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Phenolic acids in caryopses of two cultivars of wheat, rye and triticale that display different resistance to pre-harvest sprouting

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Abstract This study confirmed the significant role of phenolics in the dormancy of cereal caryopses. Investigations were conducted on two cultivars of wheat (Elena and Alba), rye (Amilo and Dańkowskie Złote), and triticale (Ugo and Bogo), with characteristic deeper or shallow dormancy, respectively. In germination studies, cultivars susceptible to sprouting (Alba, Dańkowskie Złote and Bogo) displayed higher germination percentages than those resistant to sprouting (Elena, Amilo and Ugo). The phenolic acid contents (i.e. free, liberated from soluble esters, and liberated from soluble glycosides) in caryopses were determined by an HPLC method. Caffeic, *p*-coumaric, ferulic and sinapic acids were the dominant phenolic acids detected. The majority of phenolic acids were found in the form of soluble esters. For all species examined, the levels of phenolic acids liberated from soluble esters and the total phenolic acid contents in caryopses showing shallow dormancy were higher than in those showing deeper dormancy. Slight differences in the UV spectra of extracts of phenolic compounds from caryopses showing shallow or deeper dormancy were noted.

Key words Phenolic acids · Wheat · Rye · Triticale · Dormancy

Introduction

A widespread and economically adverse phenomenon associated with dormancy in cereal grains is pre-harvest sprouting [1, 2]. Precocious germination can occur, especially in gumless caryopses such as rye, triticale and wheat, as early as 2–3 weeks after flowering, up until harvest [3–5]. The interruption of dormancy of cereal caryopses by growth stimulators [3, 4, 6] and low temperatures [3], as well as the demonstrated "dormancy" of isolated deve-loping embryos [3, 4, 7], have indicated that decisive roles may be played by phytohormones, such as gibberellic and abscisic acids [1, 8], and/or inhibitors, such as phenolic compounds, in germination.

Several experiments have shown that phenolic compounds, especially phenolic acids, can act as germination inhibitors [9–11]. The presence of catechol, chlorogenic, salicylic and syringic acids can cause dormancy in small seeds of *Atriplex triangularis.* However, these phenolic acids were not present in larger seeds [10]. A positive correlation between length of dormancy and the content of phenolic compounds was noted for developing and ripening barley caryopses [11]. Exogenous application of phenols to crop and weed seeds was found to inhibit the germination of seeds [10, 12] and embryos [11].

Decreased levels of free, esterified and glycosylated phenolic acids were found for rye, triticale, barley and oat during several months of storage in a dry state [13]. Ferulic and sinapic acids at 1 mM concentration retarded the germination of cereal embryos [14].

The aim of this study was to compare the composition of phenolic acids, including free, esterified and glycosylated forms, in two different cultivars (with deeper or shallow dormancy) of three cereal species (wheat, rye and triticale).

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Materials and methods

The study was conducted on caryopses of wheat with deeper and shallow dormancy (cv. Elena and cv. Alba), rye (cv. Amilo and cv. Dańkowskie Złote), and triticale (cv. Ugo and cv. Bogo) grown in an experimental field of the University of Agriculture and Technology in Olsztyn, Poland.

For the germination test, fresh caryopses were washed with water followed by 70% (v/v) methanol. Germination studies were then carried out by incubating the caryopses on moistened filter paper in petri dishes (120×15 mm) at 21–22 °C for 14 days. All operations were conducted in a laminar hood. There were six replicates per sample date.

Immediately after harvesting the caryopses, phenolic compounds were extracted twice with 80% (v/v) methanol for 15 min at 80 °C [15]. After evaporating the organic solvent in a rotary evaporator at 45 °C, the remaining aqueous solution was lyophilized. The concentration of phenolic compounds in the extract was determined using the Folin-Ciocalteau reagent [16] and (+)-catechin was used as a standard. UV spectra of the extracts were recorded with a Beckman DU 7500 diode array spectrophotometer.

