0	36	22.0	26	72.2	6	16.7	6	16.7
	6	32	12.0	10	31.2	25	78.1	9	28.1	40	10.0	2	5.0	35	87.5	2	5.0	40	10.0	9	22.5	12	30.0	3	7.5	36	12.0	13	36.1	16	44.4	8	22.2
	8	32	0	0	0	24	75.0	0	0	40	10.0	3	7.5	38	95.5	3	7.5	40	10.0	3	7.5	11	27.5	0	0	36	10.0	3	8.3	24	66.7	3	8.3
SH_2	4	32	15.0	14	43.8	10	31.3	7	21.9	40	10.0	1	2.5	17	42.5	1	2.5	40	11.4	7	17.5	3	7.5	1	2.5	40	14.0	22	55.0	8	20.0	8	20.0
	6	32	10.0	3	9.4	20	62.5	3	9.4	40	0	0	0	27	67.5	0	0	40	10.0	5	12.5	11	27.5	2	5.0	40	10.0	3	7.5	14	35.0	3	7.5
	8	32	0	0	0	20	62.5	0	0	40	0	0	0	32	80.0	0	0	40	0	0	0	13	32.5	0	0	40	0	0	0	17	42.5	0	0
SH_3	4	32	12.0	15	46.9	15	46.9	10	31.3	40	10.0	2	5.0	16	40.0	2	5.0	40	10.0	6	15.0	6	15.0	4	10.0	40	8.0	25	62.5	14	35.0	11	27.5
	6	32	10.0	7	21.9	21	65.6	6	18.8	40	10.0	4	10.0	31	77.5	4	10.0	40	11.1	9	22.5	15	37.5	8	20.0	40	10.0	7	17.5	24	60.0	7	17.5
	8	32	0	0	0	21	65.6	0	0	40	10.0	2	5.0	35	87.5	2	5.0	40	10.0	1	2.5	15	37.5	1	2.5	40	15.0	4	10.0	25	62.5	4	10.0
SH_4	4	35	10.0	14	43.8	12	37.5	9	28.1	40	0	0	0	19	47.5	0	0	40	12.5	4	10.0	8	20.0	0	0	40	22.0	20	50.0	8	20.0	7	17.5
	6	35	25.0	2	6.3	23	71.9	2	6.3	40	10.0	1	2.5	27	67.5	1	2.5	40	10.0	3	7.5	10	25.0	2	5.0	40	0	0	0	15	37.5	0	0
	8	35	0	0	0	24	75.0	0	0	40	30.0	1	2.5	33	82.5	1	2.5	40	0	0	0	11	27.5	0	0	40	0	0	0	18	45.0	0	0

Table 10 Effects of myo-inositol and sucrose on greenspot and root formation in different basal media. (continue)

MEDIUM	Age	RD 23								RD 25								KDML								NSPT							
		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
W_1	4	36	15.5	11	30.6	15	41.7	7	19.4	40	13.3	12	30.0	22	55.0	10	25.0	40	10.0	4	10.0	6	15.0	1	2.5	40	30.0	33	82.5	18	45.0	15	37.5
	6	36	26.3	24	66.7	25	69.4	22	61.1	40	15.6	18	45.0	20	50.0	14	35.0	40	10.9	23	57.5	15	37.5	11	27.5	40	45.0	36	90.0	18	45.0	17	42.5
	8	36	31.9	21	58.3	26	72.2	18	50.0	40	14.0	15	37.5	31	77.5	9	22.5	40	18.8	25	62.5	18	45.0	12	30.0	40	48.1	36	90.0	26	65.0	22	55.0
W_2	4	36	17.1	17	47.2	20	55.6	14	38.9	40	18.0	5	12.5	17	42.5	5	12.5	40	10.0	8	20.0	11	27.5	4	10.0	40	21.0	29	72.5	10	25.0	11	27.5
	6	36	17.5	12	33.3	27	75.0	12	33.3	40	10.0	2	5.0	26	65.0	2	5.0	40	10.0	8	20.0	15	37.5	3	7.5	40	12.7	11	27.5	20	50.0	7	17.5
	8	36	33.3	3	8.3	25	69.4	3	8.3	40	40.0	1	2.5	35	87.5	1	2.5	40	10.0	3	7.5	23	57.5	0	0	40	10.0	2	5.0	22	55.0	2	5.0
W_3	4	36	14.2	12	33.3	15	41.7	9	25.0	40	0	0	0	18	45.0	0	0	40	10.0	3	7.5	5	12.5	1	2.5	40	20.0	24	60.0	8	20.0	6	15.0
	6	36	10.0	4	11.1	21	56.3	4	11.1	40	0	0	0	21	52.5	0	0	40	0	0	0	5	12.5	0	0	40	10.0	4	10.0	20	50.0	4	10.0
	8	36	10.0	3	8.3	21	58.3	3	8.3	40	0	0	0	31	77.5	0	0	40	0	0	0	5	12.5	0	0	40	0	0	0	22	55.0	0	0
W_4	4	32	11.2	17	53.1	14	43.8	9	28.1	40	10.0	6	18.0	17	42.5	5	12.5	40	10.0	3	7.5	5	12.5	2	5.0	40	26.5	34	85.0	9	22.5	8	20.0
	6	32	32.3	30	93.8	25	78.1	25	78.1	40	13.3	18	45.0	20	50.0	14	35.0	40	11.5	20	50.0	8	20.0	9	22.5	40	30.0	40	100	17	42.5	17	42.5
	8	32	45.7	28	87.5	25	78.1	23	71.9	40	15.6	18	45.0	28	70.0	16	40.0	40	12.9	17	42.5	17	42.5	10	25.0	40	33.1	36	90.0	17	42.5	15	37.5
W_5	4	32	16.4	11	34.4	15	46.9	7	21.9	40	10.0	3	7.5	16	40.0	3	7.5	40	10.0	2	5.0	3	7.5	1	2.5	40	19.0	30	75.0	10	25.0	10	25.0
	6	32	12.5	12	37.5	28	87.5	11	34.4	40	10.0	2	5.0	22	55.0	1	2.5	40	10.0	4	10.0	9	22.5	3	7.5	40	10.8	12	30.0	18	45.0	6	15.0
	8	32	10.0	2	6.3	21	65.6	2	6.3	40	12.5	4	10.0	27	67.5	2	5.0	40	0	0	0	12	30.0	0	0	40	10.0	3	7.5	20	50.0	3	7.5

