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Impact of *L*-phenylalanine ammonia-lyase inhibition on winter triticale (×*Triticosecale* Wittmack) at early developmental stages

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A b s t r a c t. Climate change, particularly increasingly frequent autumn droughts, poses a significant threat to winter triticale by limiting seedling emergence and early development. Various strategies are being investigated to enhance winter triticale at the early stages of development. Therefore, this study aimed to investigate the impact of *L*-phenylalanine ammonia-lyase (PAL) inhibition on seed germination and very early seedling development, with a particular focus on carbon allocation towards carbohydrate biosynthesis and its subsequent utilization in growth processes. 4-Hydroxybenzoic acid hydrazide (HBH) was used as a PAL inhibitor. Osmotic potential, phenolic and carbohydrate content, photosynthetic pigments, antioxidant potential, blue fluorescence intensity, chlorophyll fluorescence, stomatal conductance, and coleoptile length were analyzed. The PAL inhibitor reduced the accumulation of phenolic compounds in the coleoptiles, accompanied by an increase in soluble sugar content and osmotic potential. The decrease in phenolic levels was supported by decrease in blue fluorescence emission. HBH lowered the antioxidant potential of coleoptiles and roots, while elevating the antioxidant potential of germinating seeds. The inhibitor suppressed coleoptile elongation and negatively affected the chlorophyll content, stomatal conductance, and the photosynthetic apparatus activity of the emerging first true leaf. Our results showed that PAL inhibition alters carbon allocation and negatively affects early seedling development.

K e y w o r d s: triticale, PAL inhibition, phenolics, carbohydrates, low-molecular antioxidants, chlorophyll fluorescence

1. INTRODUCTION

Triticale (× *Triticosecale* Wittmack) is an artificially developed cereal species, an intergeneric hybrid combining traits of wheat and rye (Różewicz, 2022). From wheat, it has inherited a high yield potential, while from rye it has gained strong tolerance to less fertile soils as well as to both biotic and abiotic stresses (Feledyn-Szewczyk *et al.*, 2020). Triticale is widely cultivated as a valuable feed grain, particularly for livestock (swine, poultry, dairy, beef cattle, sheep), due to its favorable nutritional profile. Consequently, it plays an important role in ensuring feed security for animal production and contributes to the stability of the agricultural food chain (Randhawa *et al.*, 2015).

Climate change, including increasingly frequent autumn droughts, poses a growing threat to winter cereal crops by limiting proper seedling emergence and early development (Olesen *et al.*, 2012). It should be emphasized that seed germination, coleoptile growth, and root system establishment determine uniform plant stand density, as well as the capacity for water and nutrient uptake, which are critical for subsequent growth, development, and yield formation (Giri and Schillinger, 2003; Vuković *et al.*, 2022). Therefore, various strategies (*e.g.*, seed imbibition, application of biostimulants, and appropriate soil management) are being investigated to enhance and secure cereal

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crops at the early stages of development, thereby improving their resilience to environmental stresses and increasing yield potential (Atkinson *et al.*, 2009; Jócsák *et al.*, 2022; Hobson *et al.*, 2023; Radzikowska-Kujawska *et al.*, 2023).

L-phenylalanine ammonia-lyase (PAL, EC 4.3.1.24) is a key enzyme initiating the phenylpropanoid pathway by catalyzing the deamination of *L*-phenylalanine to trans-cinnamic acid (Koukol and Conn, 1961; Urban and Hura, 2023). PAL activity determines the synthesis of secondary metabolites such as phenolic acids, flavonoids and lignin, which play crucial roles in stress adaptation (Kumar *et al.*, 2023), cellular signaling (Shalaby and Horwitz, 2015), and in shaping the mechanical properties of plant tissues (Rodriguez-Arcos *et al.*, 2002). Therefore, phenolic compounds are an important and interesting object of study in the context of growth and developmental regulation in plants (Kanjana *et al.*, 2024). On the other hand, their biosynthesis depends on the availability of carbohydrates (Hura *et al.*, 2023), which play essential roles in plant stress responses, signaling pathways, and developmental processes (Saksena *et al.*, 2020). Thus, modulation of PAL activity may affect the allocation of carbon between primary carbohydrate metabolism and secondary phenolic metabolism, which can have significant implications for plant growth and development (Peiser *et al.*, 1998; Klejdus *et al.*, 2013; Feduraev *et al.*, 2021).

