THE INDUCTION OF ROOT AND SHOOT MORPHOGENESIS IN *Phaseolus vulgaris* TISSUE CULTURES*

O.J. CROCOMO**
J.E. PETERS***
W.R. SHARP***

ABSTRACT

Exogenous concentrations of bean seed extract prepared from seeds pretreated in aerated water, homogenized in Veliky and Martin's 67–V salt solution, filtered, and added to the culture medium at proper concentrations promote callus proliferation, root morphogenesis, and shoot morphogenesis in leaf explants of *Phaseolus vulgaris* var. Bico de Ouro. The activity of the bean seed factor is dependent upon the period of pretreatment in aerated water.

INTRODUCTION

If the science of plant tissue culture is to be applied to the advancement of crop improvement, the induction and control of morphogenesis must be obtained. HILDEBRANDT et al. (1963) attempted to establish tissue cultures of 32 species of agricultural plants, and although success was obtained in gaining callus from these species, only pea and endive resulted in differentiated plantlets. Tissue cultures of *Phaseolus vulgaris* proved especially difficult with no differentiation and no chlorophyl production. Since this work, *Phaseolus* tissue culture has been limited to metabolic studies (LAMPORT, 1964; GAMBORG, 1966; MEHTA et al., 1967; LIAU & BOLL, 1971; VELIKY, 1972) except for a study of vascular differentiation controlled by indole acetic acid (IAA) and sucrose (JEFFS & NORTHCOTE, 1967). At the Eucarpia Conference, Aseptic Culture Methods in Plant Breeding held at the University of Leeds, Leeds, England, July 9–13, 1973, it was recognized that the potential application of cell culture technique to economic plant improvement is limited in most tissue culture systems because of our inability to control morphogenesis.

Recent work in *Phaseolus* using various auxin/cytokinin combinations demonstrated control of callus growth and limited control of root morphogenesis, but no shoot formation was induced (CROCOMO, SHARP & PETERS, 1976). Excellent control of root morphogenesis was obtained with the addition of exogenous nicotine to the nutrient when kinetin was removed (PETERS et al., 1976). Unsuccessful attempts

* Received for publication in 10/10/1975. Supported by grants from Comissão Nacional de Energia Nuclear (CNEN) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) - Brazil.

** Department of Chemistry and CENA - E.S.A. "Luiz de Queiroz" – USP, Piracicaba, SP - Brazil.

*** Department of Microbiology, The Ohio State University, Columbus, Ohio 43210 – U.S.A.

at obtaining shoot morphogenesis with a defined nutrient prompted the return to undefined nutrient supplements. This report pertains to the addition of a been seed extract for induction of plantlet morphogenesis. Similar experimentation has been performed using a plant extract supplement for the induction of embryogeneis in tomato cell cultures (DEBERGH & NITSCH, 1973).

MATERIALS AND METHODS

Leaves from 2-wk old *Phaseolus vulgaris* var. Bico de Ouro were surface sterilized in 20% v/v hypochlorite for 15 min. Following two rinses in sterile distilled H_2O, the leaves were sectioned into 5 mm squares and inoculated in 20 x 150 mm test tubes containing ten ml of nutrient. Cultures were grown for 8 weeks under constant environmental conditions (25°C; 12-h photoperiod; illumination from cool-white fluorescent lamps giving ca. 200 ft-c outside the culture bottles).

All nutrient contained Veliky and Martin's (1970) 67–V salts and vitamins. Following addition of auxin, cytokinin and bean extract the medium was adjusted to a final pH of 4.5, solidified with 1% agar and sterilized for 15 min at 121°C.

The extracts consisted of *Phaseolus vulgaris* var. Carioca bean seeds homogenized in 67–V salts at concentrations ranging from 1/100 bean seed/ml to 1 bean seed/ml. Seeds were soaked in aerated tap water prior to homogenization and the extract was subsequently filtered through cheese cloth.

The following tests were designed with variations in the auxin/cytokinin concentration, and in the concentration of bean seed extract of various ages.

1 – Test of effect of various kinetin concentrations in 67–V medium plus bean extract. In this test, IAA was constant at a concentration of 1.0 mg/l and kinetin varied from 0–10 mg/l. The extract addition consisted of a homogenate of bean seeds soaked for one day at a constant concentration of 1/4 bean/ml.

