# AMINO ACIDS IN THE CALLUS GROWTH AND ROOT MORPHOGENESIS OF BEAN (*Phaseolus vulgaris*) LEAVES CULTURED *in vitro*[1]

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**Resumo**

*Sementes de feijão, (*Phaseolus vulgaris *L.) cv. Carioca, foram germinadas em areia lavada e mantidas, posteriormente, em solução nutritiva nº 2 de Hoagland e Arnon. Do primeiro par de folhas das plantas de feijoeiro foram retirados, assèpticamente, explantes de aproximadamente 4 x 4 mm e inoculados em meio de cultura. O meio de cultura básico utilizado foi meio sólido contendo sais minerais, vitaminas e sacarose como fonte energética. Como fatores de crescimento foram fornecidos ao meio cinetina e IAA ou cinetina, IAA, NAA e 2,4–D. O pH dos meios foi acertado para 5.6 após a adição de todos os nutrimentos. Aminoácidos isolados ou em grupos foram adicionados ao meio básico para observar a eficiência destas substâncias em promover crescimento de calos e morfogênese de raízes em feijão. Dois meios de cultura considerados como referência foram mantidos. Um deles contendo caseina hidrolizada como fonte de nitrogênio reduzido (meio E); e o outro meio (meio G) contendo apenas o meio básico sem aminoácidos e sem caseina hidrolizada.*

*Durante o desenvolvimento das culturas foram feitas avaliações periódicas do crescimento de calos e desenvolvimento de morfogênese. No final de cada ensaio o peso fresco e o peso seco foram medidos. Com base nos dados obtidos foi calculado o IM (Indice de Morfogênese), IC (Indice de Crescimento) e os resultados foram submetidos a tratamento estatístico. Pela análise dos resultados observou-se que o meio de cultura que determinou melhor morfogênese foi o meio A contendo o meio básico acrescido de cinetina (1 mg/l), IAA (5 mg/l) e um grupo de três aminoácidos, arginina (60 mg/l), ácido aspártico (50 mg/l) e cisteina (10 mg/l). O meio B constituído de meio básico, acrescido de cinetina (1 mg/l), IAA (5 mg/l) e um grupo de três aminoácidos, ácido glutâmico, (65 mg/l), glicina (25 mg/l) e histidina (10 mg/l) ocasionou uma drástica inibição tanto no crescimento como na morfogênese. Dentre os aminoácidos deste último, o ácido glutâmico revelou-se como sendo a substância que ocasionou uma repressão do crescimento.*

*Quando foram testadas dezesseis concentrações de ácido glutâmico, mantendo glicina e histidina constantes, os resultados permitiram concluir que não foi a presença de ácido glutâmico, no meio B, a causa da repressão, nas a concentração usada (65 mg/l). Assim, pode-se observar que concentrações baixas de ácido glutâmico proporcionam um bom crescimento em culturas de tecido de folhas de feijoeiro e que em concentrações elevadas o crescimento decai. O melhor crescimento de calos foi obtido no meio $B_4$ em que o meio básico foi acrescido de quatro hormônios, cinetina (0.2 mg/l), IAA (2 mg/l), NAA (1 mg/l), e 2,4–D (1 mg/l) e um grupo de três aminoácidos, ácido glutâmico (0.5 mg/l), glicina (25 mg/l) e histidina (10 mg/l).*

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## Introduction

It is well known that reduced nitrogen stimulates growth and morphogenesis when added to the nutrient medium as a mixture of amino acids, such as hydrolyzed casein or yeast extract (18). Gamborg *et al.* (9) have presented evidence that hydrolyzed casein and coconut milk can substitute for amino acids in suspension cultures of soybean root cells and that the hydrolyzed casein can be replaced by the mixture of the amino acids, lysine, arginine, histidine, aspartic acid and glutamic acid. Cell suspension cultures from sugarcane stalk parenchyma can be grown in the synthetic medium consisting of inorganic salts, vitamins, 2,4–D and a mixture of 13 amino acids where arginine, aspartic acid and glutamic acid (15) become the most important constituents. Arginine and tyrosine have been reported to stimulate the growth of conifer callus (10). However, single amino acids and/or mixtures of amino acids can be inhibitory to the growing of cells in cultures as well. Lysine is inhibitory to the growth of carrot cells in culture while glutamic acid and proline are respectively inhibitory to the growing of cultured tobacco and soybean cell cultures (2). Similar studies have been made by other investigators (18, 20).

