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ORIGINAL PAPER

Phenolic compounds profiles of different barley varieties under the action of nanocomposite complex bacterial preparation Azogran in conditions of abiotic stress

Iryna Skorochod[1] · Ulziijargal Erdenetsogt[1,2] · Budsuren Dondov[3] · Maxim Kharkhota[1]

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Abstract

Hordeum vulgare L. is a highly valuable cereal crop worldwide. However, its yield is decreasing due to increasing abiotic stresses. The prolonged action of oxidants creates an imbalance in the functioning of antioxidant systems. One important way to stabilize the redox homeostasis of plants is through the use of microbial preparations that enhance the synthesis of antioxidant compounds. At inoculation of seeds of the barley varieties (Burkhant, Virazh, and Copeland) with the nanocomposite complex bacterial preparation Azogran in plants, the levels of the most identified phenolic acids and flavonoids in the free and bound fractions were increased. Whereas in plants whose seeds were stressed with hydrogen peroxide (H_2O_2) and inoculated with Azogran, phenolic compounds (Ph-OH) with a high ability to inactivate the harmful effects of H_2O_2 dominated. In particular, in the plants of the Burkhant barley variety, the concentration-free chlorogenic (by 33.1%), syringic, benzoic, *p*-coumaric acids, rutin and bound chlorogenic, benzoic and *trans*-ferulic acids increased. In the plants of the Virazh barley variety, the levels of free caffeic, syringic acids, quercetin and bound 4-hydroxyphenylacetic (4-HPA), *trans*-ferulic, sinapic, *trans*-cinnamic acids, quercetin, and kaempferol increased. In plants of the Copeland barley variety, only the content of 4-HPA and *trans*-ferulic (by 79.9%) acids in the free fraction and syringic acid in the bound fraction was raised. Thus, despite the unequal response of different varieties of barley to the action of the bacterial preparation Azogran, the synthesis of those Ph-OH, which are an effective buffer against peroxide stress, increased in their plants.

Keywords Bacterial preparation Azogran · Barley · Flavonoids · High-performance liquid chromatography · Peroxide stress · Phenolic acids

Introduction

One of the important tasks of modern crop production is to increase the resistance of valuable agricultural crops to the influence of abiotic stress factors (soil drought, frost, salinity, heavy metals, UV radiation, herbicides, and flooding) and biotic stress factors (phytopathogens, phytoviruses) [1, 2]. The increase in their intensity leads to an increase in the level of reactive oxygen species (ROS), which are aggressive stress agents. Exceeding the generation of oxidants over the cell's ability to eliminate them leads to hyperoxidation—oxidative stress [3]. Oxidative stress determines the state in which the "prooxidant-antioxidant" balance is disturbed in the cell, which leads to hydroxylation of nucleic acids, protein denaturation, lipid peroxidation, and apoptosis [4].

Among the members of the Poaceae family, barley (*Hordeum vulgare* L.) is one of the most economically important grain crops [5], since this cereal is grown in countries whose climatic conditions differ dramatically [6].

In recent years, the increase of anomalies in the environment leads to the accumulation of oxidants in the organism of plants. The high damaging capacity of ROS, the initiators of phytostress, poses a threat to important biomolecules (DNA, proteins, enzymes, lipids, etc.) of plant cells. The redox-homeostasis of phytoobjects is supported by a

✉ Iryna Skorochod
aphalina.77@gmail.com

[1] Zabolotny Institute of Microbiology and Virology of National Academy of Sciences of Ukraine, Zabolotny Str., 154, Kiev 03143, Ukraine

[2] National Center for Public Health, Peace Ave 17, Bayanzurkh District, Ulaanbaatar 13381, Mongolia

[3] Plant Protection Research Institute of Mongolia, 11 Khoroo, Khan-Uul District, Ulaanbaatar 17024, Mongolia

complex of antioxidants (AN). The balanced operation of high- and low-molecular protectors during different phases of oxidative stress plays an important role in maintaining the viability of plants. However, prolonged exposure to oxidants disrupts redox cycles [7].

Thus, the functioning of the plant organism's defense system largely depends on the concentration of oxidants. First, high levels of ROS damage protective enzymes (catalase, peroxidase, superoxide dismutase), which leads to a decrease in their activity. Second, the action of stress agents depletes the pool of low-molecular-weight antioxidants. As a result, these disturbances in the normal work of AN lead to the death of plant cells [8].

One of the stabilization ways of redox homeostasis in the plant organism is their mutually beneficial relationships with rhizosphere microorganisms [9]. Such microbes include representatives of the genera *Bacillus, Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Serratia*, and others [10, 11]. Plant growth-promoting rhizobacteria (PGPR) are of interest in the creation of biological plant protection agents, as they are characterized by the formation of long-term protection of the macroorganism against various stress factors [12]. PGPR contribute to the development of stress tolerance in phytoobjects through various mechanisms [13]. One of which is the activation of the synthesis of various antioxidant compounds in the plant organism [14].

Among them, compounds of phenolic nature (Ph-OH), in particular phenolcarboxylic acids and flavonoids, are of particular interest. These secondary metabolites play an important role in plant metabolism. However, their main mechanisms are related to direct and indirect antioxidant action. That is, Ph-OH acts as electron donors for oxidants, inactivate free radicals, and chelate metal ions that initiate ROS formation reactions [15]. And they can also indirectly activate antioxidant enzymes and inhibit enzymes that induce pro-oxidant effects [16].

In the crop sector of Ukraine, Mongolia, and Canada, barley is one of the dominant cereal crops. The most promising varieties in these countries include Virazh (Ukraine), Burkhant (Mongolia), and Copeland (Canada). They are widely used in production and consumption, and therefore have a high research value.

Accordingly, the main goal of the presented work was to compare the differences that were founded in the composition and content of free and bound phenolic compounds in different barley varieties, the seeds of which were exposed to the action of hydrogen peroxide and post-treatment with the nanocomposite complex bacterial preparation Azogran. This study is important in understanding the role of complex microbial preparations in modern agricultural biotechnologies.

Materials and methods

Research objects

Bacterial strains

- *Bacillus subtilis* IMV B-7023 was isolated from black soil (Cherkasy region, Ukraine). The strain is supported at the Depositary of the Zabolotny Institute of Microbiology and Virology, NAS of Ukraine. This strain is a component of the nanocomposite complex bacterial preparation Azogran for crop production [17].
- *Azotobacter vinelandii* IMV B-7076 was isolated from the rhizosphere of sugar beet in the Department of Microbiological processes on solid surfaces, Zabolotny Institute of Microbiology and Virology, NAS of Ukraine. This strain is a component of the nanocomposite complex bacterial preparation Azogran for crop production [18].

  – Nanostructured mineral bentonite is a variety of minerals of the montmorillonite group. We used bentonite from the Dashukovsky deposit (Cherkasy region). The size of bentonite nanoparticles was 28.92–99.21 nm;
  – Grain seeds of spring barley: Virazh (Ukraine), Burkhant (Mongolia), Copeland (Canada) varieties;
  – Nanocomposite complex bacterial preparation Azogran. The bacteria that make up the biological product are active producers of amino acids, enzymes, organic acids, antibiotics, phytohormones, vitamins, phenolic compounds, and other biologically active components [19].

The cultivation conditions of bacteria

B. subtilis IMV B-7023 was cultivated in modified glucose-mineral liquid nutrient medium [20], g L^{-1}: $(NH_4)_2SO_4$—0.5; $MgSO_4 \times 7H_2O$—0.3; NaCl—0.3; KCl—0.3; $CaCO_3$—5.0; $MnSO_4 \times 7H_2O$—0.001; $FeSO_4 \times 7H_2O$—0.001; glucose—10.0; sodium glycerophosphate—2.0; distilled water—1.0 L; pH 7.0–7.2.

The number of viable cells (colony-forming units (CFU)) was determined by the method of seeding a suspension of bacteria on a potato agar medium from serial tenfold dilutions. The composition of potato agar medium [21], g L^{-1}: peeled potatoes—200.0; $CaCO_3$—0.2; $MgSO_4 \times 7H_2O$—0.2; agar–agar—15.0; tap water—1 L; pH 6.8–7.2.

A. vinelandii IMV B-7076 was cultivated in liquid Burke's medium [22], g L^{-1}: $K_2HPO_4 \times 3H_2O$—0.64;

KH_2PO_4—0.16; NaCl—0.2; $MgSO_4 \times 7H_2O$—0.2; $CaSO_4 \times 2H_2O$—0.05; $Fe_2(SO_4)_3$—0.005; $FeSO_4 \times 7H_2O$—0.003; $Na_2MoO_4 \times 2H_2O$—0.001; sucrose—20.0; distilled water—1.0 L; pH 7.0–7.2.

The number of viable cells (colony-forming units (CFU)) was determined by the method of seeding a suspension of bacteria on an Ashby's solid nutrient medium [22], g L^{-1}: sucrose—20.0; $K_2HPO_4 \times 3H_2O$—0.2; $MgSO_4 \times 7H_2O$—0.2; NaCl—0.2; K_2SO_4—0.1; $CaCO_3$—5.0; distilled water—1.0 L. In this medium was added the 1 mL of microelement solution (according to Fedorov) of the following composition: H_3BO_3—5.0; (NH_4) $2MoO_4 \times 2H_2O$—5.0; $ZnSO_4 \times 7H_2O$—0.2; KJ—0.5; NaBr—0.54; $Al_2(SO_4)_3 \times 18H_2O$—0.3; distilled water—1.0 L; pH 7.2–7.3.

Cultivation of the studied bacterial strains in liquid nutrient media was carried out on rotary shakers ($n = 240$ rpm) in Erlenmeyer flasks with a volume of 750 mL (100 mL of medium) or in microbiological test tubes into which 20 mL of the medium was added. A daily culture of bacteria was used as an inoculum. The number of viable cells was determined by seeding bacterial suspensions from serial tenfold dilutions onto agar media. After cultivating the inoculations at a temperature of $+28 \pm 1$ °C, the colonies (colony-forming units, CFU) were counted on the surface of the agar medium in the dilution where their number ranged from 30 to 300.