Phenolic acids (i.e. free and those liberated from soluble esters and from soluble glycosides) were isolated from the extract according to a previously described method [17, 18]. An aqueous suspension of the extract (800 mg in 20 ml water) was adjusted to pH 2 (6 M HCl), and free phenolic acids were extracted 5 times into 20 ml diethyl ether using a separating funnel. The ether extract was evaporated to dryness under vacuum at room temperature. The aqueous solution was neutralized and then lyophilized. The residue was dissolved in 20 ml of 2 M NaOH and hydrolyzed for 4 h under a nitrogen atmosphere at room temperature. After acidification to pH 2 using 6 M HCl, phenolic acids released from soluble esters were extracted from the hydrolysate 5 times into 30 ml diethyl ether using a separating funnel. To the water solution, 15 ml of 6 M HCl was added, and the solution obtained was placed under a nitrogen atmosphere and hydrolyzed for 1 h in a water bath at 100 °C. Phenolic acids released from soluble glycosides were separated from the hydrolysate 5 times into 45 ml diethyl ether. After ether evaporation, the dry residue was dissolved in 10 ml methanol and filtered through a 0.45-μm nylon filter. The sample obtained was injected onto an HPLC column. A Shimadzu HPLC system was employed (LC–6A pump, SPD–6AV UV–VIS spectrophotometric detector, SCL–6B system controller and CR 501 Chromatopac). The conditions of the separations were as follows: pre-packed LiChrospher 100 RP–18 column (5 μm, 4×250 mm; Merck); mobile phase, water-acetonitrile-acetic acid (88:10:2, v/v/v) [19]; flow rate, 1 ml/min; injection volume, 20 μl; the detector was set at 300 nm.

Results and discussion

The percentage germination of caryopses from two cultivars of wheat, rye and triticale is shown in Figure 1. During the entire period of the test, the cultivars susceptible to sprouting (Alba, Dańkowskie Złote and Bogo) displayed a higher percentage germination than those resistant to sprouting (Elena, Amilo and Ugo). In addition, the percentage germination was also different for the selected species; for example, the germination of rye caryopses was lower than that of wheat and triticale.

The free phenolic acids found in the investigated caryopses were: caffeic, *p*-coumaric, ferulic, and sinapic acids. Futhermore, trace amounts of free syringic acid were detected (Table 1). The concentrations of free phenolic acids in caryopses of wheat and rye with deeper or shallow dormancy were practically the same: 3.44 μg/g (d.m) in wheat cv. Elena, and 3.52 μg/g (d.m) in wheat cv. Alba; 15.01 μg/g (d.m) in rye cv. Amilo, and 14.62 μg/g (d.m) in rye cv. Dańkowskie Złote. The higher concentration of free phenolic acids in caryopses

