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Observations of amino acid changes in young roots after inhibition of elongation by some agents

B. Cireli

(Botany Department, Ege University, Izmir, Turkey)

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Investigations carried out under conditions which stimulate and inhibit root elongation started a considerable time ago. The fact that the speed of differentiation increases under conditions inhibiting the elongation of young roots emphasized the importance of work in this field. Various agents were tried in order to obtain inhibition of elongation in young roots. Various investigators decapitated the roots or treated them with certain concentrations of inhibiting substances such as colchicine, coumarin, illuminating gas and subsequent to these treatments studied the root growth with the employment of different methods.

The first investigation related to the effect of decapitation on the young roots was carried out by Wiesner at 1884. Later, Cholodny (1926), and Bünning (1928) put forward new ideas in this field. More recently Younis (1954) on *Vicia faba,* Bara (1957) on *Vicia faba* and *Lens culinaris,* and Vardar and Tözün (1958) on *Vicia faba* and *Lens culinaris* studied the various aspects of decapitation. They observed that decapitation caused the inhibition of elongation, but stimulated the thickening of young roots. Likewise, it has been reported that colchicine, coumarin, and illuminating gas treatments brought about the same sort of modifications in the roots.

Audus (1948), Goodwin and Taves (1950), San Antonio (1952), Torrex (1953), and Burström (1956) studied the effects of coumarin on the root growth of *Pisum sativum* and *Lepidium sativum, Avena, Melilotus alba, Pisum sativum,* and *Triticum* seedlings, respectively.

The various aspects of effects of colchicine on young root, which is also an inhibitor of root elongation, have been investigated by some workers. Garrigues (1940), Levin and Lein (1941), and Davidson (1965, 1969) emphasized the effects of colchicine on the root growth of *Vicia faba, Allium cepa,* and *Vicia faba,* respectively. Northen (1950) studied the modifications in structural viscosity of protoplasm after colchicine treatment; Sedar and Wilson (1951) investigated the mitotic appearances in the root tip cells of colchicine-treated and untreated *Allium cepa* roots with the employment of electron microscope.

Illuminating gas is also an inhibitor of elongation of young roots, like colchicine and coumarin. Neljubow (1911) found that the elongation of the different organs of the plants kept in laboratory atmosphere decreased while their thickening increased. Later Crocker, Hitchcock and Zimmerman (1935), Borgström (1939), Vardar (1957), Vardar and Bara (1959) investigated the effects of illuminating gas on the various organs of plants.

Wrisher (1960) studied the effects of carbon dioxide and nitrogen gases on the root tips of *Elodea canadensis* and *Sinapis alba,* and Dolzman and Ullrich (1966) investigated the effects of nitrogen dioxide on *Phaseolus vulgaris* leaves in regard to ultrastructure.

Cireli (1965), Cireli and Vardar (1969) treated young *Lens culinaris* and *Vicia faba* roots with the four above-mentioned agents. They observed, along with the stimulation of thickening of roots, typical increases in volumes of epidermal and cortical cells and their nuclei.

Cireli (1970) investigated the ultrastructural differentiation in the cortical cells of the roots treated in the above-mentioned manner and made comparisons with the normal root cortical cells. The organelles of the cells belonging to the treated roots appeared to be of very different types in comparison with the untreated cells.

The aim of the present work has been to obtain some evidence on the problem of the possible modifications in the amino acid contents of roots, of which the elongation has been inhibited and thickening has been stimulated by various inhibiting substances.

Material and Method

Experimental objects used were young *Lens culinaris* and *Vicia faba* roots.

In order to obtain homogenous material to serve our purpose, *Lens culinaris* was treated according to Vardar and Tözün (1958), and *Vicia faba* was treated according to Younis (1954).

Lens culinaris seedlings with 9 mm long roots and *Vicia faba* seedlings with 12 mm long roots were selected and divided into five groups. The plants in the first group remained untreated. The roots of those in the second group were decapitated, that is, 1 mm from the tip of *Lens culinaris* roots and 2 mm from the tip of *Vicia faba* roots were discarded.

