SHOOT-BUD DIFFERENTIATION IN STEM-CALLUS TISSUE OF *CITRUS GRANDIS* AND CORRELATED CHANGES IN ITS FREE AMINO ACID CONTENT

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ABSTRACT

Stem-callus tissue of *Citrus grandis*, free from shoot-bunds (undifferentiated tissue), produced numerous shoot-buds (differentiated tissue) in the medium supplemented with 0·25 mg/l BAP + 0·1 mg/l NAA. Undifferentiated and differentiated tissue showed quantitative as well as qualitative differences in their free amino acid content. In general, the amount of free amino acids increased considerably in the differentiated tissue.

IT is well known that under the influence of cytokinins, or a combination of cytokinin and auxin, many plant tissues in culture may be induced to differentiate organized structures[1–2]. However, nothing is precisely known about the intervening biochemical changes taking place in the cells of the cultured tissue, between the application of the phytohormones and organogenesis. Qualitative and quantitative changes in respect of particular proteins (enzymes) might play decisive role in morphogenesis. Hence, it may also be worthwhile to study the amino acid pool of the cultured tissue, in relation to the formation of organized structures. Qualitative analysis of free amino acids in undifferentiated and differentiated carrot root-callus tissue does not show any significant differences[3]. On the other hand, there are reports that differentiation of organs in the cultured tissue has some influence on its biosynthetic potentiality[4–6]. We have found both qualitative and quantitative differences in the free amino acids of undifferentiated and differentiated stem-callus tissue of *Citrus grandis* (L.) Osbeck, which are reported here.

EXPERIMENTAL PROCEDURE

Tissue explants were taken from 2 to 2½-year-old stem-callus type-A tissue of *C. grandis*, which had been maintained in modifications of Murashige and Skoog's medium[7].

For organogenesis, the tissue explants were cultured in another variant of the basal medium[7] with supplements of 0·25 mg/l 6-benzylaminopurine (BAP) + 0·1 mg/l α-naphthaleneacetic acid (NAA).

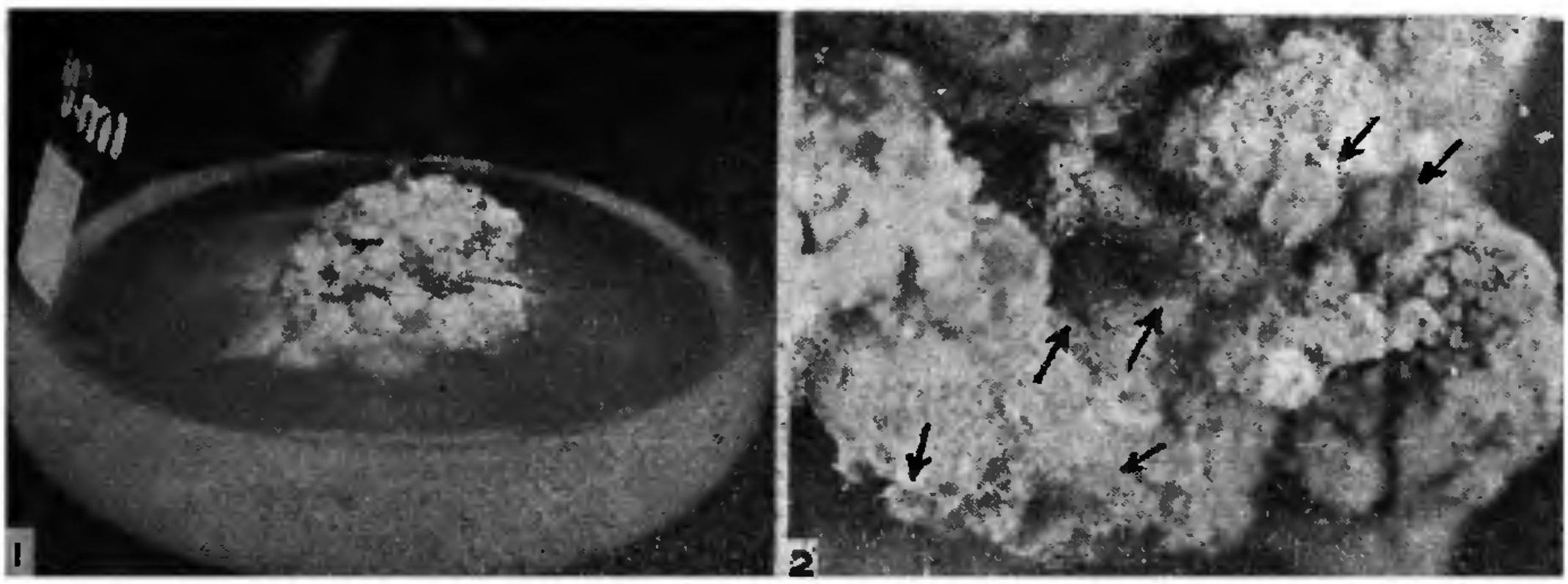
Sterilization procedure and other cultural conditions were as reported earlier[8]. Quantitative estimation of free amino acids of callus tissue, at the time of inoculation (undifferentiated tissue) and after its 60–70 days' incubation in the medium supplemented with 0·25 mg/l BAP+0·1 mg/l NAA (differentiated tissue), was made by paper chromatography adopting the following procedure: known amounts of fresh tissues were extracted in 100 ml 80% ethyl alcohol at room temperature for a week. The extracts were concentrated under reduced pressure to about ¼th of the initial amount. Each sample was spotted separately on Whatman No. 1 chromatographic paper, which was run first with *n*-butanol : acetic acid : water : : 40 : 10 : 50 in one dimension, and phenol : acetic acid : water : : 74 : 1 : 19·2 in the second dimension. The chromatogram was sprayed with 0·4% ninhydrin in acetone and allowed to develop for 30 min at 60° C. The ninhydrin positive spots were cut and extracted separately in 5 ml 75% ethyl alcohol saturated with $CuSO_4$. The extracted colour, after 30 min, was read in Spectronic-20 colorimeter at 540 mμ, and compared with the standard samples of amino acids subjected to the same treament.

