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Alkylresorcinols in rye (*Secale cereale* L.) grains *

II. Dependence of alkylresorcinols level on weight and specific weight of grains

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Abstract

A relation was found between the alkylresorcinols content and the weight of single grains, their specific weight and situation in the ear. In the group of grains with high specific weight (above 1.300 g/cm³) the relative alkylresorcinols content proved to be a constant value, whereas the total content was positively correlated with the grain weight. A negative correlation was found between the specific weight and the relative alkylresorcinols content decreasing from tip to base of ear. In the apical zone of growth grains with low specific weight (below 1.260 g/cm³) constitute 70 per cent, and in the middle and base of the ear 30 per cent of all grains in the given zone.

INTRODUCTION

Taking into account the two essential physical parameters — weight and specific weight — it is possible to calculate the volume of the grain. This volume, in view of the definite shape of the grain, is a trait strictly correlated with the surface dimensions. Under the assumption that the alkylresorcinols content falling to a surface area unit is a constant value, the size of this surface area is decisive for their total content in the grain.

In grains with similar specific weights, that is when the ratio of weight to volume ratio is constant, the relative alkylresorcinol content will be a constant trait characteristic for the given population, and their total content in all grains will increase with grain weight.

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In grains with different specific weight, and thus with a variable weight to volume ratio, the relative alkylresorcinol content should also be a variable character, negatively correlated with the specific weight of the grain. There is no distinct correlation between the weight of the particular grains and total alkylresorcinols content.

The purpose of the present study was to check the above expounded assumption. Investigations were performed on genetically definite seed material in the period of anabiosis, and on ears freshly collected during harvest. The correlations were determined between specific weight and weight of the particular grains and the total and relative resorcinol content.

MATERIAL AND METHODS

Rye of the variety Dańkowskie Złote, of the 1973 harvest was taken from the experimental Plant Breeding Station in Rogaczewo Breeding Section Choryń and from the Breeding Station at Smolec. Purity was 99.9 per cent, germination power 90, generation — original, the grain was air dry. The investigations were carried out after a year's storage of the material.

The rye ears of the variety Dańkowskie Złote, with 17 tiers, generation — original, from the Experimental Station of the Wrocław Agricultural University in Swojec. Date of harvest August, 1974.

Milling fractions: fine and coarse bran, and rye flour (1974 harvest, Laski) generation — original were obtained by grinding the grains in Büchler type mill. The material was received owing to the courtesy of doc. dr A. Biskupski, head of he Department of Cereals Technology, Agricultural University, Wrocław.

The alkylresorcinol standard was obtained by the methanol procedure from acetone exctracts from rye Dańkowskie Złote grains (Choryń). The preparation was homogeneous in thin-layer chromatography in several solvent systems (W. Mejbaum-Katzenellenbogen et al., 1975).

Rye grain specific weight was measured in water-sucrose solutions: two basic solutions were prepared — solution A of 1.200 g/cm^3 density and solution B with density 1.470 g/cm^3 containing 541 g and 1311 g sucrose in 1 l. of solution respectively. The required density of the working solution was obtained by mixing solution A with solution B in appropriate proportions by volume. Density was measured at 20° C with a densitometer.

Alkylresorcinols were determined in single rye grains by the p-nitroaniline method. Single grains were weighed on a torsion balance (Techniport — Warszawa), carrying capacity 100 mg, sensitivity 0.5 mg. The weighed grain was placed in a calibrated test tube of 10 ml volume and 1 ml of acetone p.a. was added (POCH, Gliwice) the test tube was placed in a water bath at 55° C with intermited shaking. After 3 h they were

taken out of the bath, cooled to room tempertaure and 1.5 ml of freshly prepared p-nitroaniline reagent (W. Mejbaum-Katzenellenbogen et al., 1975) was added. After 30 min 7.5 ml acetone was added and after

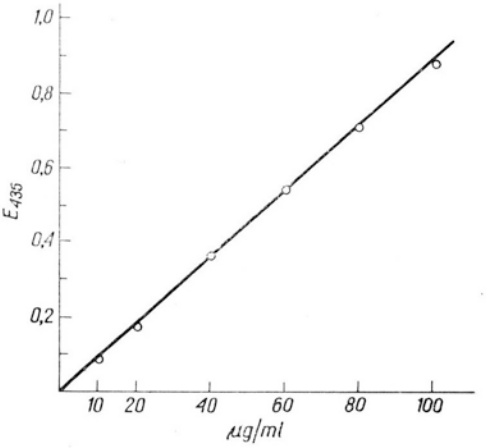

Fig. 1. Standard curve for alkylresorcinols determination. Standard — alkylresorcinol preparation from rye

60 min. extinction was read in a 1 cm layer at 435 nm in a Spekol apparatus. From the standard curve shown in Fig. 1 the alkylresorcinol content in one grain was read.