One of the research approaches presented in this study involves the application of an inhibitor of *L*-phenylalanine ammonia-lyase (Zucker, 1968), *e.g*. 4-hydroxybenzoic acid hydrazide (HBH) (Bhuiyan *et al.*, 2009). The chemical structure of HBH is similar to *L*-phenylalanine, the natural substrate of *L*-phenylalanine ammonia-lyase, and it acts as a competitive inhibitor by binding to the enzyme's active site, thereby blocking its catalytic function (Urban and Hura, 2023).

Therefore, the aim of this study was to investigate the effect of HBH on PAL activity during the early seedling development of winter triticale, with particular focus on the variability of selected physiological parameters potentially influenced by the modulation of PAL activity. It was hypothesized that PAL inhibition would modify carbon allocation toward carbohydrate synthesis and its subsequent utilization in growth processes. To test this hypothesis, key physiological parameters were assessed, including osmotic potential, phenolic compound content, blue fluorescence intensity, carbohydrate levels, antioxidant potential, and coleoptile length. Additionally, the contents of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids), stomatal conductance, and photosynthetic apparatus activity were analyzed.

2. MATERIALS AND METHODS

Seeds of winter triticale (*cv*. 'Moderato') were surface-sterilized in 70% ethanol (2 min) and 3% NaOCl (5 min), followed by four rinses in sterile distilled water. The seeds were then placed in sterile Petri dishes containing filter papers moistened either with distilled water (control) or with a 10^{-3} M solution of the PAL inhibitor, 4-hydroxybenzoic acid hydrazide (HBH) (Bhuiyan *et al.*, 2009). Each treatment consisted of six Petri dishes, each containing 25 seeds (150 seeds per treatment). Filter papers were replenished with either water or the inhibitor solution as needed to maintain a moist filter papers. Seed germination and seedling growth were conducted in climatized *in vitro* growth chamber at a constant temperature of 22°C (day/night), with a photosynthetic photon flux density (PPFD) of 100 μmol m^{-2} s^{-1} and 10 h/14 h (day/night) photoperiod.

Measurements were performed at two developmental stages: BBCH 10 – first leaf emerging through the coleoptile (osmotic potential, biochemical analyses, seedling length) and BBCH 11 – first leaf unfolded (chlorophyll *a*, chlorophyll *b*, carotenoids, stomatal conductance, chlorophyll fluorescence). Coleoptiles and seedlings of winter triticale were randomly selected for each measurement from the six Petri dishes within each treatment.

The total phenolic content (TPC) was determined using the Folin-Ciocalteau method of Singleton and Rossi (1965) after extraction with 96% ethanol. The absorbance was measured at 760 nm with a spectrophotometer (Ultrospec 2100 Pro, Amersham Biosciences, Cambridge, UK). Chlorogenic acid was used as a standard. The measurements were taken with ten replicates.

About 10 mg of plant material was homogenized and extracted with 2.5 ml of 96% aqueous ethanol. The homogenates were centrifuged at 1500 x g for 15 min at 4°C. Chlorophyll present in supernatant was removed by several extractions with hexane until no green color was visible (Hura *et al.*, 2006). The intensity of blue fluorescence emission (IF_{blue}) from ethanol solutions was recorded with Perkin-Elmer LS 50B spectrofluorometer (Norwalk, CT, USA) between 350 and 650 nm. Samples were excited at 340 nm. The slit width was set to 10 nm for excitation and to 15 nm for emission (Hura *et al.*, 2018). Measurements were carried out at room temperature in seven replicates.

Total soluble carbohydrate (TSC) content was determined with anthrone (dissolved in concentrated sulfuric acid) (Ashwell, 1957) added to aqueous extracts of leaf samples and incubated for 15 min at 90°C. Absorbance was measured spectrophotometrically (Ultrospec 2100 Pro, Amersham Biosciences, Cambridge, UK) at 620 nm. Glucose was used as a standard. The measurements were taken with seven replicates.

