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Localization of the alkylresorcinols in rye and wheat caryopses

F. TŁUŚCIK

Institute of Biochemistry, Wrocław University, Wrocław

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Abstract

A histological method for detecting of 5-n-alkylresorcinols in grains sections using the staining with Fast Blue B was developed. Taking advantage of that method it was found that alkylresorcinols are localized exclusively in the outer cuticle, but both germs and starchy endosperm of rye and wheat seeds are entirely free of these compounds.

INTRODUCTION

It is believed that alkylresorcinols are present in the external parts of the caryopse, in the pericarp or else in the aleurone, W i e r i n g a (1967) was the only author who attempted the location of alkylresorcinols and established their presence in the strongly adhering cuticular layers situated outside the aleurone and hyalin layers. He did not, however, report any closer morphological and quantitative data.

The present study was undertaken in an attempt to determine quantitatively the alkylresorcinol content in the endosperm, embryo' pericarp, testa and in the aleurone layer.

MATERIAL AND METHODS

Rye of the Dańkowskie Złote variety, original seed of the 1976 harvest from the Plant Breeding Station Rogaczewo and wheat of the Grana variety, elite seed, from the 1976 harvest from the same Station were used for the experiments.

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Preparation of pericarps (and rye and wheat grains deprived of pericarps)

The caryopses (10 g) were flushed with 85 per cent ortho-phosphoric acid and left to stand at room temperature. After 48 h the seeds were thoroughly washed with water and dried. The washings containing the macerated pericarps were collected, neutralized with 20 per cent sodium hydroxide and dried.

Preparation from seeds deprived of pericarp of section with a definite surface area

Seeds were selected from among those deprived of the pericarp which were flat in the central part on the side of the brush. The outer layer was cut off this part and from this layer squares were made with the use of parallel gilette blades. The dimensions of the squares were checked under a micrometric ocular. Squares with rough borders or not parallel sides were discarded. For alkylresorcinol content determination 25 squares 2.12×2.12 mm were chosen. Their over-all surface area was 110.2 mm^2 (1.102×10^8 μm^2).

Preparation of flour from rye and wheat grains

The grains were placed in a beaker with water and put into a boiling water bath. After 15 min they were taken out, cut in halves and placed again in water. After 24 h the flour was pressed out of the grains and air-dried.

Preparation of the embryos from rye and wheat grains

The grains were immersed in water of temperature around 40°C and kept at this temperature for 1 h. Then the embryos were isolated out free-hand with a scalpel. The 40 isolated rye or wheat embryos were inspected under a stereoscopic microscope.

Measurement of outer cuticle thickness

The measurements were routinely performed with the use of an objective standard and a micrometric PZO ocular. The measurements were performed on 10 randomly chosen caryopses on the cross section in the middle part and in the grain groove.

Staining of grain section for phenol derivatives content

The grains were placed in a moist chamber at room temperature. After 3-4 days sections were free-hand prepared in cross-section. They were stained at 30-40°C with a 0.1 per cent Fast Blue B solution in 0.1 M phosphate buffer of pH 7.4 for 1-3 min. After staining the sections were washed with water. As control material served preparations from grains deprived of alkylresorcinols. For this purpose the grains were repeatedly extracted at 56°C with 3 volumes of acetone for 3 h.

Alkylresorcinol determination by the diazo method of Mejbaum-Katzenellenbogen et al. (1975)

Thin-layer chromatography of the acetone extracts from rye and wheat grain was run on aluminium foil plates covered with silica gel Silufol (Kavalier). The chromatograms were developed in a chloroform--acetone 95:5 system and colour was developed by 0.2 per cent aqueous Fast Blue B solution. The standard was a chromatographically pure alkylresorcinol preparation from whole grains obtained after Mejbaum-Katzenellenbogen et al. (1975).

Reagents. Fast Blue B was a Chemapol, Czechoslovakia product, p-nitroaniline was from Loba-Chemie, Austria, the remaining reagents were supplied by POCH (Gliwice).