2 – Test of age of soaked bean and bean extract concentration. Auxin and cytokinin concentrations were constant (IAA = 2.0 mg/l, NAA = 1.0 mg/l and kinetin = 0.2 mg/l). Bean extract additions consisted of concentrations ranging from 1/100 bean seed/ml to 1 bean seed/ml. A second variable in bean extract composition was the age of the bean. Beans soaked for 0 (BEC-0), 1 (BE-1), 3 (BE-3) and 7 (BE-7) days were added at the various concentrations.

Ten inoculations were conducted for each test medium variation. Following 3 wks of growth all tests were evaluated for callus growth and root morphogenesis. A scale of 0–7 (growth index) was used for scoring callus with 0 being scored for no growth (Fig. 1). Before scoring, standards were selected and used as a reference to visual scoring. Cultures were scored randomly with no reference to nutrient or other results. Approximate wet weight of the different levels of the scale were as follows: 0 = no growth, 1 = 0.10 g, 2 = 0.25 g, 3 = 0.75 g, 4 = 1.25 g, 5 = 2.50 g, 6 = 3.5 g, and 7 = 4.50 g. A similar scale ranging from 0–3 was designed for levels of root induction.

Scores for callus proliferation and root production were averaged and the standard error of the mean calculated. In the case of root morphogenesis, the mean was used as a "root morphogenesis index" since it represents both percentage of root induction and amount of growth.