Measurement of osmotic potential (Ψ_O) was taken with a psychrometer HR 33T (WESCOR, Inc., Logan, UT, USA) equipped with C-52 sample chambers (WESCOR).

Analyses were done for the sap squeezed out of the coleoptile and the emerging first leaf tissue with a syringe. Filter paper discs (∅ = 5 mm) soaked in sap were placed in the chambers and left for 30 min. The measurements were taken in the dew point mode with five replicates.

Antioxidant potential was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Hura *et al.*, 2022). Absorbance was measured at 517 nm (Ultrospec 2100 Pro, Amersham Biosciences, Cambridge, UK), 30 min after the reaction was initiated. Antioxidant potential was assessed in coleoptiles ($DPPH_C$), roots ($DPPH_R$), and emerging seeds ($DPPH_{ES}$). The percent of inhibition that determined capability of the extract antioxidants to counteract oxidation was calculated as:

$$inhibition = \frac{100(A_0 - A)}{A_0},$$

A_0 – absorbance of DPPH radical solution, A – absorbance of the investigated extracts. The measurements were taken with ten replicates.

The length of winter triticale seedlings was measured at the BBCH 10 stage, *i.e.*, during the emergence of the first leaf. The measurements were taken with twenty five replicates.

Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Crts) were quantified spectrophotometrically (Ultrospec 2100 Pro, Amersham Biosciences, Cambridge, UK) after extraction with 96% ethanol. Absorbances at 663, 646 and 470 nm were read and the concentration of chlorophylls and carotenoids was then calculated according to Lichtenthaler and Wellburn (1983). The measurements were taken with seven replicates.

Chlorophyll fluorescence measurements were done with the use of fluorometer Handy PEA (Hansatech Ltd., Kings Lynn, UK). They were performed after 25 min of leaf adaptation to darkness. F_v/F_m (quantum yield of PSII) was calculated according to van Kooten and Snel (1990). Additionally other parameters were calculated per excited leaf cross-section (CS_m): ABS/CS_m (energy absorption by antennas), PI (overall performance index of PSII photochemistry), DI_O/CS_m (energy amount dissipated from PSII), RC/CS_m (number of active reaction centers), Et_0/CS_m (amount of energy used for the electron transport) and TR_O/CS_m (amount of excitation energy trapped in PSII reaction centers), ψ_O (exciton transfer efficiency to the electron transport chain), and φ_{Eo} (quantum yields of photoinduced electron transport in PSII reaction center from Q_A^- to plastoquinone). Parameters calculation was based on the theory of energy flow in PSII and the JIP test (Strasser and Tsimilli-Michael, 2001; Tsimilli-Michael and Strasser, 2008; Strasser *et al.*, 2010). The measurements were taken with twenty replicates.

The measurements of stomatal conductance (g_S) were done with a leaf porometer (SC-1, Decagon Devices, Pullman, WA, United States). The seedlings were additionally illuminated. PPFD (photosynthetic photon flux density) of about 180 μmol m^{-2} s^{-1} (QSPAR Quantum Sensor, Hansatech Instruments LTD, Kings Lynn, England) was provided by high pressure sodium lamps (400 W, Philips SON-T AGRO, Brussels, Belgium). The measurements were taken with seven replicates.

Statistica 13.0 (Stat-Soft, Inc., Tulsa, OK, USA) was used for data analysis. Statistical differences between the experimental groups were evaluated using Student's *t*-test. The data were represented as means ± standard error (SE). Asterisk indicates statistical significance at $p \leq 0.05$.

3. RESULTS

3.1. Biochemical and growth responses of winter triticale coleoptiles at the BBCH 10 stage under PAL inhibition

The application of 4-hydroxybenzoic acid hydrazide (HBH) significantly affected the physiological traits of winter triticale coleoptiles (Table 1). Total phenolic content was significantly reduced in HBH-treated coleoptiles compared with the control (7.54 *vs*. 11.87 μg mg^{-1} (d.w.)). Decrease in phenolic level was consistent with the pronounced decline in blue fluorescence intensity (IF_{blue}), which dropped by approximately 50% under HBH treatment (1.38 (r.u.)) relative to the control (2.77 (r.u.)). Moreover, the wavelength corresponding to the maximum IF_{blue} emission exhibited a significant shift from 426.86 nm in control samples to 419.90 nm in HBH-treated coleoptiles, further supporting alterations in phenolic composition. In contrast, HBH significantly enhanced the total soluble carbohydrate content, which increased by nearly 47% compared with the control (31.51 *vs.* 21.40 μg mg^{-1} (d.w.)) (Table 1). This was accompanied by an increase in osmotic potential in HBH-treated coleoptiles (from -0.61 to -1.09 MPa).

