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Comparative studies on the carbohydrate, protein and acid phosphatase contents in seeds of some rye (*Secale cereale*) varieties

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Abstract

The contents of sugars, proteins and acid phosphatase extracted with 0.1 M acetate buffer, pH 5.1, from some rye varieties were determined. The total sugar level amounted to 3.25—9.70 g per 100 g of seeds; the estimates for pentoses were 1.7—2.9 g and those for proteins 0.91—1.60 g per 100 g of seeds. Acid phosphatase showed and activity level between 0.18 and 1.26 units/mg protein.

After disc electrophoresis proteins were separated into 10 to 11 bands at pH 9.4 or into 4—7 bands at pH 3.8. Essential variety differences were expressed in protein patterns after electrophoresis at pH 3.8.

Acid phosphatase was separated into 5 and 4 activity bands at pH 9.4 and 3.8, respectively. No variations in zymogram patterns were observed in respect to variety differences or cultivation in various climate and soil conditions.

INTRODUCTION

The carbohydrate content in rye caryopses is on the average 69.5 per cent and of proteins, 11.5 per cent of dry seed mass (Kent-Jones 1954). Prolamines and glutelines constitute 70—90 per cent and albumins and globulins 10—20 per cent of the total protein content in the seeds (Neurath and Bailey, 1954). The enzymatic activity occurring in seeds is bound with the group of proteins readily soluble in water and aqueous salt solutions. Lorenc-Kubis and Wieczorek (1973) demonstrated that the use of 0.1 M acetate buffer pH 5.1 for some grass varieties of the *Gramineae* family leads to the extraction of proteins soluble in aqueous salt solution with preservation of their enzymatic activity.

In the present study the contents of sugars and proteins and acid phosphatase activity were investigated in rye caryopses extracted into acetate buffer. It was thought interesting to establish to what extent the content of the above mentioned components and their electrophoretic heterogeneity depend on the variety and cultivation conditions of rye.

MATERIAL AND METHODS

Seeds of the following varieties of crop rye were studied: Dańkowskie Złote, Dańkowskie Selekcyjne, Chrobre, Garczyńskie and Smolickie from the 1973 harvest and 3 varieties of Dańkowskie caryopses (Złote, Srebrne and Selekcyjne) as well as Pancerne from the 1974 harvest. The seeds of Dańkowskie and Pancerne from the 1974 harvest were received from the Breeding Stations in Jeleniec, Uczyce and Laski. The germinating power of the caryopses was 90—95 per cent.

After grinding the caryopses were exhaustively extracted into 0.1 M acetate buffer, pH 5.1, and into water with 0.9 per cent sodium chloride. The fluor (2 or 4 g) was shaken mechanically for 30 min with a 10-fold volume of the solvent (w/v). The homogenates were centrifuged for 30 min at 15 000 g at 5° C. After filtration through Whatman no. 1 paper protein was determined in the supernatant by the turbidimetric tannin micromethod according to Mejbaum-Katzenellenbogen (1955), total sugars by the phenol method (Whistler et al., 1962) and pentoses by the orcin method (Mejbaum, 1939). Acid phosphatase activity was determined in the presence of sodium p-nitrophenylphosphate by measuring the p-nitrophenol released after 10 min at 37°C. As unit of enzymatic activity was adopted the international unit — μmoles of p-nitrophenol released during 1 min at 37° C and pH 5.1. Electrophoretic protein separation in polyacrylamide gel at pH 9.5 was run after Davis (1964) and at pH 3.8 after Reisfeld et al. (1962). Protein in the gel was stained with 1 per cent amide black solution in 7 per cent acetic acid. Acid phosphatase activity was detected in the gel by the diazo coupling technique. After electrophoretic separation the gels were incubated in 0.2 M acetate buffer, pH 5.1 at 4°C for 15 min. Twofold incubation was applied in the case of electrophoresis at pH 9.5, then the gel was incubated at 37°C in 5 ml of 0.2 M acetate buffer, pH 5.1, containing 5 mg of alpha-naphthol phosphate and 5 mg of Fast blue B. The stained gels were stored in 7 per cent acetic acid.

