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Free amino acids in the environment of the proembryo during the inhibition phase of its growth (*Monocotyledonuos plants*)

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Abstract:

Analyses of the central vacuole sap in the ovules of *H. Katharinae* and *Clivia miniata* (during the inhibition phase of proembryo growth) for free amino acids were carried out by means of a Micro Column Amino Acid Analyser.

Some similarities and differences have been established for both speciesas regards the change of total concentration and concentration changes of particular amino acids.

INTRODUCTION

The results concerning amino nitrogen concentration, the number and concentrations of free amino acids in the central vacuole sap during the development of the ovule (dicotyledonous plants) have been published in a previous papers (Ryczkowski 1964, 1971). These investigations were carried out during the inhibition and exponential phase of embryo growth.

Data referring to the amino nitrogen and free amino acids in the environment of the proembryo in monocotyledonous plants have not been published.

This paper summarizes the results of studies carried out in order of: a) to determine the number and concentrations of free amino acids in the central vacuole sap (environment) during the inhibition phase of the proembryo growth in ovules of *Haemanthus Katharinae* and *Clivia miniata*, b) to establish whether there are any similarities or differences in the composition and concentration changes of free amino acids in the central vacuole sap in the examined ovules between these two species.

MATERIAL AND METHODS

The ovules of *Haemanthus Katharinae* Bak., and *Clivia miniata* RGL. were used as experimental material in the present investigations. The number of days elapsing from the day when the perianth wilted (*H. Katharinae*) or dropped (*Clivia miniata*)

to the day when material was taken for analyses, and the sizes of the ovules and embryos were the criteria adopted as the measure of the age of ovules and embryos.

The technique of the sap collecting from the central vacuole and the procedure of measuring the dimensions of ovules and embryos are given in previous papers (Ryczkowski 1960 a, b; 1962 a).

Before extraction of free amino acids the sap was centrifuged for 10 minutes at 3375 G at 4°C. For the extraction of free amino acids from the sap a mixture of ethanol and thiodiglycol (99 ml 75% ethanol + 1 ml thiodiglycol) was used; 250–500 µl of the sap was mixed with 5 ml of this solution. As internal standard 100 µl norleucine (3 µmoles norleucine/ml) was added to each sample.

Free amino acids were determined by means of the Micro Column Amino Acid Analyser (Technicon Instruments Co., Ltd. Chertsey, Surrey, England). For further details describing the procedure of extraction of free amino acids and their analysis see the papers by Ryczkowski et al., 1971; Linskens and Tupy, 1966. The accuracy of this method is within ±2,75%. The material for analyses was taken between 8 and 9 a.m.

The results are given in Tables 1 and 2 and present mean values of two analyses. For technical reasons threonine, serine, asparagine and glutamine (T+S+A+G) were estimated together. The very low and and high concentrations of some amino acids and ammonia are denoted in Tables I and II by + resp. ++.

RESULTS

Haemanthus Katharinae. It has been established that the central vacuole sap during the inhibition phase of the proembryo growth (ovules 9–33 days old) contains 22 free amino acids (Table 1). Two of these: α-aminobutyric acid and cystine have been found in the sap of ovules 9–18 days old and the latter one in 33 day-old ovules. If we assume that threonine, serine, asparagine and glutamine (T+S+A+G; determined together) are present in the sap, the number of identified free amino acids in the environment of the proembryo increases to 25 plus ammonia (Table 1).

The total concentration of free amino acids in the central vacuole sap (ovules 9–33 days old) decreases from 18717 to 8328 nmoles/ml sap. Ammonia concentration probably changes in an analogical way.

The nine amino acids: glycine, α-aminobutyric acid, valine, cystine, methionine, isoleucine, leucine, phenylalanine, and ethanolamine are characterized by a continual decrease of their concentration in the central vacuole sap in ovules 9–33 days old (Table 1). Alanine concentration changes in an analogical way, though in the sap of 33 dayold ovules it distinctly increases.

The concentrations of proline, lysine and arginine in the sap of young ovules (9–15 days old) increase to a determined level and in older ones (18–33 days) they drop (Table 1).