The design of peroxide stress

Plants of three barley cultivars were grown under greenhouse conditions (the temperatures around 18 °C during day and 14 °C at night, a photoperiod of 12 h and 50–70% humidity). The seed material previously was subjected to different treatment options:

- Seeds were soaked in sterile distilled water (H_2O) during 1 h;
- Seeds were bacterized with 3 ml of nanocomposite complex bacterial preparation Azogran for 1 h (Nano-CP);
- Seeds were treated with 33% hydrogen peroxide for 30 min. (H_2O_2);
- Seeds were exposed to 33% hydrogen peroxide (30 min.) and bacterized with 3 ml of nanocomposite complex bacterial preparation Azogran for 1 h (H_2O_2 + Nano-CP).

The seeds of each barley variety were sowed in 4 replications, 50 seeds per row.

Extraction of free and bound phenolic compounds from different varieties of barley

Plants were selected in phase of stem elongation and dried at room temperature (22 °C) without access to direct light to constant weight. The samples were ground to a powder-like state by a SaturnST-CM1031 electric coffee grinder (220–240 V, 50 Hz, China). A sample (1 g) was took from each variant and divided into two parts of 0.5 g each:

- First part (extraction of the free phenolic compounds): a sample (0.5 g) of each of the samples of barley plants was placed in round-bottom flasks under reflux and extracted twice with methanol (CH_3OH) (50 mL per sample) at a water bath (67.4 °C) during 2 h. The total volume of the extract (100 mL) was filtered through No. 1 filter paper in the Buchner funnel. The resulting filtrate was evaporated to dryness on an IR-1M2 rotary evaporator (Production Association Khimlaborpribor, USSR). The dry residue was redissolved in 2 mL of methanol and analyzed by HPLC;
- Second part (extraction of the hydrolysis fraction of phenolic compounds): a weighed portion (0.5 g) of each of the samples of barley plants was introduced into round-bottom flasks under reflux and was subjected to acid hydrolysis by adding 30 mL of a mixture of 2 M HCl:CH_3OH (1:1). The presence of methanol prevents the destruction of some phenolic acids [23].

Hydrolysis was carried out at 90 °C for 2 h. The hydrolysates were filtered through No. 1 filter paper in the Buchner funnel. The extraction was repeated three times by ethyl acetate ($CH_3COOC_2H_5$) (30 mL per sample) during 30 min. The extracts were evaporated to dryness on an IR-1M2 rotary evaporator (Production Association Khimlaborpribor, USSR). The dry residue was redissolved in 2 mL of methanol and analyzed by HPLC. The content of bound phenolic compounds was determined from the difference between the amount of total and free polyphenols in the hydrolysis fraction.

HPLC analysis of phenolic acids

High-performance liquid chromatography (HPLC) (Agilent 1200, USA) was used to assess the composition of phenolic acids extracted from the barley samples. Methanol (CH_3OH) (A) and 0.1% formic acid (H-COOH) in water (B) were used as the mobile phase. The settings for the elution gradient were as follows: 0 min—A (25%): B (75%); 25 min—A (75%): B (25%); 27 min—A (100%): B (0%); 35 min—A (100%): B (0%). Separation was carried out on a Zorbax SB-Aq column (4.6 mm × 150 mm, 3.5 µm) (Agilent Technologies, USA), flow rate was 0.5 mL/min, thermostat temperature was 30 °C, injection volume was 4 µL. Detection was carried out using a diode array detector with signal registration at 250 nm and 275 nm and fixation of absorption spectra in the range of 210–700 nm [24]. Identification and quantitative analyses were carried out

using standard solutions of phenolic compounds: gallic acid, 4-hydroxyphenylacetic acid (4-HPAA), chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, *trans*-ferulic acid, sinapic acid, *trans*-cinnamic acid. The values are expressed as μg g^{-1} DW (dry weight).

HPLC analysis of flavonoids

High-performance liquid chromatography (HPLC) (Agilent 1200, USA) was used to assess the composition of flavonoids extracted from the barley samples. Acetonitrile (A) and 0.1% formic acid in water (B) were used as the mobile phase. The settings for the elution gradient were as follows: 0 min—A (30%): B (70%); 20 min—A (70%): B (30%); 22 min—A (100%): B (0%); 30 min—A (100%): B (0%). Separation was carried out on a Zorbax SB-C18 column (4.6 mm × 150 mm, 3.5 μm) (Agilent Technologies, USA), flow rate was 0.25 mL/min, thermostat temperature was 30 °C, injection volume was 4 μL. Detection was carried out using a diode array detector with signal registration at 280 nm and 365 nm and fixation of absorption spectra in the range of 210–700 nm [25–27]. Identification and quantitative analyses were carried out using standard solutions of flavonoids: rutin, quercetin-3-b-glucoside, naringin, neohesperidin, quercetin, naringenin, kaempferol, luteolin, apigenin. The values are expressed as μg g^{-1} DW.

Statistical analysis

Microsoft Excel (Microsoft Corporation, USA) was used to analyze the data on the average of the three replicates (± SE) obtained from the three independent experiments. Differences were compared with the statistical significance at a *P* level less than 0.05 ($P < 0.05$) [28]. Tukey's test was used to determine significant ($P < 0.05$) differences between the samples.

Results and discussion

Composition and content of the free phenolic acids of different barley varieties

Modern agricultural biotechnologies are closely related to microbial biopreparations for crop production, in particular those of complex action. They are one of the important components of ecological agriculture [29, 30]. The mechanism of formation of plants stress tolerance with the help of biological preparations is closely related to the metabolites of bacteria included in their composition. A number of these compounds are considered as triggers that start a cascade of plant-specific biosynthetic processes that increase resistance to the damaging effects of oxidants [31, 32]. One of these processes can be the activation of the production of phenolic compounds in the organism of phytoobjects [14].

We established significant differences in the qualitative and quantitative composition of phenolic compounds in the plants of the studied varieties of barley, the seeds of which were subjected to hydrogen peroxide (H_2O_2) treatment and post-treatment with Azogran.

The 4-hydroxyphenylacetic, chlorogenic, caffeic, syringic, benzoic, *p*-coumaric, *trans*-ferulic, sinapic and *trans*-cinnamic acids were identified in the variant with the treatment of seeds of different varieties of barley with distilled sterile water (dH_2O). Their highest content was observed in the free fraction of phenolic compounds obtained from Copeland variety barley plants—1488 μg g^{-1} DW (Fig. 1, Table 1). No significant differences in the quantitative composition were found in the extracts obtained from plants of two other varieties of barley (Table 1).

Whereas, the content of phenolic acids in the free fraction obtained from Virazh variety barley plants increased significantly when seeds were treated with the nanocomposite complex bacterial preparation Azogran. Accordingly, the concentration of sinapic acid increased by 97.9%, *trans*-cinnamic acid by 106.9%, syringic acid by 116.4%, caffeic acid by 117.9%, benzoic acid by 123.9%, 4-HPAA by 124.6%, chlorogenic acid by 158.8%, *p*-coumaric acid by 230.2%, compared to the sample where the seeds were treated with sterile distilled water (Table 1).

As for the other varieties, only in the Copeland variety plants, the content of sinapic acid was increased by 1.6%, syringic acid by 8.8%, caffeic acid by 13.9%, and *trans*-ferulic acid by 104.2%, compared to the variant where barley seeds were treated with dH_2O (Table 1). The concentration of phenolic acids in Burkhant variety plants practically did not differ from this sample where seeds were treated with dH_2O. No changes in the qualitative composition of these compounds in the plants of the studied barley varieties were recorded (Table 1).

The results obtained on the effect of PGPR on the levels of phenolic acids in plants were consistent with a number of other studies. In particular, the content of these compounds in the *Piper betle* L. after inoculation with *Serratia marcescens* NBRI1213 bacteria [33] and in the *Tagetes minuta* after inoculation with *Pseudomonas fluorescens* WCS417r and *Azospirillum brasilense* [34] was increased. Singh et al. [35] reported that mono- or complex inoculation of chickpea seeds with *Ps. fluorescens* Pf4 and *Ps. aeruginosa* Pag strains activated the synthesis of gallic, ferulic, and chlorogenic acids in plants.

Under the influence of an aggressive stress agent (H_2O_2) on barley seeds, the total content of phenolic acids in the free fraction obtained from Copeland variety and Burkhant variety plants decreased by 15.4% and 78.9%, compared to the variant where the seeds were treated with