Table 1. Changes in total phenolic content (TPC), intensity emission of blue fluorescence (IF_{blue}), wavelength at maximum IF_{blue}, total soluble carbohydrates (TSC) and osmotic potential (Ψ_O) of winter triticale seedlings at BBCH 10 stage under control conditions (water) and treatment with the PAL inhibitor 4-hydroxybenzoic acid hydrazide (HBH). Mean values± SE (n=5 for Ψ_O, n=7 for IF_{blue}, wavelength, TSC, n = 10 for TPC). Asterisks indicate significant differences at $p<0.05$ *vs*. control (Student's *t*-test) within measured parameters

Parameter	Control	Inhibitor
TPC (μg mg^{-1}(d.w.))	11.87 ± 0.46	7.54 ± 0.40*
IF_{blue} (r.u.)	2.77 ± 0.08	1.38 ± 0.10*
Wavelength at maximum IF_{blue} (nm)	426.86 ± 1.70	419.90 ± 0.10*
TSC (μg mg^{-1}(d.w.))	21.40 ± 1.01	31.51 ± 1.57*
Ψ_O (MPa)	-0.61 ± 0.03	-1.09 ± 0.09*

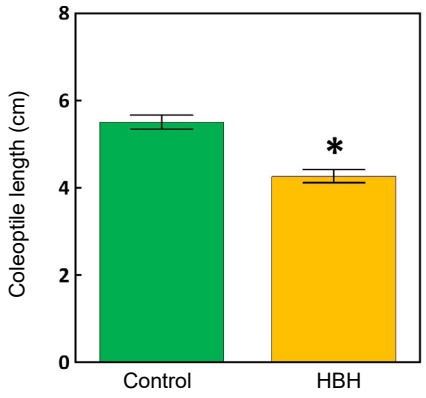

Fig. 1. Coleoptile length of winter triticale seedlings at BBCH 10 stage under control conditions (water) and treatment with the PAL inhibitor 4-hydroxybenzoic acid hydrazide (HBH). Mean values ± SE (n = 25). Asterisk marks significant differences at $p < 0.05$ *vs.* control, Student's *t*-test.

Coleoptile length was significantly reduced under HBH treatment compared with the control (Fig. 1). Under control conditions an average coleoptile length exhibited 5.5 cm, whereas HBH-treated was about 4.3 cm indicating that inhibition of PAL negatively affected early coleoptile elongation of winter triticale seedlings.

The antioxidant potential of coleoptiles ($DPPH_C$), roots ($DPPH_R$), and germinated seeds ($DPPH_{GS}$) is shown in Table 2. In coleoptiles, the $DPPH_C$ value significantly decreased from 50.45% in the control to 37.63% in the presence of the inhibitor. Similarly, roots exhibited a pronounced decline in $DPPH_R$ values, from 32.91% in the control to 18.92% under HBH treatment. Interestingly, in germinated seeds, the $DPPH_{GS}$ value significantly increased from 4.54% in the control to 14.37% in the presence of the inhibitor.

3.2. Biochemical and physiological responses of winter triticale coleoptiles at the BBCH 11 stage under PAL inhibition

The application of HBH markedly affected the pigment composition of winter triticale seedlings (Table 3). Chlorophyll *a* (Chl *a*) content was significantly reduced under PAL inhibition (6.08 μg mg^{-1}(d.w.)) compared to the control (8.87 μg mg^{-1} (d.w.)). Although the decline in chlorophyll *b* (Chl *b*) content was not statistically significant (control: 1.96 μg mg^{-1} (d.w.), inhibitor: 2.33 μg mg^{-1} (d.w.)), the total chlorophyll content (Chl *a*+*b*) exhibited a significant reduction in HBH-treated seedlings (8.03 μg mg^{-1}(d.w.)) in relation to the control (11.21 μg mg^{-1}(d.w.)). Furthermore, carotenoids level (Crts) significantly decreased in response to PAL inhibition (1.88 μg mg^{-1} (d.w.)) in comparison to untreated plants (2.52 μg mg^{-1} (d.w.)).