Table 10 Effects of myo-inositol and sucrose on greenspot and root formation in different basal media. (continued)

MEDIUM	Age	RD 23								RD 25								KDML								NSPT							
		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R		N	A %	GS		R		GS & R	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
W_6	4	36	18.6	21	58.3	18	50.0	14	38.9	40	10.0	1	2.5	15	37.5	1	2.5	40	10.0	5	12.5	7	17.5	3	7.5	40	18.2	22	55.0	6	15.0	6	15.
	6	36	16.4	11	30.6	30	83.3	10	27.8	40	0	0	0	18	45.0	0	0	40	0	0	0	12	30.0	0	0	40	10.0	4	10.0	8	20.0	1	2.
	8	36	10.0	4	11.1	27	75.0	4	11.1	40	0	0	0	29	72.5	0	0	40	0	0	0	16	40.0	0	0	40	10.0	2	5	12	30.0	1	2.
W_7	4	36	20.0	18	50.0	11	30.6	7	19.4	40	10.0	4	10.0	22	55.0	3	7.5	40	10.0	3	7.5	10	25.0	2	5.0	40	20.3	34	85.0	10	25.0	9	22.
	6	36	28.6	29	80.6	26	72.2	22	61.1	40	12.9	17	42.5	28	70.0	13	32.5	40	10.0	16	40.0	11	27.5	10	25.0	40	30.9	35	87.5	21	52.5	18	45.0
	8	36	31.9	26	72.2	23	63.9	17	47.2	40	18.8	25	62.5	35	87.5	21	52.5	40	10.0	11	27.5	14	35.0	8	20.0	40	27.6	28	70.0	21	52.5	9	22.5
W_8	4	36	22.1	14	38.9	14	38.9	8	22.2	40	10.0	7	17.5	23	57.5	6	15.0	40	10.0	5	12.5	7	17.5	3	7.5	40	20.9	31	77.5	8	20.0	7	17.5
	6	36	13.3	12	33.3	32	88.9	12	33.3	40	0	0	0	28	70.0	0	0	40	10.0	3	7.5	9	22.5	1	2.5	40	10.0	9	22.5	20	50.0	7	17.5
	8	36	12.2	9	22.5	32	88.9	9	25.0	40	0	0	0	36	90.0	0	0	40	0	0	0	12	30.0	0	0	40	10.0	2	5.0	25	62.5	2	5.0
W_9	4	36	13.8	13	36.1	11	30.6	4	11.1	40	10.0	2	5.0	16	40.0	2	5.0	40	10.0	3	7.5	3	7.5	2	5.0	40	18.3	18	45.0	9	22.5	5	12.5
	6	36	16.7	3	8.3	14	38.9	3	8.3	40	0	0	0	16	40.0	0	0	40	0	0	0	3	7.5	0	0	40	10.0	3	7.5	13	32.5	2	5.0
	8	36	10.0	2	5.6	17	47.2	2	5.6	40	0	0	0	28	70.0	0	0	40	0	0	0	6	15.0	0	0	40	10.0	1	2.5	13	32.5	1	2.5

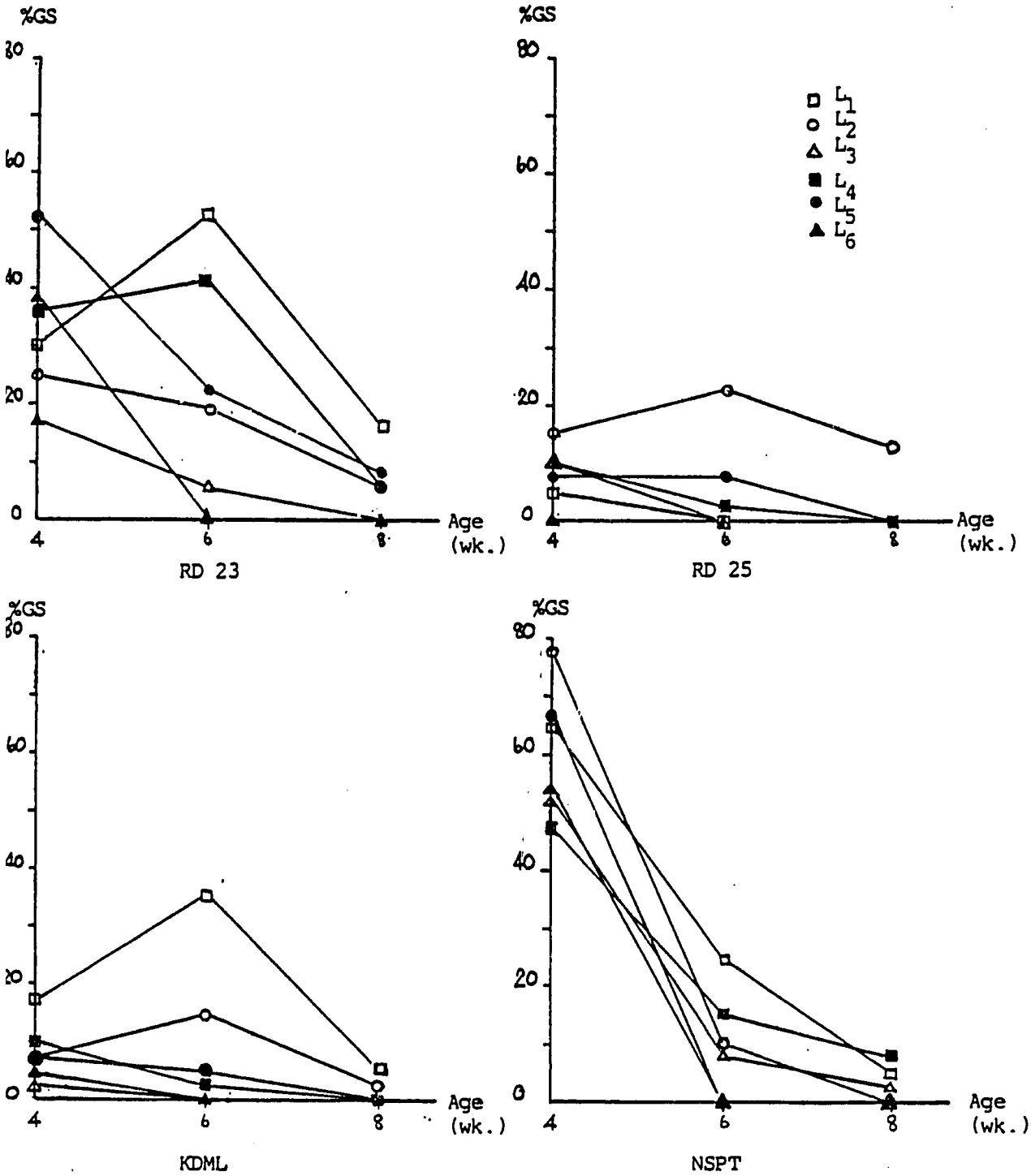

Figure 1 Effects of concentration of myo-inositol and sucrose on greenspot formation. (based on LS medium).

