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CONTENT OF PHENOLIC ACID AND YIELDING OF HYBRID WHEAT IN RESPONSE TO GROWING YEAR UNDER INTEGRATED MANAGEMENT

– Research paper –

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Abstract: Wheat grain has health-promoting properties that are related to the content of phenolic acids in its composition, among which ferulic acid, syringic acid, p-coumaric acid and vanillic acid predominate acting as potent natural antioxidants. The study evaluated the phenolic acid (PA) content and grain yield of hybrid wheat genotypes (Hyland, Hywin) compared to the common variety (Patras), grown under an integrated system at two nitrogen rates of 60 (N_{60}) and 120 (N_{120}) kg·ha^{-1}, under varying hydrothermal conditions during three growing seasons. The effects of genotype, cultivar and the interaction of cultivar and hydrothermal conditions on the phenolic acid profile, except for synapic acid, p-hydroxybenzoic acid and salicylic acid, and grain yield were confirmed in favour of the hybrid cultivars. The concentration of total phenolic acids (TPAs) and ferulic acid in the grain of the hybrid cultivar Hyland was higher and, in addition, greater yield stability of this cultivar under varying hydrothermal conditions was shown in the analysed years of the study. The grain of the common variety Patras was characterised by advanced content of p-hydroxybenzoic acid, and that of the hybrid variety Hywin by p-coumaric, caffeic and syringic acids. Higher TPAs content and lower grain yield of the varieties were observed in the 2018/2019 drought season. Higher nitrogen fertilisation (N_{120}) compared to N_{60} significantly increased TPAs content and grain yield of Hyland and Hywin hybrid varieties. Fertilisation with N_{120} compared to N_{60} increased TPAs content by 4.2% and promoted higher concentrations of ferulic and syringic acids in the wheat cultivars tested.

Keywords: hybrid wheat; phenolic acids; hydrothermal conditions, nitrogen fertilization, yield

INTRODUCTION

Cereals constitute a large share in human nutrition due to their availability and scale of consumption and due to their high content of complex carbohydrates, they are an important source of energy provided with food (Gupta et al., 2019; Li et al., 2008, Tobiasz-Salach et al. 2019). Among agricultural crops, wheat is the primary, most frequently cultivated species among cereals, with a production all over the world of 794 million tonnes in 2022 (FAOSTAT 2022).

The high scale of wheat production means that, in addition to its energy value, wheat grain provides protein, macronutrients and micronutrients, B vitamins and dietary fibre in the daily diet. Furthermore, whole grain products from wheat are a primary and valuable source of numerous fractions of phenolic compounds, among which phenolic acids are of particular health-promoting importance (Shewry et al., 2012). In wheat grain, ferulic acid is detect in the highest amounts (representing 85.3-89.3% of the total phenolic acids), which is mainly found in the seed coat, aleurone layer and embryo, and only in trace amounts present in the starchy endosperm of the kernels. In addition, vanillic, synapic, caffeic and also p-coumaric, p-hydroxybenzoic, salicylic and syringic acids, among others, have been discovered in wheat grains (Van Hung 2016).

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Wheat grain yield and quality are impacted by both varietal, agrotechnical and environmental elements (Klikocka et al., 2016; Podolska 2008). High and qualitatively technologically good wheat yields are possible when, during the growing season, all the above-mentioned factors are balanced and optimal, although the yield level obtained and the quality and technological value of the grain may still be variable. The results of studies on the impact of different cropping cultivation technology, especially conventional and organic cultivation technology, on the content and profile of phenolic acids in wheat grain are diverse and inconclusive (Fares et al., 2019; Stracke et al., 2009).

Some scientific studies confirm that the organic cultivation technology, compared to the conventional cultivation technology, improves the antioxidant properties and the amount of phenolic acids in both common and hard wheat grains (Pandino et al., 2020; Żuchowski et al., 2011). Phenolic compounds are formed in plants under the impact of various biotic and abiotic stresses, so in an organic cultivation technology where plant protection products and mineral fertilisers are not used, environmental stresses can stimulate wheat plants to synthesise phenols more intensively. The organic system, despite the higher content of secondary metabolites in the grain and thus better nutritional value, usually results in lower wheat yield (Żuchowski et al., 2009).

An alternative to organic and conventional cropping systems, is the integrated cropping system, which seeks to reduce negative environmental impacts by limiting the use of pesticides and reducing mineral fertilisation rates mainly with nitrogen, while achieving good grain quality and optimal yield levels in wheat (Buczek et al. 2023). Is obvious that nitrogen fertilisers are, a very significant element affecting wheat grain yield and quality, with increases in N fertilisation from 51 to 150 kg·ha^{-1} and even up to 300 kg·ha^{-1} likely to result in higher concentrations of phenolic acids including mainly ferulic acid, vanillic acid and synapic acid, by up to 40% especially compared to unfertilised facilities (Ma et al., 2015). However, some studies show that N rates do not change the phenolic acid content of wheat grain, and that an increase in N fertilisation in the range of 0 to 180 kg·ha^{-1} leads to a drop in the concentration of soluble phenolic acids (Stumpf et al., 2019).