RESULTS

The alkylresorcinol content in whole rye and wheat grains and in those deprived of the pericarp is shown in table 1. The results obtained indicate that removal of the pericarp does not diminish the alkylresorcinol content, this being evidence that these substances are not present in this layer. Additional attempts were undertaken to demonstrate directly alkylresorcinols in the pericarp, but the result was negative. In concentrated acetone extracts from isolated pericarps thin-layer chromatography did not show any fraction with R_f corresponding to alkylresorcinols. The chromatograms were sprayed with a Fast Blue B solution which reveals alkylresorcinols in 0.05 μg quantities.

Neither was a fraction corresponding to alkylresorcinols found by thin-layer chromatography in concentrated acetone extracts from flour obtained from wheat and rye grains. The same was true for the isolated embryos. It may, therefore, be assumed that alkylresorcinols are localized in the testa or in the aleurone layer.

For establishing whether the alkylresorcinols are uniformly distributed on the seed surface, the particular grains were cut in two parts of equal weight along 3 different axes and in both halves alkylresorcinols were determined.

Table 1

Alkylresorcinol content in whole rye and wheat grains and in those deprived of pericarp. The pericarps were removed with 85% ortho-phosphoric acid as described in methods. Determinations were done in samples of 30 grains

	Alkylresorcinols µg/30 grains	
	rye	wheat
Whole grains n=6	1000 ± 23	703 ± 34
Grains deprived of pericarp n=6	1000 ± 16	716 ± 41

As seen from the data in Table 2, these substances are not distributed uniformly, 40 per cent of them being present in the part around the rye and wheat germ, and 60 per cent of their total content in the part next to the brush.

The microscopic picture of a caryopse cut transversely and stained with a Fast Blue B solution is shown in Photo 1. A dark line is seen running through the pigment cells layer. This line corresponds to the external cuticle containing alkylresorcinols, because it stains purple with Fast Blue B solution. It is absent in the control preparations from seed deprived of alkylresorcinols by extraction with acetone (Photo 2).

Photo 1. Cross section through caryopse with visible cuticle cell layer containing alkylresorcinols

Table 2

Alkylresorcinols distribution in the particular parts of the seed
a. part close to centre, b. part close to brush. Each determination was done in 30 corresponding grain halves

	Direction of cutting		Alkylresorcinols µg/gramme	% Alkylresorcinols in the given half %
rye		a	930	46
		b	1100	54
		a	860	41
		b	1240	59
		a	800	41
		b	1130	59
wheat		a	590	42
		b	800	58
		a	540	40
		b	780	60
		a	570	42
		b	770	58

A further step was the attempt to establish the thickness of the alkylresorcinol layer by determining the content of these substances in isolated sections of the surface with known dimensions. The volume divided by the surface area would give the thickness of the layer. In the case of rye 112 µg of alkylresorcinols were extracted from a surface area of 110.2 mm^3 (1.102×10^8 μm^2). Since 1 µg of alkylresorcinols has a volume of 1×10^6 μm^3 (under the assumption that the specific weight of these substances is 1.0) the thickness of the layer may be assumed as 1.1 µm. The mean thickness of the outer cuticle both in rye and in wheat in the central part of the grain is 2.5 µm. Since the calculated thickness of the

alkylresorcinol layer is 1.1 μm, and the measured outer cuticle thickness is 2.5 μm, it may be assumed that almost 40 per cent of the cuticle volume consists of alkylresorcinols. Measurements of wheat were carried out analogously and they demonstrated that alkylresorcinols occupy only 20 per cent of the cuticle volume in the latter case.