RESULTS

Table 1 shows the relative alkylresorcinols content in several randomly taken samples of rye milling products from rye Dańkowskie Złote: in

Table 1

Total and relative alkylresorcinol content in milling products of rye 'Dańkowskie Złote'; generation — Original; 1974 harvest; Laski

Milling products	Weighed sample mg	Alkylresorcinol content	
		Content in sample mg	%
Coarse bran	372	1.40	0.376
	618	2.20	0.356
	398	1.50	0.369
Fine bran	256	0.320	0.125
	225	0.280	0.124
	238	0.290	0.122
Flour	1010	0.090	0.007
	1217	0.090	0.007
	1048	0.080	0.008

coarse and fine bran and in fluor. The coarse and fine bran contains practically all the alkylresorcinols extractable with acetone. In both milling fractions representing the outer layers of the caryopsis, the pericarp and the aleurone the relative alkylresorcinols content is 0.540 per cent. The richest in alkylresorcinols is the coarse bran milling fraction.

A statistical characteristic of the grains of Dańkowskie Złote rye is given in Table 2 in the generation — original (1973, Smolec), thus in well definite seeding material. The mean weight of single grains and the total and relative alkylresorcinol content were determined. The group of numerical results obtained shows a normal distribution. The mean weight of a grain is 43.3 ± 1.3 mg the total alkylresorcinol content is 0.040 ± 0.002 mg and the relative content 0.093 ± 0.005 per cent. The low variability coefficient 5.3 per cent indicates the homogeneity of the material.

Table 2

Total and relative alkylresorcinol content in single rye grains in dependence on their weight

Rye 'Dańkowskie Złote' variety; grains air-dry; after one-year storage (Smolec 1973)

Group	n	Mean weight $\bar{X} \pm s$ (mg)	Mean content	
			$\bar{X} \pm s$ (mg)	$\bar{X} - s$ (per cent)
Norm	600	43.3 ± 1.3*	0.040 ± 0.002	0.093 ± 0.005**
heavy grains (A)	10	45.1 ± 1.2	0.042 ± 0.009	0.093 ± 0.019
Light grains (B)	10	25.3 ± 0.9	0.034 ± 0.004	0.136 ± 0.011
Difference A—B		19.8	0.008 (t=0.14)	0.043 (t=2.89)

* — variability coefficient = 3.0%
** — variability coefficient v = 5.3%

From the whole sample (10 kg) 10 grains weighing 45 mg each (group A) and 10 weighing 25 mg each (group B) were chosen. The grains of group A do not differ from the norm either in weight or in total and relative alkylresorcinols content. In group B (25 g) the grains show significant differences as regards weight and a higher relative alkylresorcinols content, whereas the total content in a single grain is the same. The lack of correlation between the weight of single grains and the total alkylresorcinols content indicates that grains weighing 25 and 45 mg have a closely similar surface area. The differences in percentual alkylresorcinols content occurring between group A and B seem to suggest that the lighter grains are not so well developed and have a lower specific weight than those weighing 45 mg.

In further experiments the distribution of specific weight of rye grains was determined and the correlation between specific weight and relative alkylresorcinols content was established in a sample weighing 15 g (435 grains).