On the other hand, amino acids can control cell differentiation under *in vitro* conditions. Glutamic acid, or a mixture of amino acids, promotes embryo formation in carrot callus (17). Moreover, glutamine or alanine, and possibly glutamic acid, can be used as the sole source of nitrogen for growth and embryogenesis of carrot cells in culture (21).

In this investigation, we present evidence of the influence that certain amino acids or mixtures of amino acids have in the stimulation or inhibition of the root's growth and morphogenesis in tissue cultures of *Phaseolus vulgaris* leaf explants.

## Materials and Methods

**Plant material** – The explants used were obtained from the primary leaves of two-week old bean (*Phaseolus vulgaris*, cv. Carioca) grown in Hoagland and Arnon's complete nutrient solution (13), maintained in a growth cabinet (Con Viron), 26/22°C under a 15/9 h photoperiod regime. Leaves were surface sterilized in 20 per cent (v/v) commercial sodium hypochlorite for 15 minutes and then rinsed twice in sterile distilled water. Explants of ca. 4 $mm^2$ were individually inoculated into 4 oz. French square culture flasks charged with 10 ml of culture medium adjusted to pH 5.6. Twenty replicate treatments were conducted for each test medium variation as described below, and placed in constant environment chambers, at 25°C, 12-h photoperiod, under ca 200 ft.–c– 2 000 Lux of cool-white fluorescent illumination outside the culture flasks. All tests were evaluated for callus growth and root morphogenesis at the 3rd, 5th and 7th weeks. In the latter, fresh and dry weights were determined. Growth and morphogenesis were scored using a visual evaluation scale of 0–6 for callus and a similar scale of 0–4 for the extent of root morphogenesis. Before scoring, standards were selected and used as a reference, and cultures were randomly scored with respect to the nutrient medium. Scores for callus growth and scores for root induction were averaged and the standard error of the mean calculated. The means were termed the callus growth index (GI) and the root morphogenesis index (MI), respectively.

### Nutrient medium

a. **Basal medium** (G medium) the basal medium used in all experiments was a modified Murashige and Skoog (14) nutrient solution (solid medium) containing mineral salts, vitamins, inositol and sucrose. The growth regulators consisted of indole acetic acid (IAA) 5 mg/l and kinetin 1 mg/l.

b. **Controls** – the tissue cultures were grown on **E medium** (basal medium + 2 g/l hydrolyzed casein) and G medium (basal medium).

c. **Amino acid supplements to the nutrient medium** – the amino acid supplements to the culture medium are based on the experimental work of Nickell and Maretzki (15):

Medium A: basal medium + arginine (60 mg/l), aspartic acid (50 mg/l), cysteine (10 mg/l);
Medium B: basal medium + glutamic acid (65 mg/l), glycine (25 mg/l), histidine (10 mg/l);
Medium C: basal medium + isoleucine (30 mg/l), methionine (20 mg/l), phenylalanine (10 mg/l);
Medium D: basal medium + proline (40 mg/l), threonine (35 mg/l), tyrosine (5 mg/l), serine (50 mg/l);
Medium F: basal medium + thirteen amino acids (15).

d. **Alterations in the media A and F** – alterations in **Medium A** were made in order to determine the effect of single amino acids, pairs of amino acids

and the interaction among the 13 amino acids of medium F. Twenty-five replicate cultures for each treatment were made.
$A^1$: arginine; $A^2$: aspartic acid; $A^3$: cysteine; $A^4$: arginine + aspartic acid; $A^5$: arginine + cysteine; $A^6$: aspartic acid + cysteine.
$F^1$: F minus arginine; $F^2$: F minus arginine and cysteine; $F^3$: F minus cysteine; $F^4$: F minus arginine and aspartic acid; $F^5$: F minus arginine and cysteine; $F^6$: F minus arginine and cysteine; $F^7$: F minus arginine, aspartic acid and cysteine.

e. **Alterations in the media B and F** – these modifications allowed for the study of the effects of single amino acids or groups of 2 amino acids in (medium B) and the interactions among the 13 amino acids of the Medium F.
$B^1$: glutamic acid; $B^2$: glycine; $B^3$: histidine; $B^4$: glutamic acid and glycine; $B^5$: glutamic acid and histidine; $B^6$: glycine and histidine.
$F^8$: F minus glutamic acid; $F^9$: F minus glycine; $F^{10}$: F minus histidine; $F^{11}$: F minus glutamic acid and glycine; $F^{12}$: F minus glutamic acid and histidine; $F^{13}$: F minus glycine and histidine.

