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SECOND

PROGRESS REPORT

ON

# ISOLATIONS OF STRAINS, CLONES AND REGENERATION OF PLANTS FROM SINGLE CELLS OF WINGED BEAN

Grant #: DPE-5542-G-SS-3056-00

for period ending July 31, 1984

Submitted to:

Project Officer Dr. Loven Schulze
Agency for International Development
S and T/AG
SA-18, 413
Washington, D.C. 20523

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Principal Investigator
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Houston, Texas 77004

Dated August 25, 1984

# ISOLATIONS OF STRAINS, CLONES AND REGENERATION OF PLANTS FROM SINGLE CELLS OF WINGED BEAN

Grant #: DPE-5542-G-SS-3056-00

## ABSTRACT

Plantlet regeneration from callus tissue and suspension cultures of the winged bean, *Psophocarpus tetragonolobus* (L.) DC, have been carried out on six different varieties. Segments from embryos, epicotyls of in-vitro grown seedlings and stems of mature field-grown plants (6-8 mm.) were used as initial explants. Callus tissue establishment was carried out either on a Murashige and Skoog basal medium or $B_5$ salt-sucrose mixture supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthalene acetic acid (NAA) or 6-benzylaminopurine (BAP), either alone or in combinations. The response of callus formation and further growth varied depending on the nature of the explants and on the composition of the culture medium. Roots, shoot-buds, leafy-shoots and fully-differentiated plantlets have regenerated from three different varieties. Filtered suspensions of single cells and cell aggregates from liquid cultures when plated on agar petri dishes formed callus masses to produce "clones" and "strains". Analyses of the effect of a gradient of NaCl concentrations (1.0% - 10.0% - w/v) incorporated into the culture medium on growth of callus cultures have shown that the winged bean callus tissue can tolerate a concentration of up to 2% (2 g/100 ml).

# ISOLATION OF STRAINS, CLONES AND REGENERATION OF PLANTS FROM SINGLE CELL OF WINGED BEAN

Grant #: DPE-5542-G-SS-3056-00

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The first progress report on the technical performance on the above grant entitled "Isolation of strains, clones and regeneration of plants from single cells of winged bean" describing the accomplishment of work carried out from August 5, 1983 to January 31, 1984 was submitted to the Project Officer, Agency for International Development, Washington, D.C. 20523 in February 1984. The following is the 2nd progress report on the continuation of research accomplished thus far for the period ending July 31, 1984. This report will describe experiments which are carried out and which are in progress and will include continuation of experiments described in the 1st report.

The main objectives and goals of this contract are towards isolation of strains, clones and regeneration of plants from single cells of the winged bean, Psophocarpus tetragonolobus (L) DC. using a variety of procedures of tissue culture technology. On the basis of these objectives, experiments during this first year of research were emphasized on establishment of callus tissue and continued maintenance of such tissues in solid media and as suspension cultures. Using plating experiments, single cell isolation, isolation of strains, clones, etc. were also carried out. Growth analyses of the cultures in various media containing different growth factors and the cytology of the cells were routinely examined. Excellent progress towards continued growth and in vitro morphogenesis, viz., induction of roots, shoots, buds, ets. in order to obtain regenera-

tion of plants by appropriate manipulations of various growth factors has been achieved. Preliminary experiments to test NaCl tolerance of callus tissue has been carried out.

Callus establishment and continued maintenance:

As indicated in Report 1, 1984, seeds of six different varieties of winged bean, Psophocarpus tetragonolobus (L) DC which are representatives of different geographical locations particularly form Thailand, Indonesia, Sri Lanka and Papua New Guinea were used. This report includes specifically experiments carried out with the varieties labeled as LBNG 3 and LBNG 1 (New Guinea). Callus tissue was established on a salt-sucrose culture medium indicated as B5 (Gamborg et al., 1968) or MS (Murashige and Skoog, 1962) - Appendix I, supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D), (0.5 - 1.0 mg/l), α-naphthalene acetic acid (NAA), (1.0 - 5.0 mg/l) and/or kinetin (0.1 - 1.0 mg/l) in different combinations (Report 1, 1984). After initial establishment of the callus tissue in a 2,4-D + kinetin medium, continued maintenance of the callus by further subcultures was routinely carried out every 4-5 weeks. On the basis of observational and other evidence, MS medium appeared better for the maintenance of the winged bean callus than the B5 medium. Therefore, for all further experiments, MS medium was used as the basal salt solution. Additional experiments were carried out to determine which growth factors and in what combination (s) will induce response in growth, organogenesis and differentiation. Table 1 shows the data of callus growth and characteristics of the winged bean in various media with varying concentrations of growth factors after 3-4 weeks' growth. The nature of callus growth and texture varied considerably (hard, compact or loose) depending on the type of culture medium containing the growth

substances. Subcultures made from the original callus maintained in light appeared green-pigmented in certain media and were white, yellow or yellowish-brown in other media and/or under dark conditions. Callus tissue growing in a 2,4-D medium and particularly without kinetin generally were less compact, friable, and loose. Differentiated cells or cell aggregates with lignified walls and tracheid-like elements were often visible when squash preparations were stained with feulgen or dyes. The general nature of the callus appearance can be seen in Fig. 1-6, 8.