Table 1

Haemanthus Katharinae Bak. Course of concentration changes of free amino acids in the central vacuole sap during the development of the ovule and proembryo. Age of ovule and proembryo in days counted form the day the perianth wilted to the day of analysis

Age of ovules, days	9	15	18	28	33
Size of ovules, mm	5,4×2,2	7,4×3,6	7,8×4,1	9,6×5,6	9,9×6,5
Size of embryos, mm	0,13×0,10	0,15×0,17	0,20×0,20	0,45×0,40	0,56×0,54
No. Component	Concentrations nmole/ml sap				
1 MetSO	+	+	+	+	+
2 Asp	641	1186	1346	1218	1359
3 T+S+A+G	2156	1714	1529	2590	2629
4 Glu	414	299	320	467	507
5 Pro	1132	1705	1523	+	+
6 Gly	467	343	281	145	34
7 Ala	5756	2688	1361	551	988
8 Aaba	37	17	+	—	—
9 Val	889	821	665	95	102
10 Cys	33	+	+	—	+
11 Met	668	119	49	38	56
12 Ileu	282	225	171	105	108
13 Leu	491	206	179	140	143
14 Tyr	162	33	37	54	72
15 Phe	2875	1799	1502	448	406
16 $EtNH_2$	238	196	149	61	91
17 Gaba	318	150	88	91	159
18 Orn	+	+	+	+	+
19 Lys	663	864	779	366	345
20 Try	161	139	269	262	303
21 His	261	254	269	242	266
22 Arg	1073	1171	1074	820	760
Total	18717	13929	11591	7693	8328
NH_3	++	++	++	5112	1970

Abbreviations: MtSO — methionine sulphoxide, Asp — aspartic acid, T+S+A+G — threonine+serine+asparagine+glutamine, Glu — glutamic acid, Pro — proline, Gly — glycine, Ala — alanine, Aaba — α-aminobutyric acid, Val — valine, Cys — cystine, Met — methionine, Ileu — isoleucine, Leu — leucine, Tyr — tyrosine, Phe — phenylalanine, $EtNH_2$ — ethanolamine, Gaba — γ-aminobutyric acid, Orn — ornithine, Lys — lysine, Try — tryptophan, His — histidine, Arg — arginine, NH_3 — ammonia.

Methionine sulphoxide, ornithine and histidine concentrations remain at a constant level in the sap of the ovules examined.

The concentrations of threonine+serine+asparagine+glutamine (T+S+A+G), glutamic acid, γ-aminobutyric acid and tyrosine in the central vacuole sap (ovules 9–18 days old) show a small decrease and in still older ovules (28–33 days old) they increase again (Table 1).

Aspartic acid concentration reached a maximum value in ovules 9–18 days old and in still older ovules it remained constant, whereas the concentration of tryptophan showed a small increase in all examined ovules (Table 1).

The unidentified peaks on the diagrams obtained for the central vacuole sap analysed for free amino acids (ovules 9–33 days old) were found in the following positions: a) between proline and glycine — 2 peaks, b) between methionine and isoleucine — 2 peaks, c) between ammonia and lysine — 3 peaks and d) between histidine and arginine from 1 to 3 peaks. In all cases the peaks were very small.

Clivia miniata. During the inhibition phase of the proembryo growth (ovules 10–36 days old) 22 free amino acids and ammonia were found in the central vacuole

Table 2

Clivia miniata RGL. Course of concentration changes of free amino acids in central vacuole sap during the development of the ovule and proembryo. Age of ovule and preombyro in days counted from the day the perianth dropped to the day of analysis

Age of ovules days	10	17	26	36
Size of ovules mm	4,3×3,3	6,6×5,1	8,2×6,4	8,7×7,6
Size of embryos mm		0,12×0,10	0,15×0,13	0,23×0,23
No. Component	Concentrations nmole/ml sap			
1 MetSO	+	+	+	+
2 Asp	307	422	529	172
3 T+S+A+G	548	919	427	279
4 Glu	243	255	75	86
5 Pro	122	175	44	44
6 Gly	77	79	67	79
7 Ala	1542	1387	430	174
8 Aaba	19	—	—	—
9 Val	565	594	369	135
10 Cys	16	23	+	—
11 Met	10	—	+	—
12 Ileu	200	120	42	13
13 Leu	73	74	21	14
14 Tyr	409	51	19	17
15 Phe	75	66	46	32
16 $EtNH_2$	97	132	91	73
17 Gaba	167	149	95	105
18 Orn	37	+	34	47
19 Lys	32	+	20	20
20 Try	599	135	41	20
21 His	22	+	62	60
22 Arg	46	+	85	22
Total	5206	4581	2413	1392
NH_3	1132	4040	3878	2956

For abbreviations see Table 1.

sap (Table 2). This number increases to 25, if we assume that threonine, serine and amides are present in the central vacuole sap. All these amino acids with the exception of α-aminobutyric acid, cystine and methionine, occured in all the examined ovules (Table 2).