f. **Influence of glutamic acid concentration** – the following glutamic acid concentrations were used: $B_a$: 0.0 mg/l; $B_b$: 0.1 mg/l; $B_c$: 0.3 mg/l; $B_d$: 0.5 mg/l; $B_e$: 1.0 mg/l); $B_f$: 5.0 mg/l; $B_g$: 10 mg/l; $B_h$; 20 mg/l; $B_i$: 30 mg/l; $B_j$: 40 mg/l; $B_k$: 50 mg/l; $B_l$: 60 mg/l; $B_m$: 65 mg/l; $B_n$: 500 mg/l; $B_o$: 1 000 mg/l; $B_p$: 2 000 mg/l. Glycine and cysteine concentrations were 25 mg/l and 10 mg/l respectively. This assay was conducted either in the presence of 2 growth regulators (IAA 5 mg/l; kinetin 1 mg/l; as in the basal medium) or 4 growth regulators (IAA 2 mg/l; kinetin 0.2 mg/l; NAA 1 mg/l; 2,4–D 1 mg/l).

## Results and discussion

### Behavior of amino acids in groups

The onset of all proliferation was delayed 2 days when the leaf explants were exposed to **Medium B** containing glutamic acid, glycine and histidine. Callus growth occurred in all treatments followed by root morphogenesis in some. The orthotropic (aerial) roots were less vigorous and at higher frequency than were the geotropic ones. Only treatment A containing arginine, aspartic acid and cysteine promoted morphogenesis and the occurrence of green pigmented regions in the callus, in all flasks. Figure 1 summarizes the growth index (GI) and morphogenesis index (MI). It can be observed that the highest MI (12.0893 ± 0.1694) occurs in cultures on **Medium A**, and a lower GI (10.8278± 0.1994) occurred when the 13 amino acids were used. The lowest GI (10.2193 ± 0.0876), and MI (1.0028 ± 0.2784) were obtained when glutamic acid, glycine and histidine (medium B) were used. The amino acids of **Medium B** are apparently inhibitory to growth and morphogenesis. The same situation has been observed in tobacco tissues growing in the presence of glutamic acid and soybean tissue in medium containing glutamine and glycine (2). Other authors have made this observation in soybean (1) and **Plumago** (16). One possible explanation is that these amino acids inhibit the assimilation of the intracellular ammonium for amino

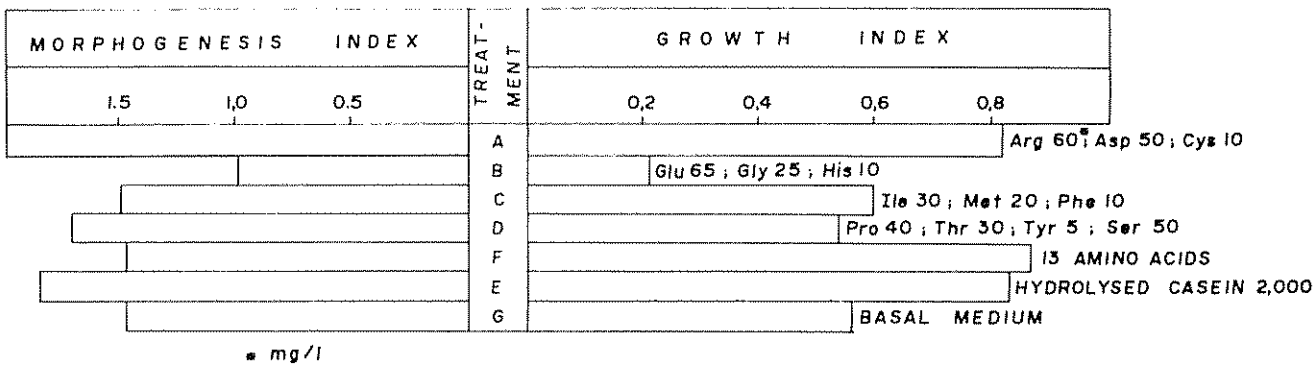


Fig 1 Influence of group of amino acids in the growth callus and root morphogenesis of *Phaseolus vulgaris* tissue culture. The number following the name of the amino acid are in mg/l of the respective amino acid.