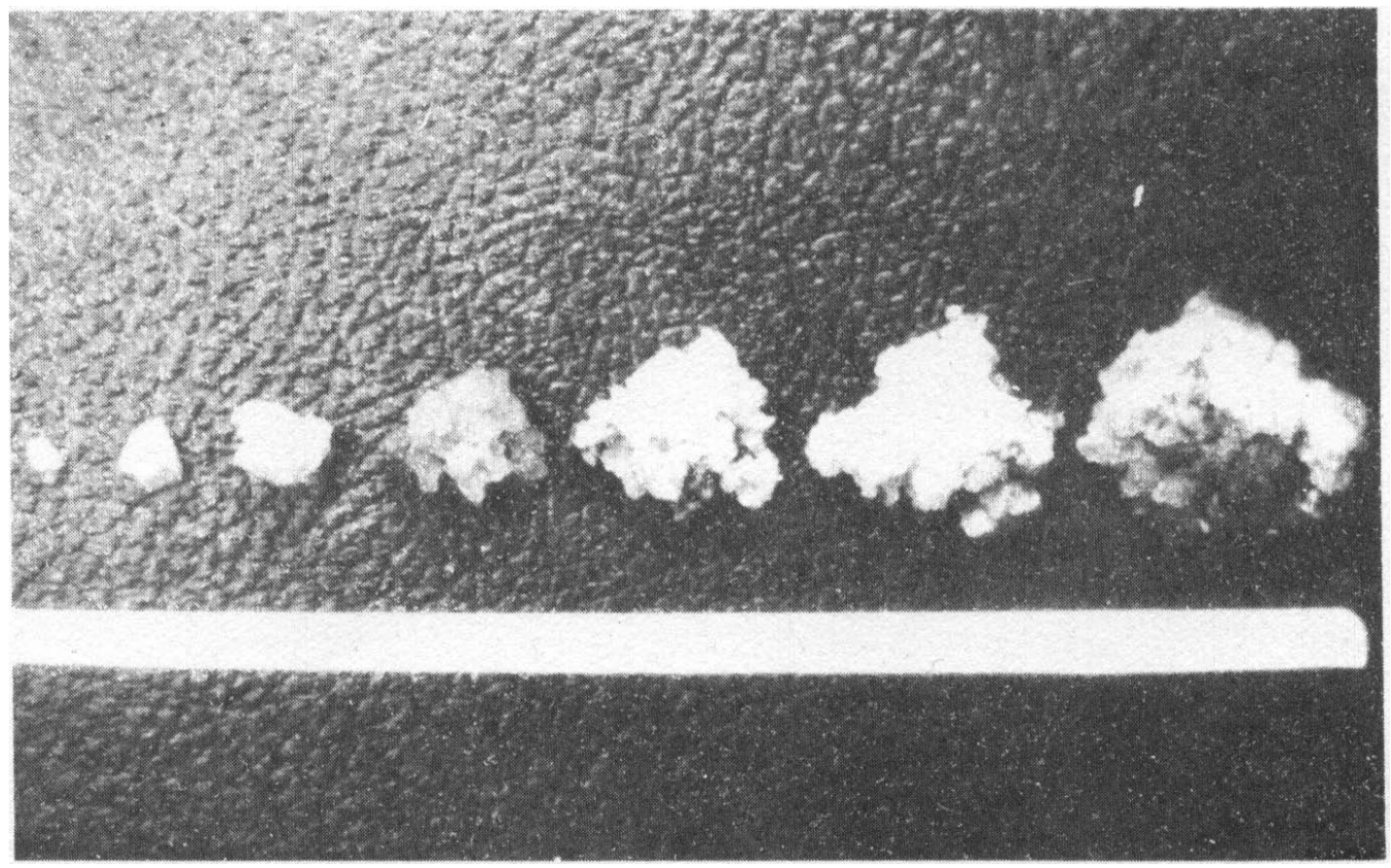

FIG. 1 – Standardization scale for scoring callus growth.

RESULTS

The addition of a bean extract to the growth medium was found to have striking effects in the enhancement of both callus growth and root morphogenesis. When leaf explants are cultured on a medium with varying amounts of kinetin (Figs. 2 and 3) callus growth increases to a maximum at 10 mg/l kinetin. Root initiation is low except for a moderate peak at 2 mg/l kinetin. With the addition of 1-day old bean seed extract at a concentration of 1/4 bean seed/ml, kinetin still exerts a concentration-dependent effect, but the levels of both callus and root growth are much higher. Callus proliferation reached a peak at 4 mg/l kinetin with a growth index of 6.37. Root induction peaked at 2 mg/l kinetin with 100% of the cultures being scored at the maximum morphogenesis index of 3.00. These results suggested that other parameters such as age and concentration of the seed extract might have significant growth and morphogenetic effects.