Note: Low inositol and without sucrose favor greenspot formation. except variety RD 25 which greenspot formation and regeneration are poor in any treatment

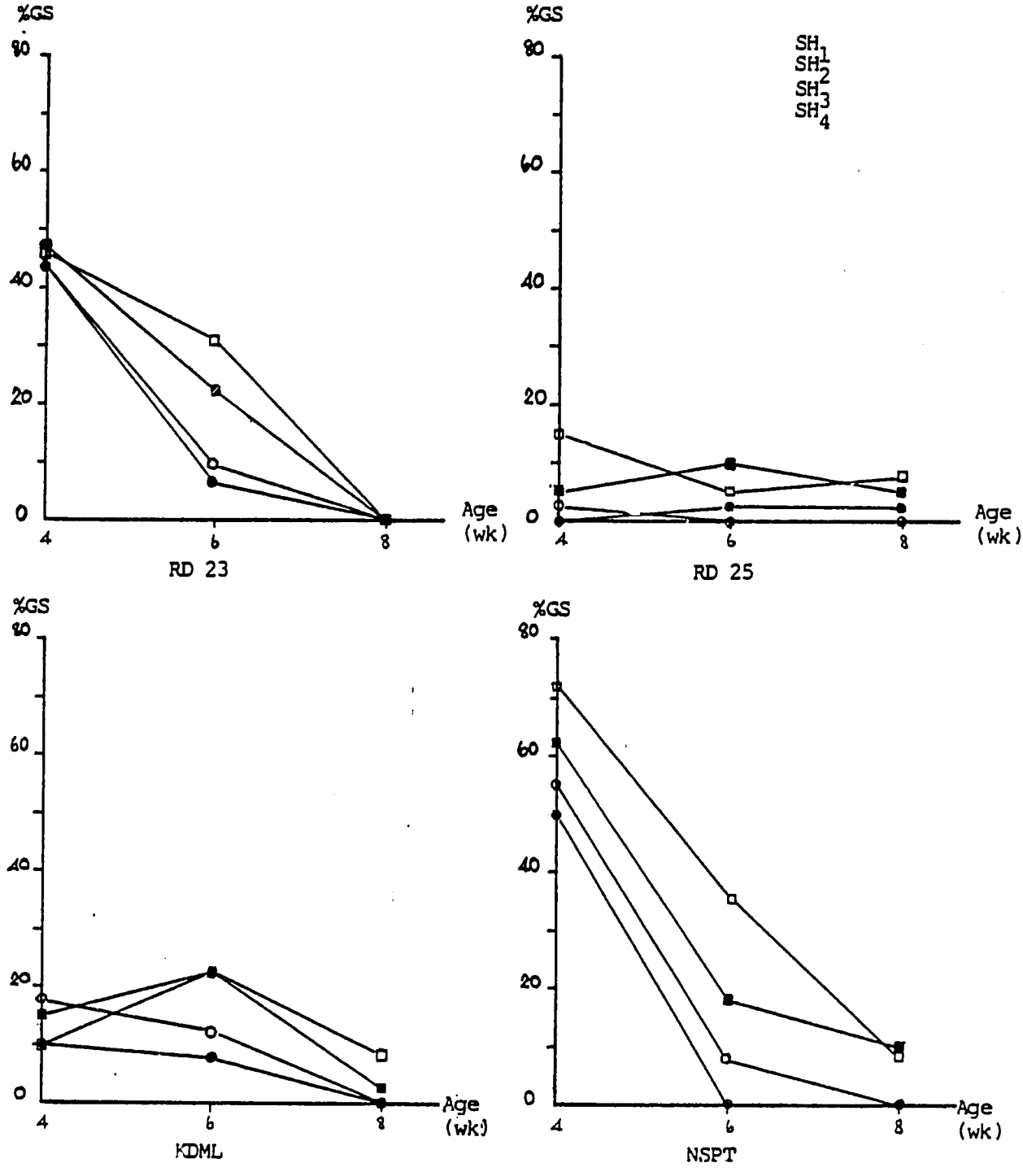

Figure 2 Effects of concentration of myo-inositol and sucrose on greenspot formation. (base on SH medium).

Note: Low concentration of sucrose (3%) in the media favors greenspot formation (SH_1. SH_3): half strength of major elements seems to be slightly better than full strengh.