Table 1. Growth charteristics of callus of Winged Bean in media with varying concentrations of growth factors after 3 weeks in culture

| Medium[a] | Growth Factors | Concn. mg/l | Texture | Pigmentation[b] |
|---|---|---|---|---|
| M.S. agar Solid | 2,4-D | 1.0 | Friable | White |
| " | 2,4-D+ | 5.0 | very friable | White-yellow |
| | Kinetin | 0.1 | | |
| " | 2,4-D+ | 1.0 | Hard | Yellow |
| | Kinetin | 1.0 | | |
| " | 2,4-D+ | 0.5 | Hard Compact | Yellow-green |
| | Kinetin | 1.0 | | |
| " | 2,4-D+ | 1.0 | Friable | Yellow |
| | NAA | 1.0/5.0 | | |
| " liquid | 2,4-D+ | 1.0/5.0 | Very friable | White-yellow |
| | Kinetin | 0.1/1.0 | | |

[a]Modified Murashinge and Skoog (1962) medium.

[b]Pigmentation varied from yellow, yellowing-brown, brown as cultures were grown for longer periods.

Suspension Cultures and Plating of Cells -- Freshly-initiated or old callus tissues were transferred to liquid medium and maintained in gyro-rotatory shaker (60 rpm) in the culture room. The callus dispersed into fine suspension of cells and cell aggregates when transferred to liquid medium every 3-4 weeks. The liquid suspension cultures were generally white, brown or yellowish-brown in color. Microscopic observations of the cells generally showed a uniform morphology with active protoplasmic streaming, distinct nucleus and various other cell organelles. After 2-3 subcultures, 5-10 ml inoculum of the suspensions containing cells and cell aggregates could initiate new cultures in fresh liquid medium. The tissues were highly friable and maintained such an active growth rate that subculturing had to be effected very frequently (every 2 weeks). Occasionally, root primordia and differentiation occurred in tissue clumps (large aggregates) growing in the liquid media. Filtration through cheese cloth or wire meshes removed big tissue pieces and 1 ml of this filtered inoculum which generally contained about 600-700 cell groups (of which approximately 70% were single cells) were plated onto agar petri plates containing 20 ml of 1% agar and maintained in dark. Colonies derived from single cells and cell groups grew into callus masses within 2 weeks and eventually covered the entire surface of the petri plate (Fig. 1). Isolated small clumps or cell masses (0.1-0-.5 cm diameter) when transferred to petri plates with agar media developed callus tissue either from one or from several regions. The new callus growing from these centers were generally white in color. They appeared very loose and friable (Fig. 2). The small callus masses, tissue pieces or colonies were routinely used for regeneration experiments.

Root and Shoot Differentiation--Tissues which were growing in solid cultures

for several months (with routine subcultures) or tissues derived from plating of cell suspensions were used. Small pieces of tissues (approximately 30 mg) or small cell aggregates from suspension cultures were inoculated on petri plates to solid agar media supplemented with 0.1, 0.5 or 1.0 mg/l NAA and 0.01 mg/l kinetin or without kinetin to induce root initiation and further development. 2,4-D was omitted in these cultures. Bud and small embryoid initiation was attempted by transferring callus pieces to agar-solid medium containing no growth hormones (viz., 2,4-D, NAA or kinetin) and maintaining them generally in such media for 2-3 subcultures.

Attempts to obtain root initiation and eventual sequence of development of well-developed roots were successful in cultures transferred to a medium containing NAA (Table 2). The various stages of development of roots are illustrated in Fig. 3-5, 6. As is evident, root primordia (RP) get initiated either single from one callus mass (CA), (Fig. 3,5,6) or several roots (R) arise from a single callus piece (Fig. 4). 2,4-D was omitted totally in all attempts to induce root organogenesis and the occasional appearance of callus on the root initiation surface may be possible due to the presence of residual growth substance in the callus or of kinetin in the medium. In general, as seen in Fig. 3-6 and Table 2, a low concentration of NAA (0.1 mg/l) induced several root organogenesis and usually from cultures maintained in dark. In one instance, from one callus mass (CA) growing in the medium containing 0.01 mg/l kinetin + 0.1 mg/l NAA, one long root was produced which was 9 cm long with 18 laterals (LA), and the longest lateral was 4 cm (Fig. 4). In several cases, many nodules (roots ?) appeared on callus surfaces. Cultures transferred to agar medium in growth jars or erlenmeyer flasks produces such intense number and amount of root growth in 3 weeks that the containers would be filled with roots growing into the medium.

TABLE 2. Growth and root organogenesis of Winged Bean callus in solid media with varying concentrations of growth factors after 3 weeks in culture.

| Medium[a]+ Growth Factors | Concn. mg/l | Callus | Roots | |
|---|---|---|---|---|
| | | | Dark | Light |
| Kinetin+ | 0.01 | ++++ | 72 (6) | 12 (4) |
| NAA | 0.10 | | | |
| Kinetin only | 0.01 | ++[b] | 4 (3) | |
| NAA only | 1.00 | ++ | -- | -- |
| NAA+ | 1.00 | | | |
| BAP[c]+ | 0.10 | +++ | -- | 12 (4) |

[a]Modified Murashige and Skoog (1962) medium.

[b]Many nodules (roots?) appear in callus.