Table 3 presents the distribution of specific weigthts, mean weight of grains in each specific weight class and the relative alkylresorcinols content. Barely 7.8 per cent of the grains exhibited a specific weight equal or lower than 1.204 g/cm³. To classes I and II jointly fell 18 per cent of the grains. Classes III, IV and V included grains with mean specific weights within the limits of 1.260—1.334 g/cm³, to this group fell 47 per cent of the grains. The last class (35%) consists of grains with specific weight equal or higher than 1.334 g/cm³. From each of these classes 30 grains were taken randomly, their weight and the content of alkylresorcinols were determined. The mean weight of single grains, notwithstanding the specific weight class was 33 ± 2 mg. The highest percentual alkylresorcinol content was found in grains with the lowest specific weight (classes I and II) which contained 0.133 per cent of these substances. The content of alkylresorcinols in grains with a moderate specific weight was markedly lower amounting to 0.110 per cent. The lowest relative alkylresorcinol content (0.095%) was noted in grains with highest specific weight.

Thus relative alkylresorcinol content is negatively correlated with

Table 3

Relative alkylresorcinol content and mean weight of rye grains in dependence on their specific weight

Grain class	Specific weight interval g/cm³	Grains		Mean weight of grain mg	Alkylresorcinols %
		number	%		
I	$d \leq 1.204$	34	7.8	32	0.135
II	$1.204 < d < 1.260$	44	10.2	31	0.130
III	$1.260 < d < 1.300$	84	19.4	32	0.100
IV	$1.300 < d < 1.326$	84	19.4	35	0.110
V	$1.326 < d < 1.334$	37	8.6	32	0.110
VI	$1.334 \leq d$	150	34.6	35	0.095
Total		433	100.0	33 ± 2 $v = 6\%$	

The 15-g sample containing 433 grains of 'Dańkowskie Złote' rye; 1973 harvest (Rogaczewo) were placed in a sucrose solution with specific weight 1.204 g/cm³.

The grains floating on the surface were considered as class I. The specific weight of the solution was gradually increased. Classes II, III, IV and V consisted of grains collected from the surface in the sucrose solution density intervals given in the table. Class VI consisted of grains suspended and lying at the bottom of the vessel in solution of 1.334 g/cm³ density. The grains of the particular classes were counted, washed with water and dried to constant weight at 105°C.

From each class 30 grains were taken randomly, weighed and their alkylresorcinol content was determined. The table gives the density interval and per cent of grains in the given specific weight class, mean weight of 1 grain in the given class and the relative alkylresorcinol content.

specific weight of the grains. It should be mentioned that the grain weight varied to a similar degree in all specific weight classes.

To supplement this experiment a trial was undertaken of characterization of grains of classes II, IV and VI. For this purpose 5 grains weighing 22 mg and five weighing 46 mg were chosen from each class. In both weight classes total and relative alkylresorcinol content was determined (Table 4). It proved that in the 3 specific weight classes investigated (II, IV and VI) grains weighing 46 mg contained significantly more alkylresorcinols than those weighing 22 mg. Grains with specific weight above 1.300 g/cm³ did not differ from one another in alkylresorcinol percentual content. Class II, grains with the lowest specific weight, show a significantly higher content of the studied substances, with a negative correlation between the single grain weight and the relative alkylresorcinol content.

Table 4

Total and relative alkylresorcinol content in single rye grains in dependence on their weight and specific weight

Rye grains Specific weight g/cm³	Weight mg	Alkylresorcynols	
		µg/grain $\bar{X}\pm s$	grain weight % $\bar{X}\pm s$
Class II	22	0.033±0.003	0.150±0.014
	46	0.055±0.006	0.121±0.013
	difference	−0.022 (t=3.21)	−0.029 (t=3.53)
Class IV	22	0.024±0.004	0.107±0.021
	46	0.043±0.007	0.094±0.016
	difference	−0,019 (t=3.93)	−0.014 (t=1.05)
Class VI	22	0.022±0.004	0.09±0.016
	46	0.044±0.014	0.095±0.032
	difference	−0.022 (t=2.98)	−0.003 (t=0.45)

Five grain weighing about 22 mg and 5 grains weighing about 46 mg were taken from each of 3 classes of specific weight.
Class II — grains with specific weight higher than 1.204 g/cm³ and lower than 1.260 g/cm³, class IV — 1.300≤1.326 g/cm³, class VI≥1.334 g/cm³

To sum up the results of this experiment it may be stated that well developed rye grains with specific weight above 1.260 g/cm³ exhibit a positive correlation between alkylresorcinols content and grain weight. This explains the increase in surface area associated with increase in weight. The relative content is a constant value characteristic for a given population.