Although the relatively high importance of agronomic factors, also environmental factors or climatic factors (rainfall and temperature) can impact and vary the phenolic acid profile. Insufficient quantity of water and rise average temperatures occurring during the grain-filling phase tend to increase the synthesis of phenolic acids. An influence element affecting phenolic acid composition is the species or genotype of the wheat variety (Tian et al., 2022). Most studies in the literature are concerned with the characterisation and content of phenolic acids in common wheat, durum wheat or spelt grain. It has been shown that the sum of phenolic acids in the grain of wheat species increases according to a trend (Li et al., 2008). There is not much information about the response of hybrid wheat varieties to environmental (temperature, precipitation) and agronomic (nitrogen rate) factors, not only on yield (yield) but also on grain health-promoting traits (Zhao et al. 2013).

Therefore, the purpose of the study was to demonstrate the effect of hybrid wheat genotype on productivity and phenolic acid content and profile in grain compared to common wheat (common). Yield levels and bioactive compounds were evaluated in the context of limited, balanced nitrogen fertilisation in an integrated cultivation technology and under different weather case during three growing seasons. The research conducted can provide important information for the quality breeding and development of cultivation technologies of hybrid wheat varieties, which can be an alternative to common wheat varieties grown and intended for consumption.

MATERIAL AND METHODS

Field experiments

Winter wheat varieties, two hybrids Hyland and Hywin (breeder Saaten Union Recherche SAS, Estrées-Saint-Denis, France) and one common Patras (breeder Deutsche Saatveredelung AG, Lippstadt, Germany), were grown in an integrated system on the experimental fields of the Experimental Station of Variety Evaluation in Przecław (50°11'N, 21°29'E, altitude 200 m). Varieties, hybrid Hyland and Hywin (quality class B) and common Patras (quality class A), are bread varieties with high grain quality and good yield potential. The two-factor experiment was implemented for three consecutive growing seasons in 2016-2019 in an equivalent block arrangement in four replicates, and the harvesting plot area was 16 m^2.

In the integrated system, mineral fertilisation rates were reduced and plant protection products were restricted. Wheat was grown in a winter rape - pea -

winter wheat rotation, with autumn application of phosphorus (50 kg $P \cdot ha^{-1}$) and potassium (70 kg $K \cdot ha^{-1}$) fertilisers. Nitrogen fertilisation rates were as follows: N_{60} - 60 $kg \cdot ha^{-1}$ (20 kg after the start of vegetation and 40 kg at the stalk shooting stage) and N_{120} - 120 $kg \cdot ha^{-1}$ (20 kg after the start of vegetation, 40 kg at the stalk shooting stage, 40 kg at the flag leaf stage and 20 kg during earing). Weeds were controlled mechanically in autumn (harrowing 1x) and spring (harrowing 1x) and herbicide was applied (metribuzin + flufenacet, dose 0.35 $l \cdot ha^{-1}$). In addition, an insecticide (lambda-cyhalothrin) at a dose of 0.35 $l \cdot ha^{-1}$ was used for plant protection. The herbicide and insecticide were performed at the developmental stages of wheat as described by the manufacturer's recommendations.

The sowing date of the wheat varieties was in the first decade of October, at 160 (Hyland and Hywin hybrid varieties) and 320 (Patras common variety) seeds/m^2. The wheat varieties were harvested at full grain maturity and the yield obtained is given in $t \cdot ha^{-1}$.

Soil and weather conditions

The experiment wasucted on a soil with a granulometric composition of silty clay (SiL; Ditzler et al., 2017), classified as Fluvic Cambisol (CMfv; IUSS 2015). The pH values specified with 1 M KCl indicate a slightly acidic or neutral soil pH (pH 6.35 to 7.42). Organic carbon content was medium (18.5-20.1 $g \cdot kg^{-1}$) and mineral nitrogen (N_{min}) in the soil was low (54.1-58.9 $kg \cdot ha^{-1}$). The soil was very high in assimilable phosphorus (101.5-134.2 $mg \cdot kg^{-1}$) and magnesium (135.1-150.8 $mg \cdot kg^{-1}$) and medium in potassium (130.1-159.2 $mg \cdot kg^{-1}$).

Weather conditions during the autumn growing season were favourable (Table 1). Only in the 2018/2019 season the amount of rainfall total was 43.3 mm lower similed to the multi-year data. The mean temperature during the winter dormancy of the plants was advanced and the total precipitation slightly lower than the multi-year data. The total rainfall during the spring-summer growing season in 2016/2017 and 2018/2019 was higher than in 2017/2018. The spring-summer growing season in 2016/2017 and 2018/2019 can therefore be classified as excessively wet (K = 2.05 and 1.82) and 2017/2018 as optimal (K = 1.08). A particularly high precipitation deficit occurred during the 2018/2019 season in June (K = 0.30) and July (K = 0.62), resulting in periodic drought during this time (Figure 1).

Table 1. Sielianinov's hydrothermic coefficients (K)

Year	Month (spring-summer period)				Mean (K)	t (°C)	p (mm)
	April	May	June	July			
2016/2017	3.69 (e/m)	2.78 (e/m)	0.81 (s/d)	0.90 (s/d)	2.05 (e/m)	6.6	616.8
2017/2018	0.40 (d)	1.33 (o)	0.92 (s/d)	1.68 (e/m)	1.08 (o)	7.5	587.8
2018/2019	2.83 (e/m)	3.53(e/m)	0.30 (d)	0.62 (s/d)	1.82 (e/m)	7.9	514.4
1956-2015	1.70 (e/m)	1.59 (e/m)	1.41(o)	1.45 (o)	1.54 (e/m)	7.3	545.2

K: < 0.5 – drought (d), 0.5–1.0 – semi-drought (s/d), 1.1–1.5 – border of optimal moisture (o), >1.5 – excessive moisture (e/m), p (mm) – sum rainfall from sowing-harvest/09–07, t (°C) – mean temperature from sowing-harvest/09–07.