[c]Benzyl-1-aminopurine.

( ) = Number of petri plates.

++ to +++ = Amount of growth (visual).

Dark vs. Light = Observations only.

When tissues growing in callus medium (2,4-D + kinetin) were transferred to medium containing no growth factors, initially the tissues showed signs of survivial with most of the regions becoming dark and appearing "dead". Occassionally, there was some callus growth, possibly due to the presence of residual growth substances in the tissues. Persistent transfer of these tissues to growth factor-free media (2-3 subcultures), revealed areas of renewed growth and appearance of several bud-like primordia (B), (Fig. 7-10). Eventually, these bud-like primordia became heavily green-pigmented and produced green leaf-like structures. In many cultures, several embryoid or embryoid-like structures (EM) with regions of mid-hypocotyl (M), root primordia (RP) and leafy structures were formed (Fig. 9, 10). A well developed root (R) was observed growing out of the basal region of a bud-like mass (Figure 10). Several of the bud-like primordia and/or embryoid-like structures (with small amount of attached callus mass) continued to grow and elongate in the medium without growth substances.

Experiments on salt tolerance:

Previous experiments carried out to study the effect of a gradient of NaCl concentrations (0.01%, 0.05%, 0.10%, 0.25%, 0.50%, 0.75% and 1.0% - w/v) on callus cultures of winged bean had shown that the growth of callus masses remained uninhibited even when grown on nutrient medium containing 1.0% w/v (Report 1, 1984). The following is a continuation of the above experiments to study the effect of NaCl at higher concentrations, viz., 1.0%, 2.0%, 5.0% and 10% - w/v) on callus growth. This was designed to examine whether callus tissue which generally is fast-growing on a control mineral salt-sucrose medium (MS + 0.5 mg/l NAA + 2.0 mg/l BAP) will tolerate NaCl concentrations from 1.0% - 10.0% - w/v) incorporated to the control medium.

Callus masses (200 - 500 mg.) each derived from established tissue cultures (derived from stem and epicotyle segments of seedlings var. LBNG 3) were transferred to MS medium + 0.5 mg/l NAA and 2.0 mg/l BAP containing NaCl concentrations of 0.0%, 1.0%, 2.0%, 5.0% and 10.0% - w/v). Appropriate quantities of NaCl were weighed and dissolved in the culture medium before autoclaving. The above medium was poured into petri plates and allowed to gel. The experimental set-up consisted of 4 petri plates with 4 individual callus pieces in each petri plate. Individual callus pieces were transferred to the petri plates and the petri plates were sealed around with parafilm. Cultures were allowed to grow in a growth chamber maintained at 24 ± 1°C with 12 hrs./day illumination. After 4 weeks, the weight of each of the individual callus mass was determined for each concentration. The results were tabulated and analysed statistically.

The histogram and Figure 11 (a-f) show the quantitative data and the growth of the callus pieces of winged bean in control medium and with different concentrations NaCl incorporated into the culture medium. The quantitative data was obtained by recording the initial and final fresh weight of each of the individual callus masses and determining the amount of growth in four weeks. The callus pieces appeared healthy and green and grew extremely well in the control medium (Fig. 11B) after four weeks of growth. Figure 11a, shows the initial callus pieces at the time of inoculation. At a concentration of 1.0% NaCl, several of the cultures grew reasonably well and appeared comparatively healthy (Fig. 11c), although the amount of growth was reduced. In comparison, at a concentration of 2.0% NaCl, the growth of the callus declined very considerably to the extent that there was very little growth. At 5.0% and 10.0% concentrations of NaCl, the callus masses turned brown and died off, (Fig. 11d and e).

These experiments suggest that the callus cultures of winged bean can tolerate NaCl concentration levels up to 2.0% and it may be possible to obtain salt-tolerant callus tissue within the concentration level of 1.0% - 2.0%. Further experiments are in progress to determine the salt tolerant level of callus both on solid medium and in suspension cultures to select salt-tolerant lines and obtain regeneration from such lines.

## REFERENCES

Gamborg, O.L., R.A. Miller and K. Ojima. 1968. Nutrient requirement of suspension cultures of soybean root cells. Exp. Cell Res. 50:151-158.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Report, 1. 1984. Progress report on Isolation of strains, clones and regeneration of plants from single cells of winged bean to AID., Washington, D.C.

Fig. 1-6. Callus growth and stages of root organogenesis from callus of winged bean. (CA, callus; LA, lateral root; R, root; RP, root primordia). 1. Callus growth of liquid suspension plated onto agar after 5 weeks. 2. Callus formation from cell clumps, 0.1-0.5 cm diameter, after 2-3 weeks with white friable callus arising from centers. 3. Root primordia emergence from callus. 4. Several roots arising and growing from callus pieces. 5-6. Long root with several laterals arising from single callus pieces. Fig. 1-2, ca. X 20; Fig. 3, ca. X 50; Fig. 4, ca. X 30; Fig. 56, ca X 15.