The 1 mm portion of the root tips of *Lens culianaris* and 2 mm portion of *Vicia faba* root tips in the third group were treated for three hours with 1/40.000 conc. of colchicine according to the method of Geissler (1950). In the fourth group the same portions of the root tips of *Vicia faba* and *Lens culinaris* seedlings were treated with coumarin according to Burström (1956). The roots in the fifth group were likewise treated with 0.02% conc. of illuminating gas in a gasometer.

Treated and untreated roots were kept in a moist chamber made of glass during the experiments.

At the end of the experiments, the undecapitated, colchicine, coumarin, and illuminating gas-treated *Lens culinaris* and *Vicia faba* roots were decapitated 1 mm and 2 mm from the root tip, respectively; so that the regions to be investigated of all the above-mentioned roots were at the same level as those of the decapitated ones. After this decapitation process 5 mm tip portions of roots were taken and dried in an incubator at 60^0 C for 3 hours. After this, material was powdered, and hydrolysed in accordance with the method of Linser, Riehle and Neumann (1966). The amino acid contents of the hydrolysates were measured with special resined 120 B Beckman amino acid analyser in accordance with Moore and Stein (1964). The total nitrogen contents of dry matter, of which the amino acid contents were determined with amino acid analyser, were measured with Kjeldahl Method.

Results and Discussion

Table I shows the total N contents of experimental material. Here a decrease in the total N contents of decapitated *Lens culinaris* roots compared to those of undecapitated roots can be seen. This decrease is more marked with colchicine, coumarin, and illuminating gas-treated roots. In *Vicia faba* roots, this decrease in the total N contents of treated plants was smaller than in *Lens culinaris* roots.

Table II shows the comparative amino acid contents of the roots which were treated in different ways and of the untreated ones. Although the total amino

Table I. **The effect of decapitation, colchicine, coumarin, and illuminating gas treatments on the N contents (after Kjeldahl) of *Lens culinaris* and *Vicia faba* roots (mg N/g dry matter).**

	Lens culinaris			*Vicia faba*		
Total	(1)	(2)	mean	(1)	(2)	mean
Undecapitated	59,0	61,5	60,2	64,3	57,6	60,9
Decapitated	56,9	59,5	58,2	60,2	59,8	60,0
Colchicine	48,1	51,9	50,0	58,1	60,1	59,1
Coumarin	50,1	54,7	52,4	57,2	53,3	55,2
Illuminating gas	58,4	58,9	58,6	57,5	60,4	58,9

Table II. **The amino acid contents of undecapitated (U), decapitated (D), Colchicin (Col), Coumarin (Cou) and illuminating gas (IG) treated *Lens culinaris* and *Vicia faba* roots (N content of each amino acid in % of total N content).**

	Lens culinaris					*Vicia faba*				
	U	D	Col	Cou	IG	U	D	Col	Cou	IG
Lysine	14,0	15,4	14,9	14,2	14,9	9,7	9,8	9,9	9,9	9,8
Histidine	8,3	9,3	9,8	9,0	9,0	5,2	5,6	5,6	6,1	5,5
Arginine	10,8	8,3	9,6	8,3	8,9	14,0	12,8	9,6	10,6	10,5
Asparagin A	13,5	17,9	17,3	19,8	17,9	13,2	15,7	24,2	21,5	13,3
Threonine	3,6	4,7	4,1	3,9	4,5	4,2	4,8	4,9	4,9	4,9
Serine	4,4	4,1	4,3	4,0	4,3	4,8	4,5	4,0	5,0	4,0
Glutamic A	9,5	8,1	9,2	8,4	7,9	11,2	10,1	8,9	9,1	11,1
Proline	3,6	3,4	4,1	5,9	4,2	3,4	4,2	3,0	5,0	4,1
Glycine	6,3	5,9	5,7	5,1	5,0	5,7	6,2	6,0	6,1	6,0
Alanine	6,4	5,9	5,8	4,5	5,9	7,6	7,0	6,9	6,9	6,9
Cystine	0,7	0,5	0,6	0,8	1,0	0,8	0,9	0,7	0,8	1,1
Valine	5,3	4,3	5,6	5,7	5,9	4,7	5,3	4,9	5,5	4,1
Methionin	0,5	0,5	0,6	0,8	1,0	0,8	0,9	0,7	0,8	0,8
Isoleucine	4,2	4,2	4,0	3,7	4,0	3,8	3,8	3,8	4,0	3,9
Leucine	5,9	5,3	5,3	4,6	4,9	5,3	5,5	4,9	5,7	4,3
Tyrosine	0,7	0,6	0,6	4,3	0,7	2,2	1,4	2,6	2,3	0,9
Phenylalanine	1,6	1,3	1,5	0,9	1,8	2,4	1,7	2,2	1,9	1,4