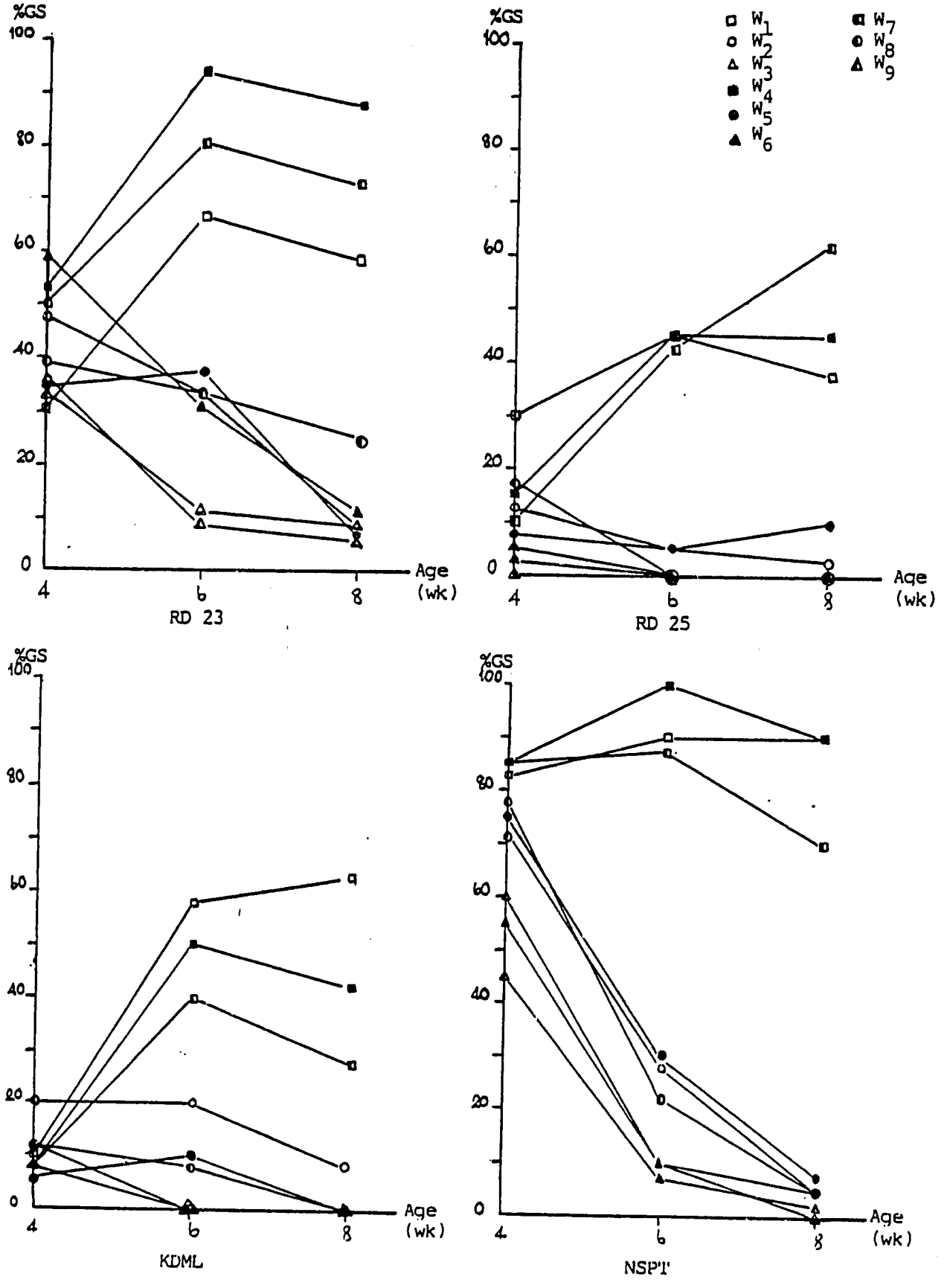

Figure 3 Effects of concentration of myo-inositol and sucroseon greenspot formation. (based on W medium).

Note: Best results obtained are among the media without sucrose (W_1, W_4, W_7)

(treatments L_1, L_4; fig. 1). Further experiment was the addition of myo-inositol to the media which supplemented with coconut water and found no difference in greenspot formation in the media with 0, 100, or 1000 mg/l myo-inositol (treatments W_1, W_4, W_7, fig.3). Therefore, the addition of myo-inositol is unnescessary where coconut water is the main organic additive because it probably containes large amount of inositol or similar compounds, which can very well support growth and differentiation of callus (Steward et al., 1969).

Experiment 3

OBJECTIVES: To re examine the effect of sucrose on plant regeneration; to study the altered physical factors that might affect plant regeneration by adding more agar or activated charcoal; to find the differences in plant regeneration under different photoperiods.

BASIC CULTURE MEDIUM: White's (1954) supplemented with 10 % CW and 0.1 ppm IAA (Table 10).

RICE VARIETIES: RD 23, RD 25, KDML

CALLUS AGES: 2 and 4 weeks.

LIGHT REGIMES: 14/10; 16/8 light/dark

NUMBER OF TREATMENT: 5 different media; 3 rice varieties.

NUMBER OF REPLICATION: 36 to 40 calli/treatment.

CONCLUSION: Media with 2 % sucrose gave poorest results both in greenspot formation and plant regeneration; solid media with 0.8 % agar gave better result than the liquid media or the harder ones (1.0 %); activated charcoal did not improve growth of callus or regeneration; fourteen or sixteen hours photoperiods gave similar results in inducing greenspot and plant regeneration.

RESULTS AND DISCUSSION: This experiment still confirmed the results of previous experiments that supplementing sucrose to medium consistently produced poorer results in greenspot formation (treatment E_4 gave poorer results than those of E_2, E_3, and E_5).

The results showed that solid media were definitely superior to liquid ones in both greenspot formation and plant regeneration. Media solidfied with 0.8 % and 1.0 % agar were not much different in supporting callus growth and greenspot formation (see table 10, 11, 12, 13 and 14). However, calli cultured in a modified White's medium prior to transplanting to the treatments gave more greenspots in the media with 0.8 % than 1.0 % agar (E_2, E_3), On the contrary, when the two week old calli were transfered directly from the callus induction medium produced more greenspots in the harder agar (E_3). The overall effects of softer agar (E_2) seemed to be better than the harder one (E_3) in plant regeneration.

Ages of calli at the beginning of the experiment had no effect on greenspot formation, but the four week old calli gave significantly higher plant regeneration than the two week old ones. This may be due to the effect of frequent subculturing, which some waste products of metabolism had been removed away, before the beginning of the treatment.

Activated charcoal at the rate of 8 grams per liter (E_5) was incorporated into media in the hope that it would retard browning of callus by aerating the media Arditti, 1982). Unfortunately, the result of the experiment seem to be slighly inferior to the control (treatment E_2, E_5). This may be due to the adsorbtion of vitamins and hormones by charcoal granules (Arditti et al. 1982). Deficiencies of these substances would certainly, interfere with normal development.

Differences in both greenspot formation and plant regeneration were not detected under 14/10 & 16/18 light/dark photoperiods.

Table 11 Media based on White's major element salts, with half strength of LS minor element salts, supplemented with 10 % coconut water and 0.1 ppm IAA. Treatments E_1 to E_5 are variation of the above.