RESULTS AND DISCUSSION

In the medium supplemented with 0·25 mg/l BAP + 0·1 mg/l NAA, numerous tiny green shoot-buds were formed on the surface of stem-callus tissue of *C. grandis* after an incubation of about 60 days (Fig. 2). Young shoot-buds showed numerous epidermal hairs. Stem-callus tissue, type-A, from which the explants were taken was compact-nodular, greenish-white, and devoid of any shoot-bud (Fig. 1).

Free amino acid analysis of the above-mentioned undifferentiated and differentiated tissue brought out not only quantitative differences, but also qualitative (*cf.* 3, Table I). Except glycine—the concentration of which remained unchanged, the amounts of all other amino acids increased in the differentiated tissue. This was strikingly high in respect of L-tyrosine which showed 5-fold increase, and L-alanine and L-threonine which showed an increase of 4-fold each. The amounts of L-proline and L-serine got tripled, whereas those of L-aspartic acid, L-gutamic acid and L-tryptophane got doubled (approx.). L-cystine and L-methionine, which could not be traced in the undifferentiated tissue, were present in good amount in the differentiated tissue. On the other hand, L-arginine and L-asparagine, which were present in the undifferentiated tissue, disappeared from the differentiated tissue.

The increase in amounts of amino acids in the differentiated tissue, as compared to the undifferentiated tissue of *C. grandis*, is indicative of higher metabolic activity in the former. There are some instances where differentiating organs in (*in vitro*) growing callus tissue do affect the concentration of plant constituents in them. Stem and leaf-callus tissue of *Atropa belladonna* does not synthesise atropine unless macroscopic roots are formed[5]. On the contrary, diosgenine content of root-callus tissue of *Dioscorea deltoidea* gets reduced following rhizogenesis[6]. In tobacco callus cultures, the high scopoletin content is correlated with a high capacity to form shoot-buds[4]. In the present study, changes in the concentration of amino acids, as also the appearance of some new amino acids and the loss of some others, are correlated with the differentiation of shoot-buds in the callus tissue. However, it cannot be concluded as to whether such quantitative and qualitative changes in the free amino acid content of the tissue cultured on the medium supplemented with BAP + NAA precede shoot-bud differentiation, or result therefrom.



FIGS. 1–2. Cultures of stem-callus tissue of *Citrus grandis*. Fig. 1. Tissue in undifferentiated state (× 1·2). Fig. 2. Tissue showing differentiation of shoot-buds in the medium supplemented with 0·25 mg/l BAP + 0·1 mg/l NAA (× 9·9).

TABLE I

Free amino acid content of stem-callus tissue of Citrus grandis

Amino acid*	Undifferentiated tissue	Differentiated tissue
L-Alanine	16·6	72·0
L-Arginine	18·7	..
L-Asparagine	45·8	..
L-Aspartic acid	32·0	72·0
Glycine	38·2	44·0
L-Glutamic acid	42·0	106·4
L-Proline	16·0	54·0
L-Serine	20·5	62·0
L-Threonine	25·0	100·0
L-Tryptophane	31·6	60·0
L-Tyrosine	12·2	60·0
L-Cystine	..	78·0
L-Methionine	..	40·8

* Quantity in μg/100 mg fr. wt. of callus tissue; data based on three chromatographs of each sample.

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