

Free amino acids in the panicles of *Dactylis glomerata* in the course of their development

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(Received: June 13, 1973.)

Abstract

The composition of free amino acids and proline content in various parts of *Dactylis glomerata* panicles at different stages of their development were determined by paper chromatography and electrophoresis. The proline level in the spikelets was found to change widely in the course of their development. The results are discussed with reference to the role of proline in the nitrogen metabolism of plants.

INTRODUCTION

Earlier studies on the free amino acid composition in various grass species (Rymowicz-Dąbrowska and Przybylska, 1970; Dąbrowska and Przybylska, 1970) demonstrated an unusually high free proline level in the inflorescences at the stage of pollen emission. As shown (Dąbrowska and Przybylska, 1970), the high proline level in the inflorescences of the grass species examined is due to a high concentration of this amino acid in the anthers. This agrees with the known fact of accumulation of free proline reserves in pollen grains.

The present investigations were undertaken in order to establish how the proline contribution to the free amino acid pool changes in various parts of the panicle in the course of its development. For these studies the species *Dactylis glomerata*, belonging to the tribe *Festuceae* was chosen.

MATERIALS AND METHODS

The object of investigations were plants growing in field conditions. The panicle branches and spikelets were analysed in the following phases of panicle development: beginning of heading, end of heading, open panicle, one day before pollen emission, milk ripeness, wax ripeness. Besides, immediately before pollen emission mature anthers and spikelets deprived of them were analysed separately. Anthers in the period of pollination (anthers after dehiscence) and spikelets in the phase of full ripeness were also analysed.

Samples for analysis were collected in 1969 — in the fourth year development of the plants.

In all samples the composition of free amino acids and free proline content were determined, and so was total nitrogen and α -amino nitrogen content.

Total nitrogen was determined in dry plant material by Kjeldahl's method in the modification of Perrin (1953).

Soluble nitrogen compounds from the plant material were extracted as described by Przybylska and Rymowicz (1965). α -Amino nitrogen was determined in plant extracts by the method of Pope-Stephen's in Albanese and Irby modification (1944).

Analysis of free amino acids was performed by means of two-dimensional chromatography and paper electrophoresis as described earlier (Przybylska, 1964; Przybylska and Rymowicz, 1965).

Free proline was determined quantitatively by the colorimetric method of Kruze and Iwańska (1965) after previous separation of the amino acid mixture by unidimensional paper chromatography in Partridge's solvent (1948).

RESULTS

A. Free amino acids various parts of the panicle

The level of free amino acid in the panicle branches and spikelets of *Dactylis glomerata* shows large differences depending on the phase of panicle development. This is manifested by changes of the α -amino nitrogen level in the examined organs in the course of development of the panicles.

In the panicle branches the contribution of α -amino nitrogen to total nitrogen is highest at the beginning of heading and at the stage of open panicle (Table 1). In the spikelets the contribution of α -amino nitrogen to total nitrogen increases gradually in the course

Table 2

Dry matter, total nitrogen, α -amino nitrogen and proline nitrogen contents in spikelets *Dactylis glomerata* at various phases of panicle development (1969)

Phase of development	Dry matter %	Total N in dry matter %	Proline in dry matter %	α -Amino N as percentage of		Proline N as percentage of	
				dry matter	total N	dry matter	total N
beginning of earing 20.V.	35.5	2.36	0.03	0.23	9.75	0.006	0.25
end of earing 26.V.	37.5	2.52	0.08	0.25	9.90	0.017	0.67
open panicle 30.V.	38.4	2.75	0.11	0.30	10.90	0.02	0.73
1 day before pollination 10.VI.	43.6	3.14	1.19	0.56	17.7	0.22	7.0
milk ripeness 18.VI.	50.8	2.98	0.16	0.25	9.7	0.029	0.97
wax ripeness 23.VI.	51.9	3.07	0.04	0.20	9.1	0.007	0.23
full ripeness 3.VII.	61.4	3.37	0.03	0.30	8.9	0.006	0.18
							2.0

of development of the plants, reaching its maximal value directly before pollination; in later phases of the development the contribution of α -amino nitrogen in the spikelets decreases (Table 2).

As shown by chromatographic analysis, several amino acids are mainly responsible for the above described changes in α -amino nitrogen. Data concerning the contribution of the particular amino acids to the total pool of free amino acids in the organs examined in the course of development of the inflorescence are given below.

Panicle branches. The main amino acids — independent the phase of development of the panicle — proved to be glutamic acid with glutamine and alanine. In all phases, with the exception of the wax ripeness stage, aspartic acid with asparagine was found in high concentrations. The composition of free amino acids in branches taken from the panicles for analysis 7 days after pollination of the plants exhibited a relatively high proline level. This compound was one of the main free amino acids.