13W

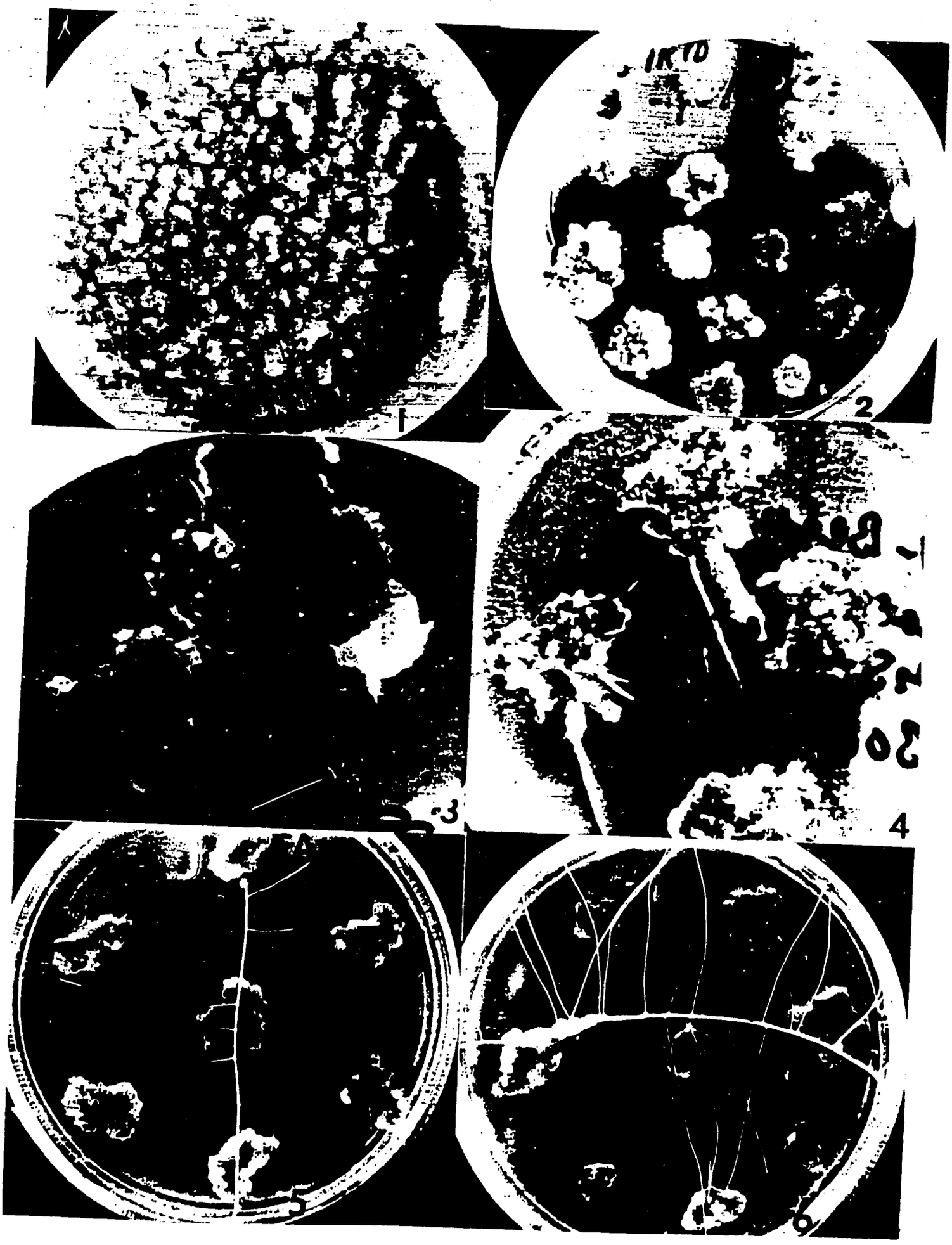
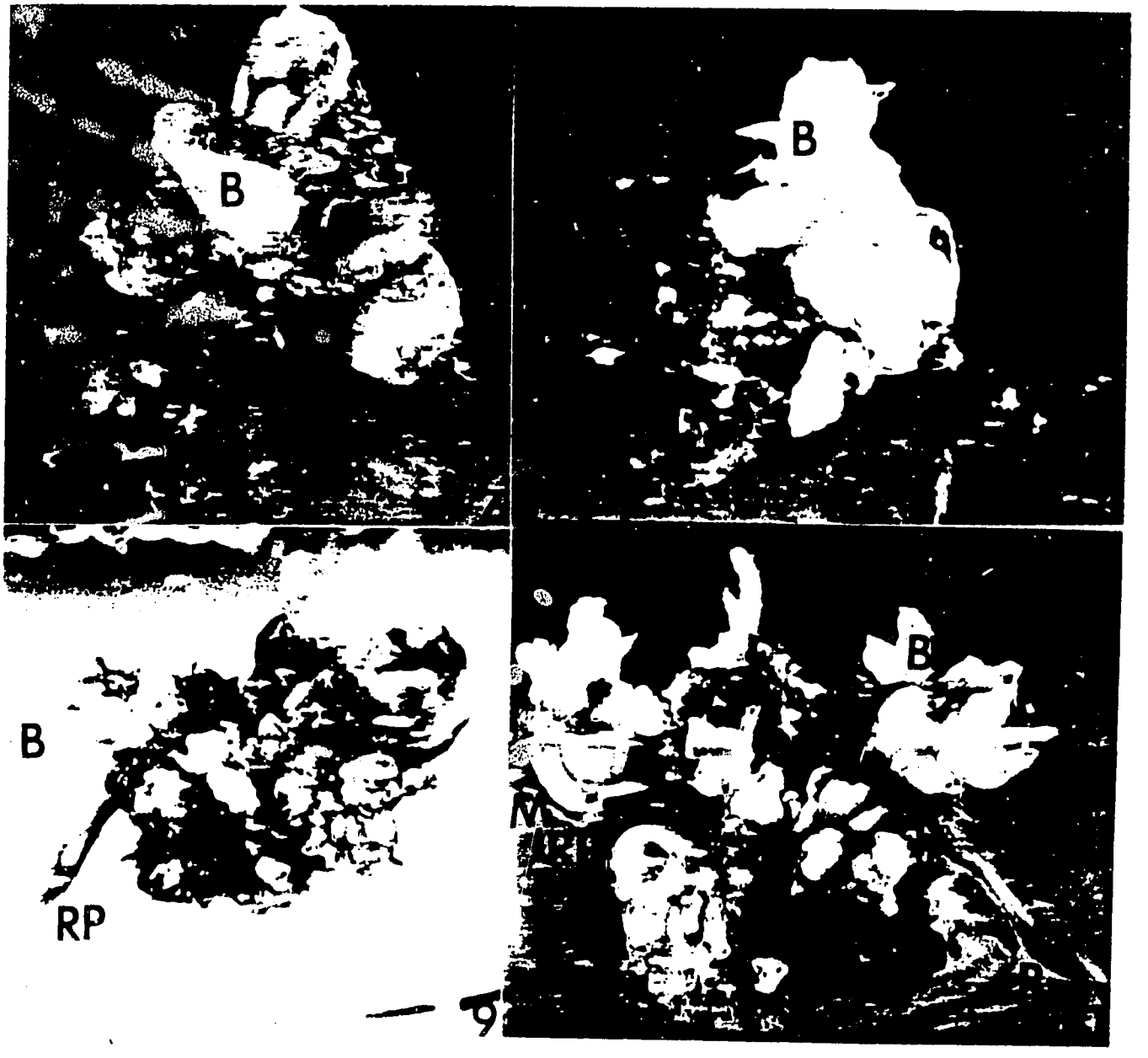