Basal medium: $NO_2HPO_4$ (7 $H_2O$) (37.7 mg/l); KI(0.05 mg/l); $NaH_2PO_4.H_2O$ (173.0 mg/l); $CoCl_2$ 6 $H_2O$ (0.25 mg/l); $Na_2MoO_4$ 2 $H_2O$ (0.25 mg/l); $MgSO_4$. 7 $H_2O$ (250 mg/l); $KNO_3$ (800 mg/l); $(NH_4)_2SO_4$ (100 mg/l); $MnSO_4$ $H_2O$ (4.5 mg/l); $ZnSO_4$ 7 $H_2O$ (2.4 mg/l); $CuSO_4$ 5 $H_2O$ (0.25 mg/l); KCl (200 mg/l); $CaCl_2$ (135 mg/l); $FeSO_4$ . 7 $H_2O$ (27.8 mg/l); Na EDTA (37.3 mg/l); Thyamine (8 mg/l); Pyridoxine (0.5 mg/l); Niacin (1.25 mg/l); Calcium Panthotenate (1.0 mg/l); Sucrose (20 g/l); Inositol (100 mg/l); Indole Acetic Acid (IAA) (5 mg/l); kinetin (1 mg/l); Agar (10 g/l)

acids synthesis of cells when growing in the presence of nitrate (2, 8). Our basal medium contains both nitrate and ammonium salts. The group of amino acids: arginine, aspartic acid, and cysteine promoted a higher MI as compared to other amino acid supplements while the GI was at least comparable to that of medium containing hydrolyzed casein (medium E) or 13 amino acids (medium F). Indeed, reduced nitrogen is necessary for growth and morphogenesis (18); arginine and aspartic acid are good promotors in soybean cell culture (9), and arginine in conifers (10).

**Interactions between arginine, aspartic acid and cysteine**

These three amino acids, individually, in groups of two, or all three together in the culture medium, in general behave in a similar manner in the promotion of growth and morphogenesis in bean cell cultures (Figure 2). About 50% of the cultures have root development being aerial and geotropic. Medium $A^1$ (arginine) did not differ statistically from medium $A^5$ (arginine and cysteine) and a single supplement of arginine has the same effect as a single supplement of aspartic acid or cysteine. Aspartic acid is more effective than cysteine or arginine + aspartic acid, and the comparison of arginine + cysteine is less effective than aspartic acid + cysteine. The pair arginine + aspartic acid is more promotive of GI than is the pair arginine + cysteine. Indeed, arginine, aspartic acid and cysteine together or individually promote growth of carrot cells in the presence of nitrate (2). In general, arginine is a good nitrogen source for the growth of tissue cultures (7, 16, 19).

While exogenous arginine and aspartic acid are incorporated into proteins, the rate of incorporation is dependent upon their concentrations in the external medium. Glutamic acid is not incorporated directly into protein; however, it influences the incorporation of other amino acids (4). Maybe this is an explanation for the two day delay in the onset of callus formation of cultures growing on glutamic acid in **Medium B** as compared to **Medium A** (arginine and aspartic acid).

When one considers the behavior of the 13 amino acids (medium F) (Figure 2), the GI is 1.0598±0.1595. It can be observed that when one amino acid is withdrawn (medium $F^1$), the GI is + 0.7220 ± 0.1228, and when two amino acids are withdrawn (medium $F^4$), the GI is 0.530 ± 0.1830. Therefore, when one of the group of 13 amino acids is absent, the GI is greater than when 2 amino acids are absent. A statistical analysis confirms these visual results: GI is lower in the absence of arginine as compared to the absence of aspartic acid or cysteine from the culture medium. Aspartic acid is more promotive of GI than is cysteine.

The response to medium $F^6$ (medium F minus Asp and Cys) on GI is higher than medium $F^4$ (medium F minus Arg and Asp) and medium $F^5$ (medium F minus Arg and Cys) which can be explained on the basis of the absence of arginine in these two media. Considering the performance of these three acids, either individually or in a group of two, or in interactions with the group of 13 amino acids, the efficiency on promotion of GI is in the following order: arginine / aspartic acid / cysteine.