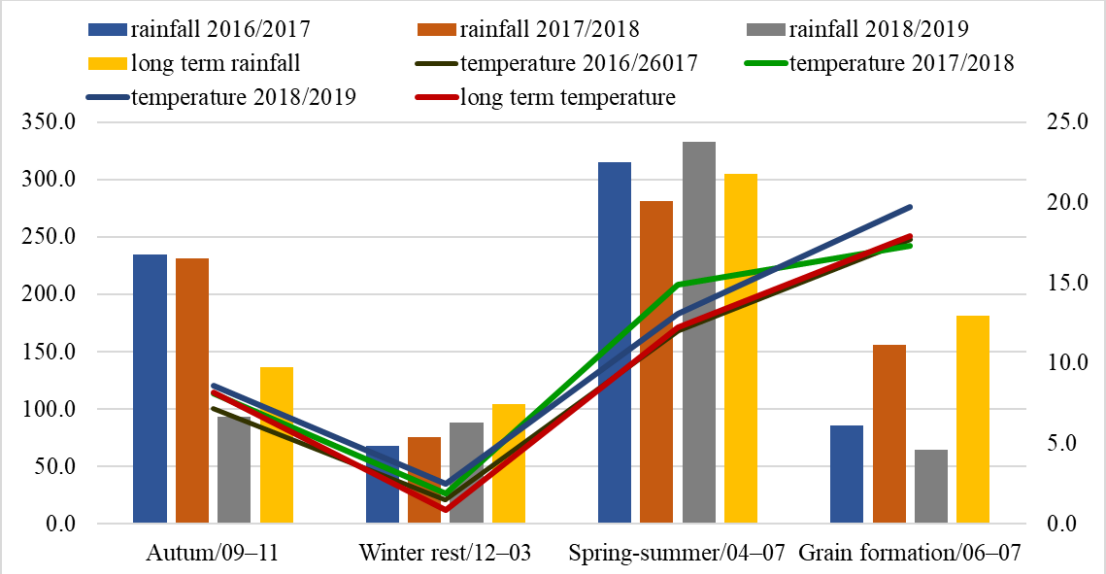

Figure 1. Weather conditions for three growing seasons (period/months)

The formula (1) was used (Skowera 2014):

$$K = [(p \times 10)/\Sigma t] \quad (1)$$

Where: K is the Selyaninov's hydrothermal coefficient for an individual month during the growing season; p – signifies the monthly sum of rainfall; Σt – monthly sum of air temperatures > 0°C from a given month.

Extraction of phenolic acids (PA)

Phenolic acids were obtained and determined using the method of Mpofu et al. (2006) as modified by Żuchowski et al. (2009). To a ground wheat sample (2.0 g), 60 ml of 4 M NaOH was added and the mixture was homogenised for 10 min at 7000 rpm on a centrifuge (Centrifuge 5427 R, Eppendorf, Individual PAs were analysed using the UPLC PDA-MS/MS method on an ACQUITY UPLC Systems chromatograph (Waters Corporation, Milford, MA, USA) combined with a Waters ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm) and equipped with a photodiode array (PDA) detector. The following capillary parameters were used: capillary voltage 3.5 kV, con voltage 30 V in negative mode, the source was kept at 250°C and desolvation temperature was 350°C. Nitrogen was used as desolvation gas (flow rate of 1000 L/h) and cone gas (flow rate of 100 L/h). Argon was used as collision gas at a flow rate of 0.3 mL/min. The mobile phase for analysis contained solvent A (0.1% formic acid in water, v/v) and solvent B (40% acetonitrile in 0.1% formic acid, v/v). The solvent gradient was programmed as follows: 5 min, 0% B; 0.5 min, 1% B; 2.5 min, 10% B; 10 min,10–100% B; 1 min, 100% B; 1 min, 100–0% B. A flow rate of 0.3 mL/min was maintained, the column temperature at 50°C, a the injection volume was 5 µL.

Nussloch, Germany). After hydrolysis, the sample was acidified with 2.5 ml of 6 M HCl at pH 2. The supernatants subjected to extraction three times using 70.0 ml of ethyl acetate. The organic phase was collected, filtered and evaporated to dryness at 45°C in a rotary evaporator (Rotavapor 100, Buchi, Flawil, Szwajcaria). The residue after evaporation was dissolved in 50% acetonitrile (2.0 ml) and stored in a refrigerator. After centrifugation (millipore, 0,45 µm), samples were diluted 10-fold with ultrapure water. Samples were extracted twice and used for further UPLC analysis.