Poorly developed grain with specific weight below 1.260 g/cm³ do not exhibit these regularities, this finding its expression in a negative correlation between specific weight and relative alkylresorcinols content.

For checking in how far biological variability effects the alkylresorcinol content, analogous investigations were performed in grains collected directly from freshly harvested ears of Złote Dańkowskie ryc (Original).

Grains were taken from the ear base (tiers 2, 3, 4) the middle (tiers 9, 10, 11) and tip (tiers 14, 15, 16) and dried to constant weight. Then each grain was weighed and alkylresorcinols were determined in it. The statistically elaborated results are shown in Table 5. The grain weight increases from the base tot he middle of the ear and then decreases towards the tip. The high variability coefficient (v = 10—23%) indicates wide differences in grain weight in each zone of growth. Total alkylresorcinols content increases with the increase of grain weight. The relative alkylresorcinols content decreases from the tip to the base of the ear. Grains of the second tier contain on the average 0.090, and of the 16th tier 0.135 per cent alkylresorcinols. The variability coefficient is also high amounting to 12—30 per cent.

Table 5

Total and relative alkylresorcinol content in single rye grains in dependence on their position in the ear

Position of grain in ear		Mean weight of grain		Mean alkylresorcinol content		
zone of growth	tier	$\bar{X}\pm s$ (mg)	v	$\bar{X}+\pm s$ (mg)	$\bar{X}\pm s$ (per cent)	v
Base	2	25.7±3.8	14.8	0.023±0.005	0.090±0.021	23.3
	3	31.6±7.4	23.4	0.032±0 .009	0.099±0.029	29.2
	4	32.1±3.8	11.8	0.032±0.010	0.099±0.031	31.3
Middle part	9	34.5±6.2	18.0	0.040±0.012	0.115±0.035	30.4
	10	37.7±4.5	11.9	0.042±0.006	0.110±0.017	15.5
	11	38.9±5.1	13.1	0.046±0.010	0.121±0.026	21.5
Tip	12	36.2±7.6	20.1	0.040±0.007	0.117±0.020	17.1
	13	33.0±7.1	21.5	0.039±0.005	0.117±0.015	12.8
	14	29.8±5.4	18.1	0.040±0.008	0.135±0.029	21.4

For determinations 2 grains were taken from each of the chosen tiers of 15 ears — for each tier 10 measurements were performed.

From each zone of growth 60 grains were taken. The distribution of specific weights was established for each zone (Table 6).

In the base of the ear grains with low specific weight (classes I and II) constitute 34 per cent, in classes III and IV 48.9 per cent. The specific weight of the remaining 16.5 per cent of grains does not exceed 1.326 g/cm³ (class IVb).

Table 6

Grain specific weight distribution in dependence on their situation in the ear

Class	Material	Specific weight interval (g/cm³)	Position of grain in ear					
			base %		middle part %		tip %	
I		1.204≥d	16.2	34.6	0.0	31.4	47.0	73.4
II		1.204<d<1.260	18.4		31.4		26.4	
III		1.260<d<1.300	26.5	48.9	44.6	61.0	9.5	19.0
IVa		1.300<d<1.326	22.4		16.4		9.5	
IVb*		d≥1.326	16.5	16.5	7.4	7.4	7.6	7.6
	Total		100		100		100	

* In the material of this experiment no grains with specific weight higher than that given here were found.

Ears of 'Dańkowskie Złote' rye; Swojec — August 1974:. The grains were taken from the particular tiers and dried to constant weight at 105°C. Each zone was represented by 60 grains taken from 5 ears.

Specific weight distribution was measured as described in Table 3. Base — tiers 2, 3, 4; middle part — 9, 10, 11; tip — tiers 14, 15, 16.

The middle zone of growth includes 31.4 per cent of grains with low specific weight (classes I and II), 61 per cent of moderately heavy (classes III and IVa) and only 7.4 per cent of grains with specific weight 1.326 g/cm³. The tip of the ear contains as much as 73 per cent of grain with low specific weight (classes I and II).