The effect of the PAL inhibitor on the photosynthetic apparatus activity was evaluated using chlorophyll *a* fluorescence parameters (Table 4). The maximum quantum

Table 2. Changes in antioxidant potential in coleoptiles ($DPPH_C$), roots ($DPPH_R$), and emerging seeds ($DPPH_{ES}$) of winter triticale seedlings at BBCH 10 stage under control conditions (water) and treatment with the PAL inhibitor 4-hydroxybenzoic acid hydrazide (HBH). Mean values ± SE (n = 10). Asterisks indicate significant differences at $p < 0.05$ *vs.* control (Student's *t*-test) within measured parameters

Parameter (%)	Control	Inhibitor
$DPPH_C$	50.45 ± 1.44	37.63 ± 2.33*
$DPPH_R$	32.91 ± 1.53	18.92 ± 1.62*
$DPPH_{GS}$	4.54 ± 0.52	14.37 ± 1.29*

Table 3. Changes in content of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), chlorophyll *a* + *b* (Chl *a* + *b*) and carotenoids (Crts) of winter triticale seedlings at BBCH 11 stage under control conditions (water) and treatment with the PAL inhibitor 4-hydroxybenzoic acid hydrazide (HBH). Mean values ± SE (n = 7). Asterisks indicate significant differences at $p < 0.05$ *vs.* control (Student's *t*-test) within measured parameters

Parameter (μg mg^{-1} (d.w.))	Control	Inhibitor
Chl *a*	8.87 ± 0.29	6.08 ± 0.37*
Chl *b*	2.33 ± 0.06	1.96 ± 0.38
Chl *a*+*b*	11.21 ± 0.35	8.03 ± 0.69*
Crts	2.52 ± 0.07	1.88 ± 0.12*

Table 4. Changes in chlorophyll fluorescence parameters (F_v/F_m - quantum yield of PSII, ABS/CS_m - energy absorption by antennas, PI - overall performance index of PSII photochemistry, Et_0/CS_m - amount of energy used for the electron transport, TR_O/CS_m - amount of excitation energy trapped in PSII reaction centers, RC/CS_m - number of active reaction centers, DI_O/CS_m - energy amount dissipated from PSII, ψ_O - exciton transfer efficiency to the electron transport chain, φ_{Eo} - quantum yields of photoinduced electron transport in PSII reaction center from Q_A^- to plastoquinone) of winter triticale seedlings at BBCH 11 stage under control conditions (water) and treatment with the PAL inhibitor 4-hydroxybenzoic acid hydrazide (HBH). Mean values ± SE (n=20). Asterisks indicate significant differences at $p < 0.05$ *vs.* control (Student's *t*-test) within measured parameters

Parameter	Control	Inhibitor
F_v/F_m	0.815 ± 0.002	0.768 ± 0.007*
ABS/CS_m	3197 ± 47	2755 ± 96*
TR_O/CS_m	2605 ± 41	2130 ± 91*
Et_0/CS_m	1389 ± 24	1039 ± 49*
RC/CS_m	1040 ± 19	829 ± 32*
DI_O/CS_m	591.7 ± 7.3	625.3 ± 7.0*
PI	1.64 ± 0.04	1.00 ± 0.06*
ψ_O	0.533 ± 0.004	0.489 ± 0.011*
φ_{Eo}	0.434 ± 0.003	0.375 ± 0.009*

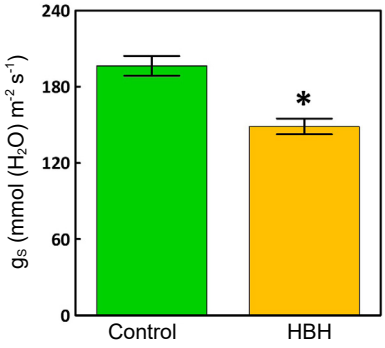

Fig. 2. Changes in stomatal conductance (g_S) of winter triticale seedlings at BBCH 11 stage under control conditions (water) and treatment with the PAL inhibitor 4-hydroxybenzoic acid hydrazide (HBH). Mean values ± SE (n = 7). Asterisk indicates significant differences at $p < 0.05$ *vs*. control, Student's *t*-test.