Supplement / Agar (gm./l)	Activated charcoal 0 gm /l		Activated charcoal 8 gm /l
	Sucrose 0 gm /l	Sucrose 20 gm/l	Sucrose 0 gm/l
0	E_1	—	—
8	E_2	E_4	E_5
10	E_3	—	—

Table 12 Greenspot and shoot formation in different media modified by altering amount of sucrose, agar, and activated charcoal under 14/10 light/dark regime (two week old calli were used).

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
E_1	2	40	4.00	0	0	0	0	0	0	40	3.70	0	0	0	0	0	0	40	4.0	0	0	0	0	0	0
	4	40	3.43	10	25	9	22.5	0	0	40	3.5	3	7.5	0	0	0	0	40	4	2	5	0	0	0	0
	6	40	3.15	23	57.5	11	27.5	0	0	40	3.4	8	20	10	25	0	0	40	4	2	5	0	0	0	0
E_2	2	40	4	0	0	0	0	0	0	40	3.88	0	0	0	0	0	0	40	4	0	5	0	0	0	0
	4	40	3.7	18	45	18	45	0	0	40	3.4	2	5	17	42.5	0	0	40	3.85	20	50	14	35	0	0
	6	40	3	38	95	19	47.5	0	0	40	3	7	17.5	23	57.5	0	0	40	3.65	33	82.5	13	32.5	1	2.5
E_3	2	40	4	0	0	0	0	0	0	40	3.95	0	0	0	0	0	0	40	3.98	0	0	0	0	0	0
	4	40	3.78	20	50	26	65	0	0	40	3.08	7	17.5	16	40	0	0	40	3.55	20	50	17	42.5	0	0
	6	40	3.83	37	92.5	33	82.5	1	2.5	40	2.75	17	42.5	20	50	3	7.5	40	3.45	36	90	17	42.5	0	0
E_4	2	40	4	0	0	0	0	0	0	40	3.88	0	0	0	0	0	0	40	3.98	0	0	0	0	0	0
	4	40	3.25	4	10	18	45	0	0	40	3.83	0	0	15	37.5	0	0	40	3.5	4	10	16	40	0	0
	6	40	2.65	8	20	26	65	0	0	40	2.45	0	0	21	52.5	0	0	40	2.93	2	5	15	37.5	0	0
E_5	2	40	4	0	0	0	0	0	0	40	3.55	[illegible]	0	0	0	0	0	40	3.98	0	0	0	0	0	0
	4	40	3.58	6	15	23	57.5	0	0	40	2.78	2	5	19	47.5	0	0	40	3.75	17	42.5	15	37.5	0	0
	6	40	2.88	28	70	30	75	0	0	40	2.20	7	17.5	26	65	0	0	40	3.7	15	37.5	15	37.5	0	0

Table 13 Greenspot and shoot formation in different media modified by altering amount of sucrose, agar, and activated charcoal under 16/8 light/dark regime (two week old calli were used).

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
E_1	2	40	4.00	0	0	0	0	0	0	40	3.90	0	0	0	0	0	0	40	4.00	0	0	0	0	0	0
	4	40	3.78	10	25.0	3	7.5	0	0	40	3.30	0	0	1	2.5	0	0	40	4.00	0	0	5	12.5	0	0
	6	40	3.40	24	60.0	2	5.0	0	0	40	2.70	0	0	1	2.5	0	0	40	3.90	0	0	5	12.5	0	0
E_2	2	40	4.00	0	0	0	0	0	0	40	3.70	0	0	0	0	0	0	40	3.93	0	0	0	0	0	0
	4	40	3.70	18	45.0	15	37.5	0	0	40	3.63	4	10.0	4	10.0	0	0	40	3.63	11	27.5	13	32.5	0	0
	6	40	3.23	34	85.0	17	42.5	0	0	40	3.10	0	0	2	5.0	0	0	40	3.23	16	40.0	17	42.5	0	0
E_3	2	40	4.0	0	0	0	0	0	0	40	3.90	0	0	0	0	0	0	40	3.90	0	0	0	0	0	0
	4	40	3.65	28	70.0	17	42.5	0	0	40	3.25	15	37.5	18	45.0	1	2.5	40	3.80	10	25.0	7	17.5	0	0
	6	40	2.98	36	90.0	24	60.0	1	2.5	40	3.05	16	40.0	19	47.5	0	0	40	3.25	21	52.5	12	30.0	0	0
E_4	2	40	4.00	0	0	0	0	0	0	40	3.98	0	0	0	0	0	0	40	3.88	0	0	0	0	0	0
	4	40	3.15	5	12.5	26	65.0	0	0	40	3.08	0	0	9	22.5	0	0	40	3.50	1	2.5	8	20.0	0	0
	6	40	2.73	2	5.0	24	60.0	0	0	40	2.78	0	0	22	55.0	0	0	40	2.80	4	10.0	12	30.0	0	0
E_5	2	40	4.00	0	0	0	0	0	0	40	3.88	0	0	0	0	0	0	40	4.00	0	0	0	0	0	0
	4	40	3.73	12	30.0	20	50.0	0	0	40	3.53	3	7.5	15	37.5	0	0	40	3.90	9	22.5	17	42.5	0	0
	6	40	3.10	20	50.0	18	45.0	1	2.5	40	3.20	9	22.5	16	40.0	0	0	40	3.43	9	22.5	20	50.0	0	0

Table 14 Greenspot and shoot formation in different media modified by altering amount of sucrose, agar and activated charcoal under 14/10 light/dark regime (four week old calli were used).