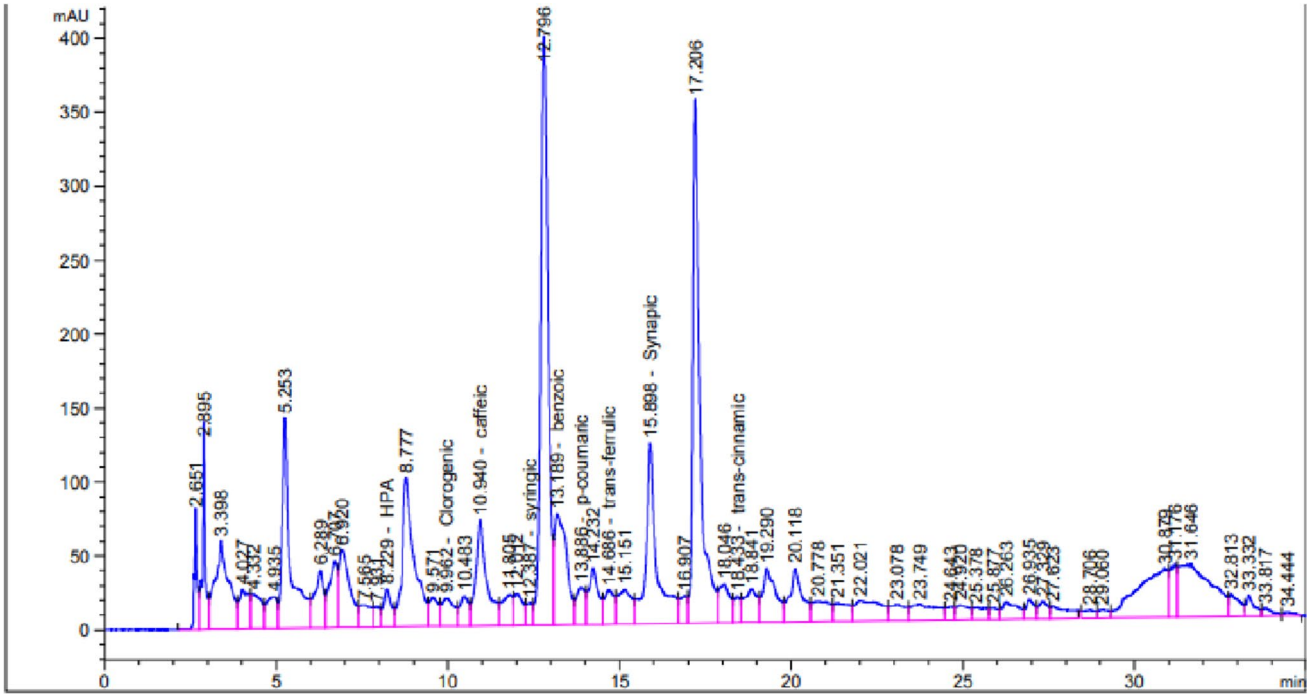

Fig. 1 HPLC analysis of phenolic acids in the free fraction that was obtained from Copeland variety barley plants after seed treatment with sterile distilled water

sterile distilled water. In particular, in the Burkhant variety plants, the concentration of chlorogenic acid decreased by 138.71 μg g^{-1} DW, caffeic acid by 175.52 μg g^{-1} DW, benzoic acid by 139.00 μg g^{-1} DW, *trans*-ferulic acid by 41.34 μg g^{-1} DW, and sinapic acid by 132.74 μg g^{-1} DW, respectively. And in the Copeland variety plants, the level of chlorogenic acid decreased by 39.04 μg g^{-1} DW, caffeic acid by 45.50 μg g^{-1} DW, benzoic acid by 116.54 μg g^{-1} DW, and sinapic acid by 57.80 μg g^{-1} DW, respectively. It was also found that syringic acid was not identified in methanol extracts obtained from plants of these barley varieties (Fig. 2, Table 1).

Elguera et al. [36] found that at growing of *Lepidium sativum* in the presence of cadmium chloride [Cd(II)], the content of chlorogenic, ferulic, and caffeic acids in the free fraction of phenolic acids obtained from plant leaves decreased. Increased levels of ROS can lead to disruption of the secondary-metabolite biosynthesis (in particular phenolic compounds) whose structural skeleton consists of carbon atoms [37].

However, the treatment of seeds of the barley Virazh variety with hydrogen peroxide increased the level of phenolic acids (free fraction) by two times in plants compared to the variant (dH_2O) (Table 1). Stress tolerance and biological activity of cereal crops significantly depend on their variety [38–40].

At post-treatment with Azogran for seeds of the tested cereal varieties, the concentration of phenolic acids (free fraction) increased only in plants of barley Burkhant variety by 27.24 μg g^{-1} DW, compared to stressed plants of the same variety. Especially the level of sinapic acid increased by 34.1%. This phenolic compound is a powerful antioxidant. Its antiradical activity (ARA) is significantly higher than that of ferulic acid [41, 42]. Chiappero et al. [43] found that inoculated with *Ps. fluorescens* WCS417 r and *B. amyloliquefaciens* GB03 drought-stressed *Mentha piperita* plants activated the synthesis of phenolic compounds. Post-treatment of stressed seeds of two other barley varieties with biological preparation Azogran did not have a pronounced stimulating effect on the content of phenolic carboxylic acids of the free fraction in their plants. Thus, for barley Virazh variety, an increase in caffeic acid by 5.7% was observed, and in plants of Copeland variety, the content of 4-HPAA increased by 15.0% and *trans*-ferulic acid by 79.9%, compared to the stressed variant (Table 1). These phenolic compounds effectively inactivate such stress agents as hydrogen peroxide and hydroxyl radical [44, 45]. Their high antioxidant activity depends on the presence of hydroxyl and methoxy groups in the chemical structure [46]. All phenolic carboxylic acids, including syringic acid (SA), were identified in the qualitative composition (Table 1). The antioxidant potentials of SA and caffeic acid are very similar [47]. And the high ARA

Table 1 Composition and content of phenolic acids in the free fraction that was obtained from plants of different barley varieties

Treatment options	Barley variety	Phenolic acids (μg g^{-1} DW)								
		4-HPAA	Chlorogenic acid	Caffeic acid	Syringic acid	Benzoic acid	*p*-Coumaric acid	*trans*-Ferulic acid	Sinapic acid	*trans*-Cinnamic acid	Total content, Σ
dH_2O	Burkhant	30.08 ± 0.17b	180.15 ± 3.59a	243.76 ± 7.72a	15.02 ± 0.90a	156.14 ± 9.35a	35.18 ± 1.82a	50.22 ± 10.45a	158.40 ± 8.8a	17.60 ± 2.25a	886.55 ± 10.42a
	Virazh	39.20 ± 0.42a	129.80 ± 2.06a	208.42 ± 2.60a	20.78 ± 0.23a	175.46 ± 2.58a	37.92 ± 3.65a	96.04 ± 0.26a	156.04 ± 3.45a	10.72 ± 1.15a	874.38 ± 12.69a
	Copeland	66.64 ± 0.69a	356.98 ± 2.95a	361.14 ± 7.71a	22.78 ± 0.26a	260.86 ± 6.17a	63.58 ± 1.22a	60.02 ± 3.87a	284.28 ± 9.80a	11.72 ± 1.79a	1488.00 ± 4.90a
Nano-CP	Burkhant	27.40 ± 1.53a	167.52 ± 1.42a	182.74 ± 1.18a	13.24 ± 0.11a	136.34 ± 6.78a	31.50 ± 0.39a	26.16 ± 2.85a	133.12 ± 4.86a	6.22 ± 0.29a	724.24 ± 9.70a
	Virazh	88.06 ± 1.02a	335.90 ± 1.04a	454.24 ± 9.25a	44.96 ± 2.62a	392.80 ± 13.23a	125.22 ± 5.24a	87.56 ± 5.44a	308.80 ± 10.14a	22.18 ± 5.29a	1859.72 ± 16.14a
	Copeland	56.7 ± 1.16a	304.50 ± 0.65a	411.48 ± 5.06a	24.78 ± 1.87a	149.98 ± 8.59a	62.90 ± 7.57a	122.56 ± 7.68a	288.76 ± 7.12a	10.84 ± 1.75a	1432.56 ± 18.41a
H_2O_2	Burkhant	7.20 ± 0.26b	41.44 ± 0.61a	68.24 ± 0.97a	Nd	17.14 ± 0.32b	15.16 ± 2.16b	8.88 ± 1.02b	25.66 ± 2.18a	2.84 ± 0.22b	186.56 ± 7.75b
	Virazh	82.18 ± 1.18a	348.78 ± 0.46a	374.12 ± 8.64a	Nd	428.60 ± 12.76a	136.22 ± 12.07a	316.40 ± 13.24a	176.30 ± 5.22a	23.26 ± 1.17a	1885.86 ± 12.63a
	Copeland	56.52 ± 0.19a	317.94 ± 0.38a	315.64 ± 7.12a	26.58 ± 0.25	144.32 ± 4.59a	79.96 ± 10.25a	53.94 ± 3.49b	226.48 ± 8.96a	38.18 ± 3.45a	1259.56 ± 15.85a
H_2O_2 + Nano-CP	Burkhant	8.84 ± 0.74a	55.16 ± 0.82a	57.44 ± 3.37a	5.00 ± 0.17a	21.30 ± 0.67a	18.68 ± 4.88b	8.48 ± 0.97a	34.40 ± 2.62a	4.50 ± 0.19b	213.80 ± 14.89a
	Virazh	82.42 ± 1.24a	339.30 ± 1.04a	395.62 ± 4.22b	40.32 ± 0.47a	379.20 ± 11.8a7	111.22 ± 2.20a	69.24 ± 0.84a	173.26 ± 4.34a	20.76 ± 1.34a	1611.34 ± 13.49a
	Copeland	65.02 ± 0.82a	316.54 ± 2.72a	283.36 ± 6.54a	24.42 ± 0.42a	120.58 ± 8.66a	70.02 ± 10.06a	97.06 ± 5.66a	171.14 ± 8.59a	33.90 ± 1.47a	1182.04 ± 19.22a

Different letters indicate the values significantly differing one from another within a column of the table based on the results of comparison using the Tukey test ($P < 0.05$)

ND not detected

of this phenolic compound is due to the presence of two methoxy groups attached to the aromatic ring at positions 3 and 5 [48, 49]. Thus, post-treatment with the nanocomposite complex bacterial preparation Azogran of hydrogen peroxide-stressed seeds of different barley varieties activated the synthesis of phenolic acids with powerful antioxidant and antiradical properties in plants.

Composition and content of the bound phenolic acids of different barley varieties

The qualitative and quantitative content of phenolic acids in the hydrolysis fraction obtained from plants of different barley varieties differed significantly from their presence in the free fraction and depended on the variant of seed treatment.

It was found that the hydrolyzed fraction of phenolic compounds obtained from plants of the Burkhant barley variety, whose seeds were soaked in sterile distilled water, contains syringic, benzoic, *p*-coumaric, *trans*-ferulic, sinapic, and *trans*-cinnamic acids (Table 2). In addition to these phenolic acids, 4-HPAA and caffeic acids were found in the plants of Virazh barley variety, but syringic acid was absent. The quantitative content of each of the phenolic acids in the bound fraction was the highest compared to the other two varieties (Fig. 3B, Table 2). In the hydrolyzed fraction of phenolic acids from plants of Copeland barley variety, all phenolic acids were present, except for gallic acid (Fig. 3C, Table 2).