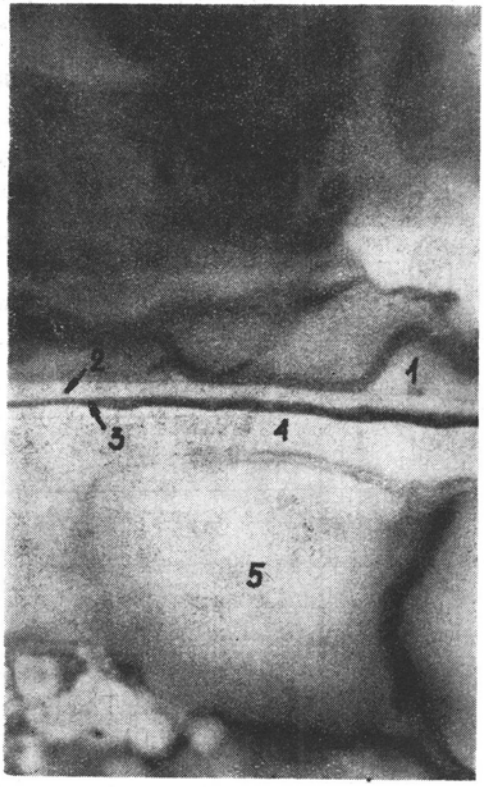

Photo 2. Cross section of caryopse deprived of alkylresorcinols (control) by extraction with acetone. Notations: 1. transverse cell, 2. outer cuticle, 3. pigment cell layer (testa), 4. hyalin cell layer, 5. aleurone cell

DISCUSSION

Wieringa (1976) used vanillin in phosphoric acid for developing thin-layer chromatograms and for staining grain sections. That means that he used a method of colour development 200 times less sensitive than that with Fast Blue B. The colour developed by vanillin in ortho-phosphoric acid is not stable and disappears after several minutes, while the cellular structures are completely destroyed.

Wieringa when assuming the localization of alkylresorcinols in the two strongly adherent cuticular layers outside the aleurone and hyalin layers, meant the whole seed coat of the caryopsis jointly with

the outer cuticle. Such a localization of alkylresorcinols, is however, not exact, since, as it results from the present study, they are situated exclusively in the external cuticle.

The present results contradict also the findings of Verdeal (1977) who reported the presence of large amounts of alkylresorcinols in the pericarp, a moderate amount in the aleurone and low in the endosperm. The fractions obtained by this author were derived from ground caryopses, and since grinding does not give pure parts of the grain separately, his results are not surprising.

Since alkylresorcinols are found exclusively in the external cuticle, it is of no use to determine their content in ground caryopses, the more so, since from ground material 10 times more lipid material is obtained in extraction with acetone and this interferes with the determinations.

In view of the fact that alkylresorcinols in rye caryopses occupy about 40 per cent of the outer cuticle volume and as much of other lipids is present in it (author's unpublished results), there remains but little room for any structural elements. These results contradict the suggestion advanced earlier by Mejbaum-Katzenellenbogen et al. (1976) that alkylresorcinols are bound with the structure of caryopses. The occurrence of alkylresorcinols in the outer cuticle exclusively and the high content of lipids in this layer exclude such a possibility.

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Author's address:
Dr. Franciszek Tłuścik
Institute of Biochemistry
Wrocław University,
Tamka 2, 50-137 Wrocław
Poland

Lokalizacja alkilorezorcynoli w ziarniakach żyta i pszenicy

Oznaczanie zawartości alkilorezorcynoli w poszczególnych częściach ziarniaka

Streszczenie

1. Opracowano chemiczną metodę oddzielania okryw owocowych ziarniaków polegającą na maceracji ziaren w 85% kwasie ortofosforowym.

2. Opracowano histologiczną metodę wykazywania obecności alkilorezorcynoli, która polega na barwieniu skrawków ziaren roztworem Fast Blue B.

3. Nie stwierdzono obecności alkilorezorcynoli w zarodkach, mące i okrywach owocowych ziarniaków żyta i pszenicy.

4. Alkilorezorcynole wykryto jedynie w kutykuli zewnętrznej ziarniaków.

5. „Stężenie" alkilorezorcynoli w kutykuli zewnętrznej jest duże, gdyż stanowi w życie około 40%, a w pszenicy 20% jej składu.