acid contents of treated roots were found to be less than those of undecapitated roots, an increase was observed in the amounts of some individual amino acids. This increase was more obvious with lysine and aspartic acid, and less marked with histidine and threonine. On the other hand, a decrease was observed in arginine, glutamic acid, alanine, and phenyl-alanine contents.

There are differences in total N and amino acid amounts between treated and untreated roots. Hence there must be a difference between the protein synthesis mechanisms of these roots. According to Levine and Lein (1941), colchicine has catalytic effects on the metabolic activities of the cells and causes modifications in the structure of protoplasm along with its inhibitive effects on the elongation of the roots. Northen (1950), observing a decrease in the structural viscosity of the protoplasm after treatment with colchicine, likewise proposed that some protein dissociations caused the disruption of the order in metaphase.

Audus (1948), who worked with *Lepidium* and *Pisum* roots, reported that coumarin, along with its inhibiting effect on root elongation, probably caused the accumulation of some metabolites, e.g. some amino acids. According to this investigator, this accumulation reaches a toxic level. Dexheimer (1966) reported that with chloramphenicol the peptide bond structure was inhibited, leading to cessation of protein synthesis and to accumulation of amino acids in cytoplasm. According to these two authors, this accumulation reached a toxic level, depending on the time. In our opinion, those four amino acid types (lysine, asparagine, histidine, threonine) of which a decrease was observed in our work are the precursors of the amino acids of which the accumulation leads to a toxic level.

Our view has been strenghtened by our previous ultrastructural observations (Cireli 1970, unpublished). In the root cells subjected to the above-mentioned four treatments, we observed degenerated wall, endoplasmic reticulum, golgi, mitochondrium, and nucleus types different from those of untreated cells. It is already known that amino acids, by virtue of the activities of nucleus, nucleolus and endoplasmic reticulum, play an important role in the protein synthesis within the cell.

In view of these results, there seems to be a partial relationship between a disturbance in the balance of amino acids in the cell, and cell expansion.

Summary

The present work has been carried out in order to investigate the modifications in the amino acid contents of young roots subjected to treatments (decapitation, colchicine, coumarin, illuminating gas) which inhibit their elongation. Amino acid contents of the hydrolysates prepared from the test material have been determined with 120 B Beckman amino acid analyser. In the experiments, young roots of 16 hour-old *Lens culinaris* and 30 hour-old *Vicia faba* seedlings have been used as the test objects.

At the end of the investigations, total nitrogen and amino acid contents of the treated roots have decreased, whereas some individual amino acids have been found to have markedly increased, in comparison with the control roots.

A possible and partial role of a disturbance in the amino acid content of the cell on the cell expansion has been suggested.

Zusammenfassung

In der vorliegenden Arbeit wurde der Aminosäurengehalt junger Wurzeln nach Hemmung des Längenwachstums (durch Entfernung der Spitze oder Behandlung mit Colchicin, Cumarin oder Leuchtgas) untersucht. Der Aminosäurengehalt der Hydrolysate 16 Std. alter Keimlinge von *Lens culinaris* und 30 Std. alter Keimlinge von *Vicia faba* wurde mit einem Aminosäurenanalysator (Beckman 120 B) bestimmt.

Am Versuchsende nahm der Gehalt der behandelten Wurzeln an Gesamtstickstoff und Aminosäuren ab; einzelne Aminosäuren nahmen jedoch deutlich zu. Die Bedeutung einer Störung des Aminosäurenstoffwechsels für die Zellausdehnung wird diskutiert.

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Dr. Bilkan Cireli
Department of Botany, University of Ege
Izmir, Turkey