At inoculation of seeds of the Burkhant barley variety with the nanocomposite complex bacterial preparation Azogran, in the bound fraction of phenolic acids obtained from plants, a high level of caffeic acid (3,4-dihydroxycinnamic acid) was found—89.88 μg g^{-1} DW (Table 2). This compound is an ortho-dihydroxyphenol with a powerful antioxidant potential and can inactivate the highly aggressive hydroxyl radical [50]. The product of the methylation reaction of caffeic acid is ferulic acid, which, together with *p*-coumaric acid, initiates the synthesis of lignin [51, 52]. This natural polymer provides plant resistance to various abiotic stressors [53].

In the bound fraction obtained from plants of Virazh barley variety, after inoculation seeds with Azogran, high content of caffeic acid (239.82 μg g^{-1} DW) and benzoic acid (264.40 μg g^{-1} DW) was detected (Table 2).

The treatment of seeds with 33% H_2O_2 negatively affected both on qualitative and on quantitative composition of phenolic acids in the bound fraction obtained from plants of the three tested barley varieties. In particular, in plants of Burkhant barley variety, the concentration of benzoic acid decreased by 145.40 μg g^{-1} DW, *p*-coumaric acid by 3.14 μg g^{-1} DW, *trans*-ferulic acid by 55.90 μg g^{-1} DW, sinapic acid by 6.60 μg g^{-1} DW, and *trans*-cinnamic acid by 9.88 μg g^{-1} DW, compared to plants of the same variety grown from seeds soaked in sterile distilled water (Table 2).

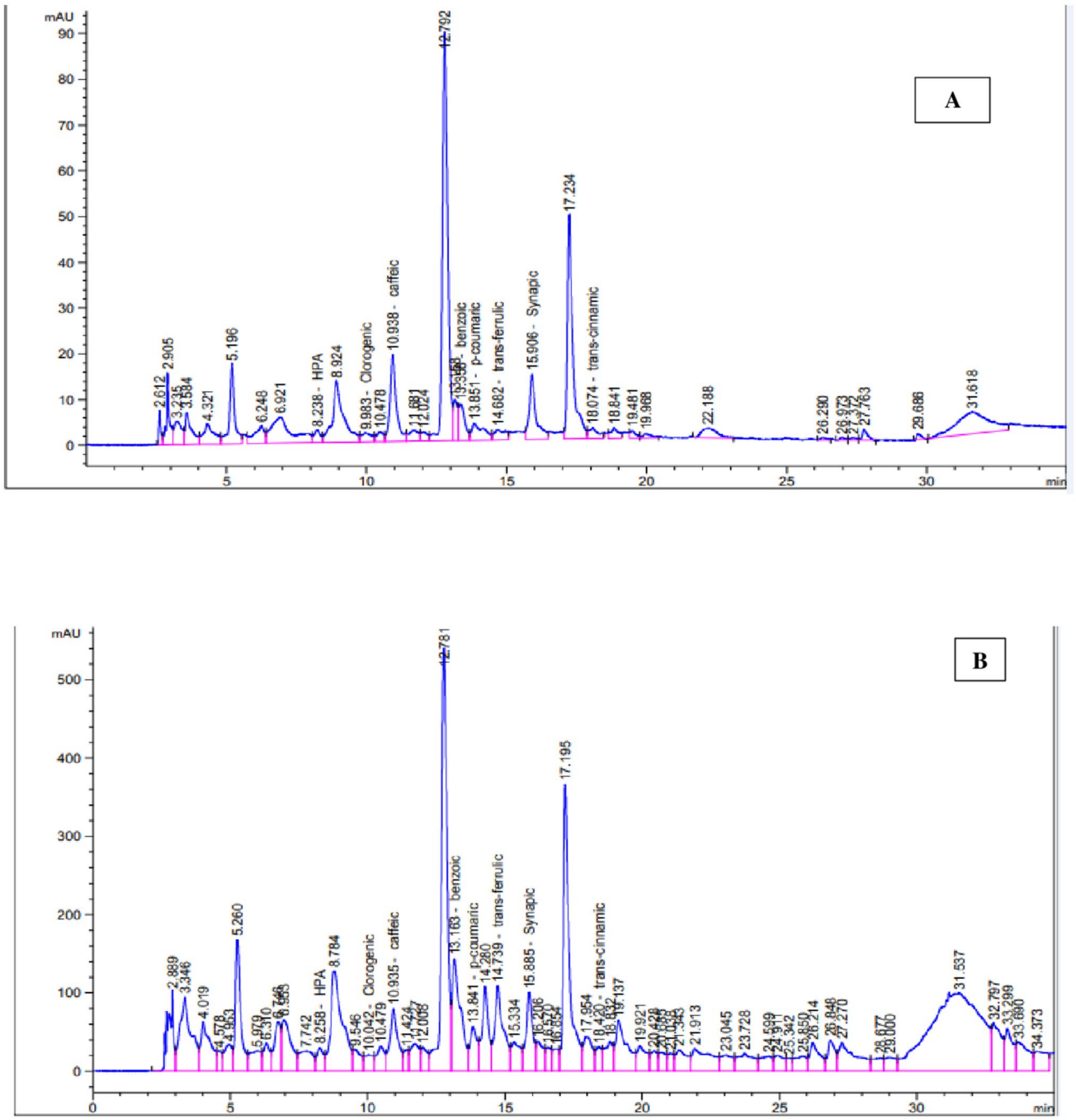

Fig. 2 HPLC analysis of phenolic acids in the free fraction that was obtained from barley plants of the Burkhant (**A**) and Virazh (**B**) varieties after treatment of seeds with the stress agent hydrogen peroxide

In plants of the Virazh barley variety, only the content of sinapic acid decreased by 55.60 μg g^{-1} DW compared to the sample which seeds were soaked in the sterile distilled water (Table 2).

Hydrogen peroxide had the most negative effect on the content of phenolic acids in the bound fraction in plants of Copeland barley variety. Accordingly, the concentration of caffeic acid decreased by 76.18 μg g^{-1} DW, benzoic acid by 64.70 μg g^{-1} DW, *p*-coumaric acid by 32.08 μg g^{-1} DW, *trans*-ferulic acid by 13.60 μg g^{-1} DW, sinapic acid by 92.44 μg g^{-1} DW, and *trans*-cinnamic acid by 7.78 μg g^{-1} DW, compared to the variant which seeds were soaked only in sterile distilled water. Also, 4-HPAA, chlorogenic, and syringic acids were not identified for this variety (Fig. 4, Table 2).

Table 2 Composition and content of phenolic acids in the bound fraction that was obtained from plants of different barley varieties

Treatment options	Barley variety	Phenolic acids (μg g^{-1} DW)									
		4-HPAA	Chlorogenic acid	Caffeic acid	Syringic acid	Benzoic acid	*p*-Coumaric acid	*trans*-Ferulic acid	Sinapic acid	*trans*-Cinnamic acid	Total content, Σ
dH_2O	Burkhant	Nd	Nd	Nd	21.06 ± 4.70	149.86 ± 10.45a	11.56 ± 4.29a	58.60 ± 8.64a	17.38 ± 5.49a	12.92 ± 3.91a	271.38 ± 13.91a
	Virazh	49.18 ± 5.76	Nd	138.44 ± 9.35	Nd	127.74 ± 8.94a	111.32 ± 6.92a	127.56 ± 13.74a	425.76 ± 22.18a	43.24 ± 0.49a	1023.24 ± 23.56a
	Copeland	33.16 ± 2.54	71.74 ± 4.88	95.90 ± 6.24	20.64 ± 7.88	89.62 ± 6.83a	35.54 ± 3.81a	28.28 ± 8.46a	168.58 ± 9.45a	16.24 ± 5.18a	559.70 ± 7.83a
Nano-CP	Burkhant	Nd	Nd	89.88 ± 5.28b	11.54 ± 3.97	69.72 ± 3.26a	25.12 ± 4.71a	36.82 ± 3.99a	100.36 ± 11.80a	3.60 ± 0.03a	344.24 ± 15.40a
	Virazh	20.56 ± 3.84	Nd	239.82 ± 13.44b	Nd	264.40 ± 17.22a	23.54 ± 3.15a	128.24 ± 12.77a	240.20 ± 18.84a	35.42 ± 7.28a	952.18 ± 17.96a
	Copeland	2.24 ± 0.17	Nd	11.96 ± 0.82b	3.08 ± 0.72	30.76 ± 2.15a	4.56 ± 1.59a	12.42 ± 0.83a	49.66 ± 9.66a	6.42 ± 0.84a	121.11 ± 14.55a
H_2O_2	Burkhant	Nd	21.28 ± 3.62	19.94 ± 2.56b	39.82 ± 4.21	4.46 ± 1.89a	8.42 ± 1.95b	2.70 ± 0.60a	10.78 ± 1.72a	3.04 ± 0.06a	110.44 ± 9.17b
	Virazh	27.52 ± 2.40	Nd	214.44 ± 16.49b	194.44 ± 15.66	244.20 ± 13.95a	135.78 ± 12.07b	215.80 ± 7.47a	370.10 ± 8.41a	29.58 ± 2.61a	1431.86 ± 19.69b
	Copeland	Nd	Nd	19.72 ± 4.77a	Nd	24.92 ± 2.18a	3.46 ± 0.56a	14.68 ± 2.55a	76.14 ± 4.19a	8.46 ± 0.18a	147.38 ± 11.84a
H_2O_2 + Nano-CP	Burkhant	Nd	35.50 ± 1.43	Nd	Nd	55.22 ± 4.17a	4.22 ± 1.03a	3.18 ± 0.07a	9.54 ± 1.63a	Nd	107.66 ± 6.18a
	Virazh	60.10 ± 7.33	Nd	184.60 ± 11.89	Nd	229.40 ± 7.72a	7.68 ± 0.48a	219.16 ± 14.28a	375.74 ± 16.58a	33.98 ± 2.49a	1110.66 ± 26.15a
	Copeland	Nd	Nd	17.82 ± 3.47	12.30 ± 5.17	19.16 ± 5.44a	2.38 ± 0.23a	14.14 ± 4.99a	67.32 ± 9.67a	4.72 ± 0.04a	137.84 ± 11.22a

Different letters indicate the values significantly differing one from another within a column of the table based on the results of comparison using the Tukey test ($P < 0.05$)

ND not detected

Thus, the effect of peroxide stress on phenolic acids in the bound fraction of all three tested barley varieties differed significantly. However, the concentration of caffeic, benzoic, *trans*-ferulic, and sinapic acids decreased most sharply (Table 2).