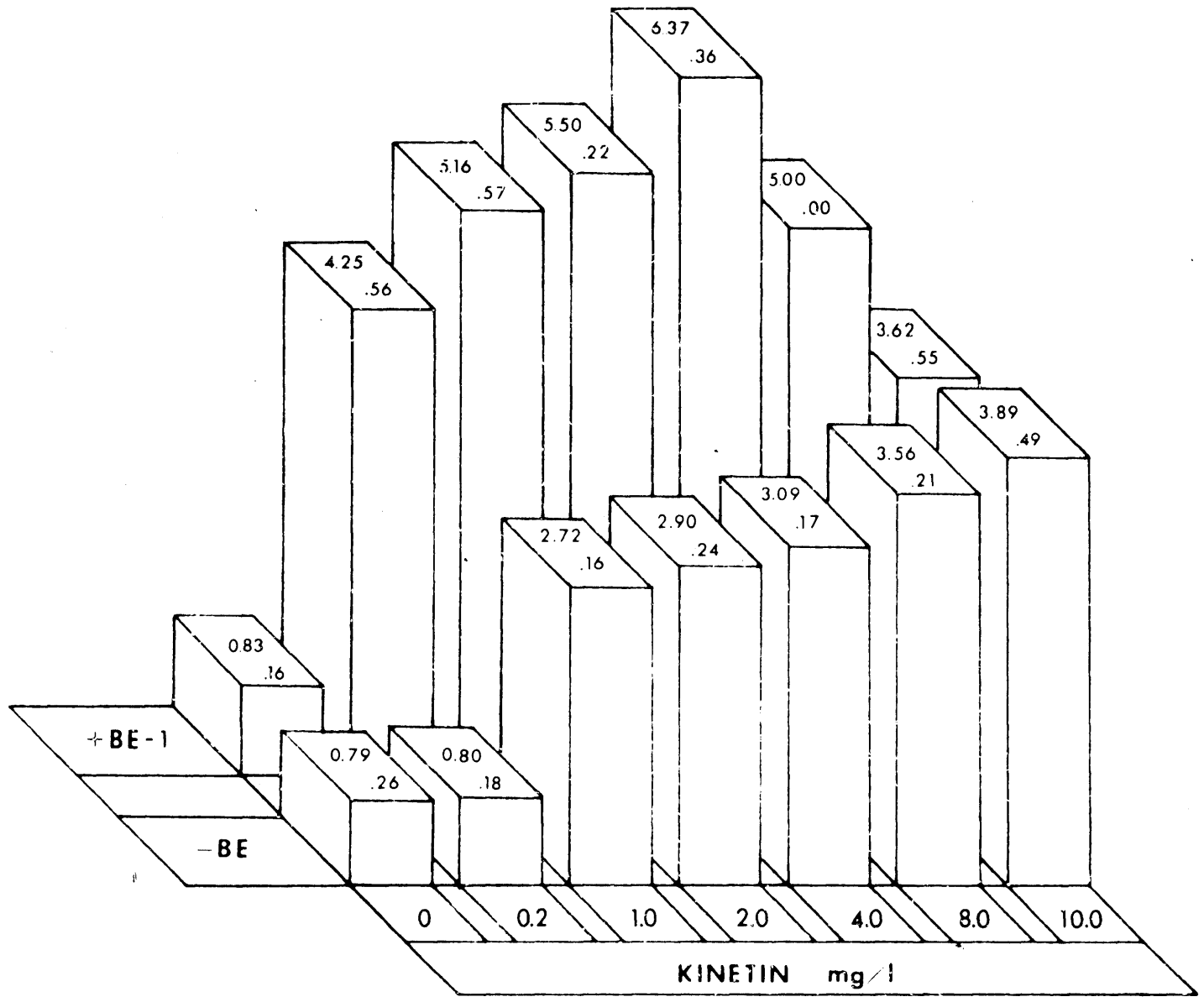

FIG. 2 – Effect of the additions of 1-day old bean seed extract (BE-1) at a concentration of ½ bean seed/ml on callus growth of leaf explants of *Phaseolus vulgaris* cultured on 67–V salts and vitamins, 1 mg/l IAA and varying concentrations of kinetin. The upper and lower numerical values of the individual histograms represent callus growth index and standard error of the mean respectively.