Spikelets. The free amino acid composition of the spikelets changed analogously to that in panicle branches. The differences concerned primarily the relative proline and arginine levels. Maximal proline contribution to the pool of free amino acids in the spikelets was observed directly before pollination (one day). It should be stressed that the highest α -amino nitrogen level is found at this stage. In the end phase of panicle development a relatively high arginine concentration was noted in the spikelets. This amino acid occurred in the panicle branches and pedicels in small or trace quantities.

In order to establish which part of the spikelet is responsible for proline accumulation, free amino acids were analysed, separately, in the anthers and in spikelets with anthers removed directly before pollination (one day).

It has been demonstrated that the relative free proline level in the anthers was several times higher than in spikelets deprived of anthers. As regards the concentrations of other free amino acids, the differences observed involved only aspartic acid with asparagine and glutamic acid with glutamine; in the anthers a particularly high concentration of aspartic acid with asparagine and in the spikelets without anthers of glutamic acid with glutamine was observed.

Analysis of free amino acids demonstrated a markedly lower proline level in the anthers after dehiscence as compared with that in anthers analysed one day before pollination. A characteristic feature of anthers after dehiscence was a very high aspartic acid and asparagine level.

B. Free proline content in various parts of the panicle

The characteristic changes in proline level in the panicle branches and spikelets observed in chromatographic analysis were confirmed by the quantitative results.

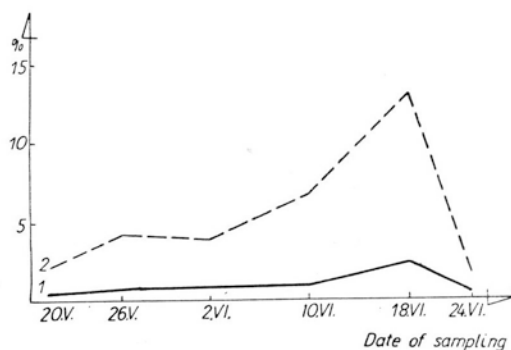


Diagram 1. Changes in levels of proline nitrogen in branches of *Dactylis glomerata* during the panicle development

1 — proline-N in per cent of total nitrogen; 2 — proline-N in per cent of α -amino nitrogen

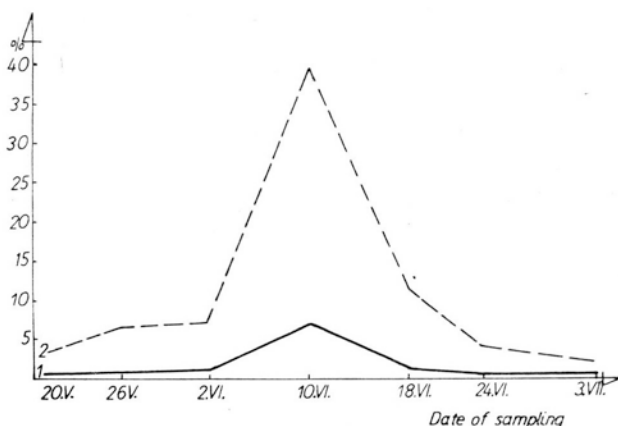


Diagram 2. Changes in levels of proline nitrogen in spikelets of *Dactylis glomerata* during the panicle development

1 — proline-N in per cent of total nitrogen; 2 — proline-N in per cent of α -amino nitrogen

The amount of proline in dry matter and the contribution of proline nitrogen to total nitrogen and α -amino nitrogen increases in the panicle branches to the milk ripeness stage and then falls drastically (Diagram 1, Table 1). In the spikelets the high proline concentration and its dominant contribution to α -amino nitrogen falls to the period immediately preceding pollination (Diagram 2, Table 2).

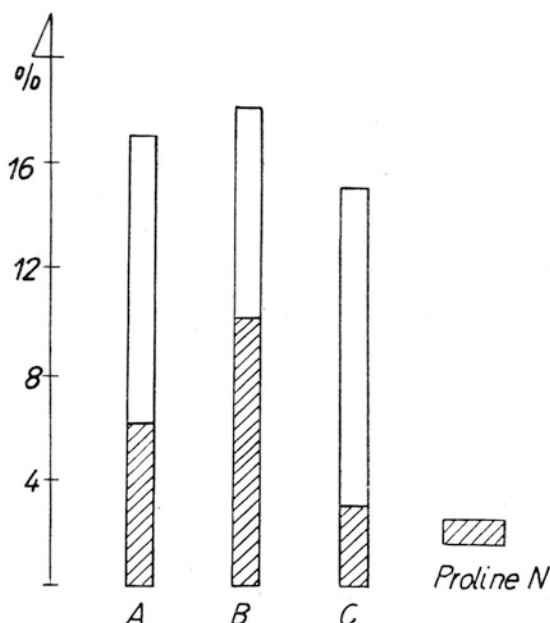


Diagram 3. Contents of α -amino nitrogen and proline nitrogen in spikelets, anthers and spikelets without anthers one day before pollination (in per cent of total nitrogen)

A — spikelets; B — anthers; C — spikelets without anthers

Quantitative analysis of proline in the spikelets, mature anthers and spikelets deprived of anthers, collected just before pollination demonstrated a very high proline level in the anthers. Proline nitrogen constituted in the anthers more than 53 per cent of α -amino nitrogen (Diagram 3).