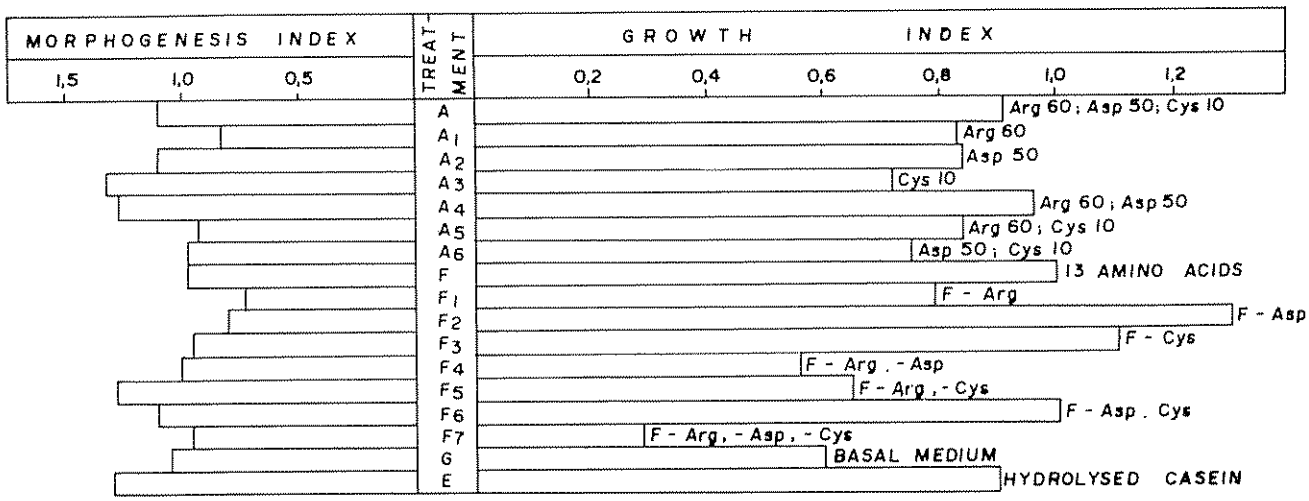


Fig. 2. Influence of arginine, aspartic acid and cysteine on the growth callus and root morphogenesis of *Phaseolus vulgaris* tissue culture

**Glutamic Acid**

Figure 3 is a representation of GI and MI when glutamic acid, glycine and histidine were added to the medium, either individually or in groups of two or in interactions with the group of 13 amino acids. Almost all cultures undergo root morphogenesis. On medium A (arginine, aspartic acid and cysteine) rooting occurs at a higher frequency; however, roots are less firm and stunted. The GI was lower in the presence of the three amino acids (medium B) than the average of the media $B^1$-$B^6$ in which the amino acids are added individually or in groups of two. Glutamic acid alone (medium $B^1$) is promotive of a lower growth index (GI = 0.1337 ± 0.1121) than glycine (medium $B^2$, GI = 0.3743 ± 0.1404) or histidine (medium $B^3$, GI = 0.3534 ± 0.1188). GI in the presence of glycine + histidine (medium $B^6$) was higher (GI = 0.3688 ± 0.1076) than the average GI in a medium containing glutamic acid + glycine (medium $B^4$, GI = 0.2703 ± 0.2664) and glutamic acid + histidine (medium $B^5$, GI = 0.1120 ± 0.0359). Similar observations have been made by other authors working with single amino acids inhibiting growth of tobacco cells in culture (11, 12). Glutamic acid supports a lower level of growth in soybean cells than other tested amino acids (1, 2, 9).

It can be observed in Figure 3 that when glutamic acid is withdrawn from the culture medium (medium $F^1$) the GI is higher (GI = 0.6098 ± 0.1709) as compared to the situation when glycine (medium $F^2$, GI = 0.5189 ± 0.1073) or histidine (medium $F^3$, GI = 0.4567 ± 0.1177) is withdrawn. On the other hand, when glycine + histidine (medium $F^6$) is withdrawn from the medium, the GI is lower (GI = 0.4505 ± 0.1065) than when glutamic acid + glycine (medium $F^4$, GI = 0.6078 ± 0.1154) or glutamic acid + histidine (medium $F^5$, GI = 0.5444 ± 0.1231) are withdrawn.

Comparing the data in Figure 2 with that in Figure 3 it can be observed that the highest GI in the latter (corresponding to medium B and alterations) was 0.6097 ± 0.1709, while the highest GI in Figure 2 (corresponding to medium A) was 1.3035 ± 0.3108. Only 3 treatments of medium A have GIs equivalent to or lower than the highest GI in medium B, reinforcing the positive effect of the amino acids arginine, aspartic and cysteine (medium A) on the bean leaf explants.

An optimal GI occurs when concentrations of glycine and histidine are maintained and glutamic acid varied at lower concentrations (Figure 4). Glu 0.1 mg/l, GI = 0.1040 ± 0.2124; Glu 0.3 mg/l, GI = 0.7848 ± 0.1835; Glu 0.5 mg/l, GI = 1.0577 ± 0.1439. Glutamic acid concentrations of 1 mg/l and higher inhibit growth while concentrations above 50 mg/l result in a GI of ca. 0. Other authors have made similar observations in different cell culture systems (5).