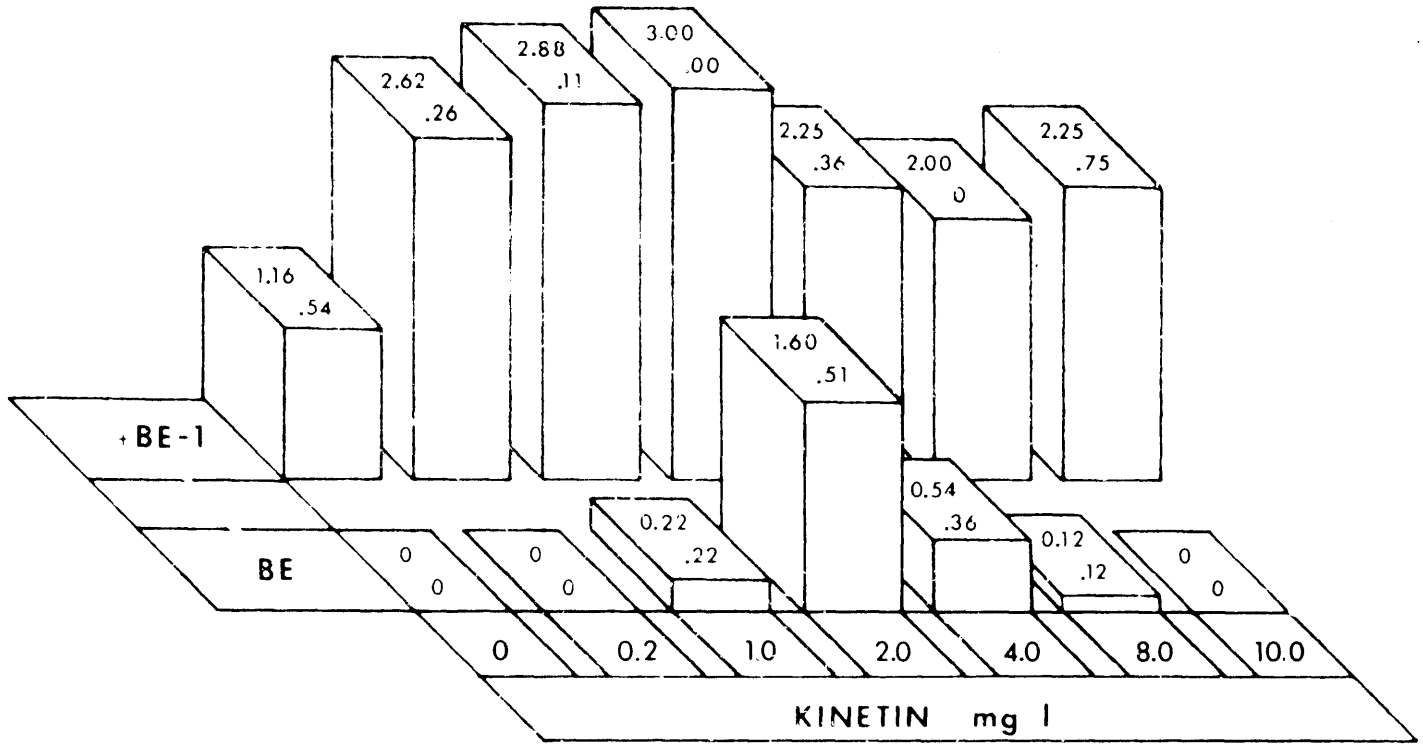

FIG. 3 – Effect of the addition of 1-day old bean seed extract (BE-1) at a concentration of ¼ bean seed/ml on root morphogenesis of leaf explants of *Phaseolus vulgaris* cultured on 67–V salts and vitamins, 1 mg/l IAA and varying concentrations of kinetin. The upper and lower numerical values of the individual histrograms represent root morphogenesis index and standard error of the mean respectively.

When exogenous auxin and cytokinin concentrations are kept constant the addition of various aged bean seed extract to the medium has little effect on callus growth (Fig. 4) except for a possible inhibition of growth with 1-day old seed extract and for the higher concentrations at 1/2, 3/4 and 1 bean seed/ml of seeds 1-day old and older. A strong inhibition of root morphogenesis is produced by concentrations greater than 1/4 bean seed per ml except for 0-day old bean seeds (Fig. 5).

The most striking and potentially significant results were obtained with extract from bean seeds soaked for two hours (BE-0) and homogenized. At a concentration of 1/4 bean seed/ml, 2 plantlets were induced in 9 cultures. These two were the only plantlets induced in these experiments representing a ca 400 cultures.

