ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXIX — nr 3
1970

Free amino acids in leaves and inflorescences of 34 grass species

T. DĄBROWSKA, J. PRZYBYLSKA

INTRODUCTION

From the standpoint of free amino acid composition grasses have so far been relatively little examined. Former studies, before application of paper chromatography as an analytical procedure, indicated an important role of glutamine in nitrogen metabolism of grasses, yet they did not provide a full characteristic of the amino acid composition (Chibnall, 1952). More recent studies are in general concerned with particular non-protein amino acids and with amino acid derivatives (Fowden, 1958; Synge, 1951; Synge and Wood, 1958).

Our preliminary investigations on free amino acid composition in grasses covered several species of *Festuceae* tribe. Apart from a high level of glutamine within all the examined organs, a high proportion of proline in the pool of free amino acids of inflorescences has proved to be the characteristic feature of the tested species (Rymowicz-Dąbrowska and Przybylska, in print). The purpose of the present studies, covering 34 species of 7 tribes, is to provide a more general survey of free amino acids in grasses. The analyses cover leaves and inflorescences — organs which were available and seemed to be especially interesting from the point of view of free amino acid composition.

MATERIALS AND METHODS

The plant material originated from the collection of the Poznań Botanical Garden; and from the collection of the Institute of Soil Science and Cultivation of Plants, Przebędowo near Poznań. The object of studies consisted of leaves and inflorescences of 34 species of grasses representing the following tribes *: *Paniceae, Phalarideae, Agrostideae, Aveneae, Chlorideae, Festuceae* and *Hordeae* (see: Table 1). Samples for analyses were collected at time of pollen shedding. Moreover, in case of *Festuca arundinacea* and *F. pratensis* there were also analysed spikelets, mature an-

* After Hegi (1964).

thers (yellow) and spikelets without anthers, collected one day before pollen shedding. All samples were collected in the year 1968.

For all the analysed samples there has been carried out a qualitative analysis and rough estimation of free amino acids; the content of free proline was evaluated semi-quantitatively. In the case of spikelets, anthers, and spikelets without anthers, the content of free proline was determined quantitatively.

The extraction of plant material was performed as reported previously (Przybylska and Rymowicz, 1965). The analysis of free amino acids was carried out by two-dimensional paper chromatography and paper electrophoresis, as described in the former papers (Przybylska, 1964; Przybylska and Rymowicz, 1965).

Semi-quantitative estimate of free proline was based on a visual comparison of the colour intensity of the respective bands on circular chromatograms prepared as described in the previous paper (Przybylska, Kociałkowski and Wiewiórowski, 1958). Beside plant extracts there were applied on chromatograms different amounts of proline, ranging from 2 to 14 μg (six levels). The chromatograms were run in n-butanol: acetic acid: water (4 : 1 : 5, by volume, upper layer; Partridge, 1948) and developed in 0.2% solution of isatin in acetone.

Quantitative determination of proline was carried out by the colorimetric method after Kruze and Iwańska (1965), the above mentioned solvent being used in chromatography.

RESULTS

A. Composition of free amino acids in leaves and inflorescences

Both in leaves and in inflorescences of all the examined species there has been found a high concentration of glutamic acid with glutamine and of alanine. The level of aspartic acid with asparagine and that of serine was in general lower. Phenylalanine, tyrosine, isoleucine, leucine, glycine, methionine and/or valine, threonine, histidine, lysine and arginine occurred in small or trace amounts.

Differences in the composition of free amino acids in leaves and inflorescences concern chiefly the relative level of proline and of γ-aminobutyric acid. In inflorescences of 28 species proline was one of the main free amino acids, while in leaves it occurred as a rule in small or trace amounts. On the contrary, γ-aminobutyric acid showed in general a high proportion in the free amino acid pool of leaves, being only a minor component in inflorescences.

It should be noted that very high levels of asparagine, or of serine, or of both these amino acids, have been observed in inflorescences of several species.

As regards non-protein amino acids — apart from the above mentioned γ-aminobutyric acid — leaves and inflorescences of all the tested species have been found to contain β-alanine. α-Aminobutyric acid was present in leaves of twelve species, namely: *Phalaris arundinacea, Phleum boehmeri, Agrostis alba, A. vulgaris, Calamagrostis tenella, Brachypodium phoenicoides, Briza media, Festuca rubra, Poa pratensis, Agropyron elongatum, A. repens* and *Elymus condensatus*. Leaves of *Brachypodium phoenicoides, Festuca rubra* and *Poa pratensis* contained α-aminoadipic acid, while leaves of *Secale montanum* and inflorescences of *Melica altissima* — pipecolic acid. All these amino acids — except γ-aminobutyric acid — occurred in small amounts.