RESULTS AND DISCUSSION

Table 1 presents the sugar and protein content and acid phosphatase activity of rye caryopses of the Dańkowskie Złote variety, extracted into water, 0.9 per cent NaCl and 0.1 M acetate buffer, pH 5.1. The highest

content of the investigated substances was found in the extracts into sodium chloride solution, whereas the highest acid phosphatase specific activity was noted in extracts into acetate buffer.

Table 1

The level of proteins, carbohydrates and acid phosphatase activities in seeds of *Secale cereale* var. Dańkowskie złote

Extraction with	Carbohydrates g/100 g of seeds		Proteins g/100 g of seeds	Acid phosphatase	
	total	pentoses		specific activity u./mg protein	total activity u./100 g of seeds
Water	8.30	1.86	1.30	0.86	1122
Sodium chloride 0.9%	9.40	2.10	1.50	1.00	1500
Acetate buffer pH 5.1	9.00	2.00	1.16	1.17	1357

Assays were performed as described in "Material and Methods".
Results are summarized from 3 independent experiments.

In contrast to the buffer extracts, the aqueous and salt ones show strong opalescence and the content of the studied substances changes within 24 h. Therefore, in further experiments the rye caryopses were extracted into acetate buffer. Table 2 gives the results of comparative studies on the sugar, and protein contents and acid phosphatase activity in 4 varieties and 3 pure lines of rye caryopses. The content of sugar soluble in acetate buffer in caryopses of the studied rye varieties was 7.80—9.70 g/100 g of seeds; pentose content was 2.30—2.90 g/100 g seeds and the acid phosphatase level 0.66—1.26 u./mg of protein. The differences between the varieties in protein content reached 30 per cent amounting to 0.91—1.35 g/100 g seeds. Variations within the pure lines are much smaller amounting to 3.00—2.40 g protein/100 g seeds. The total sugar and pentose levels differed widely both within varieties and pure breeding lines of rye caryopses. The differences in acid phosphatase specific activity reached 50 per cent depending on the variety. Table 3 lists the results of sugar, protein and acid phosphatase determination in 3 Dańkowskie rye varieties and Pancerne rye from 3 different climate and soil sites. The mean protein content in the Dańkowskie varieties was 1.37 g, of total sugar 5.11 g, of pentoses 2.74 g per 100 g seeds and the acid phosphatase level was 0.61 u./mg of protein. In the seeds of Pancerne rye from various climate and soil sites the mean protein level was 1.53 g/100 g seeds and was much higher than the mean protein level in the 3 Dańkowskie varieties cultivated under the same climate and soil conditions.

Table 2

The level of proteins, carbohydrates and acid phosphatase activities from four varieties and three uniform plots of *Secale cereale* seeds extracted with 0.1 M acetate buffer pH 5.1

Varieties	Carbohydrates g/100 g of seeds		Proteins g/100 g of seeds	Acid phosphatase	
	total	pentoses		specific activity u./mg protein	total activity u./100 g of seeds
Dańkowskie złote	8.10	2.40	1.30	0.85	1079
Dańkowskie selekcyjne	8.60	2.90	1.17	0.66	772
Chrobre	9.00	2.80	0.91	1.00	1001
Garczyńskie	8.90	2.30	1.20	0.75	900
mean value	8.65	2.60	1.15	0.82	938
Uniform plots of Smolickie rye seeds					
S-5	7,80	2.60	1.35	1.10	1485
S-7	9.70	3.00	1.10	1.26	1386
S-15	8.70	2.40	1.30	0.97	1261
mean value	8.73	2.67	1.25	1.11	1377

The mean value of the remaining components investigated was much lower than in the Dańkowskie rye caryopses, amounting for total sugar to 3.87, and pentoses to 1.97 g/100 g seeds. Mean specific activity of acid phosphatase in Pancerne rye caryopses was one half that in the Dańkowskie variety.