Similar results were obtained by different groups of scientists in the study of the profile of phenolic compounds in different plant species under abiotic stress. In scientific papers [54, 55], it was showed that *Vitis vinifera* plants grown under cold stress conditions had decreased levels of esters and glycosides of the caffeic, ferulic, and *p*-coumaric acids compared to the control. According to a study by Kovacik et al. [56], among the 14 identified phenolic acids in the leaf rosette of *Matricaria chamomilla*, the content of chlorogenic and caffeic acids decreased sharply under NaCl stress. The decrease in the content of phenolic acids may be the result of inhibition by stress agents of the activity of enzymes involved in their biosynthesis [57].

Post-treatment of seeds of the different barley varieties with nanocomposite complex bacterial preparation Azogran caused an increase in the content of only some phenolic acids in stressed plants. Accordingly, in the bound fraction obtained from barley plants of the Burkhant variety, the concentration of chlorogenic acid increased by 14.22 μg g^{-1} DW and benzoic acid by 50.76 μg g^{-1} DW, compared to plant varieties where seeds were treated only with hydrogen peroxide (Table 2). Chlorogenic acid (CGA) is a phenolic derivative with a unique chemical structure that is a combination of caffeic and quinic acids. This allows CGA to effectively neutralize R in plant cells [58, 59]. Benzoic acid, in turn, ensures the resistance of phytoobjects to various abiotic and biotic stresses: drought, cold [60], phytopathogenic fungi [61].

In the same fraction from plants of the Virazh barley variety, the concentration of 4-HPAA increased by 32.58 μg g^{-1} DW, *trans*-ferulic acid by 3.36 μg g^{-1} DW, and sinapic acid by 4.40 μg g^{-1} DW compared to the variant where seeds are treated with a stress agent only (Table 2). 4-Hydroxyphenylacetic acid, in addition to being an antioxidant [62], effectively inhibits the development of some phytopathogenic microbes: *Fusarium culmorum* 50536, *Fusarium solani* 50666, *Alternaria alternate* 16765 [63]. The mechanism of antioxidant action of the ferulic acid is difficult and is aimed at inhibiting the surge of ROS and neutralizing free radicals in living cells. Also, this phenolic compound is responsible for chelation of protonated metal ions (Cu (II) and Fe (II)), the initiators of the Fenton reaction [64, 65], the product of which is a highly reactive hydroxyl radical. Ferulic acid not only converts free radicals (R·) into neutral molecules, but also inhibits enzymes that catalyze R· generation [66]. No significant changes were found in the content of phenolic acids in the bound fraction obtained from plants of Copeland barley variety. In addition, syringic acid was

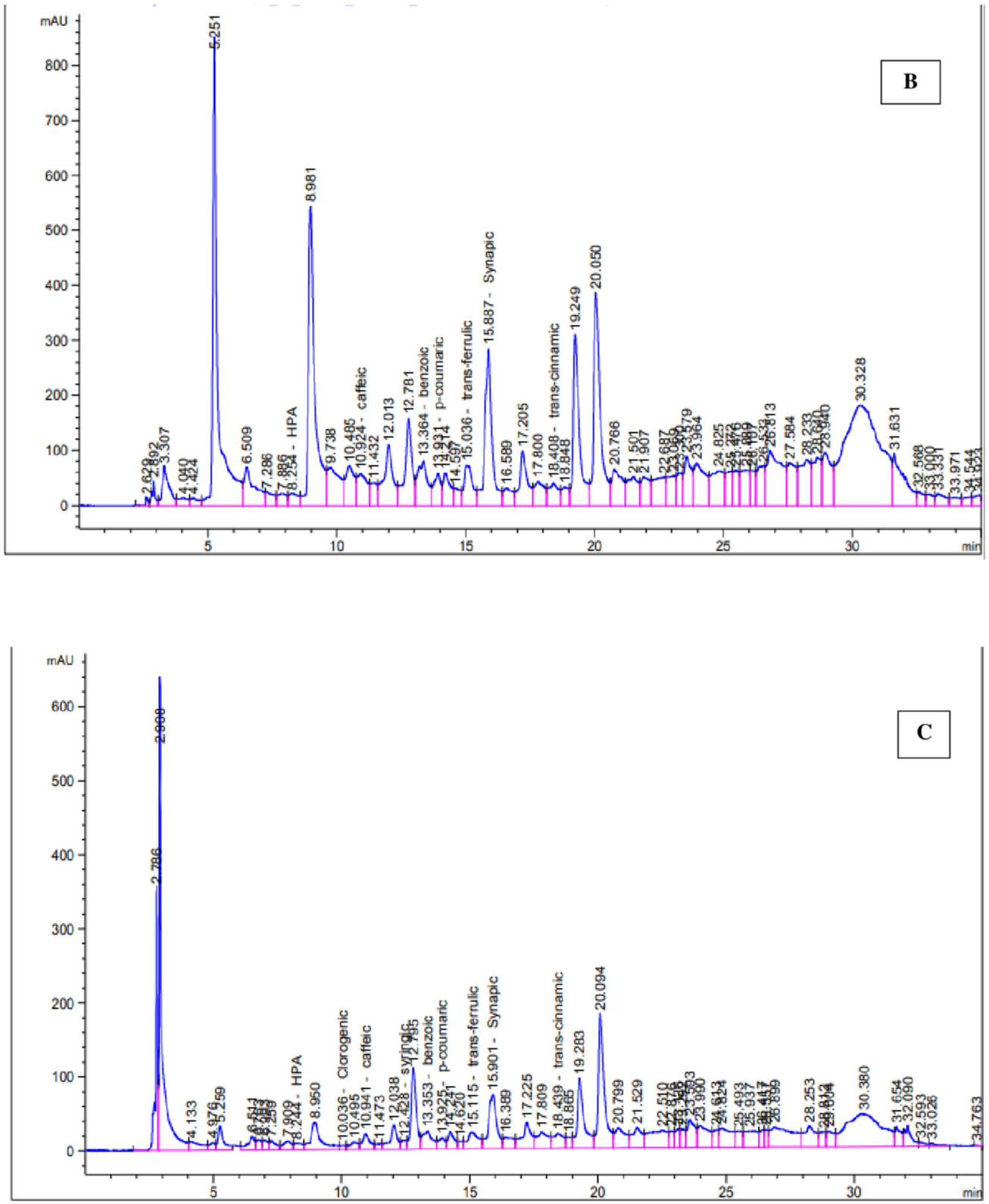

Fig. 3 HPLC analysis of phenolic acids in the hydrolyzed fraction that was obtained from plants of Virazh (**B**) and Copeland (**C**) barley varieties after soaking of seeds in sterile distilled water

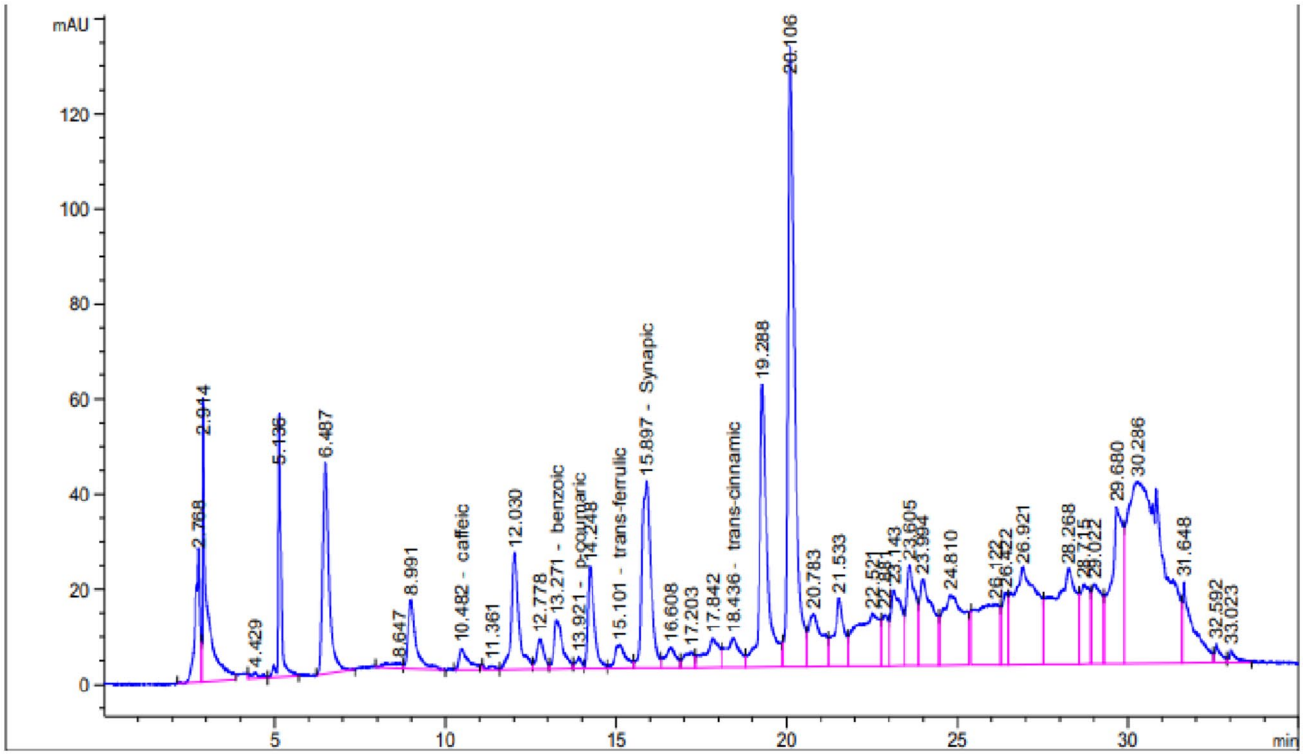

Fig. 4 HPLC analysis of phenolic acids in the hydrolyzed fraction that was obtained from plants Copeland barley variety after treatment of seeds with a stress agent—hydrogen peroxide

identified, which was absent in barley plants that developed from stressed seeds (Table 2). Tiwari et al. [67] reported that inoculation of wheat (*Triticum aestivum*) seeds with *Bacillus pumilus* under salt stress conditions promoted the accumulation of syringic acid in plants.