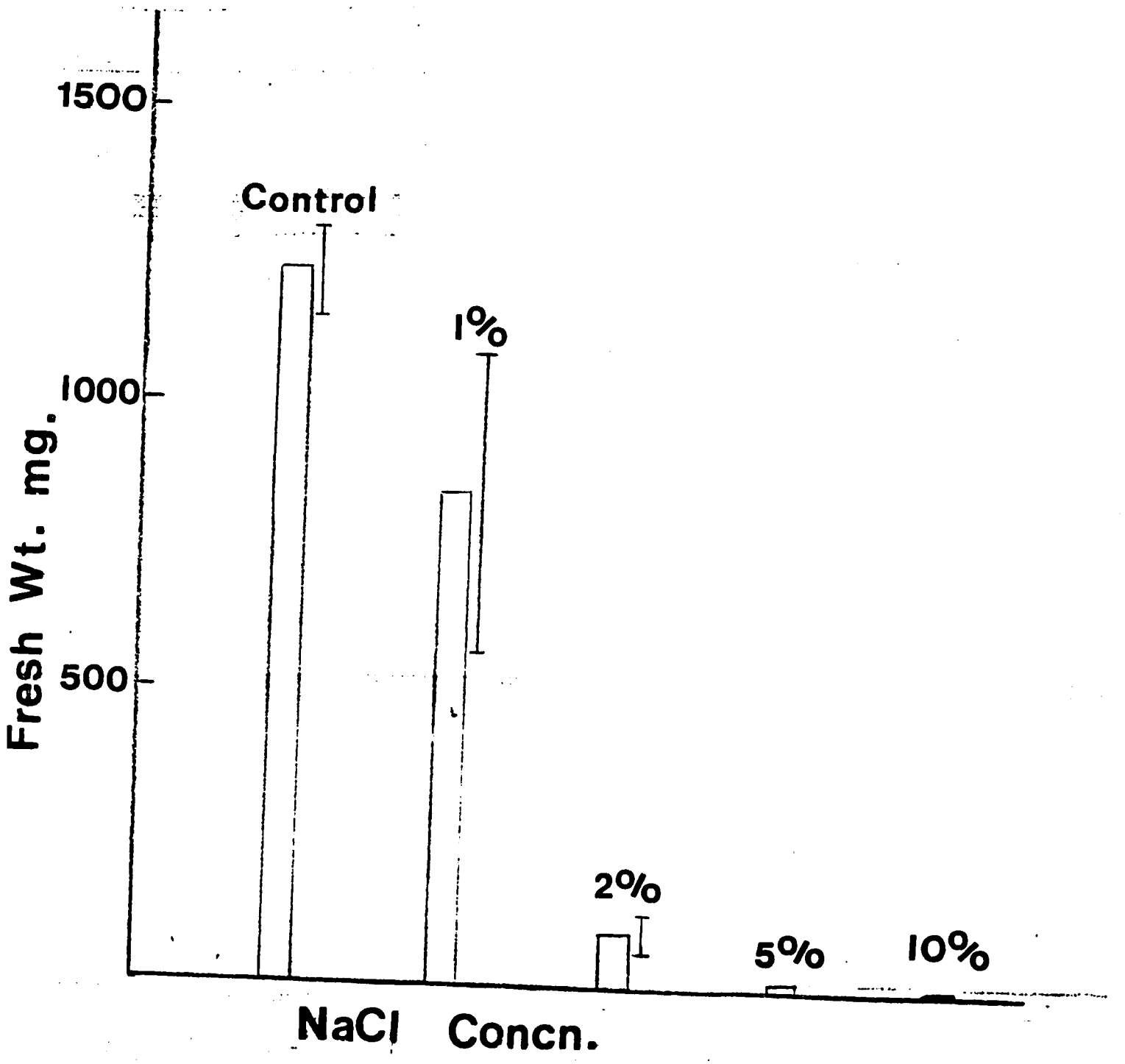
Fig. 7-10. Development of bud-like primordia and embryoids from callus of winged bean. (B, bud, bud-like primordia; EM, embryoid; M, mid-hypocotyl; R, root; RP, root primordia). 7-8. Bud or bud-like primordia at apex of callus mass. 9-10. Several bud-like structures growing out of the callus mass with embryoid structure, root primordia and root. Fig. 7-9, ca. X 20; Fig. 10, ca. X 40.