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
E_1	4	40	3.80	19	47.5	21	52.5	0	0	36	3.67	17	47.2	22	61.1	0	0	40	4.00	5	12.5	9	22.5	0	0
	6	40	3.10	31	77.5	24	60.0	4	10.0	36	2.78	20	55.6	23	63.9	0	0	40	3.50	8	20.0	11	27.5	2	5.0
	8	40	3.00	22	55.0	25	62.5	2	5.0	36	2.67	19	52.8	25	69.4	0	0	40	3.10	5	12.5	15	37.5	1	2.5
E_2	4	40	3.08	20	50.0	28	70.0	0	0	40	2.05	17	42.5	32	80.0	0	0	40	3.73	4	10.0	2	5.0	0	0
	6	40	2.58	35	87.5	33	82.5	4	10.0	40	2.40	23	57.5	37	92.5	4	10.0	40	3.40	25	62.5	17	42.5	4	10.0
	8	40	2.40	36	90.0	33	82.5	3	7.5	40	2.13	19	47.5	37	92.5	3	7.5	40	3.05	35	87.5	16	40.0	5	12.5
E_3	4	40	3.03	25	62.5	21	52.5	0	0	36	2.83	14	38.9	31	86.1	1	2.8	40	3.78	6	15.0	8	20.0	0	0
	6	40	2.78	34	85.0	30	75.0	1	2.5	36	2.53	22	61.1	33	91.7	2	5.6	40	3.18	27	67.5	19	47.5	0	0
	8	40	2.48	33	82.5	32	80.0	3	7.5	36	2.14	21	58.3	33	91.7	2	5.6	40	2.93	31	77.5	21	52.5	2	5.0
E4	4	40	3.80	12	30.0	22	55.0	0	0	40	2.78	11	27.5	31	77.5	0	0	40	3.78	5	12.5	9	22.5	0	0
	6	40	2.68	21	52.5	35	87.5	0	0	40	2.33	4	10.0	39	97.5	0	0	40	3.06	10	25.0	20	50.0	0	0
	8	40	2.15	18	45.0	37	92.5	1	2.5	40	2.10	2	5.0	40	100	0	0	40	2.85	5	12.5	18	45.0	0	0
E_5	4	40	3.60	15	37.5	16	40.0	0	0	40	2.98	14	35.0	28	70.0	0	0	40	3.83	4	10.0	8	20.0	1	2.5
	6	40	2.85	29	72.5	28	70.0	2	5.0	40	2.55	23	57.5	34	85.0	2	5.0	40	3.50	23	57.5	23	57.5	1	2.5
	8	40	2.33	32	80.0	29	72.5	2	5.0	40	2.23	19	47.5	34	85.0	1	2.5	40	3.15	23	57.5	27	67.5	1	2.5

Table 15 Greenspot and shoot formation in different media modified by altering amount of sucrose, agar, and activated charcoal under 16/8 light/dark regime (four week old calli were used).

MEDIUM	Age	RD 25								KDML								NSPT							
		N	C	GS		R		Sh		N	C	GS		R		Sh		N	C	GS		R		Sh	
				n	%	n	%	n	%			n	%	n	%	n	%			n	%	n	%	n	%
E_1	4	40	3.60	12	30.0	16	40.0	0	0	40	3.69	11	27.5	13	32.5	0	0	40	3.90	2	5.0	10	25.0	1	2.5
	6	40	3.10	24	60.0	23	57.5	0	0	40	2.90	21	52.5	30	75.0	0	0	40	3.40	6	15.0	13	32.5	2	5.0
	8	40	3.10	21	52.5	25	57.5	2	5.0	40	2.80	22	55.0	25	62.5	4	10.0	40	3.30	0	0	14	35.0	1	2.5
E_2	4	40	3.43	20	50.0	18	45.0	0	0	40	2.98	24	60.0	32	80.0	0	0	40	3.88	13	32.5	9	22.5	1	2.5
	6	40	2.98	36	90.0	28	70.0	6	15.0	40	2.45	32	80.0	39	97.5	1	2.5	40	3.13	31	77.5	17	42.5	2	5.0
	8	40	2.98	34	85.0	28	70.0	5	12.5	40	2.20	24	60.0	36	90.0	1	2.5	40	3.10	29	72.5	16	45.0	2	5.0
E_3	4	40	3.58	15	37.5	16	40.0	0	0	40	3.03	14	35.0	32	80.0	0	0	40	3.88	5	12.5	5	12.5	1	2.5
	6	40	3.05	36	90.0	33	82.5	1	2.5	40	2.65	25	62.5	35	87.5	1	2.5	40	3.15	22	55.0	13	32.5	1	2.5
	8	40	2.83	34	85.0	32	80.0	2	5.0	40	2.25	18	45.0	34	85.0	1	2.5	40	3.03	21	52.5	13	32.5	1	2.5
E_4	4	40	3.05	16	40.0	20	50.0	0	0	40	3.05	9	22.5	25	62.5	0	0	40	3.78	5	12.5	12	30.0	0	0
	6	40	2.65	15	37.5	30	75.0	1	2.5	40	2.43	12	30.0	36	90.0	1	2.5	40	3.03	5	12.5	20	50.0	0	0
	8	40	2.30	9	22.5	30	75.0	1	2.5	40	2.25	12	30.0	38	95.0	1	2.5	40	2.68	1	2.5	21	52.5	0	0
E_5	4	40	3.65	12	30.0	19	47.5	0	0	40	2.93	6	15.0	30	75.0	0	0	40	3.73	4	10.0	9	22.5	1	2.5
	6	40	2.85	29	72.5	31	77.5	2	5.0	40	2.50	21	52.5	36	90.0	0	0	40	3.03	16	40.0	19	47.5	2	5.0
	8	40	2.60	25	62.5	30	75.0	1	2.5	40	2.28	20	50.0	37	92.5	2	5.0	40	2.93	14	35.0	21	52.5	2	5.0

Table 16 Shoot formation in different media modified by altering amount of sucrose, agar and activated charcoal under 14/10 light/dark regime and 16/8 light/dark regime (two week old calli were used)

Light condition	Variety	Age (wk)	MEDIUM				
			E_1	E_2	E_3	E_4	E_5
14/10	RD 23	2	2				
		4					
		6			↓		
	RD 25	2					
		4					
		6			↓ ↓ ↓		
	KDML	2					
		4					
		6		↓			
16/8	RD 23	2					
		4					
		6			↓		
	RD 25	2					
		4					
		6			↓		
	KDML	2					
		4					
		6					

Table 17 Shoot formation in different media modified by altering amount of sucrose, agar and activated charcoal under 14/10 light/dark regime and 16/8 light/dark regime (four week old calli were used).

