ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 49, nr 1/2; 85—90
1980

Comparative studies on the proteins, carbohydrates, acid phosphatase and ribonuclease in caryopses of two orchardgrass (*Dactylis glomerata* L.) varieties *

ELEONORA WIECZOREK, ANNA BIJAK, BRONISŁAWA MORAWIECKA

Department of Molecular Biochemistry, Institute of Biochemistry, University of Wrocław, ul. Tamka 2, 50-137 Wrocław, Poland

(Received: June 15, 1979)

Abstract

Comparative studies were carried out on some biochemical components in caryopses of two varieties of *Dactylis glomerata*. The determined protein content depended upon the solvent and amounted to 0.22-0.39 g, with sugar amounting to 2.7-3.6 g and pentoses to 0.30-0.67 g per 100 g of caryopses. The level of pentoses, acid phosphatase and ribonuclease activity was about twice higher in the caryopses of the 'Motycka' variety than that in 'Nakielska'. Similar differences in the two varieties were found in the enzyme activity in the seed and chaff of the *Dactylis glomerata* caryopses.

INTRODUCTION

Previous studies showed that the content of proteins and carbohydrates, as also the level of the activity of some enzymes in rye caryopses depend upon the variety and on soil-climatic conditions (Morawiecka et al., 1976). Of the numerous grass species, *Dactylis glomerata* is capable of developing in unfavorable environmental conditions, and its 'Nakielska' and 'Motycka' varieties are the two most frequently cultivated in Poland (Falkowski et al., 1974). The caryopses of these varrieties do not differ morphologically. It was therefore interesting to investigate whether this similarity is reflected in the content of certain biochemical components. With this in mind the content of protein and sugar was determined, as also acid phosphates (AcPase) and ribonuclease (RNase) activity in whole caryopses, the seed, and in the chaff.

* This study has been financed from MR-II.1.2.9.

MATERIAL AND METHODS

The investigations were conducted on caryopses of the grass *Dactylis glomerata* L. (orchardgrass, cocksfoot) of the 'Motycka' and 'Nakielska' varieties harvested in 1976.

Caryopses extracts — 5 g of ground caryopses were extracted into distilled water, 0.1 M acetate buffer, pH 5.1, or into a 0.9% solution a sodium chloride according to Wieczorek et al. (1978).

Seed and chaff — 1 g of seed or chaff were triturated in a mortar with 0.1 M acetate buffer, pH 5.1, at a proportion of 1:10 (w/v) for ten minutes. The whole was centrifuged and filtered as in the case of caryopses extracts.

Protein was determined by means of the turbidimetric tannin micromethod according to Mejbaum-Katzenellenbogen (1955).

Total sugars were determined by the phenol method according to Whistler and Wolfram (1962), and pentoses by means of the orcin method according to Mejbaum (1939).

Phosphatase activity was determined by measuring the p-nitrophenol released from sodium p-nitrophenylphosphate, as described in a previous study (Wieczorek et. al., 1978). A unit of acid phosphatase activity was expressed as an activity of the enzyme which liberates 1 μmol of p-nitrophenol during 1 min at 37°C. Specific activity was defined as the number of units per 1 mg of protein.

Ribonuclease activity was determined by means of the Anfinsen et al. (1954) method in 0.1 M acetate buffer, pH 5.1. A unit of ribonuclease activity coresponds to the activity of enzyme which causes an absorption increase by 0.1 at 260 nm during 10 minutes. Specific activity was expressed by the number of units per mg of protein.

Electrophoretic protein separation in polyacrylamide gel was carried out after Reisfeld et al. (1962). Samples containing from 100 μg to 200 μg protein were deposited on gel and separated for 90 minutes. Protein was stained with a 1% solution of amide black 10B in 7% acetic acid. Acid phosphatase activity was determined in the gels by the diazo coupling method in the presence of alfasodium naphthylphosphate and Fast Blue B.

Ribonuclease activity in the gels was localized after Wolf (1968).

Staining for sugars was carried out according to the Zacharius et al. (1969) method.

RESULTS AND DISCUSION

The protein level in the caryopses extracts of both varieties ranged from 0.22 to 0.39 g/100 grams depending upon the extracting factor. A higher protein content was found in extracts of up to 0.9% NaCl than

in extracts into water or 0.1 M acetate buffer, pH 5.1. A similar picture was observed with proteins extracted from rye caryopses (Morawiecka et al., 1976). Proteins of timothy (*Phleum pratense*), redtop (*Agrostis alba* L.) and *Avena elatior* L. caryopses extract easier into water than into salt solutions (Lorenc-Kubis, Wieczorek, 1973). Total sugar content in the extracts under study ranged from 2.7 to 3.6 g/100 g of caryopses, and pentose from 0.30 to 0.67 g/100 g. Irrespective of the method of extraction, caryopses of 'Motycka' variety showed a 55% higher pentose content than caryopses of the 'Nakielska' variety. Significant differences between the two varieties were likewise noted in acid phosphatase and ribonuclease activity (Table 1). Specific acid phosphatase activity in the caryopses of the 'Motycka' variety of orchardgrass ranged from 2.4 to 3.1 units per mg of protein, whereas in the 'Nakielska' variety it was lower by an average of 52% ranging from 1.2 to 1.4 units per mg of protein. Specific ribonuclease activity in the 'Motycka' variety ranged from 22.4 to 36.2 units per mg of protein, and was lower in the 'Nakielska' variety by 32% ranging from 14.7 to 26.2 units per mg of protein. A higher activity of the enzymes under study was observed in acetate extracts, pH 5.1, than in extracts into water or 0.9% NaCl. A similar correlation between enzymatic activity and solvents used was likewise observed for four other varieties of grasses (Lorenc-Kubis, Wieczorek, 1973).