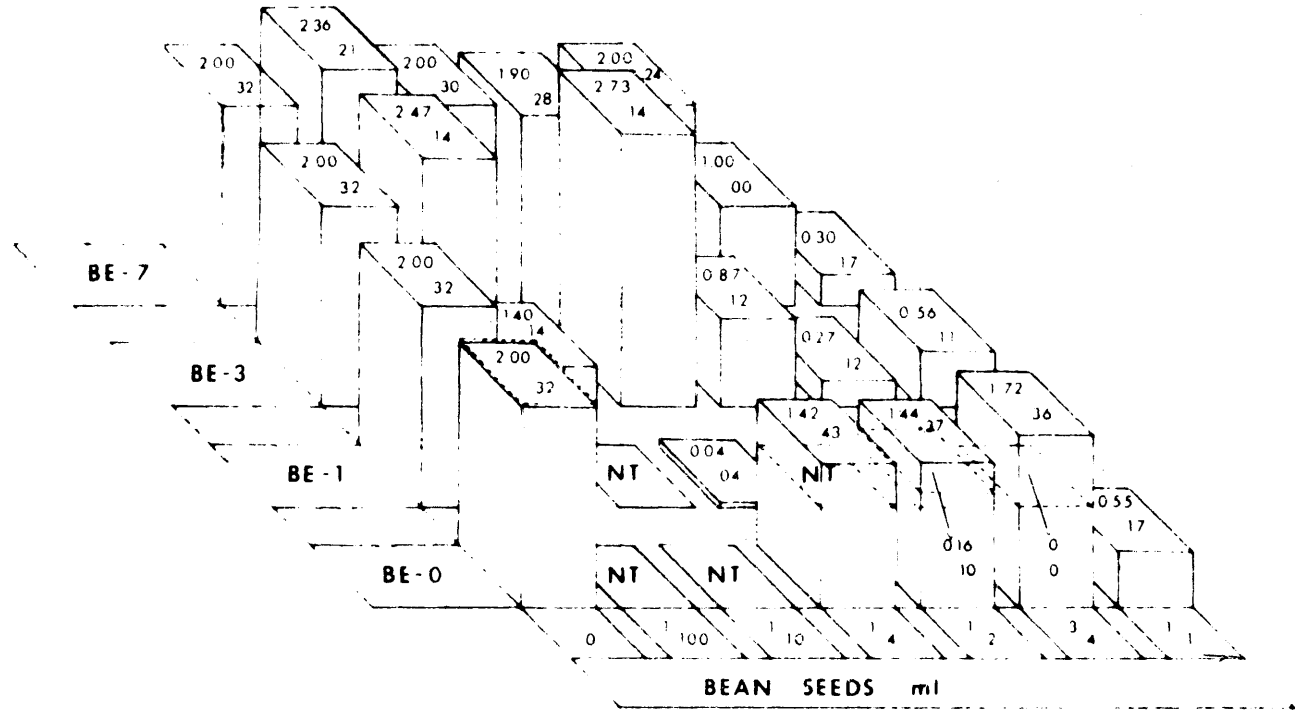

FIG. 4 – Effect of age of bean seed used for extract (BE-0 = 0-day old, BE-1 = 1-day-old, BE-3 = 3-day old and BE-7 = 7-day old) and concentration of extract on callus growth of *Phaseolus vulgaris* leaf explants cultured on 67–V salts and vitamins, 2 mg/l NAA and 0.2 mg/l kinetin. NT = No test at concentration. The upper and lower numerical values of the individual histograms represent callus growth index and standard error of the mean respectively.

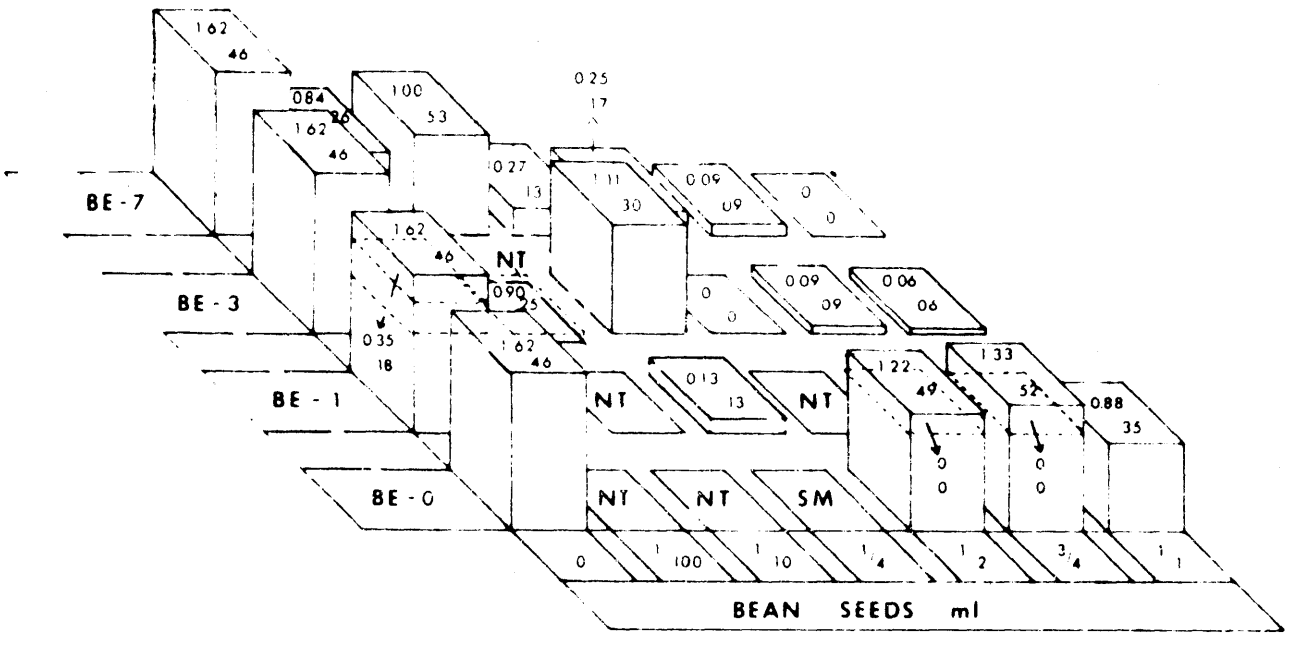

FIG. 5 – Effect of age of bean seed used for extract (BE-0 = 0-day old, BE-1 = 1-day old, BE-3 = 3-day old and BE-7 = 7-day old) and concentration of extract on root morphogenesis of *Phaseolus vulgaris* leaf explants cultured on 67–V salts and vitamins, 2 mg/l IAA, 1 mg/l NAA and 0.2 mg/l kinetin. SM = Two plantlets induced at this concentration. NT = No test at concentration. The upper and lower numerical values of the individual histograms represent root morphogenesis index and standard error of the mean respectively.