Light condition	Variety	Age (wk)	MEDIUM				
			E_1	E_2	E_3	E_4	E_5
14/10	RD 23	4					
		6					
		8					
	RD25	4					
		6					
		8					
	KDML	4					
		6					
		8					
16/8	RD 23	4					
		6					
		8					
	RD 25	4					
		6					
		8					
	KDML	4					
		6					
		8					

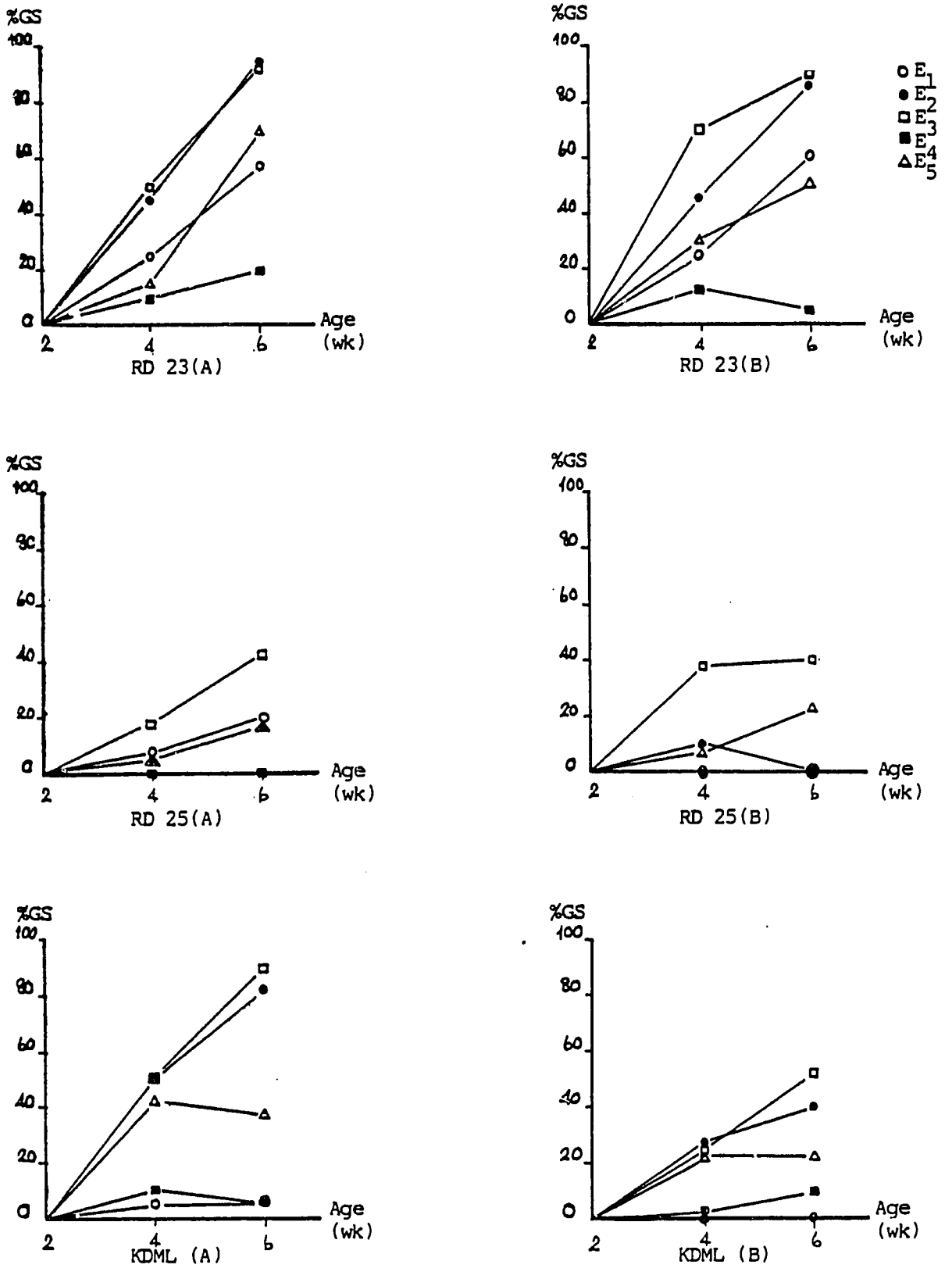

Figure 4 Effects of concentration of agar. sucrose and activated charcoal On greenspot formation (based on White's medium).