The total concentration of all free amino acids in the central vacuole sap (ovules 10–36 days old) decreases from 5206 to 1392 nmoles/ml sap (Table 2).

The concentrations of nine amino acids (alanine, α-aminobutyric acid, valine, isolencine, leucine, tyrosine, phenylalanine, γ-aminobutyric acid and tryptophan) decreased in the central vacuole in the ovules examined (Table 2).

Aspartic acid, threonine+serine+amides (determined together), proline and ethanolamine have their maximum concentrations in the sap of 17-26-day-old ovules. In older ovules their concentrations drop (Table 2).

The concentrations of glycine, ornithine and lysine were constant, whereas the concentrations of histidine and arginine were less regular and glutamic acid concentration was much lower in 26–36 days ovules than in young ones (10–17 days old; Table 2).

Methionine sulphoxide, cystine and methionine were present in the central vacuole sap in low concentrations. The concentrations of cystine and methionine changed irregularly in the sap.

On the diagrams representing analyses of the central vacuole sap for free amino acids the unidentified peaks were found between the following amino acids: a) proline and glycine —1 peak, b) methionine and isoleucine —1–2 peaks, c) between ammonia and ornithine —1 peak.

DISCUSSION

Analyses of the central vacuole sap (from ovules *H. Katharinae* and *Clivia miniata*) for free amino acids during the inhibition phase of proembryo growth were carried out. On the basis of the results obtained some similarities and differences could be established in the composition and changes of free amino acids in the central vacuole sap of both species.

Similarities

a) In the central vacuole sap of both species 22 identical free amino acids were found. Taking into account that T+S+A+G (determined together) is represented in the sap by 4 amino acids (Tulecke et al. 1961; Baptist 1963; Grzesiuk et al. 1962; Jennings and Morton 1963), the number of free amino acids increases to 25.

b) The total concentration of free amino acids decreases in the central vacuole sap in the examined ovules (Tables 1 and 2).

c) Analogical concentration changes in the central vacuole sap during the development of the ovule have been found in the following amino acids: methionine sulphoxide, proline, alanine, valine, cystine, isoleucine, leucine, phenylalanine, γ-aminobutyric acid, ornithine and probably α-aminobutyric acid, methionine and histidine.

d) Aspartic acid concentration is higher as compared with the concentration of glutamic acid in all examined ovules.

Differences

a) The nine amino acids (aspartic acid, T+S+A+G, glutamic acid, glycine, tyrosine, ethanolamine, lysine, tryptophan and arginine) are characterized by different concentration changes in the central vacuole sap in both species.

b) The total concentration of free amino acids in the sap from ovules of *H. Katharinae* is much higher as compared with the concentration determined in the central vacuole sap from ovules of *Clivia miniata*. This difference in concentration is particularly wide between both species as regards methionine.

c) The central vacuole sap in ovules of *H. Katharinae* contains a greater number (8–10) of unidentified compounds (amino acids) than the sap in ovules of *Clivia miniata* (3–4 compounds). All these compounds are represented in diagrams by small peaks.

Threonine, serine and amides have been found in coconut water (Tulecke et al. 1961; Baptist 1963), seeds of *Vicia faba* (Grzesiuk et al. 1962) and in the endosperm of *Triticum* (Jennings and Morton 1963). Thus, it has been assumed that these amino acids and amides (determined together) are present in the central vacuole sap in ovules of both investigated species.

Basing upon the results of Oreskes et al., 1965; 1967 the author assumed that in the total concentration (T+S+A+G), glutamine may constitute (after chromatographic separation) 30% of its real initial value in the sap. During chromatographic separation glutamine undergoes transformation into pyrrolidine carboxylic acid and in a very small degree (1,8%) into glutamic acid, therefore the glutamic acid concentrations determined represent real values.