These results indicate that the content of the relevant organic substances varies widely both in dependence on variety, pure line and climate and soil conditions of rye cultivation. The differences observed in protein content and acid phosphatase specific activity in the examined varieties prompted us to investigate the electrophoretic heterogeneity of proteins and acid phosphatase. Electrophoretic separation of protein from Dańkowskie, Chrobry and Garczyńskie rye caryopses is illustrated in Fig. 1.

Under conditions of disc electrophoresis at pH 9.4 proteins from rye seeds separate into 10—11 fractions. In all proteinograms two fractions with intermediate anodic mobility prevail. Slight differences are visible only between the bands moving slowest towards the anode. Significant differences were observed in protein separation by disc electrophoresis at pH 3.8. As seen in Fig. 2, the proteins of the examined rye caryopses separate into 4—7 fractions. In the anodic zone of the proteinograms there occurs a single band in the case of all rye varieties. In the intermediate

Table 3

The content of proteins, carbohydrates and acid phosphatase activities from three varieties of Dańkowskie and Pancerne rye seeds cultivated in various climate and soil conditions

Varieties	Locality	Carbohydrates g/100 g of seeds		Proteins g/100 g of seeds	Acid phosphatase	
		total	pentoses		specific activity u./mg protein	total activity u./100 g of seeds
Dańkowskie selekcyjne	Jeleniec	5.25	2.40	1.10	0.56	616
	Uszyce	4.25	2.30	1.10	0.65	715
	Laski	5.37	3,55	1.10	0.90	990
Dańkowskie złote	Jeleniec	4.00	2.85	1.05	0.80	840
	Uszyce	6.37	4.20	1.05	0.75	788
	Laski	4.25	2.55	1.10	0.87	957
Dańkowskie srebrne	Jeleniec	4.12	2.10	1.00	0.37	370
	Uszyce	4.25	2.60	1.60	0.18	288
	Laski	4.00	2.10	1.60	0.37	592
mean value		5.11	2.74	1.37	0.61	684
Pancerne	Jeleniec	4.12	2.15	1.50	0.33	495
	Uszyce	4.25	2.05	1.50	0.25	375
	Laski	3.25	1.70	1.60	0.37	592
mean value		3.87	1.97	1.53	0.32	487

Each figure represents four independent estimates.

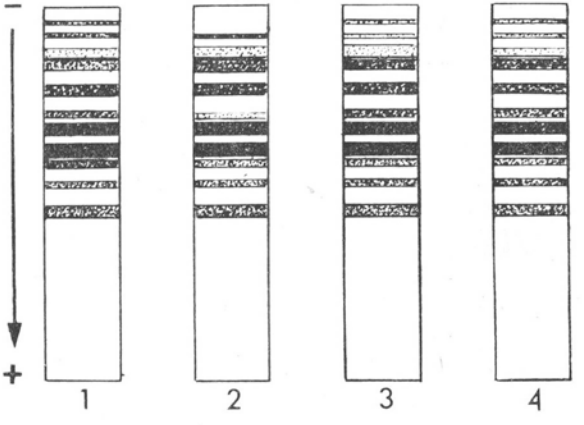

Fig. 1. Separation of proteins from *Secale cereale* seeds on 7,5% polyacrylamide gel pH 9.5

Proteinograms: 1 — Dańkowskie złote, 2 — Dańkowskie selekcyjne, 3 — Chrobre, 4 — Garczyńskie Electrophoresis was performed in tris-glycine buffer pH 8.4. 120 μg protein were subjected to electrophoresis

zone 3 fractions occur for both Dańkowskie varieties, for Chrobry 2 fractions and in the case of Garczyńskie in this zone of the proteinograms no protein fractions were noted. In the cathodic zone of the proteinograms each of the rye caryopse varieties showed 3 protein fractions. In this zone like in the anodic one differences in staining intensity with amide black of the particular bands were observed.