Quantification and identification of phenolic acids (PA)

Identification of phenolic acids was made using by comparing UV spectra and peak retention times in wheat extracts for standard compounds. Standard curves were used for calculation of the concentration of phenolic acids in the samples. The following phenolic acids were quantified: ferulic acid (Fer), sinapic acid (Sin), p-coumaric acid (p-Cou), caffeic acid (Caf), va-nillic acid (Van), syringic acid (Syr), p-hydroxybenzoic acid (p-Hb), protocatechuic acid (Pca) and salicylic acid (Sa). Determinations were performed in duplicate.

Statistical analysis

Statistical analysis of the results was performed using the ANOVA method, using the statistical software Analwar-5.1FR (Bialystok University of Technology, Poland) and Statistica 13.3. (TIBCO Software Inc. Palo Alto. CA. USA). The considerable of differences between results was verified using the Tukey's test (LSD), at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

The effect of genotype (C) and nitrogen fertilization (N) on the content of phenolic acids (PA)

The phenolic acid (PA) content of wheat grain can be modified by environmental and agronomic factors including nitrogen fertilisation, with the cultivar genotype and the interaction of cultivar genotype and environment having a decisive influence (Tian et al., 2022; Ma et al., 2015). In the experiment conducted, statistical analysis showed a considerable effect of variety (C) and hydrothermal conditions in the test years (Y) and their C x Y interaction on the content (TPAs) and PA profile in wheat grain (Table 2). Fertilisation rates (N) also caused variable modification of the composition of TPAs and PA in the grain of the wheat varieties tested. The average TPAs content was 672.45 $\mu g \cdot g^{-1}$, which was not different from the TPAs value (556.0 to 654.0 $\mu g \cdot g^{-1}$) found by Horvat et al. (2020), and significantly different from the very high TPAs values reported (1709.0 to 1990.0 $\mu g \cdot g^{-1}$) by Mpofu et al. (2006).

In our study, nine phenolic acids were analysed in the grain, including ferulic acid (Fer), sinapic acid (Sin), p-coumaric acid (p-Cou), caffeic acid (Caf), vanillic acid (Van), syringic acid (Syr), p-hydroxybenzoic acid (p-Hb), protocatechuic acid (Pca) and salicylic acid (Sa) (Table 3).

Table 2. Total content of phenolic acids (TPAs) of wheat grain depending on wheat cultivars, N fertilization and growing years (μg·g^{-1})

Factor	Cultivar (C)			Mean
	Patras	Hyland[1]	Hywin [1]	
Nitrogen (N)				
N_{60}	593.41^{d} ±14.01	709.72^{b} ±26.67	670.90^{c} ±26.48	658.01^{b} ±24,12
N_{120}	598.10^{d} ±42.10	745.11^{a} ±9.58	717.45^{b} ±16.40	686.89^{a} ±18,26
		p N x C *		*p nitrogen (N)* *
Year (Y)				
2016/2017	592.08^{d} ±8.86	716.85^{b} ±36.26	683.04^{c} ±29.15	663.99^{b} ±28.75
2017/2018	590.51^{d} ±16.43	710.85^{b} ±41.42	686.76^{c} ±33.23	662.71^{b} ±25.65
2018/2019	604.69^{d} ±16.10	754.55^{a} ±30.18	712.74^{b} ±25.47	690.66^{a} ±32.85
		p C x Y ***		*p year (Y)* **
Mean	595.76^{c} ±38.60	727.42^{a} ±32.49	694.18^{b} ±25.71	672.45 ±57.15
		p cultivar (C) ***		

[1] – hybrid cultivars, ± standard deviation, Different letters in the same column indicate significant differences [p<0.05], Significance at: *p < 0.05; *** p < 0.001

Table 3. Individual phenolic acids composition (μg/g) of wheat grain [mean for cultivars (C) and N fertilization (N)]

Factor	Fer	Sin	p-Cou	Caf	Van	Syr	p-Hb	Pca	Sa
Cultivar (C)									
Patras	517.15^{c} ±12.04	38.93^{a} ±3.15	10.81^{b} ±1.22	1.02^{b} ±0.37	10.21^{a} ±1.10	7.18^{b} ±1.17	7.67^{a} ±2.14	1.66^{b} ±0.40	0.28^{a} ±0.04
Hyland [1]	652.38^{a} ±35.27	38.89^{a} ±3.31	9.13^{b} ±1.38	1.18^{b} ±0.61	10.18^{a} ±1.40	7.95^{b} ±2.18	5.18^{b} ±1.20	1.90^{a} ±0.37	0.25^{a} ±0.04
Hywin [1]	613.92^{b} ±22.21	39.45^{a} ±5.20	12.25^{a} ±2.45	2.36^{a} ±0.72	9.36^{b} ±1.51	9.64^{a} ±2.49	5.26^{b} ±1.60	1.16^{b} ±0.32	0.25^{a} ±0.05
p cultivar (C)	***	*ns*	*	*	**	**	**	*	*ns*
Nitrogen (N)									
N_{60}	582.24^{b} ±54.29	38.64^{a} ±3.18	10.59^{a} ±1.74	1.50^{a} ±0.57	9.52^{a} ±1.00	7.43^{b} ±1.90	5.24^{b} ±1.60	1.44^{a} ±0.47	0.25^{a} ±0.04
N_{120}	606.72^{a} ±45.10	39.54^{a} ±3.51	10.87^{a} ±1.62	1.54^{a} ±0.31	10.31^{a} ±1.29	9.09^{a} ±1.21	6.84^{a} ±1.42	1.71^{a} ±0.51	0.27^{a} ±0.05
p nitrogen (N)	**	*ns*	*ns*	*ns*	*	**	**	*ns*	*ns*
Mean	594.48	39.09	10.73	1.52	9.92	8.26	6.04	1.57	0.26