The concentrations of asparagine and aspartic acid owing to their high stability (Vickery et al. 1935) are real values occurring in the central vacuole sap in both species.

The different course of concentration changes of particular amino acids in the central vacuole sap in ovules of one species have been discussed in the previous paper (Ryczkowski 1971). When considering the results for two different species another factor should be taken into account, i.e. the specificity of the species.

The drop of the total concentration of free amino acids in the central vacuole sap in ovules of *H. Katharinae* (with the exception of 9 day-old ovules) is in agreement with the decrease of the osmotic value in it (Ryczkowski 1960 a, b).

In ovules of *Clivia miniata* the decrease of total free amino acids concentration in the sap is shifted forward in relation to the drop of the osmotic value of this sap and sugar concentration in it (ovules of *Clivia* sp.; Ryczkowski 1960 b, 1962 b). This shift is probably connected partly with the fact that the *Clivia sp.* taken for experiments fruited in March — a long period of the ovule development as compared with the period of development of the ovule of *Clivia miniata* which flowered and fruited in summer.

It could be presumed that maximum total concentration of free amino acids in the central vacuole sap appears (in both species) before the maximum of its osmotic value and highest concentration of sugars in it (McKee et al. 1955). This shift would be in contrast with analogical results obtained for the central vacuole sap in ovules of dicotyledonous plants (Ryczkowski 1964).

The drop of total concentration in the central vacuole sap (ovules of *H. Katharinae* and *Clivia miniata*) is in agreement with the decrease of amino nitrogen in it (unpublished data) and is most probably connected with the uptake of free amino acids by the intensively developing endosperm tissue.

The increase of concentrations of some free amino acids in the sap (ovules of *H. Katharinae*, 33 days old) may be a result of injury to the endosperm tissue and outflow of these amino acids into the central vacuole sap.

The unidentified peaks occurring in the diagrams obtained as the result of analyses of the central vacuole sap for free amino acids suggests that the environment of the proembryo contains more than 25 free amino acids.

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SUMMARY

Analyses of the central vacuole sap were carried out in ovules of *H. Katharinae* and *Clivia miniata* (during the inhibition phase of the proembryo growth) for free amino acids.

The free amino acids were determined by means of the Micro Column Amino Acid Analyser (Technicon Instruments Co. Ltd. Chertsey, Surrey, England). The accuracy of this method is within ±2,75%.

The central vacuole sap in ovules of both species contains 22 identical free amino acids. If we assume that T+S+A+G (threonine+serine+asparagine+glutamine — determined together) is represented by 4 amino acids, the number of identified free amino acids increases to 25.

The total conentration of free amino acids in the central vacuole sap decreases in the ovules of both species. Some similarities and differences have been established for both species as regards the change of total concentration and concentration changes of particular amino acids.

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Wolne aminokwasy w środowisku prozarodka podczas fazy zahamowania jego wzrostu (rośliny jednoliścienne)

Streszczenie

Wykonano analizy soku centralnej wakuoli zalążków *H. Katharinae* i *Clivia miniata* (podczas fazy zahamowania wzrostu prozarodka) na zawartość wolnych aminokwasów.

Wolne aminokwasy oznaczono z pomocą mikrokolumnowego analizatora aminokwasów (Technicon Instrumenst Co. Ltd. Chertsey, Surrey, England). Dokładność stosowanej metody $\pm 2{,}75\%$.

Sok centralnej wakuoli u obu gatunków zawierał po 22 wolne iedntyczne aminokwasy. Ponieważ przyjęto, że T+S+A+G (treonina+seryna+asparagina+glutamina — oznaczane sumarycznie) są reprezentowane przez 4 wymienione związki, liczba wolnych zidentyfikowanych aminokwasów w soku centralnej wakuoli (u każdego gatunku) wzrasta do 25.

Całkowite stężenie wolnych aminokwasów w soku centralnej wakuoli obniża się u przebadanych zalążków obu gatunków. Ustalono pewne podobieństwa i różnice (dla obu gatunków) zarówno w odniesieniu do zmiany stężenia globalnego wolnych aminokwasów, jak też w stosunku do zmian stężeń poszczególnych aminokwasów w soku centralnej wakuoli podczas fazy zahamowania wzrostu prozarodka.