It must be mentioned that extracts of leaves and inflorescences of all the analysed species contained several unidentified ninhydrin-positive compounds. As judged by the colour intensity of the respective spots on the paper chromatograms, two of these compounds occurred in high concentration.

B. Composition of free amino acids in spikelets, anthers and spikelets without anthers

As revealed for *Festuca arundinacea* and *F. pratensis*, the relative level of free proline in anthers is very high. In spikelets without anthers the proportion of proline in free amino acid pool was much lower. As regards the concentration of other free amino acids, the observed differences concerned merely asparagine and glutamine; in the anthers an exceptionally high level of asparagine, while in spikelets without anthers a high concentration of glutamine is to be stressed. The composition of free amino acids within entire spikelets was similar to that within inflorescences.

C. Content of free proline in leaves, inflorescences and spikelets

As revealed by semi-quantitative analysis, the content of free proline on a dry matter basis is in general much higher in inflorescences than in leaves. Only in the case of a few species both types of organs show the same proline level (Table 1).

Quantitative determination of free proline in spikelets, anthers and spikelets without anthers, has shown the percentage content of proline on a dry matter basis to be much higher in anthers than in spikelets without anthers; the level of proline within entire spikelets is intermediate. Percentage contents of free proline on a dry matter basis found for spikelets, anthers and spikelets without anthers in *Festuca arundinacea* worked out as follows: 0.42, 0.85 and 0.16. The corresponding values obtained for *F. pratensis* were: 1.3, 2.4 and 0.2, respectively.

Table 1

Semi-quantitative estimation of the content of free proline in leaves and inflorescences of different grass species. Details in the text

Tribe	Species	Free proline (dry wt. %)	
		leaves	inflorescences
Paniceae	Penisetum japonicum	—	0.4
Phalarideae	Phalaris arundinacea	< 0.1	0.2
	Phalaris tuberosa	0.1	0.3
Agrostideae	Phleum boehmeri	< 0.1	0.1
	Phleum pratense	< 0.1	0.2
	Agrostis alba	0.2	0.2
	Agrostis vulgaris	< 0.1	0.5
	Calamagrostis epigeios	—	0.4
	Calamagrostis tenella	< 0.1	< 0.1
	Calamagrostis varia	—	0.2
	Apera spica-venti	0.1	0.4
Chlorideae	Becmania eruciformis	< 0.1	0.7
Aveneae	Deschampsia caespitosa	< 0.1	0.5
Festuceae	Brachypodium phoenicoides	< 0.1	0.2
	Briza media	0.3	0.8
	Dactylis aschersoniana	0.2	0.5
	Festuca arundinacea	< 0.1	0.3
	Festuca gigantea	< 0.1	0.2
	Festuca pratensis	0.1	0.2
	Festuca rubra	< 0.1	< 0.1
	Festuca tatrae	< 0.1	< 0.1
	Koeleria gracilis	< 0.1	0.1
	Melica altissima	< 0.1	0.1
	Poa pratensis	< 0.1	0.2
Hordeae	Agropyron cristatum	< 0.1	0.2
	Agropyron elongatum	< 0.1	0.3
	Agropyron intermedium	—	< 0.1
	Agropyron junceum	< 0.1	0.2
	Agropyron pectiniforme	< 0.1	0.3
	Agropyron repens	< 0.1	0.3
	Elymus canadensis	< 0.1	0.3
	Elymus condensatus	< 0.1	0.1
	Secale montanum	< 0.1	0.1
	Secale vavilovi	< 0.1	0.4

— unfound

DISCUSSION

The above presented studies indicate that the high level of free proline found in inflorescences of a vast majority of the examined species is due to a very large proportion of this compound in anthers. This is consistent with a well known fact of accumulating of free proline in pollen grains. It is supposed that proline is transported from the vegetative organs to pollen grains where it is utilized during processes of pollination, fertilization, and perhaps even of embryogenesis (Auclair and Jamieson, 1948; Bathurst, 1954; Bellartz, 1956, Britikov *et al.*, 1964; Tupy, 1963, 1964). In grasses which develop particularly high quantities of pollen, a high level of free proline in inflorescences is striking; yet it should be reminded that flowers of legumes are likewise characterized by a relatively high proportion of free proline (Przybylska and Rymowicz-Dąbrowska, in print I, II; Simola, 1968). The latter might be due to a high concentration of proline in pollen grains; yet it cannot be excluded that during the transport of proline towards pollen grains the compound accumulates temporarily in other parts of flower.