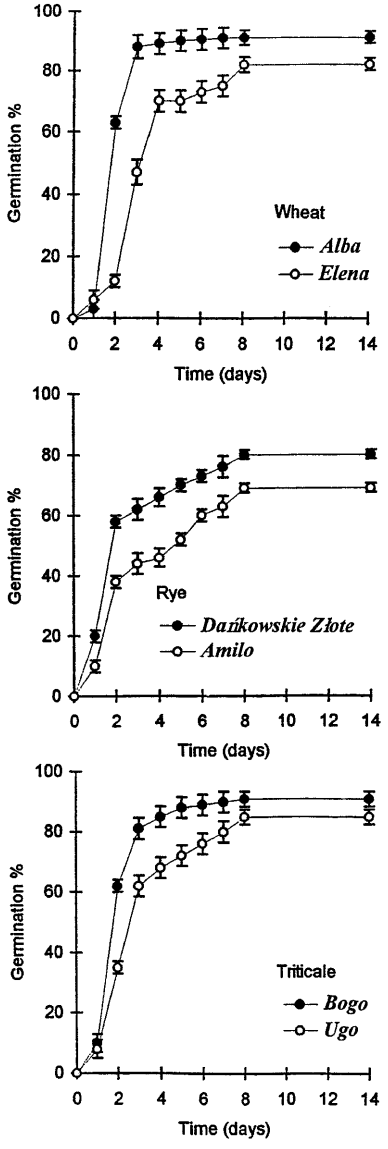

Fig. 1 Germination of wheat, rye, and triticale caryopses

of triticale cv. Ugo with deeper dormancy compared to that of triticale cv. Bogo with shallow dormancy [5.71 and 8.38 μg/g (d.m.), respectively] was due to a high level of caffeic acid in the former [3.42 μg/g (d.m.)].

All caryopses with deeper dormancy possessed a high concentration of phenolic acids in the form of their soluble esters (Table 2). The most notable differences were for rye caryopses: 151.92 μg/g (d.m.) for Amilo with deeper dormancy, and 89.91 μg/g (d.m.) for Dańkowskie Złote with shallow dormancy. For all three cereals, the concentrations of ferulic and sinapic acids, and in the case of wheat and rye also *p*-coumaric, were higher in caryopses with deeper dormancy than in caryopses with shallow dormancy. For example, the concentration of the soluble esters of ferulic acid for wheat cv. Elena with deeper dormancy was almost twice as high as that for wheat cv. Alba with shallow

Table 1 Concentrations of free phenolic acids [μg/g (d.m.)] in cereal caryopses

Phenolic acids	Wheat		Rye		Triticale	
	Alba[a]	Elena[b]	Dańkowskie Złote[a]	Amilo[b]	Bogo[a]	Ugo[b]
Gentisic	–	–	–	–	–	–
Caffeic	0.53	0.48	2.39	2.12	0.67	3.43
Syringic	Trace	Trace	Trace	Trace	Trace	Trace
p-Coumaric	0.70	Trace	3.35	3.37	1.08	1.09
Ferulic	1.99	2.00	6.08	6.11	3.57	3.50
Sinapic	0.30	0.18	2.80	3.41	0.39	0.36
Salicylic	–	–	–	–	–	–
o-Coumaric	–	–	–	–	–	–
Total	3.52	3.44	14.62	15.01	5.71	8.38

[a] Cultivars with shallow dormancy
[b] Cultivars with deeper dormancy

Table 2 Concentrations of phenolic acids liberated from soluble esters [μg/g (d.m.)] in cereal caryopses

Phenolic acids	Wheat		Rye		Triticale	
	Alba[a]	Elena[b]	Dańkowskie Złote[a]	Amilo[b]	Bogo[a]	Ugo[b]
Gentisic	3.33	0.71	0.86	Trace	Trace	0.73
Caffeic	1.00	1.07	1.90	5.23	2.09	0.91
Syringic	1.72	1.20	0.51	2.90	1.42	1.03
p-Coumaric	2.38	10.61	17.33	30.20	7.56	2.54
Ferulic	13.51	24.91	33.22	57.16	24.56	32.91
Sinapic	20.47	28.21	32.00	55.43	27.36	43.06
Salicylic	0.14	2.98	3.01	–	0.29	1.14
o-Coumaric	0.38	0.20	1.08	1.00	0.65	3.44
Total	42.93	69.89	89.91	151.92	63.93	85.76

[a] Cultivars with shallow dormancy
[b] Cultivars with deeper dormancy

Table 3 Concentrations of phenolic acids liberated from soluble glycosides [μg/g (d.m.)] in cereal caryopses