[1] – hybrid cultivars, Fer – ferulic acid, Sin – sinapic acid, p-Cou – p-coumaric acid, Caf – caffeic acid, Van – vanillic acid, Syr – syringic acid, p-HB – p-hydroxybenzoic acid, Pca – protocatechuic acid, Sa – salicylic acid, ± standard deviation, Different letters in the same column indicate significant differences [p<0.05], Significance at: *p < 0.05; **p < 0.01; *** p < 0.001; ns – not significant

The average PA content of the total phenols (TPAs) decreased in the order Fer (88.5%) < Sin (5.8%) < p-Cou (1.6%) < Van (1.5%) < Syr (1.2%) < P-Hb (0.9%) < Caf (0.2%) = Pca (0.2%) < Sa (0.1%). The highest amount of Fer acid was obtained in the grains of the varieties tested, with an average Fer acid content in the total phenolic acids (TPAs) ranging from 86.6 for the common variety Patras to 89.7% for the hybrid Hyland. According to Irmak et al. (2008) and Kurasiak-Popowska et al. (2016), the Fer acid contents of the grains of wheat cultivars show great variation, with the proportion of Fer acid in TPAs being dominant. The contents of p-Cou, Caf, Van and Syr acids in the grain of hybrid cultivar Hyland compared to common Patras were not statistically different. Similarly, there were no considerably differences in the amount of p-Hb acid between Hyland and Hywin hybrid varieties and Pca for Patras and Hywin varieties. There were no static intervarietal differences in the amount of Sin and Sa acids. Among the cultivars, the hybrid cultivar Hyland had a considerably higher content of TPAs compared to the hybrid cultivar Hywin and the common cultivar Patras, by 4.6 and 18.1%, respectively. A study by Czaban et al. (2013) shows that the ranges of the content of the more important PAs determined in wheat grain are a genotypic trait of the varieties and can fluctuate within wide limits taking the following values: Fer (545.7-645.8 μg·g^{-1}), Sin (43.3-50.2 μg·g^{-1}), Van (10.6-13.4 μg·g^{-1}), p-Cou (10.9-12.5 μg·g^{-1}) and Syr (8.1-10.9 μg·g^{-1}).
Fertilisation of wheat varieties with N_{120} compared to N_{60} favoured the accumulation of mainly p-Hb (by 23.4%), Syr (by 18.8%), Van (by 7.7%) and Fer (4.0%) acids in the grain. The lower dose of N_{60} resulted in a 4.2% limited in TPAs compared to

N_{120}. No statistical differences were found between N fertilisation rates in the content of Sin, p-Cou, Caf, Pca and Sa acids. They obtained similar research results by Tian et al. (2022), where N rates (from 0 to 168 kg N·ha^{-1}) significantly differentiated the contents of only para-coumaric, sinapic and cis-ferulic acids, while they did not affect the amounts of TPAs. There is a statement that the obtained results suggest that varying the N dosage influenced the hidden metabolic pathway of various Pas. Therefore, according to Shewry et al. (2012), the aim of modern wheat breeding is to obtain new varieties with better health properties which seems to be a practical solution. Also according to Stumpf et al. (2018), phenolic acids (PA) including Fer acid and total phenolic acids (TPAs) did not show significant changes in wheat grain fertilised with different N rates (0 to 180 kg N·ha^{-1}).

Our own research showed an interaction between variety (C) and nitrogen (N) fertilisation with the exception of Caf, Van, Pca and Sa acid content (Table 4). A higher dose of N_{120} compared to N_{60} influenced the increase in TPAs content of hybrid varieties Hyland and Hywin without differentiating TPAs in common variety Patras. The N_{120} dose favoured an increase in Fer and Syr acid content in all cultivars. In addition, Sin and p-Cou acids in the grain of the hybrid variety Hyland and the common Patras variety, and p-Hb acid in both hybrid cultivars without differentiating its content in the common Patras variety. In our study, the content of Sin and p-Cou acids in the grain of the hybrid variety Hywin was lower for the N_{60} application rate compared to N_{120}, indicating a differential response of this cultivar to N fertilisation. According to Giorgi et al. (2009) when N application is limited, plants transform their metabolism production purpose carbon-containing compounds such as starch, cellulose and N-free compounds, including phenolic acids.

The effect of hydrothermal conditions on the content phenolic acids (PA)

The PA content of wheat grain is differentiated by the weather pattern (temperature, precipitation) during the growing season (Laddomada et al., 2021), which was confirmed in our own study. Hydrothermal conditions significantly modified the total (TPAs) and phenolic acids (PA) profile of wheat grain (Table 2). The highest phenolic acids (PA) and their sum (TPAs) were contained in wheat grain from the 2018/2019 season, in which a large rainfall deficit in June (K = 0.30) and July (K = 0.62) caused periodic drought (Table 5).