It is hard to explain the high level of γ-aminobutyric acid in leaves of the tested grass species. It may result from an increased activity of glutamic acid decarboxylase which was observed in aging leaves of various plant species (Khavkin, 1964; Weinberger and Clendenning, 1952; Weinberger and Godin, 1964). It should, however, be emphasized that γ-aminobutyric acid — widely distributed in plants — does not always arise as the product of glutamic acid decarboxylation; formation of γ-aminobutyric acid, proceeding through transamination of succinic acid semialdehyde has been demonstrated with the aid of enzyme preparations (Dixon and Fowden, 1961; Kretovich *et al.*, 1967). Moreover, it needs to be pointed out that considerable amounts of γ-aminobutyric acid have been found in organs, or within tissues, with a high metabolic rate (Thompson *et al.*, 1953; Przybylska and Rymowicz-Dąbrowska, in print: II) and this in itself might suggest an important role of this compound in nitrogen metabolism.

As regards the occurrence of non-protein amino acids, there have not been observed any characteristic differences between species. All the non-protein amino acids noticed in the analysed grass species are widely distributed in plants. Regarding several unidentified ninhydrin-positive compounds, they were observed in all the analysed species and therefore also cannot serve as a criterion of taxonomical significance.

There should be emphasized the difference between *Gramineae* and *Papilionaceae*. The latter are well known to synthesize a great number of unusual amino acids which are characteristic for tribes, genera or even groups of species within a genus (Przybylska, 1963, 1965). It is possible, that in the course of evolution *Papilionaceae* have developed different enzymatic systems responsible for the biosynthesis of uncommon amino acids. The possibility of the occurrence in plants of very small amounts of so called „unusual" amino acids, which may be easily overlooked, must however be taken into regard. In such case the difference between *Gramineae* and *Papilionaceae* would not be a matter of synthetic capability, but of metabolic blocks resulting in accumulation of particular non-protein amino acids.

SUMMARY

Free amino acid composition of leaves and inflorescences of 34 grass species, collected at time of pollen shedding, has been determined with the use of paper chromatography and paper electrophoresis. The studies have led to the following conclusions:

1. Free amino acid composition of leaves is characterized by a high level of γ-aminobutyric acid.

2. Free amino acid composition of inflorescences is characterized by a high proportion of proline, which is due to the accumulation of proline in anthers.

3. No unusual characteristic amino acids have been found in the analysed species. Non-protein amino acids observed in the examined material, namely γ-aminobutyric acid, β-alanine, α-aminobutyric acid, α-aminoadipic acid and pipecolic acid, are widely distributed in plants.

Acknowledgements.

The authors are indebted to Dr S. Sulinowski of the Experimental Station of the Institute of Soil Science and Cultivation of Plants, Przebędowo near Poznań, for the valuable information concerning developmental biology of grasses, as well as for providing the plant material. Skilfull technical assistance of B. Czerwińska, Sc. B. also requires to be acknowledged.

This work was supported in part by a research grant from the U.S. Department of Agriculture.

Polish Academy of Sciences, Poznań
Institute of Plant Genetics

(Entered: January 26, 1970)

Wolne aminokwasy w liściach i kwiatostanach 34 gatunków traw

Streszczenie

Przy pomocy chromatografii i elektroforezy bibułowej określono skład wolnych aminokwasów liści i kwiatostanów 34 gatunków traw, pobranych w okresie pylenia roślin. Wyniki badań prowadzą do następujących wniosków:

1. Skład wolnych aminokwasów liści charakteryzuje się wysokim poziomem kwasu γ-aminomasłowego.

2. Skład wolnych aminokwasów kwiatostanów charakteryzuje się dużym udziałem proliny; wysokie stężenie tego związku wynika z nagromadzania się proliny w pylnikach.

3. U analizowanych gatunków nie wykryto rzadko spotykanych, specyficznych aminokwasów. Niebiałkowe aminokwasy, obserwowane w badanym materiale, mianowicie: kwas γ-aminomasłowy, β-alanina, kwas α-aminomasłowy, kwas α-aminoadypinowy oraz kwas pipekolinowy, są szeroko rozpowszechnione w roślinach.

Zakład Genetyki Roślin PAN
w Poznaniu

REFERENCES

Auclair J. L. and Jamieson C. A., 1948, A qualitative analysis of amino acids in pollen collected by bees, Science 108: 357—358.

Bathurst N. O., 1954, The amino-acids of grass pollen, J. Exp. Bot. 5: 253—256.

Bellartz S., 1956, Das Pollenschlauchwachstum nach arteigener und artfremder Bestäubung einiger Solanaceen und die Inhaltsstoffe ihres Pollens und ihrer Griffel, Planta 47: 588—612.

Britikov E. A., Musatova N. A., Vladimirtseva S. V. and Protsenko M. A., 1964, Proline in the reproductive system of plants. [In:] Linskens H. F. (ed.). Pollen physiology and fertilization. North Holland Publishing Co., Amsterdam, pp. 77—85.