It is interesting to notice the negligible stimulation of morphogenesis at lower concentrations of glutamic acid (Figure 4), while at higher concentrations morphogenesis is promoted. The peak occurs at concentrations 20-40 mg/l, the same in which callus growth is repressed. From these concentrations on, the inhibition of morphogenesis becomes clear. Indeed,

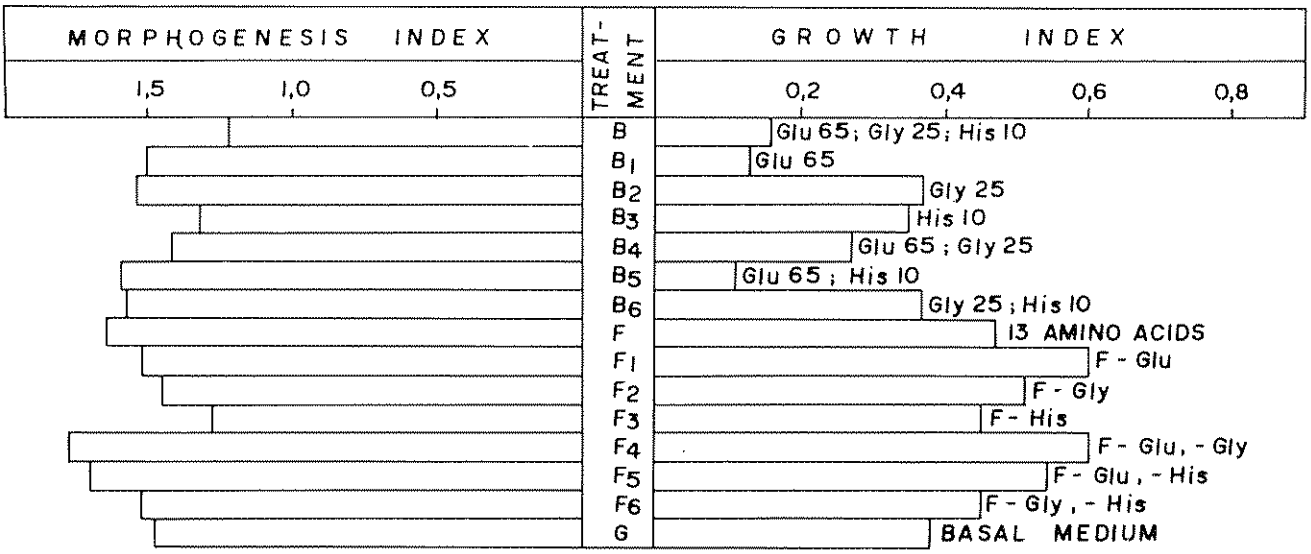


Fig 3. Influence of glutamic acid, glycine and hystidine on the growth callus and root morphogenesis of *Phaseolus vulgaris* tissue culture