DISCUSSION

The above described investigations demonstrated that the dynamic rise of free proline level in the spikelets of *Dactylis glomerata* observed before pollination is connected with the accumulation of this imino acid in the anthers. An unusually high proline level in mature anthers of some grass species has also been reported in our earlier studies (Dąbrowska and Przybylska, 1970) and in those of other authors (Natrova, 1968, Petrovskaya and Tsinger, 1961). As demonstrated by Natrova (1968) in mature anthers of spring barley the content of free proline may exceed 70 per cent of the sum of free amino acids. These data also agree with the well known fact of the free proline accumulation in pollen grains of various plant species (Auclair and Jamieson, 1948; Bathurst, 1954;

Bellartz, 1956; Britikov and Musatova, 1964; Britikov, Musatova, Vladimirtseva and Protsenko, 1964; Cełujko, 1968; Linskens and Tupy, 1966; Tupy, 1963; 1964; Virtanen and Kari, 1955).

It is at present suggested that proline is transported to the pollen grains from the vegetative organs (Britikov and Musatova, 1964; Britikov, Vladimirtseva and Musatova, 1965; Linskens and Schrauwen, 1969) and utilized in the processes of pollination and fertilization.

If the composition of free amino acids in mature anthers of *Dactylis glomerata* before pollination is compared with that after dehiscence, it appears that much less free proline and several times more free aspartic acid with asparagine is contained in the anthers after dehiscence than before. These differences are in the first place the result of loss of pollen by the anthers, this confirming additionally that proline is accumulated mainly in pollen grains. Moreover, these differences may be also associated with the loss of fertility of the pollen which remains in the anthers. A number of authors observed quantitative changes in the composition of free amino acids in pollen which has lost or is losing its fertility. In general, in such pollen the proline and alanine level is reduced, while the relative free aspartic acid level with asparagine increases (Diakonu, 1961; Fukasava, 1954, 1959; Khoo and Stinson, 1957).

The relatively high free proline level in the branches and spikelets of *Dactylis glomerata* at the stage of milk ripeness indicates that this imino acid plays a rather important role also in the transport of nitrogen to the developing caryopses. The absence of proline in ripe caryopses shows in turn that this compound is transported to them for metabolic purposes and not as a compound storing nitrogen.

It should be stressed that the data obtained in this Laboratory for a number of leguminous plants also indicate to the important role of proline in the nitrogen metabolism of developing seeds (Przybylska and Rymowicz, 1965; Przybylska and Rymowicz-Dąbrowska, 1970). In the literature, numerous papers report high free proline concentrations in fast-growing and regenerating tissues (Barnard and Oaks, 1970; Breyhan, Heilinger and Fischnich, 1959); Duranton and Maille, 1962; Durzan and Steward, 1963; Raveux, Bové and Bové, 1957; Shvedskaya and Kruzhlina, 1966; Simola, 1968). This is evidence of the high physiological activity of this imino acid and of the possibility of its multi-purpose utilization in metabolic processes in plants. According to some authors, proline is utilized not only as a component of structural and enzymatic proteins, but also as a valuable

source of nitrogen and energy in various metabolic processes (Britikov, Musatova and Vladimirtseva, 1966; Britikov, Vladimirtseva and Musatova, 1965; Britikov, Schrauwen and Linskens, 1970; Britikov and Linskens, 1970). It is also supposed that proline is used in chlorophyll synthesis (Britikov, Schrauwen and Linskens, 1970; Duranton and Maille, 1962; Perdrizet, Macquaire, 1963).

I wish to express my cordial thanks to Docent dr J. Przybylska for her valuable guidance in the course of this work.

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Wolne aminokwasy w wiechach Dactylis glomerata w trakcie ich rozwoju

Streszczenie

Przeprowadzono szczegółową analizę składu wolnych aminokwasów w różnych częściach wiech zebranych w różnych fazach ich rozwoju.

Z uzyskanych danych na podkreślenie zasługują zmiany poziomu wolnej proliny w kłoskach kupkówki. Poziom proliny w kłoskach dynamicznie wzrasta w trakcie rozwoju kwiatostanu, osiągając najwyższą wartość bezpośrednio przed pyleniem. Zjawisko to związane jest z nagromadzeniem się znacznych ilości proliny w pylnikach, co zgodne jest z dobrze znanym faktem kumulowania znacznych rezerw wolnej proliny w ziarnach pyłku różnych gatunków roślin.

Wysoki stosunkowo poziom wolnej proliny w gałązkach wiech oraz w kłoskach w fazie dojrzałości mleczej oraz brak tego iminokwasu w dojrzałych ziarniakach wskazuje na udział proliny w doprowadzaniu azotu do rozwijających się ziarniaków dla celów metabolicznych.

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