Table 4 . Effect of wheat varieties and N fertilization on composition of phenolic acids (μg·g^{-1})

Nitrogen (N)	Cultivar (C)	Fer	Sin	p-Cou	Caf	Van	Syr	p-Hb	Pca	Sa
N_{60}	Patras	514.19^{b} ±11.14	37.59^{b} ±2.34	11.59^{a} ±1.41	0.98^{a} ±0.32	11.28^{a} ±1.01	6.89^{b} ±0.63	7.40^{a} ±2.11	1.54^{a} ±0.42	0.27^{a} ±0.02
	Hyland [1]	638.26^{c} ±22.15	37.85^{b} ±4.76	9.31^{c} ±2.21	1.36^{a} ±0.35	8.98^{b} ±0.71	7.67^{b} ±2.34	3.52^{b} ±1.02	1.79^{a} ±0.21	0.23^{a} ±0.02
	Hywin [1]	594.27^{d} ±27.00	40.48^{a} ±3.10	10.88^{b} ±0.52	2.17^{b} ±0.32	8.31^{b} ±1.02	7.74^{b} ±2.19	4.80^{b} ±1.19	0.97^{b} ±0.34	0.26^{a} ±0.04
N_{120}	Patras	520.11^{e} ±41.10	40.27^{a} ±1.55	10.03^{b} ±0.90	1.06^{a} ±0.74	9.15^{b} ±1.05	7.48^{a} ±1.74	7.94^{a} ±0.44	1.77^{a} ±0.25	0.29^{a} ±0.03
	Hyland [1]	666.50^{a} ±4.37	39.93^{a} ±4.11	8.96^{b} ±1.45	1.00^{a} ±0.21	11.38^{a} ±1.34	8.24^{a} ±0.54	6.84^{a} ±2.10	2.00^{a} ±0.40	0.27^{a} ±0.04
	Hywin [1]	633.56^{a} ±10.01	38.41^{b} ±3.24	13.63^{d} 2.12	2.56^{b} ±0.24	10.41^{a} ±0.58	11.55^{c} ±0.45	5.73^{c} ±0.70	1.35^{c} ±0.14	0.25^{a} ±0.05
p N x C		**	**	**	*ns*	*ns*	*	*	*ns*	*ns*

Legend: as in Table 3.

Table 5. Individual phenolic acids composition (μg·g^{-1}) of wheat grain [mean for growing years (Y)]

Factor	Fer	Sin	p-Cou	Caf	Van	Syr	p-Hb	Pca	Sa
Year (Y)									
2016/2017	588.22^{b} ±51.30	38.78^{b} ±3.22	10.41^{b} ±1.70	1.51^{a} ±0.62	9.64^{b} ±1.33	7.99^{b} ±2.14	5.74^{b} ±1.70	1.45^{b} ±0.34	0.23^{a} ±0.04
2017/2018	588.05^{b} ±57.30	37.40^{b} ±3.70	9.91^{b} ±1.74	1.33^{b} ±0.70	9.17^{b} ±1.40	7.69^{b} ±2.43	5.77^{b} ±1.69	1.42^{b} ±0.53	0.27^{a} ±0.05
2018/2019	607.19^{a} ±48.21	41.09^{a} ±3.95	11.88^{a} ±1.56	1.73^{a} ±0.68	10.95^{a} ±1.09	9.10^{a} ±2.40	6.61^{a} ±1.52	1.84^{a} ±0.36	0.28^{a} ±0.04
p year (Y)	***	**	**	*	*	**	*	*	*ns*
Mean	594.48	39.09	10.73	1.52	9.92	8.26	6.04	1.57	0.26

Legend: as in Table 3.

Heat stress during wheat maturation may interfere with this process and therefore more PA w occurs in wheat grain (Liu et al., 2018). An rise in the accumulation of phenolic acids in wheat grain as an effect of drought stress is pointed out by Stumpf et al. (2019). In contrast, weather conditions in the 2016/2017 and 2017/2018 seasons influenced the lower accumulation of TPAs in wheat grain and the analysed acids with the exception of Caf acid. The years of study did not modify the content of Sa acid. Stracke et al. (2009) showed that crop year was the most important element during the three-year study period under organic farming cultivation technology that influenced the variation in PA.

The profile of PAs and TPAs in wheat grain was shaped by the interaction of variety (C) and years of experiment (Y) (Table 6). Hyland and Hywin hybrid varieties showed higher TPAs content in the 2018/2019 season than in the other years of the study. In the 2018/2019 season, the grains of the hybrid cultivars had the highest content of p-Cou, Caf and Syr acid (Hywin) and Pca acid (Hyland), the common Patras variety p-Hb acid, and the Patras and Hyland varieties Van acid. The interaction between C x Y showed that the hybrid variety Hyland, compared to Hywin and Patras, had a higher Fer acid content over the years of the study. Also, Stumpf et al. (2019) and Tian et al. (2022) demonstrated that final PA concentrations in wheat grains were determined by the cooperation effect between wheat cultivars and milieu conditions (test years, location).

Grain yield variability

In the experiments conducted, the average grain yield was 6.94 $t \cdot ha^{-1}$ (Table 7).