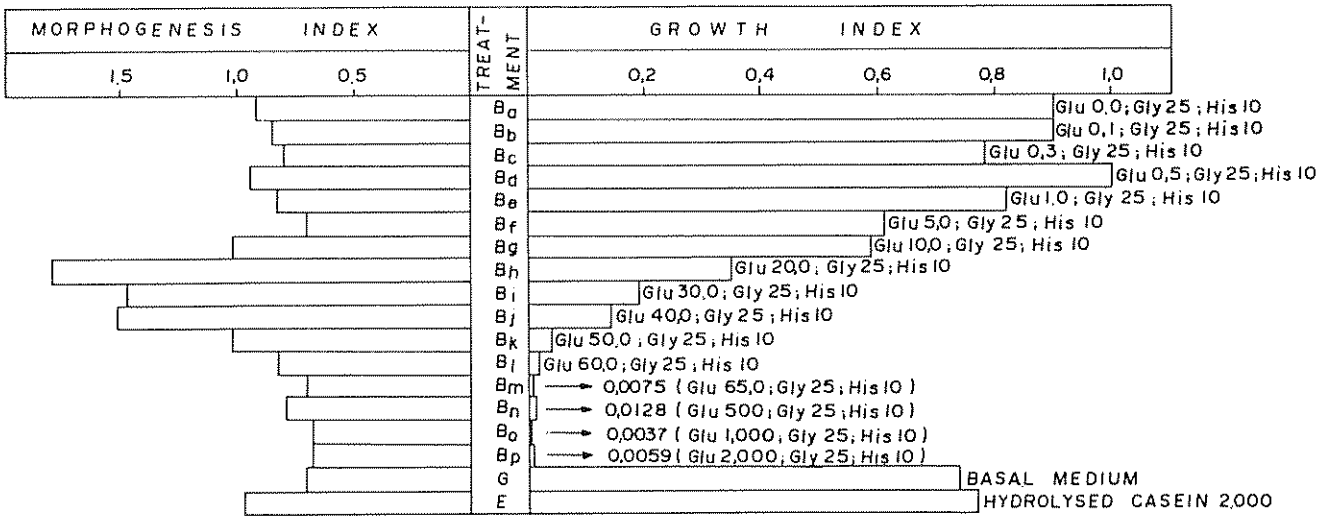


Fig 4 Influence of different concentrations of glutamic acid on the growth callus and root morphogenesis of *Phaseolus vulgaris* tissue culture.

glutamic acid promotes and increases the formation of secondary roots (6) and concentrations of 20 mg/l increase the growth of *Datura tatula* roots in culture (6).

Table 1 summarizes the growth and morphogenetic indices of the cultures growing in presence of different concentrations of glutamic acid and the growth regulators IAA + kinetin or IAA + kinetin + 2,4–D + NAA. Dramatic differences occur between these two kinds of treatments (2 or 4 growth regulators), mainly in concentrations of 0.1, 0.3 and 5.0 mg/l glutamic acid. These concentrations in the presence of NAA, 2,4–D and IAA promote better

Table 1. Growth and morphogenesis indexes of bean leaf explants growing in the presence of different concentrations of glutamic acid and 2 or 4 growth factors*

| Glutamic Acid | IAA + Kinetin** | | IAA + Kinetin + 2,4–D + NAA*** | |
|---|---|---|---|---|
| mg/l | GI | MI | GI | MI |
| 0.0 | 0.9006 ± 0.1941 | 0.9899 ± 0.5803 | 2.8069 ± 0.3439 | 0.8839 ± 0.5000 |
| 0.1 | 0.9040 ± 0.2124 | 0.8839 ± 0.4830 | 3.1691 ± 0.5312 | 0.7071 ± 0.0918 |
| 0.3 | 0.7848 ± 0.1835 | 0.8081 ± 0.3780 | 3.0782 ± 0.5645 | 0.8106 ± 0.2182 |
| 0.4 | 1.0577 ± 0.1439 | 0.9716 ± 0.5428 | 3.1763 ± 0.5929 | 1.0789 ± 0.6148 |
| 1.0 | 0.8299 ± 0.1721 | 0.7803 ± 0.2273 | 2.4852 ± 0.9743 | 0.7071 ± 0.0876 |
| 5.0 | 0.6120 ± 0.1834 | 0.7071 ± 0.1010 | 2.0269 ± 0.7656 | 0.8642 ± 0.4714 |
| 10.0 | 0.5940 ± 0.2991 | 1.1187 ± 0.6395 | 1.1153 ± 0.3291 | 0.7071 ± 0.0765 |
| 20.0 | 0.3567 ± 0.0908 | 1.7827 ± 0.5952 | 1.4921 ± 0.4607 | 1.4264 ± 0.6370 |
| 30.0 | 0.1913 ± 0.1381 | 1.4786 ± 0.7054 | 0.8734 ± 0.8180 | 1.2061 ± 0.6930 |
| 40.0 | 0.1479 ± 0.0952 | 1.5127 ± 0.7081 | 1.1736 ± 0.3568 | 1.1435 ± 0.6023 |
| 50.0 | 0.0676 – | 1.1334 ± 0.6556 | 0.7004 ± 0.7714 | 1.0906 ± 0.5910 |
| 60.0 | 0.0214 – | 0.8560 ± 0.4459 | 0.1169 ± 0.0721 | 0.8939 ± 0.4044 |
| 65.0 | 0.0077 – | 0.7071 ± 0.0706 | 0.5408 ± 0.4360 | 1.1493 ± 0.6128 |
| 500.0 | 0.0128 – | 0.8543 ± 0.2716 | 0.0199 + – | 0.7810 ± 0.1956 |
| 1 000.0 | 0.0037 – | 0.7071 ± 0.0690 | 0.0040 – | 0.7071 ± 0.0853 |
| 2 000.0 | 0.0059 – | 0.7071 ± 0.071 | 0.0010 – | 0.7071 ± 0.0795 |
| Medium G | 0.7392 ± 0.1907 | 0.7646 ± 0.1674 | 3.5034 ± 0.4157 | 0.8810 ± 0.1830 |
| Medium E | 0.7725 ± 0.2148 | 0.9774 ± 0.5569 | 2.2492 ± 0.6127 | 0.7718 ± 0.3359 |

* Glycine 25 mg/l; hystidine 10 mg/l

** IAA 5 mg/l; kinetin 1 mg/l

*** IAA 2 mg/l; kinetin 0.2 mg/l; NAA 1 mg/l; 2,4-D 1 mg/l

development of calluses. In conifers, a supplement of amino acids to a medium with more than one auxin promoted increased growth (10).