14a




— 9

# HISTOGRAM



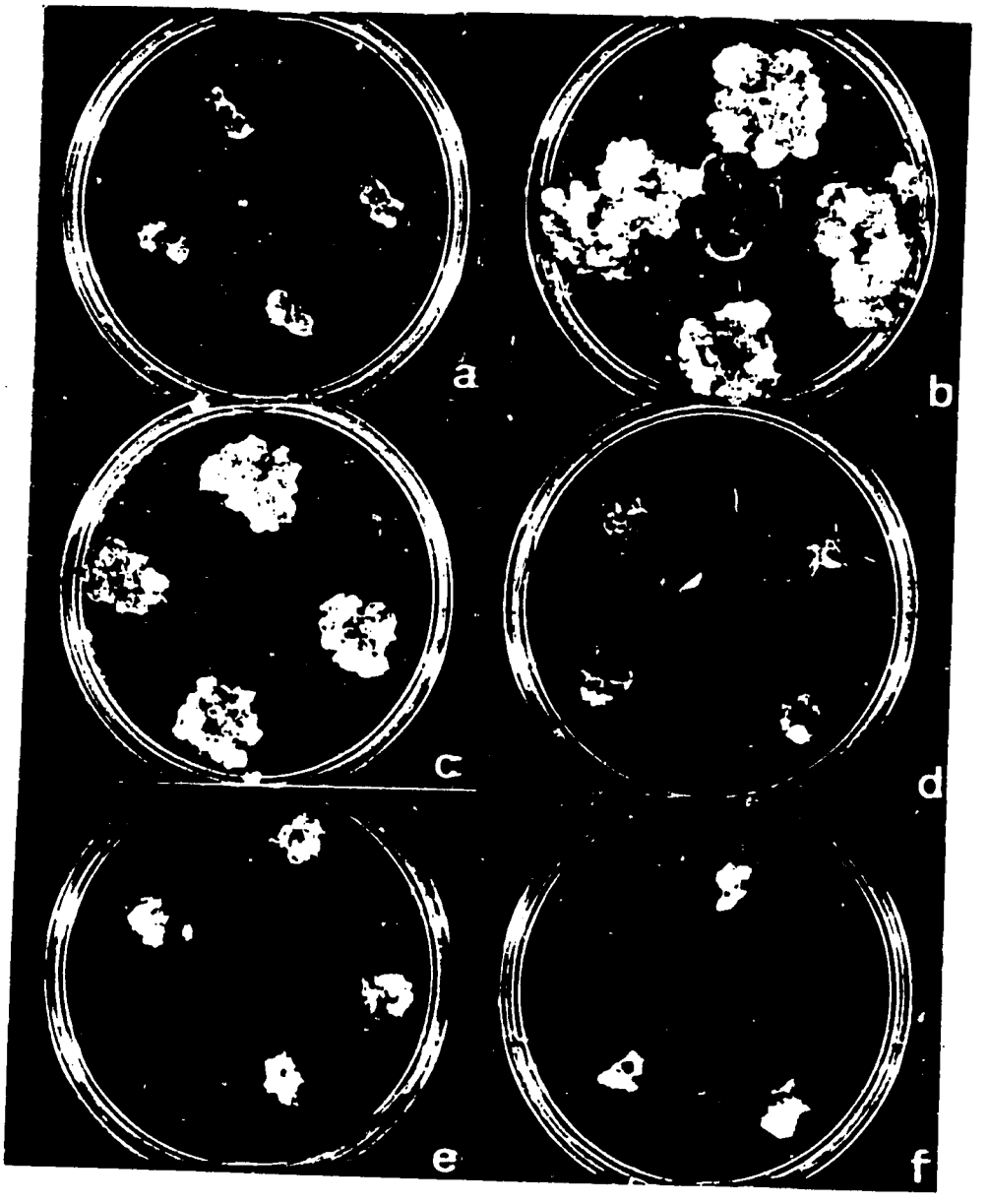



Figure 11: Effect of NaCl concentrations on callus cultures of winged-bean.

a-: Callus masses at the time of inoculation.

b-: Callus cultures at the end of four weeks of growth on MS + α-NAA (0.5 mg/l) + 6-BAP (2.0 mg/l) with no NaCl -- CONTROL.

c-f: Callus cultures at the end of four weeks of growth on the above media + NaCl concentration of 1.0, 2.0, 5.0, and 10.0% (w/v).