DISCUSSION

The induction of shoot morphogenesis and the enhancement of callus growth and root induction by the addition of a bean seed extract are significant advancements in the culture of *Phaseolus vulgaris*. In extensive tests using 67–V salts and the auxin, indole acetic acid (IAA) or napthalene acetic acid (NAA) accompanied by the cytokinins kinetin or zeatin, no plantlet morphogenesis was induced in more than 3,000 cultures (CROCOMO et al., 1976). Both callus growth and root morphogenesis could be controlled by auxin and cytokinin concentration, but the levels obtained with the addition of bean seed extract are significantly increased.

The work testing the age of the bean seed extract although preliminary in nature, is both exciting and promising. It is our belief that morphogenesis in *Phaseolus vulgaris* is very desirable and should be sought even if the return to undefined nutrient is necessary. The use of the bean extract has been the most promising area of our attempts, although another potentially significant morphogen in our system has been the addition of nicotine to induce and control root morphogenesis (PETERS et al., 1976).

The potential application of tissue culture in the production of improved *Phaseolus* varieties has been limited by the lack of morphogenesis. Since the significance of this plant as a source of protein is becoming increased with the increased world food shortages, the induction of morphogenesis is very important. Haploid callus has been obtained in *Phaseolus vulgaris* (SHARP et al., unpublished) and the control of morphogenesis in this system supplemented by promising protoplast work offers unlimited possibilities for the production of new varieties.

LITERATURE CITED

CROCOMO, O.J., PETERS, J.E. & SHARP, W.R., 1976. Interactions of Phytohormones on the control of growth and root morphogenesis in cultured *Phaseolus vulgaris* leaf explants. Turrialba, 26:232-236.

DEBERGH, P. & NITSCH., C., 1973. Premiers résultats sur la culture in vitro de grains de pollen isolés chez la Tomate. C.R. Acad. Sc. Paris t. 276 Serie D. 1281-1284.

GAMBORG, O.L., 1966. Aromatic metabolism in plants. II. Enzymes of shikimate pathway in suspension cultures of plant cells. Can. J. Biochem., 44:791-799.

HILDEBRANDT, A.C., WILMAR, J.C., JOHNS, H. & RIKER, A.J., 1963. Growth of edible chlorophyllous plant tissues in vitro. Amer. J. Bot., 50:248-254.

JEFFS, R.A. & NORTHCOTE, D.H., 1967. The influence of IAA and sugar on the pattern of induced differentiation in plant tissue culture. J. Cell. Sci., 2:77-88.

LAMPORT, D.T.A., 1964. Cell suspension cultures of higher plants: isolation and growth energetics. Exp. Cell Res., 33:195-206.

LIAU, D.F. & BOLL, W.G., 1971. Growth and patterns of growth and division of cell suspension cultures of bush beans (*P. vulgaris* cv. Contender). Can. J. Bot., 49:1131-1139.

MEHTA, A.R., HENSHAW, G.G. & STREET, H.E., 1967. Aspects of growth in suspension cultures of *Phaseolus vulgaris* L. and *Linum usitatissium* L. Indian J. Plant Physiol., 10:44-53.

PETERS, J.E., CROCOMO, O.J. & SHARP, W.R., 1976. Effect os caffeine and nicotine on the callus growth and root morphogenesis of *Phaseolus vulgaris* tissue cultures. Turrialba, 26: 337-341.

VELIKY, I.A., 1972. Synthesis of carboline alkaloids by plant cell cultures. Phytochemistry, 11:1405-1406.

VELIKY, I.A. & MARTIN, S.M., 1970. A fermenter for plant cell suspension cultures. Can. J. Microbiol., 16:223-226.