Composition and content of the free flavonoids of different barley varieties

In plants of barley varieties, whose seeds were subjected to different treatments, the content of flavonoids in free and bound fractions was determined. As a result, significant differences were found.

The free fraction of flavonoids extracted from plants Burkhant barley variety, whose seeds were treated with sterile distilled water, contained rutin, quercetin-3-β-glycoside, quercetin, and luteolin (Fig. 5A, Table 3). The pronounced antioxidant, anti-inflammatory, and antitumor properties of quercetin and luteolin are due to the high similarity of their chemical structure. Only the presence of a hydroxyl group in the quercetin molecule at position 3 distinguishes these two flavonoids [68].

In the same fraction obtained from plants Virazh barley variety, in addition to the above flavonoids, neohesperidin was identified. The total content of these compounds was the highest and amounted to 30.72 μg g^{-1} DW (Fig. 5B, Table 3). Only two flavonoids were detected in Copeland plants—quercetin-3-β-glycoside and quercetin (Fig. 5C, Table 3). The flavonoid content is an important indicator of the antioxidant potential of plants and also determines the health benefits of functional foods [69].

The treatment of seeds of different barley varieties with the nanocomposite complex bacterial preparation Azogran increased the concentration of flavonoids in the free fraction obtained from plants of Burkhant and Virazh varieties by 9.52 μg g^{-1} DW and 11.64 μg g^{-1} DW, respectively, compared to the previous variant. The study [70] showed that inoculation of the roots of two broccoli varieties with *Paraburkholderia graminis* PHS1, *P. hospita* mHSR1, and *P. terricola* mHS1 strains contributed to the accumulation of secondary metabolites, including flavonoids, in plants.

In addition, naringin was identified in Burkhant plants (Fig. 6, Table 3). This flavanone is a glycoside of naringenin and is able to effectively inactivate hydroxyl and superoxide radicals, thus protecting DNA from oxidative stress [71, 72].

Under the action of 33% hydrogen peroxide on barley seeds, the synthesis of flavonoids in the free fraction in barley plants of the Burkhant variety was significantly reduced. Among the previously detected flavonoid compounds, only quercetin-3-β-glycoside was identified—4.30 μg g^{-1} DW. Glycosylated flavonoids with a catechol group at the 3′–4′

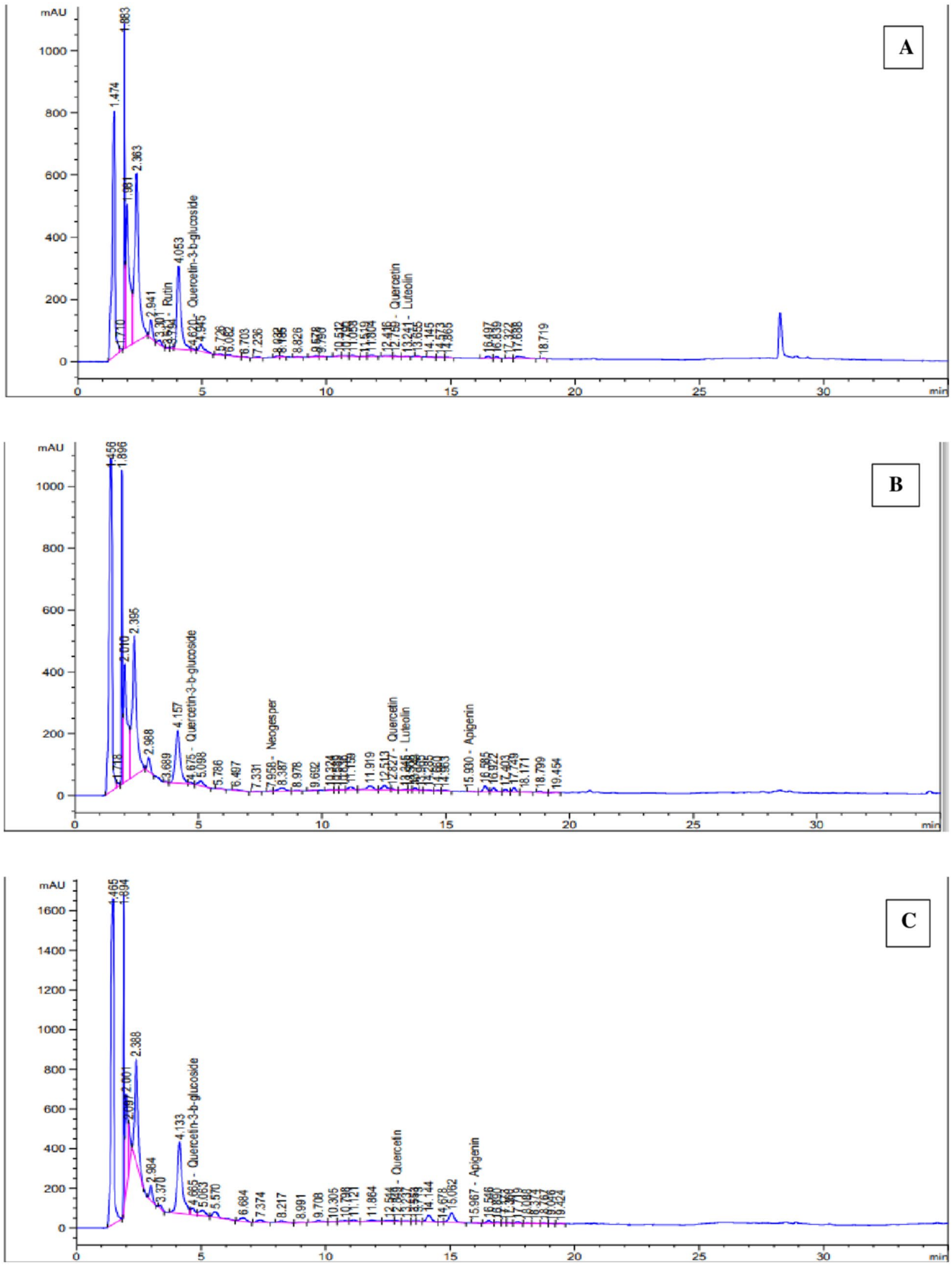

Fig. 5 HPLC analysis of flavonoids in the free fraction that was obtained from barley plants of Burkhant (A), Virazh (**B**), and Copeland (**C**) varieties after seed treatment with sterile distilled water

Table 3 Composition and content of flavonoids in the free fraction that was obtained from plants of different barley varieties

Treatment options	Barley variety	Flavonoids (μg g^{-1} DW)								
		Rutin	Quercetin-3-β-glycoside	Naringin	Neohesperidin	Quercetin	Luteolin	Naringenin	Apigenin	Kaempferol	Total content, Σ
dH_2O	Burkhant	3.33 ± 0.19	4.69 ± 0.11	Nd	Nd	7.37 ± 0.47	6.77 ± 0.03	Nd	Nd	Nd	22.16 ± 4.12a
	Virazh	Nd	4.98 ± 0.18	Nd	6.38	9.21 ± 0.05	10.15 ± 0.05	Nd	Nd	Nd	30.72 ± 6.60a
	Copeland	Nd	10.77 ± 0.08	Nd	Nd	9.76 ± 0.17	Nd	Nd	Nd	Nd	20.53 ± 1.75a
Nano-CP	Burkhant	3.32 ± 0.73	5.00 ± 0.04	11.05 ± 0.08	Nd	5.49 ± 0.03	6.55 ± 0.15	Nd	Nd	Nd	31.41 ± 8.42a
	Virazh	3.75 ± 0.14	7.31 ± 0.26	Nd	Nd	14.52 ± 0.34	16.78 ± 1.11	Nd	Nd	Nd	42.36 ± 2.84a
	Copeland	Nd	Nd	Nd	5.52 ± 0.87	Nd	8.44 ± 0.22	Nd	Nd	Nd	13.96 ± 3.16a
H_2O_2	Burkhant	Nd	4.30 ± 0.16	Nd	Nd	Nd	Nd	Nd	Nd	Nd	4.30 ± 0.88b
	Virazh	5.34 ± 0.29	6.59 ± 0.24	Nd	Nd	15.34 ± 0.18	19.30 ± 3.75	Nd	Nd	Nd	46.57 ± 5.84a
	Copeland	Nd	6.01 ± 0.57	Nd	Nd	8.08 ± 0.15	8.45 ± 1.64	Nd	Nd	Nd	22.54 ± 1.87a
H_2O_2 + Nano-CP	Burkhant	3.51 ± 0.05	4.39 ± 0.04	Nd	Nd	Nd	Nd	Nd	Nd	Nd	7.90 ± 0.35a
	Virazh	Nd	6.31 ± 0.14	Nd	Nd	16.54 ± 0.48	17.51 ± 4.20	Nd	Nd	Nd	40.36 ± 6.24a
	Copeland	Nd	5.96 ± 0.37	Nd	Nd	6.20 ± 0.23	8.20 ± 1.62	Nd	Nd	Nd	20.36 ± 2.75a

Different letters indicate the values significantly differing one from another within a column of the table based on the results of comparison using the Tukey test ($P < 0.05$). As there is a lot of Nd, then significant ($P < 0.05$) differences were calculated only for the total content

ND not detected

position are characterized by a high antioxidant potential [73]. A number of experimental studies [74–76] have shown that the concentration of quercetin glycosides in different plant species remained high in response to various abiotic stresses.