Phenolic acids	Wheat		Rye		Triticale	
	Alba[a]	Elena[b]	Dańkowskie Złote[a]	Amilo[b]	Bogo[a]	Ugo[b]
Gentisic	Trace	Trace	Trace	Trace	Trace	Trace
Caffeic	0.46	1.17	1.46	1.00	0.37	0.27
Syringic	Trace	Trace	Trace	Trace	Trace	Trace
p-Coumaric	0.30	1.83	2.97	3.23	0.82	0.31
Ferulic	1.33	3.47	7.01	6.60	3.63	2.11
Sinapic	1.59	3.20	5.43	6.26	3.68	2.08
Salicylic	–	0.16	–	0.36	0.34	0.23
o-Coumaric	Trace	–	–	Trace	Trace	0.20
Total	3.68	9.83	16.87	17.45	8.84	5.20

[a] Cultivars with shallow dormancy
[b] Cultivars with deeper dormancy

dormancy; the concentration of sinapic acid in triticale cv. Ugo with deeper dormancy was 43.06 μg/g (d.m.), whereas in Bogo with shallow dormancy it was only 27.36 μg/g (d.m.).

A high level of phenolic acids liberated from soluble glycosides correlated with the dormancy of caryopses only for wheat (Table 3). The concentration in the wheat cv. Elena with deeper dormancy was 9.83 μg/g (d.m.), whereas for Alba with shallow dormancy it was only 3.68 μg/g (d.m.). Caryopses of rye cv. Dańkowskie Złote and cv. Amilo showed almost the same amounts of phenolic acids in the form of soluble glycosides: 16.87 and 17.45 μg/g (d.m.), respectively.

The sum of phenolic acids which were free, liberated from soluble esters, and liberated from soluble glycosides was higher for cereals with deeper dormancy than those with shallow dormancy (Fig. 3). For wheat and rye these differences were greater than those for triticale. However, the differences in the concentrations of total phenolics were less marked for cereal cultivars with different levels of dormancy (Fig. 4). Caryopses of wheat cv. Elena with deeper dormancy showed even a slightly lower concentration of total phenolics than those of Alba with shallow dormancy.

A comparison of the results in Figs. 3 and 4 showed that for caryopses of both cultivars of rye, triticale and

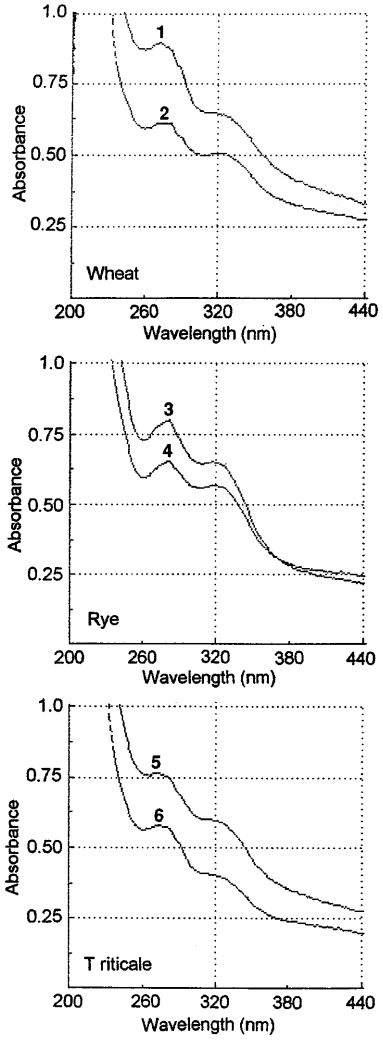

Fig. 2 UV spectra of extracts from caryopses of wheat, rye and triticale

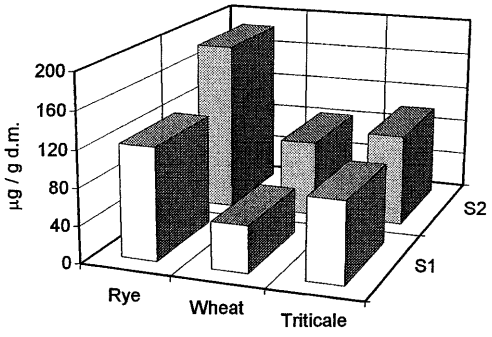

Fig. 3 Total phenolic acids (free, liberated from soluble esters and glycosides) in cereal caryopses. *S2* Cultivars with deeper dormancy (Amilo, Elena, Ugo), *S1* cultivars with shallow dormancy (Dańkowskie Złote, Alba, Bogo)