The distribution of specific weight in the base and the middle part of the ear is similar, low specific weight grains constituting 1/3, whereas in the top part they amount to 3/4 of all grains. Wide differences in specific weight in dependence on the zone of growth may be attributed to the highest percentual content of alkylresorcinols in the grains of the ear tip.

DISCUSSION

The main aim of the present study was to find evidence supporting the assumptions advanced at the beginning that alkylresorcinol content is directly dependent on the surface dimensions of the grain. The establishment of the relations between weight and specific weight, that is weight and volume and total alkylresorcinol content as index of surface area of the grain is the basic condition for correct interpretation of the changes occurring in the alkylresorcinol content under the influence of breeding and genetic factors.

The investigations were carried out on one rye variety in the original generation, thus on grains derived from a genetically definite population in two physiological states, in the period of anabiosis and in the end phase of ripening in the ear.

The rye grain sample was divided into 6 groups differing in specific weight. It was found that in all classes of specific weight the mean weight of grain does not differ from the standard determined for the sample as a whole. It is clear from this experiment that the volume of the grains decreases with the increase in specific weight. Thus, there is a negative correlation between the grain surface area and the specific weight. The sample contained 80 per cent of well developed grains with high specific weight and barely 20 per cent of grain of inferior quality with low specific weight.

In connection with the assumption that the alkylresorcinol level is an index of the grain surface area, the content of these substances was determined in grains of known weight and specific weight. The specific weight of the grains proved to be negatively correlated with the relative alkylresorcinol content. A further confirmation of the correctness of the initial assumption concerning the localization of alkylresorcinols in the surface layers of the grain is the positive correlation between the weight of grains with similar specific weight and the total alkylresorcinol content in the grain.

Only selection of grain from the aspect of one physiological trait such as their specific weight may give groups with constant relative alkylresorcinol content, in which the total content of these compounds increases with the weight increment of the grain. The only exception proved to be grains with low specific weight in which total alkylresorcinol content increased significantly with grain weight, but the percentual content diminished. These groups differ from normal not only by the physiological character of their weight to volume ratio, and thus by their surface area, but also by their chemical character, that is the alkylresorcinol level.

In order to establish in how far the physiological state of grains affects their specific weight and the constance of the alkylresorcinol index, an analogous analysis of grains taken directly from the ears during the harvest was performed. The grains from the base and middle zone of the ear contained about 30 per cent of grains with low specific weight, and those of the top part as much as 70 per cent of such grains. In the light of the foregoing analyses performed on stored grain it was not surprising that grain from the tip of the ear show the highest alkylresorcinol level, and those from the base the lowest. The high variability coefficient for the weight of the particular grains and for the relative alkylresorcinol content are additional evidence for the wide differences in the degree of maturity of grains in the particular zones of ear growth.

The variability in alkylresorcinols content observed in the grains of one rye variety, both in the period of storage and during the harvest, is a secondary character, since it does not indicate directly the changes occurring in the concentration of these compounds, but it indirectly reflects the variability of the weight to surface ratio of the grain.

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Alkilorezorcinole w ziarniakach żyta (Secale cereale L.

II. Poziom alkilorezorcinoli w zależności od masy i ciężaru właściwego ziaren

Streszczenie

1. Przeprowadzono analizę rozkładu ciężarów właściwych, całkowitej i względnej zawartości alkilorezorcynoli w ziarnach żyta odmiany Dańkowskie Złote w stanie anabiozy i w końcowej fazie dojrzewania w czasie żniw.

2. Stwierdzono zależność zawartości alkilorezorcynoli od ciężaru pojedynczych ziaren, ich ciężaru właściwego oraz od położenia w kłosie.

3. W grupie ziaren o wysokim ciężarze właściwym (powyżej 1,300 g/cm^3) względna zawartość alikilorezorcynoli okazała się wartością stałą, natomiast zawartość całkowita była dodatnio skorelowana z wagą ziarna.

4. Wykazano ujemną korelację pomiędzy ciężarem właściwym a względną zawartością alkilorezorcynoli.

5. Względna zawartość alkilorezorcynoli maleje od wierzchołka do podstawy kłosa.

6. W wierzchołkowej strefie wzrostu ziarna o niskim ciężarze właściwym (poniżej 1,260 g/cm^3) stanowią 70%, a w środku i podstawie kłosa 30% ziaren danej strefy.