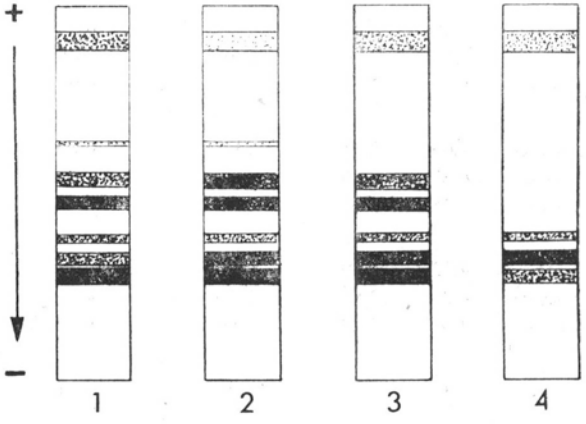

Fig. 2. Separation of proteins from *Secale cereale* seeds on 7.5% polyacrylamide gel. pH 3.8

Proteinograms: 1 — Dańkowskie Złote, 2 — Dańkowskie selekcyjne, 3 — Chrobre, 4 — Garczyńskie Electrophoresis was performed in tris-HCl buffer, pH 4.3. 120 μg protein were subjected to electrophoresis

The electrophoretic heterogeneity of acid phosphatase is shown in Fig. 3. In disc electrophoresis at pH 9.4 acid phosphatase of all the examined varieties or pure lines of rye caryopse separated into 5 fractions,

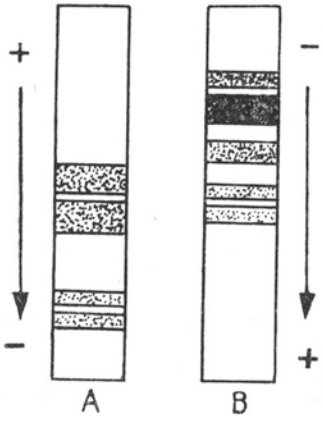

Fig. 3. Zymograms of acid phosphatases in acetate extracts of rye seeds

A — zymogram of acid phosphatases separated in disc electrophoresis at pH 3.8; B — zymogram of acid phosphatase separated in disc electrophoresis at pH 9.5

The amount of protein applied to the gel acid and alkaline pH values was 60 μg and 150 μg, respectively

whereas at pH 3.8 into 4 fractions located in the intermediate and cathodic zone of the zymograms. The electrophoretic heterogeneity of acid phosphatase from rye caryopses does not coincide with the variety differences found in the proteinograms (Fig. 2) of the caryopses.

The above described differences in carbohydrates, proteins and the heterogeneity of the proteinograms may be a valuable indication in selection for valuable traits of rye caryopses.

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Porównawcze badania nad zawartością cukrów, białek oraz fosfatazą kwaśną w kilku odmianach żyta (Secale cereale)

Streszczenie

Porównano zawartość cukrów, białek i aktywności fosfatazy kwaśnej w kilku odmianach uprawnych nasion żyta ekstrahowanych do 0,1 M buforu octanowego o pH 5,1. Poziom cukrów całkowitych w badanych ziarniakach żyta wynosi od 3,25 g do 9,7 g/100 g nasion; pentoz od 1,7 g do 2,9 g/100 g nasion; białka od 0,91 g do 1,60 g/100 g nasion, a fosfatazy kwaśnej od 0,18 do 1,26 jedn./mg białka. Heterogenność elektroforetyczna białek wyraża się obecnością od 10 do 11 frakcji w elektroforezie dyskowej w pH 9,4 oraz od 4 do 7 frakcji w pH 3,8. Istotne różnice odmianowe zaobserwowano w heterogenności białek w dyskowej elektroforezie w pH 3,8. Heterogenność elektroforetyczna aktywności fosfatazy kwaśnej nie zależy od odmiany ani warunków klimatyczno-glebowych i wyraża się obecnością 5 frakcji w pH 9,4 i 4 frakcji w pH 3,8 elektroforezy dyskowej.