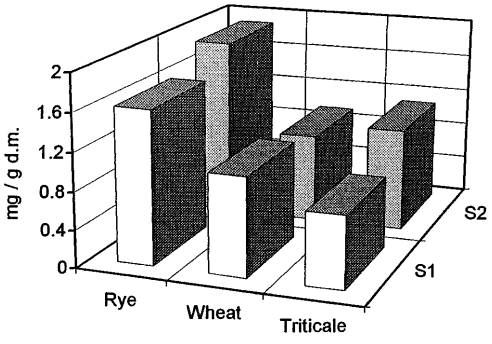

Fig. 4 Total phenolic compounds in cereal caryopses. *S2* Cultivars with deeper dormancy (Amilo, Elena, Ugo), *S1* cultivars with shallow dormancy (Dąkowskie Złote, Alba, Bogo)

wheat, phenolic acids constituted approximately 10% of the total phenolic fraction.

Our investigation indicated that phenolic acids in the form of esters most probably dictate the dormancy period of wheat, rye and triticale caryopses. Weidner et al. [13] confirmed the role of phenolic acids in the dormancy of barley, oat, rye and triticale, as the concentrations of free phenolic acids in dormant caryopses (in all species examined) were much higher than those in non-dormant ones, even when analysed after 6 months of storage in a dry state. During this time esters of phenolic acids could have been hydrolyzed enzymatically to yield free acids.

UV spectra of phenolic compounds extracted from cereal caryopses are presented in Fig. 2. These spectra are characterized by maxima or shoulders ranging from 313 to 324 nm (i.e. characteristic of phenolic acids [20, 21]) and by maxima at shorter wavelenths attributed to other phenolic compounds. Slight differences in the UV spectra of extracts from caryopses with different levels of dormancy were noted. For wheat cv. Elena with deeper dormancy the maximum was noted at 324 nm, whereas for wheat cv. Alba with shallow dormancy only a shoulder was noted at this wavelength. The shift in the maxima of the UV spectra between cultivars with different levels of dormancy was 2 and 3 nm for rye and triticale, respectively.

Comparing our results with those from the literature is difficult because the concentrations of phenolic acids in cereal grains has not been adequately reported. Some variations in results in the literature could have been caused by different methods used for analysis [22]. *P*-Hydroxybenzoic, protocatechuic, syringic, *p*-coumaric, ferulic, gentisic, caffeic and isoferulic acids were present in wheat, with ferulic acid being the most abundant [23, 24]. Pussayanawin and Wenzel [25] reported at concetrations of 500 µg/g and 41 µg/g in whole wheat and its flour, respectively. Sosulski et al. [23] reported ferulic acid at a concentration of 64 µg/g in freshly milled wheat flour, whereas Rybka et al. [24] found as much as 199 µg/g in the same material. Rybka et al. [24] reported that the dominant phenolic acid in rye grain was ferulic acid, although isoferulic, *p*-coumaric, syringic, *p*-hydroxybenzoic and caffeic acids were also detected in minor amounts by TLC. Maga

and Lorenz [26] found *p*-hydroxybenzoic, salicylic, gentisic, protocatechuic, vanillic, syringic, *p*-coumaric, *o*-coumaric, ferulic and isoferulic acids in whole triticale and in the bran, shorts, and flour. Our knowledge about the composition of the flavonoid fraction in cereals is fragmentary. Two di-*C*-glycosylflavones were isolated from the bran of several varieties of wheat [27]. In wheat bran, the total flavonoid concentration varied from 149 to 406 μg/g [28]. Cyanidin 3-glucoside and peonidin 3-glucoside were found as the main anthocyanins of rye [29] and wheat [30].

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