As concerns gel electrophoresis (Fig. 1) three molecular forms of acid

Table 1

Contents of proteins, carbohydrates, acid phosphatase (AcPase) and ribonuclease (RNase) activity in two varieties of *Dactylis glomerata* caryopses as depending upon the solvents

Variety	Solvent	Proteins	Carbohydrates		Activity	
			total	pentoses	AcPase	RNase
		g/100 g caryopses			U/mg protein	
'Motycka'	Water	0.23	2.8	0.63	2.4	22.4
	Acetate buffer pH 5.1	0.28	3.6	0.65	3.1	36.2
	Sodium chloride 0.9%	0.39	3.6	0.67	2.6	29.8
'Nakielska'	Water	0.24	2.7	0.30	1.2	14.7
	Acetate buffer pH 5.1	0.22	3.1	0.38	1.4	26.2
	Sodium chloride 0.9%	0.39	3.2	0.41	1.2	19.4

phosphatase and four molecular forms of ribonuclease were found in both the 'Nakielska' and the 'Motycka' varieties of *Dactylis glomerata*. Glycoproteid was found to be the acid phosphatase with the lowest catode mobility, similarly as phosphatase F_1 of *Avena elatior* L. caryopses (Wieczorek et al., 1977). The electrophoretic heterogenity of acid phosphatase and ribonuclease is a characteristic trait of many grasses (*Graminae*) varieties.

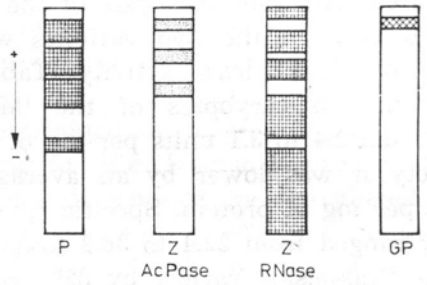

Fig. 1. Electrophoresis in 15% polyacrylamide gel at pH 4.5

Proteins of caryopses extracted with 0.1M acetate buffer, pH 5.1 were subjected to electrophoresis. P — proteinogram, Z — zymograms of acid phosphatase (AcPase), ribonuclease (RNase) GP — glycoproteinogram. For other details see "Material and Methods"

It was of interest to investigate whether the variety differences described above likewise appear in the investigated components in the seeds and chaff of the selected caryopses. It was shown that seed of the 'Motycka' variety constitutes 30% and of the 'Nakielska' variety 54% of the wieght of the caryopses. Seed and chaff was extracted by using 0.1 M acetate buffer, pH 5.1, and protein and sugar content as also acid phosphatase and ribonuclease activity determined in the extracts (Table 2). The content of the components investigated in the seed of both varieties of *Dactylis glomerata* was two to three times higher than in the chaff. The level of protein and acid phosphatase activity in the

Table 2

Contents of proteins, carbohydrates, acid phosphatase (AcPase) and ribonuclease activity in seeds (S) and chaff (Ch) of two varieties of *Dactylis glomerata* caryopses

Material		Proteins	Carbohydrates		Activity	
			total	pentoses	AcPase	RNase
		g/100 g			U/mg protein	
'Motycka'	S	0.26	2.9	0.31	2.6	23.4
	Ch	0.10	0.74	0.16	1.0	14.7
'Nakielska'	S	0.16	2.5	0.29	1.3	17.3
	Ch	0.09	0.47	0.13	0.48	9.3

seed of the 'Motycka' variety was twice higher than in the seed of the 'Nakielska' variety. Chaff of the 'Motycka' variety likewise contains a twice higher level of total sugars, acid phosphatase and ribonuclease activity than the chaff of the 'Nakielska' variety.

These studies showed that differences in the content of some biochemical components in the caryopses of varieties 'Motycka' and 'Nakielska' of *Dactylis glomerata* refer principally to acid phosphatase and ribonuclease activity, as likewise to pentoses.

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Porównawcze badania nad białkami, węglowodanami oraz aktywnością fosfatazy kwaśnej i rybonukleazy w dwóch odmianach ziarniaków Dactylis glomerata (L.).

Streszczenie

Zawartość białek w ziarniakach *Dactylis glomerata* odmiany 'Motycka' i 'Nakielska' wynosiła od 0,22 do 0,39 g/100 g masy w zależności od czynnika ekstrahującego, cukrów całkowitych od 2,7 do 3,6 g. Pentozy w odmianie 'Nakielska' stanowią od 0,30 do 0,41 g, a w odmianie 'Motycka' od 0,63 do 0,67 g/100 g ziarniaków. Aktywność fosfatazy kwaśnej w ziarniakach odmiany 'Motycka' wynosiła od 2,4 do 3,1 j./mg białka, a w odmianie 'Nakielska' od 1,2 do 1,4 j./mg białka. Aktywność

RNazowa w ziarniakach odmiany 'Motycka' wynosiła od 22,4 do 36,2 j./mg, a w odmianie 'Nakielska' od 14,7 do 26,2 j./mg białka. Badania porównawcze nad zawartością niektórych składników biochemicznych w ziarniakach *Dactylis glomerata* wykazały, że odmiany 'Motycka' i 'Nakielska' różnią się poziomem aktywności fosfatazy kwaśnej i rybonukleazy oraz zawartością pentoz. W nasionach pozbawionych plewek oraz w plewkach ziarniaków różnice odmianowe dotyczą jedynie badanych aktywności enzymatycznych.