Appexdix I - Compositions of plant culture media

| | MS (mg/l) | B5 (mg/l) |
|---|---|---|
| $NH_4NO_3$ | 1650 | |
| $KNO_3$ | 1900 | 2500 |
| $CaCl_2$-$2H_2O$ | 440 | 150 |
| $MgSO_4$-$7H_2O$ | 370 | 250 |
| $KH_2PO_4$ | 170 | |
| $(NH_4)_2SO_4$ | | 134 |
| $NaH_2PO_4$-$H_2O$ | | 150 |
| KI | 0.83 | 0.75 |
| $H_3BO_3$ | 6.2 | 3.0 |
| $MnSO_4$-$4H_2O$ | 22.3 | |
| $MnSO_4$-$H_2O$ | | 10 |
| $ZnSO_4$-$7H_2O$ | 8.6 | 2.0 |
| $Na_2MoO_4$-2 $H_2O$ | 0.25 | 0.25 |
| $CuSO_4$-$5H_2O$ | 0.025 | 0.025 |
| $CoCL_2$-$6H_2O$ | 0.025 | 0.025 |
| $Na_2EDTA$ | 37.3 | 37.3 |
| $FeSO_4$-$7H_2O$ | 27.8 | 27.8 |
| Supplements: | | |
| Inositol | 100 | 100 |
| Nicotinic acid | 0.5 | 1.0 |
| Pyridoxine HCl | 0.5 | 1.0 |
| Thiamine HCl | 0.1 | 10.0 |
| Glycine | 2.0 | |
| Growth Substances[a] | 1-5 ppm | 1-5 ppm |
| Sucrose (gram) | 30 | 20 |
| Agar (gram) | 8 | 8 |

2,4-dichlorophenoxyacetic acid (2,4-D); and α-naphthalene acetic acid (NAA); 6-Benzyl-1-aminopurine (BAP) at appropriate concentrations.

## APPENDIX II

The Initial and Final Fresh Weight of Callus Culture of Winged Bean. on Control medium and + 1.0% and 2.0% of NaCl.

Control

| Medium Composition | Fresh Wt. of Callus at The Time of Inoculation | Fresh Wt. of Callus After Four Weeks mg. | Growth Increase mg. |
|---|---|---|---|
| M.S. + 0.5 mg/l NAA | 500 mg | 2,500 | 2,000 |
| 2.0 mg/l BAP | " | 2,200 | 1,700 |
| (control) | " | 2,500 | 2,000 |
| " | 200 mg | 1,530 | 1,330 |
| | " | 1,490 | 1,290 |
| | " | 1,330 | 1,130 |
| | " | 942 | 742 |
| " | 200 mg | 1,470 | 1,270 |
| | " | 1,210 | 1,010 |
| | " | 980 | 780 |
| | " | 1,355 | 1,155 |
| " | 200 mg | 985 | 785 |
| | " | 1,221 | 1,021 |
| | " | 870 | 670 |
| | " | 1,520 | 1,320 |

Mean Fresh Weight = 1,213 mg

(S.E.) of the mean = ± 64

APPENDIX II (cont)

Control + 1% NaCl

| Medium Composition | Fresh Wt. of Callus at The Time of Inoculation | Fresh Wt. of Callus After Four Weeks mg. | Growth Increase mg. |
|---|---|---|---|
| M.S. + 0.5 mg/l NAA | 200 mg | 900 | 400 |
| 2.0 mg/l BAP | " | 800 | 300 |
| 1.0% NaCl | " | 800 | 300 |
| | 200 mg | 1,066 | 866 |
| | " | 937 | 737 |
| | " | 957 | 757 |
| | " | 826 | 626 |
| | " | 1,165 | 965 |
| | " | 960 | 760 |
| | " | 1,453 | 1.253 |
| | " | 1,121 | 921 |
| | " | 1,502 | 1,302 |
| | " | 994 | 794 |
| | " | 1,043 | 1,843 |
| | " | 1,045 | 845 |

Mean Fresh Weight = 844 mg

(S.E.) of the mean = ± 532

APPENDIX II (cont)

Control + 2% NaCl

| Medium Composition | Fresh Wt. of Callus at The Time of Inoculation | Fresh Wt. of Callus After Four Weeks mg. | Growth Increase mg. |
|---|---|---|---|
| M.S. + 0.5 mg/l NAA | 200 mg | 600 | 100 |
| 2.0 mg/l BAP | " | 700 | 200 |
| 2.0% NaCl | " | 600 | 100 |
| | 200 mg | 232 | 32 |
| | " | 244 | 044 |
| | " | 270 | 070 |
| | " | 330 | 130 |
| | 200 mg | 334 | 134 |
| | " | 219 | 19 |
| | " | 237 | 37 |
| | " | 209 | 9 |
| | 200 mg | 364 | 164 |
| | " | 380 | 180 |
| | " | 399 | 199 |
| | " | 369 | 169 |

Mean Fresh Weight = 112 mg

(S.E.) of the mean = ± 76

Collaborative efforts:

Correspondence is being continued with Prof. Dr. Walter Herath, Deputy Director General of the International Dambala (Winged Bean) Institute Sri Lanka, and Profs. H.P.M. Gunasena and Y.D.A. Senanayake of University of Peradeniya, Peradeniya, Sri Lanka to recruit two students from Sri Lanka so that they can be sent to my laboratory for tissue culture training.