A=14/10 light/dark regime. B=16/8 light/dark regime

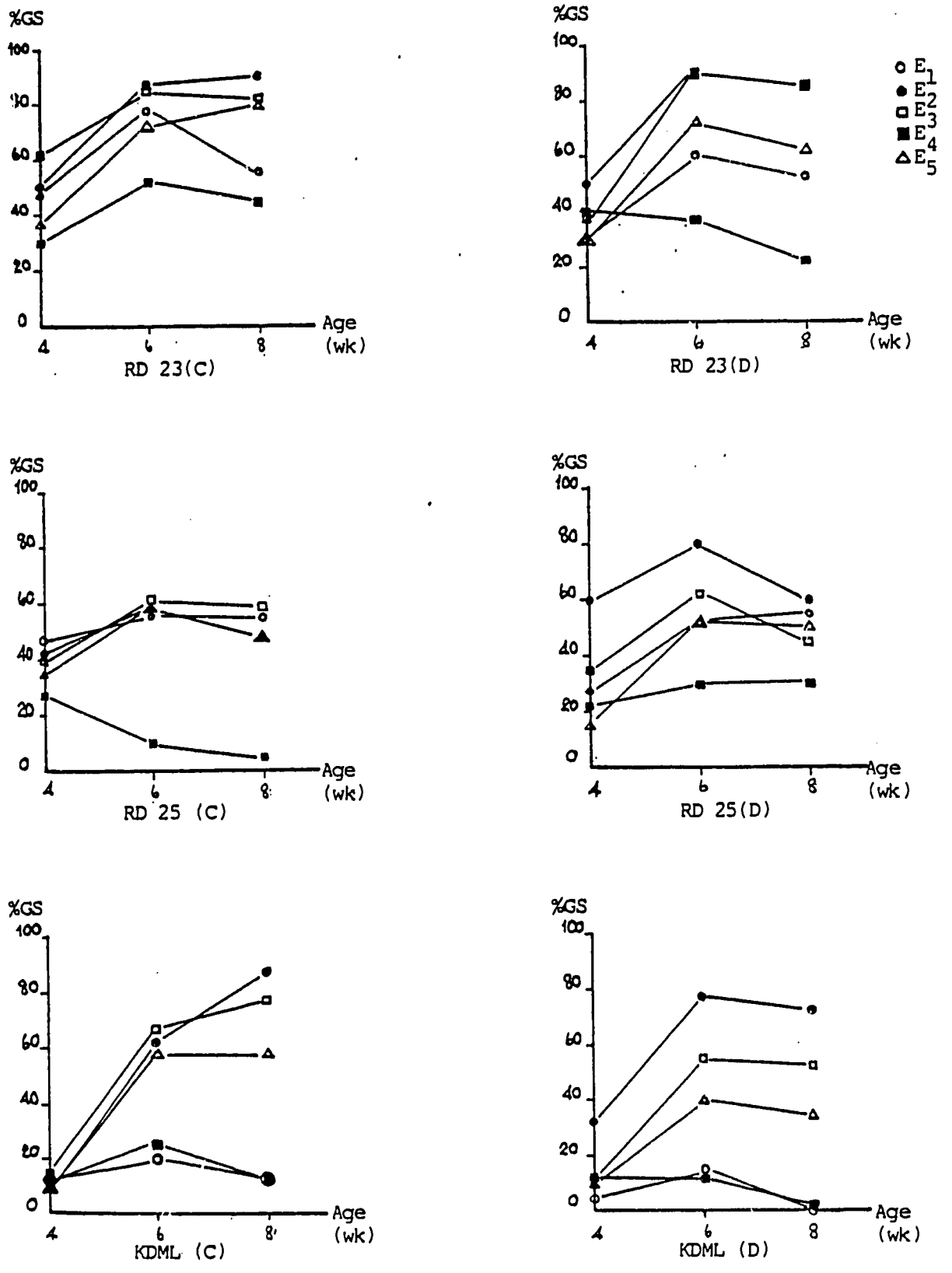

Figure 5 Effects of concentration of agar, sucrose and activated charcoal on greenspot formation (based on White's medium).

C=14/10 light/dark regime D=16/8 light/dark regime.

Experiment 4

OBJECTIVES: To reexamine the effects of IAA and kinetin on plant regeneration from callus; to find a suitable ratio and concentration of IAA and kinetin for plant regeneration; to reexamine the effects of increased potassium and nitrogen level in the media on plant regeneration; to find a suitable callus age for plant regeneration.

BASIC CULTURE MEDIUM: White's (1954) supplemented with 10 % CW, in one treatment (H_5) KNO_3 was increased ten times (Table 18).

RICE VARIETIES: RD 23, LPT, KDML.

CALLUS AGES: 2 and 4 weeks.

LIGHT REGIME: 16/8 light/dark.

NUMBER OF TREATMENT: 28 media, 3 rice varieties.

NUMBER OF REPLICATION: 36 to 40 calli/treatment.

CONCLUSION: Ratio of IAA to kinetin used in the media is important 1 ppm IAA to 3 ppm kinetin gave good overall results. Two week old calli regenerate more plants than the four week old ones. High concentration of potassium and nitrogen favor plant regeneration.

RESULTS AND DISCUSSION: Ratios of IAA to kinetin required for plant regeneration differred from variety to variety (Table 19). The RD 23 responded well to the ratios of 1:3 and 1:5 , with 1:3 was slightly better; while the LPT could regenerate in all ratio tested, but 1:2 being the best. The four week old calli regenerated only when the ratio were 1:1, 1:2 and 1:3 where as the two week old calli also responded to lower ratios (1:4, 1:5 and 1:30). The younger calli could not only regenerate in wider range of IAA to kinetin ratios but also at a given ratio they produce more plants than those of the older ones.

In general, 1 ppm IAA and 3 ppm kinetin are the recommended concentrations for regeneration of plants from calli when White's basic salts supplemented by 10 % CW is used.

Table 18 Seven different modified White's media used in experiment 4.

Treatment	IAA ppm	Kinetin ppm
H_1	0.1	3
H_2	1	3
H_3	1	4
H_4	1	5
* H_5	1	3
H_6	1	2
H_7	1	1

* H_5 KNO_3 was increased 10 times.

Table 19 Root and shoot formation from calli cultured in seven different modified White's media; calli were two or four weeks old at the beginning of the experiment.

MEDIUM	Callus age after culture (weeks)	Callus age at the beginning of the experiment. 4 weeks		4 weeks		4 weeks		2 weeks	
		RD 23		LPT		KDML		KDML	
		R	SH	R	SH	R	SH	R	SH
H_1	0	42.5	0	17.5	0	10.0	0	0	0
	2	47.5	0	55.0	15.0	35.0	0	35.0	0
	4	65.0	0	60.0	40.0	25.0	0	50.0	2.5
H_2	0	17.5	0	27.5	0	0	4.2	0	0
	2	35.0	5.0	50.0	22.5	29.2	12.5	45.0	7.5
	4	55.0	10.0	50.0	30.0	20.8	8.3	55.0	15.0
H_3	0	40.0	0	22.5	0	5.6	0	0	0
	2	37.5	2.5	52.5	25.0	36.1	0	25.0	0
	4	70.0	0	52.5	35.0	22.2	0	40.0	7.5
H_4	0	22.5	0	17.9	0	6.3	0	0	0
	2	35.0	0	42.9	28.6	25.0	0	30.0	5.0
	4	52.5	7.5	42.9	39.3	28.1	0	27.5	5.0

Table 19 (continue)

MEDIUM	Callus age after culture (weeks)	Callus age at the beginning of the experiment: 4 weeks, RD 23		4 weeks, LPT		4 weeks, KDML		2 weeks, KDML	
		R	SH	R	SH	R	SH	R	SH
H_5	0	-	-	30.0	2.5	27.8	0	-	-
	2	-	-	45.0	37.5	44.4	8.3	-	-
	4	-	-	45.0	40.0	33.3	8.3	-	-
H_6	0	-	-	17.5	2.5	21.9	0	-	-
	2	-	-	50.0	35.0	59.4	3.1	-	-
	4	-	-	47.5	45.0	40.6	3.1	-	-
H_7	0	-	-	2.5	0	15.6	0	-	-
	2	-	-	22.5	32.5	37.5	6.3	-	-
	4	-	-	22.5	37.5	34.4	6.3	-	-

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