Chibnall A. C., 1952, Metabolizm białek w roślinie, P.W.R.i L., Warszawa, pp. 160—161.

Dixon R. O. D. and Fowden L., 1961, γ-Aminobutyric acid metabolism in plants, part 2. Metabolism in higher plants, Ann. Bot. 25: 513—530.

Fowden L., 1958, δ-Acetylornithine: a constituent of some common grasses, Nature 182: 406—407.

Hegi G., 1964, Illustrierte Flora von Mittel-Europa, Carl Hanser (Verlag) München, Bd. I, 234—520.

Khavkin E. E., 1964, Age-dependent changes in the content of free amino acids in bean leaves and the accumulation of γ-aminobutyric acid, Fizioł. Rastienij 11: 862—866.

Kretovich W. L., Kariakina T. I. and Luibimova N. V., 1967, Metabolism of succinic semialdehyde and γ-aminobutyric acid in plants, Fizioł. Rastenij 14: 919—925.

Kruze D. and Iwańska J., 1965, Ilościowe oznaczanie proliny, I. Warunki oznaczania, Chemia Analityczna 10:237—241.

Natrova Z., 1968, Quantitative analysis of free amino acids and carbohydrates in spring barley anthers at different stages of maturity, Biol. Plant. 10: 118—125.

Partridge S. M., 1948, Filter-paper partition chromatography of sugars, Biochem. J. 42: 238.

Przybylska J., 1963, Wolne aminokwasy, ich rola w metabolizmie i znaczenie w taksonomii roślin, Post. Biochem. 9: 105—127.

Przybylska J., 1964, The role of tingitanine in nitrogen metabolism of Lathyrus tingitanus, Acta Soc. Bot. Polon. 33: 211—241.

Przybylska J., 1965, Niebiałkowe aminokwasy w pokrewnych rodzajach *Lathyrus* i *Vicia* oraz ich działanie na organizm zwierzęcy, Post. Nauk. Roln. 3(93): 51—66.

Przybylska J., Kociałkowski Z. and Wiewiórowski M., 1958, Przyczynek do metodyki chromatograficznego oznaczania aminokwasów na bibule, Roczn. Nauk Roln. 79-A-1:1—17.

Przybylska J. and Rymowicz T., 1965, Free amino acids in different organs of 16 *Lathyrus* species, Genetica Polonica 6: 91—124.

Przybylska J. and Rymowicz-Dąbrowska T., Wolne aminokwasy w roślinach pastewnych. I. Wolne aminokwasy w różnych organach niektórych gatunków jednorocznych roślin motylkowych, Roczn. Nauk Roln. (in print).

Przybylska J. and Rymowicz-Dąbrowska T., Wolne aminokwasy w roślinach pastewnych. II. Wolne aminokwasy w różnych organach niektórych gatunków wieloletnich roślin motylkowych, Roczn. Nauk Roln. (in print).

Rymowicz-Dąbrowska T. and Przybylska J., Wolne aminokwasy w roślinach pastewnych. III. Wolne aminokwasy w różnych organach niektórych gatunków traw, Roczn. Nauk Roln. (in print).

Simola L. K., 1968, Comparative studies on the amino acid pools of three *Lathyrus* species, Acta bot. fenn. 81: 1—62 (separatum).

Synge R. L. M., 1951, Methods for isolation ω-amino-acids: γ-aminobutyric acid from ryegrass, Biochem. J. 48: 429—435.

Synge R. L. M. and Wood J. C., 1958, Bound amino acids in protein-free extracts of Italian ryegrass, Biochem. J. 70: 321—339.

Thompson J. F., Pollard J. K. and Steward F. C., 1953, Investigations of nitrogen compounds and nitrogen metabolism in plants. III. γ-Aminobutyric acid in plants, with special reference to potato tuber and a new procedure for isolating amino acids other than α-amino acids, Plant Physiol. 28: 401—414.

Tupy J., 1963, Free amino acids in apple pollen from the point of view of its fertility, Biol. Plant. 5: 154—160.

Tupy J., 1964, Metabolism of proline in styles and pollen tubes of Nicotiana alata. [In:] Linskens H. F. (ed.). Pollen physiology and fertilization, North Holland Publishing Co., Amsterdam, pp 86—94.

Weinberger P. and Clendenning K. A., 1952, Glutamic carboxylase of the mature wheat leaf, Can. J. Bot. 30: 755—763.

Weinberger P. and Godin C., 1964, Glutamic decarboxylase in relation to leaf ontogeny and vernalization, Can. J. Bot. 42: 329—332.