While for barley variety Virazh, an increase in the concentration of some flavonoids in the free fraction was observed. In particular, the content of quercetin increased by 6.13 μg/g DW and luteolin by 9.15 μg/g DW, compared to plants of the same variety, whose seeds were treated with sterile distilled water (Fig. 7, Table 3). On the one hand, the impact of stress factors on plants can impair their ontogeny and productivity in general, and on the other hand, it can activate the metabolism of important biologically active compounds [40].

No significant changes in the quantitative and qualitative content of flavonoids in the free fraction of the barley variety Copeland were found.

Post-treatment of the stressed seed material of the studied cereal crop with the biological product Azogran had no significant effect on the level of flavonoids in the free fraction in plants of all three barley varieties (Table 3).

Composition and content of the bound flavonoids of different barley varieties

In barley samples, the content of flavonoids in the bound fraction was checked and significant differences between varieties were found. The concentration of flavonoids in the bound fraction was higher than in the free fraction. It should be noted that in plants of different varieties of blue Highland barley, the content of flavonoids in the free fraction significantly exceeded the content of bound flavonoids [77]. While, in buckwheat, wheat, rice, corn, and oats, flavonoids prevailed in the bound fraction [78]. At treatment seeds with sterile distilled water, the total content of these compounds in plants of Burkhant variety was 190.52 μg g^{-1} DW, Virazh variety was 47.76 μg g^{-1} DW, and Copeland variety was 20.55 μg g^{-1} DW. Differences were also found in the qualitative composition. Rutin, quercetin-3-β-glycoside, quercetin were identified in barley plants of Burkhant variety; quercetin, kaempferol in Virazh variety; quercetin-3-β-glycoside, quercetin in Copeland variety (Fig. 8; Table 4). This difference is related to the genotype of each of the barley varieties under study. For example, Xi-Juan with co-authors [77] found that in blue Highland barley plants, naringenin and hesperidin predominated in the bound fraction of flavonoids. While Kim with co-authors [79] showed that in colored barley, the main flavonoid was myricetin.

Treatment of seeds with nanocomposite complex bacterial preparation was accompanied by an increase in the concentration of flavonoids only in plants of the Virazh variety

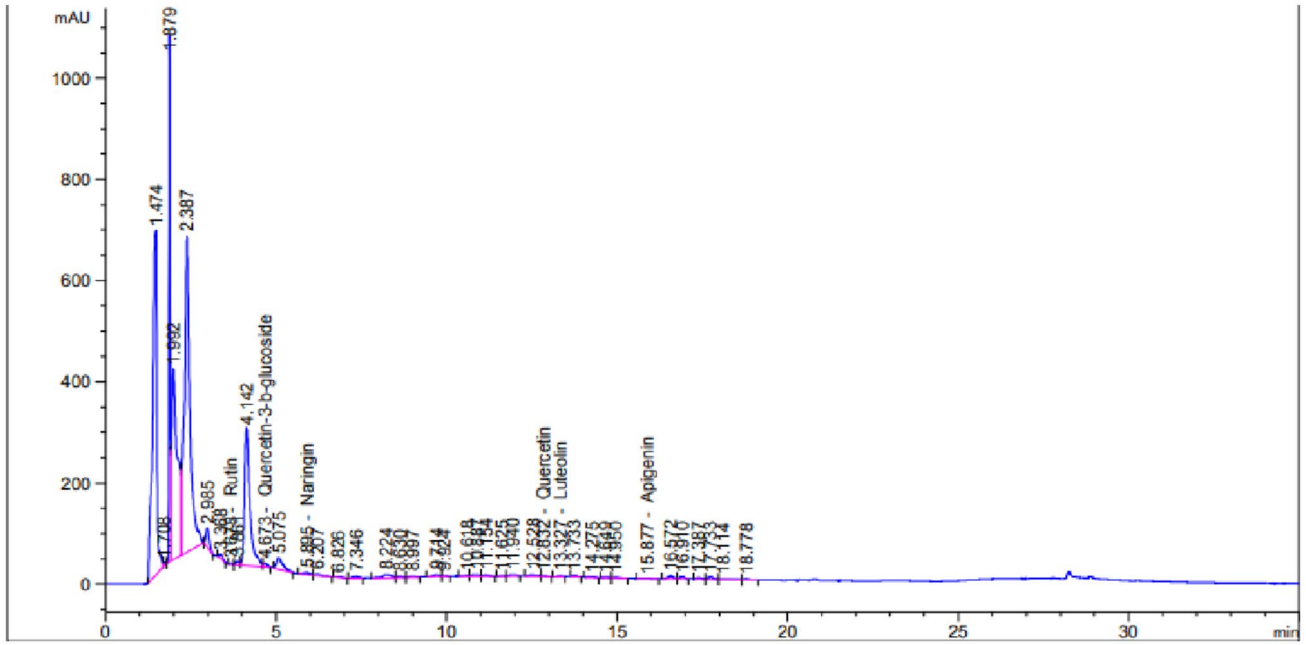

Fig. 6 HPLC analysis of flavonoids in the free fraction that was obtained from Burkhant barley plants after seed treatment with nanocomposite complex bacterial preparation Azogran

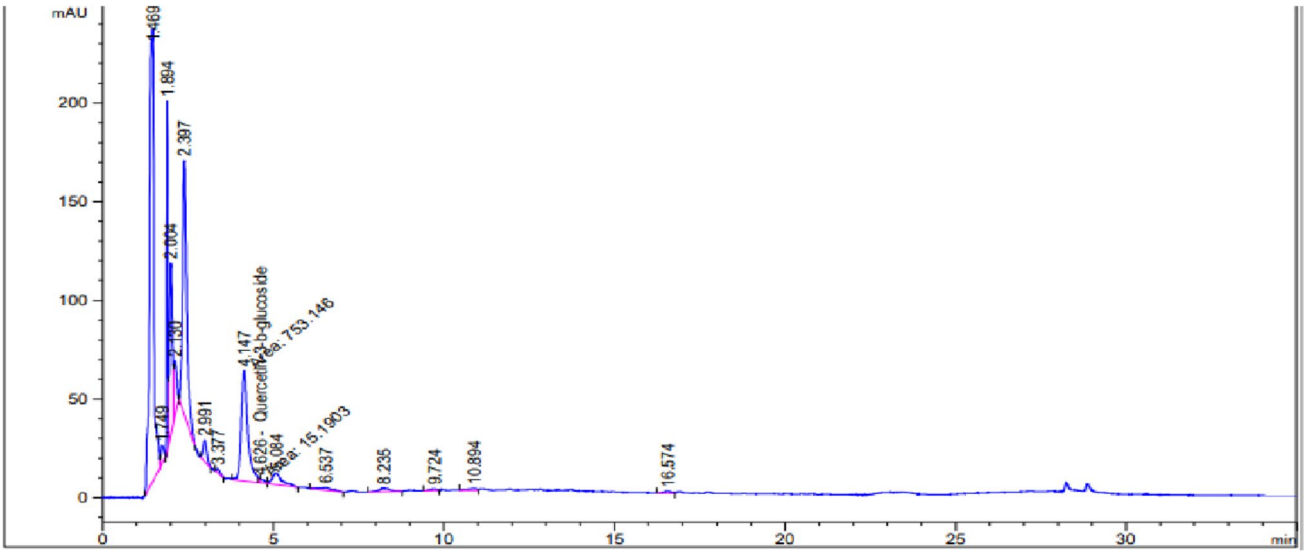

Fig. 7 HPLC analysis of flavonoids in the free fraction obtained from Burkhant barley plants after seed treatment with the stress agent hydrogen peroxide

to 125.27 µg g^{-1} DW (Table 4). Ali et al. [80] found that the treatment of *Arabidopsis thaliana* with the microbial preparation Soil Builder™-AF increased the induction of the transcriptional profile of genes of the phenylpropanoid pathway, which contributed to the accumulation of flavonoids in the leaves of plants.

The stimulating effect of Azogran on the qualitative and quantitative composition of flavonoids in the bound fraction in plants of the other two varieties was not detected. In particular, for Burkhant variety, a decrease in flavonoid content by 170.69 µg g^{-1} DW was recorded compared to the variant in which the seeds were treated with sterile distilled water (Table 4). This may be due to the specifics of the development of each of the studied cereal varieties, when their seeds were treated with the nanocomposite complex bacterial preparation Azogran. The effect