Table 6. Effect of wheat varieties and growing years on composition of phenolic acids ($\mu g \cdot g^{-1}$)

Cultivar (C)	Year (Y)	Fer	Sin	p-Cou	Caf	Van	Syr	p-Hb	Pca	Sa
Patras	2016/2017	516.68^{c} ±7.41	38.43^{b} ±2.12	10.41^{b} ±1.17	1.01^{d} ±0.22	9.75^{b} ±1.05	6.86^{f} ±0.68	7.14^{c} ±2.01	1.52^{bc} ±0.31	0.24^{b} ±0.03
	2017/2018	514.85^{c} ±15.15	37.01^{b} ±2.16	9.80^{b} ±1.24	0.80^{e} ±0.30	9.43^{c} ±1.06	6.75^{f} ±0.75	7.50^{b} ±2.52	1.62^{bc} ±0.44	0.28^{b} ±0.05
	2018/2019	519.93^{c} ±10.41	41.36^{a} ±3.20	12.23^{a} ±1.12	1.25^{c} ±0.38	11.46^{a} ±1.01	7.94^{d} ±1.10	8.38^{a} ±1.89	1.84^{b} ±0.31	0.32^{a} ±0.04
Hyland [1]	2016/2017	643.84^{a} ±32.47	38.38^{b} ±2.52	8.90^{cb} ±0.94	1.20^{c} ±0.60	9.94^{b} ±1.31	7.71^{d} ±1.78	4.94^{e} ±1.13	1.72^{b} ±0.20	0.23^{b} ±0.06
	2017/2018	639.57^{a} ±40.20	37.69^{b} ±2.40	8.40^{c} ±1.01	1.00^{d} ±0.74	9.06^{d} ±1.35	7.26^{e} ±1.95	4.94^{e} ±1.00	1.59^{bc} ±0.18	0.27^{b} ±0.05
	2018/2019	673.73^{a} ±26.37	40.60^{a} ±3.28	10.10^{b} ±1.23	1.35^{c} ±0.51	11.55^{a} ±1.15	8.90^{c} ±2.40	5.68^{d} ±1.12	2.39^{a} ±0.35	0.26^{b} ±0.04
Hywin [1]	2016/2017	604.13^{b} ±19.15	39.54^{b} ±4.14	11.93^{cd} ±2.61	2.33^{a} ±0.52	9.22^{c} ±1.31	9.42^{b} ±2.35	5.14^{e} ±1.71	1.12^{e} ±0.28	0.24^{b} ±0.05
	2017/2018	609.72^{b} ±22.14	37.51^{b} ±5.11	11.53^{cd} ±2.61	2.19^{b} ±0.61	9.01^{d} ±1.60	9.07^{c} ±2.67	4.86^{e} ±1.43	1.07^{e} ±0.38	0.27^{b} ±0.04
	2018/2019	627.91^{b} ±18.11	41.30^{a} ±5.62	13.30^{a} ±2.21	2.58^{a} ±0.66	9.85^{b} ±1.11	10.45^{a} ±2.28	5.79^{d} ±1.38	1.30^{d} ±0.28	0.26^{b} ±0.06
p C x Y		***	*	***	***	*	***	***	*	*ns*

Legend: as in Table 3.

Table 7. Grain yield depending on wheat cultivars, N fertilization and growing years ($t \cdot ha^{-1}$)

Factor	Cultivar (C)			Mean
	Patras	Hyland [1]	Hywin [1]	
Nitrogen (N)				
N_{60}	6.53^{b} ±0.89	6.91^{c} ±0.92	6.46^{b} ±0.79	6.64^{b} ±0.72
N_{120}	6.95^{c} ±0.95	7.64^{a} ±1.02	7.16^{c} ±0.93	7.25^{a} ±0.81
		p N x C *		*p nitrogen (N)* ***
Year (Y)				
2016/2017	6.07^{e} ±1.01	6.92^{c} ±1.01	6.62^{d} ±1.01	6.54^{b} ±0.72
2017/2018	8.27^{a} ±1.01	8.35^{a} ±1.01	7.79^{b} ±1.01	8.13^{a} ±1.01
2018/2019	5.89^{e} ±1.01	6.57^{d} ±1.01	6.03^{e} ±1.01	6.16^{b} ±0.85
		C x Y ***		*p year (Y)* ***
Mean	6.74^{b} ±0.86	7.28^{a} ±1.15	6.81^{b} ±0.78	6.94 ±1.10
		p cultivar (C) ***		

[1] – hybrid cultivars, ± standard deviation, different letters in the same column indicate significant differences [p<0.05], Significance at: *p < 0.05; *** p < 0.001; ns – not significant