Through the efforts of these collaborators, two students have been selected and they have applied to the Graduate School for admission. They will be trained in this laboratory on tissue culture techniques and will carry out research assignments as part of this AID project. After completing their assignments, they are expected to return to Sri Lanka and join the International Dambala (Winged Bean) Institute at Sri Lanka.

Another Sri Lanka student, Mr. Athula Wikramanayake, who is a graduate student in our Department already, worked in my laboratory this summer for three months. He was trained in all the basic techniques of plant tissue culture. He plans to use this training in his future graduate work.

Copies of relevant correspondence is included.

# INTERNATIONAL DAMBALA (Winged Bean) INSTITUTE

T'Phone: 08-23568

PALLEKELE
KUNDASALE,
SRI LANKA.

23rd June, 1984

Professor S.Venketeswaran
Department of Biology
Central Campus
University of Houston
Texas 77004
U.S.A

Dear Prof.Venketeswaran,

Thank you so much for your letter dated May 15, 1984. Sorry for not being able to send you a prompt reply. As you know I am up to my neck with work.

I am pleased to hear that you have got a Sri Lankan, Atula Wickremanayake to help you on Winged Bean research. We are trying our best to send two students this year.

The position is that we have selected three possible candidates and have asked the first two to apply for admission to the University. Copies of their application forms are anclosed herein for your information. They have sat for the TOEFL examination and will inform you as soon as the results are released.

Hope you will get a chance to follow up on these applications on your return.

Did you had an enjoyable and profitable visit to Germany and Sweden? This letter should reach you before your return to Houston on July 2nd.

Please let me know the quantities of seed and the number of accessions you require. I could send them from here. Lou is expected on the 30th of June. He will spend one month with us.

Contd /2.....

# INTERNATIONAL DAMBALA (Winged Bean) INSTITUTE

T'Phone: 08-23568

PALLEKELE
KUNDASALE,
SRI LANKA.




23rd June, 1984.

Thanks for the copy of your first progress report. The proceedings of the symposium have not been published yet but we hope to do so soon.

With my best regards to you and your family.

Yours sincerely,

Prof.Walter Herath
DEPUTY DIRECTOR GENERAL

Encl.

WH:smf.

PS. I am enclosing copy of only one application. The other will be sent soon.

Yours. Walter

University of Peradeniya. Sri Lanka
DEPARTMENT OF AGRICULTURAL BIOLOGY.

12.06.1984.

Dr. S. Venketeswaran
Dept. of Biology
University of Houston
Houston, Texas.

Dear Sir,

We are writing this letter to you Sir, on the recommendation of Prof. W. Herath of the International Winged Bean Institute to forward our applications for training in Tissue culture of winged bean leading to Masters at your esteemed institution.

Prof. Herath asked us to send the applications direct to you Sir, as the time is running out for the admission in fall 1984.

Sir, we are enclosing our original applications and other documents in seperate cover for the evaluation of our suitability and then to be forwarded to the Admissions Office.

We have directed educational testing service (ETS) to send the TOEFL and GRE scores and University of Peradeniya to forward the academic transcripts.

We regret the delay in making the application and thus the inconvenience caused to you. Please pardon.

Thanking you for your kind interest shown in recruiting people from Sri Lanka and wishing you all the best we beg to remain in silence.

Yours faithfully,

M.A.D.L. Dias

L.L. Ranasinghe.

Please
Department of Agric. Biology,
Faculty of Agriculture
University of Peradeniya, Peradeniya, Sri Lanka.

Telephone:
(08) 88301
Ext 320/6

Sirisepa,
Hiripathwella,
Wadakada,
Polgahawela,
Sri Lanka,
June 9, 1984.

Prof. S. Venketaswaran,
Dept. of Biology,
University of Houston,
Central Campus,
Houston,
TEXAS 77004,
U. S. A.

Dear Prof. Venkateswaran,

I am one of the three candidates selected by the Winged-Bean Institute of Sri Lanka for pursuing a project on Tissue Culture of the Winged Bean at your University. Out of the three selected, it has been decided that the two who get the highest marks at the G.R.E. will finally be recommended for proceeding to your University to undertake the culture experiments.

Dr. Walter Herath, the Deputy Director of the Winged Bean Institute has advised me to write to you immediately and request for a set of application forms as only two have been received. Thus it will enable you to process the applications of the three of us who are selected in the first round pending the results of the G.R.E. test which are likely to be released only after the final date for processing applications at Houston UNiversity.

Therefore in order to enable my candidature also to be taken into consideration please be good enough to send by return of post a set of application forms which I will return to your University post haste, having completed them, immediately I receive them from you.

I thank you in anticipation.

Yours faithfully,

W. M. W. Weerakoon,