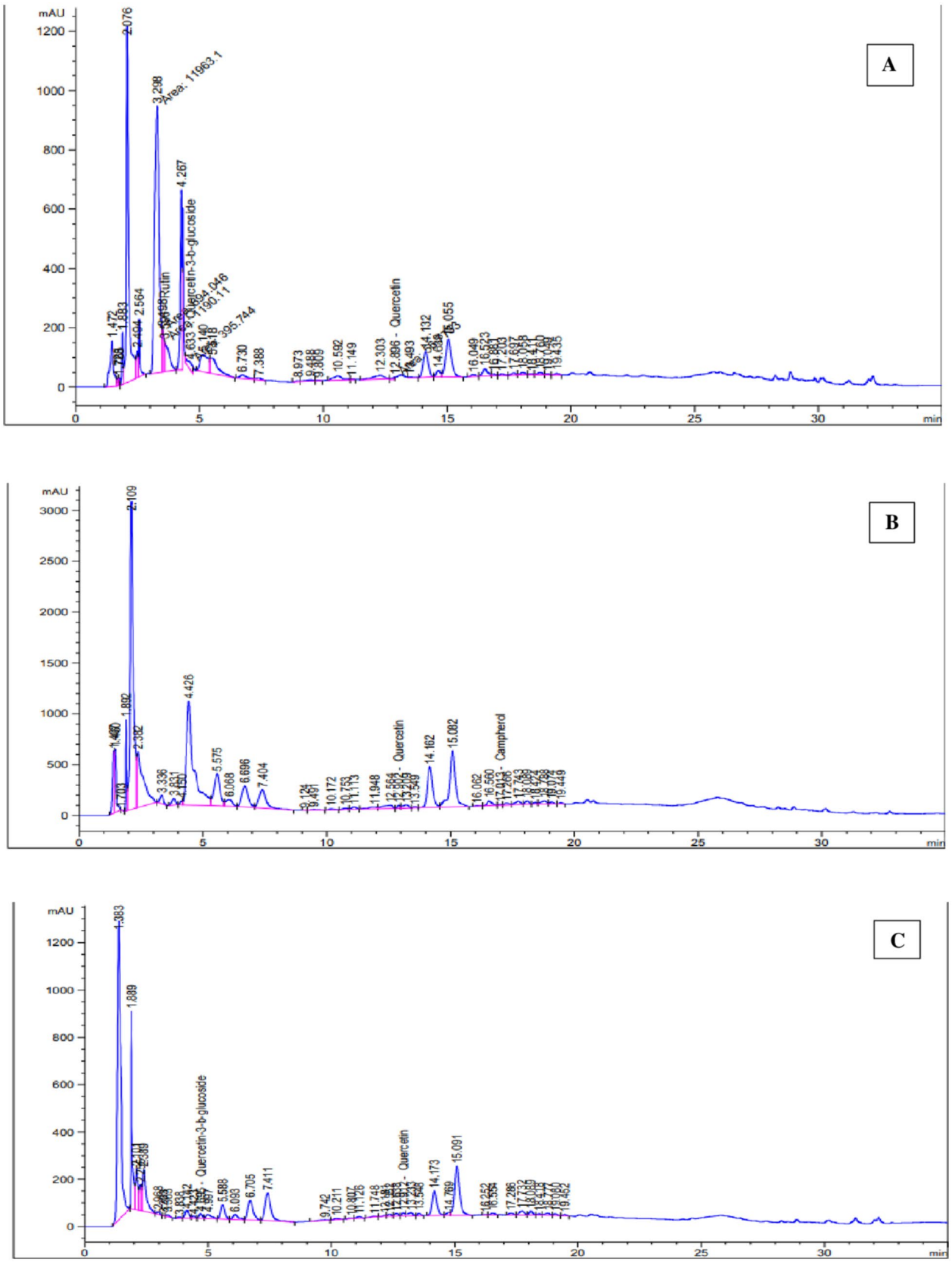

Fig. 8 HPLC analysis of flavonoids in the hydrolysis fraction obtained from barley plants of Burkhant (A), Virazh (**B**), and Copeland (**C**) varieties after treatment of their seeds with sterile distilled water

Table 4 Composition and content of flavonoids in the bound fraction that was obtained from plants of different barley varieties

Treatment options	Barley variety	Flavonoids (μg g^{-1} DW)									
		Rutin	Quercetin-3-β-glycoside	Naringin	Neohesperidin	Quercetin	Luteolin	Naringenin	Apigenin	Kaempferol	Total content, Σ
dH_2O	Burkhant	178.20 ± 9.21	5.72 ± 1.88	Nd	Nd	6.60 ± 0.15a	Nd	Nd	Nd	Nd	190.52 ± 13.38a
	Virazh	Nd	Nd	Nd	Nd	44.25 ± 5.21a	Nd	Nd	Nd	3.51 ± 0.19	47.76 ± 3.99a
	Copeland	Nd	7.23 ± 0.88	Nd	Nd	13.32 ± 0.18a	Nd	Nd	Nd	Nd	20.55 ± 1.34a
Nano-CP	Burkhant	11.00 ± 0.32	5.85 ± 0.18	Nd	Nd	2.98 ± 0.10a	Nd	Nd	Nd	Nd	19.83 ± 0.16a
	Virazh	Nd	83.70 ± 6.29	Nd	Nd	40.28 ± 3.99a	Nd	Nd	Nd	1.29 ± 0.02	125.27 ± 9.06a
	Copeland	Nd	8.73 ± 0.98	Nd	Nd	16.92 ± 0.94a	Nd	Nd	Nd	Nd	25.65 ± 1.16a
H_2O_2	Burkhant	Nd	0.36 ± 0.04	19.66 ± 2.27	Nd	5.31 ± 0.50a	Nd	Nd	Nd	Nd	25.33 ± 1.02a
	Virazh	Nd	56.30 ± 2.19	Nd	Nd	38.95 ± 1.68a	Nd	Nd	Nd	0.66 ± 0.05	95.91 ± 5.29a
	Copeland	Nd	7.01 ± 0.26	Nd	12.49 ± 0.34	16.36 ± 0.21a	Nd	Nd	Nd	Nd	35.86 ± 0.16a
H_2O_2 + Nano-CP	Burkhant	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	Virazh	Nd	36.78 ± 5.16	Nd	Nd	42.63 ± 6.39a	Nd	Nd	Nd	0.94 ± 0.08	80.35 ± 7.02a
	Copeland	Nd	5.03 ± 0.78	Nd	4.61	9.88 ± 0.62a	Nd	Nd	Nd	Nd	19.52 ± 0.34a

Different letters indicate the values significantly differing one from another within a column of the table based on the results of comparison using the Tukey test ($P < 0.05$)

ND not detected

of PGPR on phenylpropanoid pathways is associated with the stimulation or inhibition of plant growth at a certain phase of their development. That is, if rhizobacteria activate growth, then the biosynthesis of flavonoids is inhibited and, conversely, with a decrease in growth, the level of these phenolic compounds in plants increases [81]. We conducted our research with barley plants in the tube stage. This is one of the most critical periods in the ontogeny of cereal spiked crops. This phase is characterized by the formation of flowers in the spikelets and active growth of the spikelet. That is, this is the transition from the vegetative to the generative phase of cereal crop development [82]. In barley plants of Burkhant and Copeland varieties, whose seeds were inoculated with Azogran, this transition was very slow. They were still growing quite actively, which may have influenced the decrease in flavonoid levels.

Under the action of hydrogen peroxide on the seed of the Virazh variety, the content of flavonoids in the bound fraction decreased by 29.36 μg g^{-1} DW, compared to plants whose seeds were treated with the nanocomposite complex bacterial preparation Azogran. Treatment of barley grain of Burkhant variety with this stress agent stimulated the synthesis of naringin in plants, its concentration was 19.66 μg g^{-1} DW, while the content of other flavonoids in the bound fraction decreased (Table 4). Hydrogen peroxide had a stimulating effect only on the flavonoid complex of Copeland plants. In addition, neohesperidin was identified. The increase in the flavonoid content may be due to the ability of H_2O_2 to regulate the expression of the genes of phenylalanine ammonia lyase, chalcone synthase, and stilbene synthase, which are involved in the synthesis of plant flavonoids [83, 84].

Post-treatment of stressed barley seeds with Azogran had the most positive effect on the flavonoid complex of the bound fraction of plants of the Virazh variety. Accordingly, a high content of quercetin-3-β-glycoside—36.78 μg g^{-1} DW and quercetin—42.63 μg g^{-1} DW was found (Table 4). For the other two varieties, this effect was not observed. Ayuso-Calles with co-authors [85] showed that in lettuce inoculated with *Rhizobium laguerreae* bacteria, which developed under salt stress, the content of flavonoids was slightly reduced compared to inoculated plants growing under normal conditions. Such effects may have different causes. First, it is the type of microorganisms-inoculants. Zapata-Sufientes et al. [86], at studying the effect of *Pseudomonas paralactis*, *Sinorhizobium meliloti*, and *Acinetobacter radioresistens* on the flavonoid content of cucumbers, found that *S. meliloti* bacteria contribute to the greatest accumulation of these compounds in the fruits of these plants. Secondly, it is the plant variety. According to a study by Jeon et al. [81], treatment of two broccoli varieties, Malibu and Coronado, with the epiphytic rhizobacterium *Paraburkholderia* led to a greater accumulation of flavonoid glycosides only in Malibu plants. Whereas Zaferanchi et al. [87] pointed out

an insignificant concentration difference of flavonoids in marigold plants of Isfahan double flower and Isfahan single flower varieties, the seeds of which were inoculated with PGPR (*Azotobacter* sp.145PI and *Azospirillum* sp.AC49I). And third, it is the level of influence of the stressor on the plant, the higher it is, the greater the imbalance of redox homeostasis and other biochemical processes in the cells.

Conclusion

The large amplitude of variation of phenolic carboxylic acids and flavonoids indicates the specificity of the interaction of different barley varieties with the bacteria components of Azogran and their different responses to the effect of the preparation under conditions of peroxide stress. For barley variety Virazh, higher results were obtained in studying the effect of the stress agent and nanocomposite complex bacterial preparation Azogran on the qualitative and quantitative content of phenolic compounds in its plants. Since the selection of this variety and its agricultural technology is carried out in Ukraine, it is more adapted to the climatic conditions and soil microbial community of this country. At that time, the Burkhant (Mongolia) and Copeland (Canada) barley varieties were first grown in Ukraine. However, the proposed treatment of native and post-treatment of stressed seeds of these barley varieties with a nanocomposite complex bacterial preparation helped to activate the synthesis of a complex of phenolic compounds in their plants.

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Author contributions IS designed experiments, performed extraction of phenolic compounds from plant material, analyzed data and wrote manuscript. UE participated in designing the experiments, experiment analysis, and interpretation of data. BD provided seeds of two barley varieties Burkhant (Mongolia) and Copeland (Canada). MK performed HPLC analysis.

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Availability of data and materials All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Compliance with ethics requirements The authors declare this study was conducted in accordance with ethical guidelines and principles.

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