The elements of the experiment significantly influenced the yield of winter wheat varieties. Also Rozbicki et al. (2015) in their study showed the differential effect of genotype, nitrogen fertilisation and weather factors and their interaction between them, on the obtained grain yield of wheat. The application of a higher N_{120} nitrogen fertilisation rate resulted in a considerable rise in wheat grain yield liken to the N_{60} rate, by an average of 0.61 t·ha^{-1}. Klikocka et al. (2016) showed no significant rise in grain yield after application of 80 kg N·ha^{-1} (5.40 t·ha^{-1}) compared to 120 kg N·ha^{-1} (5.59 t·ha^{-1}). In a study by Podolska (2008), the application of 120 kg N·ha^{-1} applied in two split doses (60+60 kg N·ha^{-1}) was more favourable for grain yield liked to the dose of N applied three times, and grain yield ranged from 7.20 to 10.50 t·ha^{-1}. In our study, the wheat was grown in an integrated system with a lower intensity of plant protection inputs, which favours environmental protection, but may have resulted in a lower range of grain yield (6.64 to 7.25 t·ha^{-1}) depending on the nitrogen dose. This is confirmed by a study by Kołodziejczyk and Szmigiel (2014), who showed a 1.49 t·ha^{-1} decrease in wheat grain yield in the integrated cultivation technology compared to the conventional cultivation technology, with a 40 kg·ha^{-1} reduction in nitrogen dose and no fungicide application. Irrespective of the nitrogen fertilisation rate applied, in our study the highest yielding variety was hybrid Hyland (7.28 t·ha^{-1}) and the lowest yielding variety was common Patras (6.74 t·ha^{-1}). The common Patras variety achieved significantly lower yields than the hybrid varieties Hyland (by 7.4%) and Hywin (by 6.5%). The increase in yield levels of hybrid wheat varieties compared to common can range from 0.5 to 1.5 t·ha^{-1}, with hybrid varieties having yield stability and better adaptation to changing environmental conditions, mainly rainfall deficiency (Gupta et al., 2019; Whitford et al., 2013; Zhao et al., 2013).

The interaction of nitrogen (N) and variety (C) fertilisation showed that the varieties analysed responded to an increase in nitrogen fertilisation at a rate of N_{120}. A similar increase in grain yield was obtained for the hybrid varieties Hyland (by 9.6%) and Hywin (by 9.8%), while the lowest was found for the common variety Patras (by 6.1%). The genotypic effect of wheat cultivars and different N share and their influence on wheat grain yield was also demonstrate in the study of Haque et al. (2017). On the other hand, Šip et al. (2013) and Mariem et al., (2020) showed varietal variation in grain yield, with the interaction of variety and nitrogen fertilisation not affecting the variation in grain yield. Zrcková et al. (2018) in their study proved the significant effect of milieu and especially weather conditions as considerable factors determining grain yield and quality in winter wheat.

In our study, temperature distribution and rainfall total that occurred during the study seasons differentiated the yield of wheat varieties. Lower grain yields were obtained in the 2016/2017 and 2018/2019 seasons (by 1.59 and 1.97 t·ha^{-1}, respectively) compared to the 2017/2018 season. The 2016/2017 season was wet (K=2.05) with a total rainfall of 616.8 mm. According to Šip et al. (2013) and Linina and Ruza (2018), excessive rainfall throughout the growing season, can be a limiting factor for yield levels because it reduces the efficiency of nitrogen fertilisation and fungicide application in wheat. In the 2017/2018 season where favourable hydrothermal conditions were recorded, especially during the spring-summer growing season (K=1.08), a considerable rise in grain yield was obtained which was 8.13 t·ha^{-1}. In addition, an interaction between variety (C) and years of study (Y) was shown, where promotable weather conditions in 2017/2018 influenced the increase in yield of the varieties tested. In the 2017/2018 season, the common variety Patras had a similar grain yield to the hybrid variety Hyland, and significantly higher (by 5.8%) than the variety Hywin. The growing season of 2018/2019, with a higher air temperature of 7.8°C and lower rainfall of 514.4 mm, resulted in lower wheat grain yield. With the Patras and Hywin varieties responding most to weather stress with a reduction in grain yield. The hybrid variety Hyland was more resistant to rainfall deficiency and achieved a higher yield level under these conditions. The favourable distribution of precipitation and temperature from the earing to maturity stage of wheat as the primary factor determining higher grain yield is also pointed out by Zrcková et al. (2018).

CONCLUSIONS

The variation of phenolic acids in grain and the yield of wheat varieties depended largely on the genotype of the variety and hydrothermal factors during the study years and on nitrogen fertilisation. The hybrid cultivar Hyland was characterised by a superior content of phenolic acids including mainly ferulic acid, and had the highest grain yield in the years of the study and in the 2018/2019 season in

which there was a drought during the grain maturation period. This demonstrates the yield stability of this variety, what could be an alternative to the conventional variety in years with periodic rainfall deficiency during the growing season. Hydrothermal conditions with smaller rainfall and higher monthly temperatures during the forming and maturing period of wheat resulted in lower yield but increased phenolic acids in the grain of the wheat varieties. The higher nitrogen rate increased the content of total phenolic acids in the grain of Hyland and Hywin hybrid varieties without differentiating this value in the common Patras variety. The interaction of variety and nitrogen fertilisation showed that the N_{120} dose compared to N_{60} favoured an rise in grain yield and content of mainly ferulic and syringic acids in all wheat varieties. The results obtained indicate that it is possible to grow hybrid varieties under varying hydrothermal conditions with high phenolic acid concentrations and stable grain yields within the principles of integrated agriculture. Analysis of the effect of cultivar interaction and hydrothermal conditions on the profile of phenolic acids in grain can provide valuable information for improving breeding work aimed at producing wheat varieties rich in health-promoting phenolic compounds. Currently, in plant production, in accordance with the assumptions of the European Green Deal, efforts should be made to reduce the use of pesticides and doses of mineral fertilization as much as possible by introducing sustainable farming systems that are more environmentally friendly and at the same time ensure food security. The basis of such a management system is integrated plant production, with a diversified crop structure based on nutrient management and the selection of selected varieties adapted to the climate and soil conditions and resistant to harmful organisms.

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