The calluses growing on IAA + kinetin are more compact and light brown in color, while those formed in the presence of IAA + kinetin + 2,4–D + NAA are cream colored and friable. Soybean callus growing in the presence of IAA and kinetin is white and compact (22), while in 2,4–D the callus is yellow and friable. Treatments with four growth regulators result in stunted roots with a reduced diameter, while roots resulting from cultures on medium with two growth regulators are elongated, aerial or geotropic and occur at a higher frequency. In beans, kinetin promotes cell division and in the presence of IAA morphogenesis is promoted as well (3). Root morphogenesis does not occur in the absence of kinetin.

The influences of glutamic acid is not the same for promotion of growth and morphogenesis (Table 1). In both treatments (2 and 4 growth factors), the best GIs were obtained when glutamic acid concentration was low, with a dramatic decrease in GI above 5.0 mg/l. On the other hand, the best MIs were obtained at intermediary concentrations of glutamic acid with a peak at 5.0 mg/l in the presence of either 2 or 4 growth regulators.

## Conclusions

Experiments on the effects of exogenous concentrations of amino acids either individually or in groups on the control of growth and morphogenesis in *Phaseolus vulgaris* tissue cultures, provide evidence that the group of amino acids: arginine, aspartic acid and cysteine regulate cell proliferation and morphogenesis. The three amino acids either individually or in groups of two or three promote increased growth and root morphogenesis. On the other hand, the amino acids glutamic, glycine and histidine are inhibitory to growth and morphogenesis. Glutamic acid, depending on the concentration, can inhibit or stimulate growth and morphogenesis with low concentration being promotive and high concentration inhibitory. Optimal callus growth was obtained in the presence of 4 growth regulators (IAA, NAA, 2,4–D and kinetin) in the presence of glutamic acid, glycine and histidine.

## Summary

Bean seeds (*Phaseolus vulgaris* L.) cv. Carioca, were germinated in sand and maintained in a Hoagland and Arnon's nutrient solution number 2. Explants from the first pair of leaves were aseptically removed and inoculated onto a modified Murashige and Skoog (1962) agar culture medium. The medium contained mineral salts, vitamins, and sucrose. Growth regulators used in the medium consisted of kinetin and IAA together or kinetin, IAA, NAA and 2,4–D. The pH of all media was adjusted to 5.6 after the addition of all the nutrients. Individual amino acids or groups of amino acids were added to the basal medium in order to study the interactions of these substances in the promotion of callus growth and root morphogenesis. Two kinds of cultures were maintained as standards. One containing hydrolyzed casein as a reduced nitrogen source (medium E) and the other containing basal medium in the absence of amino acids and casein hydrolysate. The MI (Morphogenesis Index) and GI (Growth Index) were calculated from the compiled data and tabulated. According to the results of the analysis, the culture medium A containing basal medium with kinetin (1 mg/l), IAA (5 mg/l) and a group of three amino acids: arginine (60 mg/l), aspartic acid (50 mg/l) and cysteine (10 mg/l) promoted optimal morphogenesis.

Medium B containing basal medium with kinetin (1 mg/l), IAA (5 mg/l) and a group of three amino acids: glutamic acid (65 mg/l), glycine (25 mg/l) and histidine (10 mg/l) was inhibitory to both growth and morphogenesis. Data resulting from the testing of 16 glutamic acid concentrations in the presence of fixed concentrations of glycine and histidine revealed that the presence of glutamic acid, *per se*, in medium B was not responsible for growth inhibition but rather the concentration of glutamic acid used (65 mg/l). One can conclude, therefore, that low concentrations of glutamic acid (0.5 mg/l) are promoters of growth, while higher concentrations are inhibitory. Optimal callus growth was obtained in medium $B^4$, in which four hormones kinetin (0.2 mg/l), IAA (2 mg/l), NAA (1 mg/l) and 2,4–D (1 mg/l) were added to the basal medium together with a group of three amino acids: glutamic acid (0.5 mg/l), glycine (25 mg/l) and histidine (10 mg/l).

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