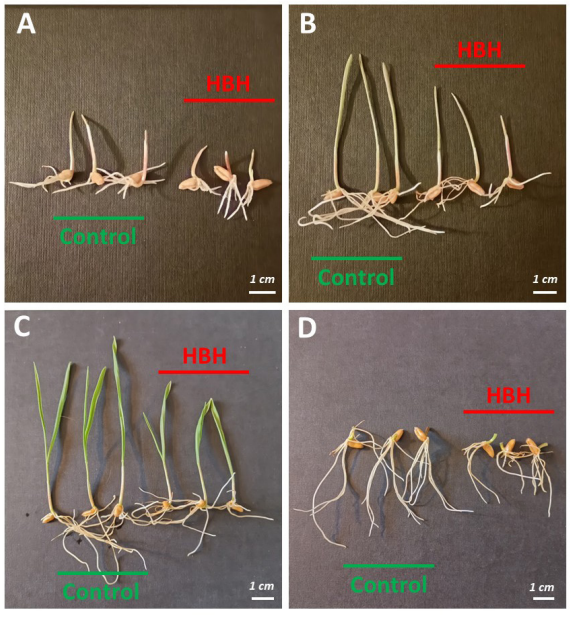

Fig. 3. The differences in growth dynamics of winter triticale seedlings under treatment with the PAL inhibitor, 4-hydroxybenzoic acid hydrazide (HBH). A – germination stage, B – coleoptiles with emerging leaf, C – seedlings, D – differences in root development.

yield of PSII (F_v/F_m) decreased significantly from 0.815 in the control to 0.768 in HBH-treated seedlings, indicating a reduction in the photochemical efficiency of PSII.

Parameters related to leaf cross-section (CS_m) also showed significant declines under inhibitor treatment. Both energy absorption (ABS/CS_m) and trapped excitation energy (TR_O/CS_m) decreased from 3 197 to 2 755 and from 2 605 to 2 130, respectively, suggesting reduced light-harvesting and trapping efficiency. Correspondingly, amount of energy used for the electron transport (Et_0/CS_m) decreased from 1 389 to 1 039, and the number of active reaction centers (RC/CS_m) dropped from 1040 to 829, reflecting impaired PSII photochemistry. The overall performance index (PI) was also strongly affected, declining from 1.64 to 1.00, which confirms a general reduction in photosynthetic performance. Additionally, energy dissipation (DI_O/CS_m) slightly increased from 591.7 to 625.3, indicating enhanced non-photochemical quenching. Additionally, the exciton transfer efficiency (ψ_O) and the quantum yield of electron transport (φ_{Eo}) decreased from 0.533 to 0.489 and from 0.434 to 0.375 ± 0.009, respectively, confirming that inhibitor treatment impairs electron flow through PSII (Table 4).

Changes in the photosynthetic apparatus activity were accompanied by alterations in stomatal conductance (g_S) (Fig. 2). In control seedlings, g_S was measured at 196 mmol (H_2O) m^{-2} s^{-1}, whereas HBH-treated seedlings exhibited a significantly lower value of 149 mmol (H_2O) m^{-2} s^{-1}.

Figure 3 illustrates the differences in growth dynamics of winter triticale seedlings under treatment with the PAL inhibitor, 4-hydroxybenzoic acid hydrazide (HBH). Distinct differences can be seen between the control and seedlings treated with HBH, which caused inhibition of both shoot and root growth.

4. DISCUSSION

The significant reduction in total phenolic content (TPC) and blue fluorescence intensity (IF_{blue}) (Table 1) in HBH-treated coleoptiles clearly indicates effective suppression of the phenylpropanoid pathway through PAL inhibition. Bhuiyan *et al.* (2009) showed that HBH inhibitor downregulates synthesis of phenolic acids and lignin precursors, ultimately affecting cell wall composition and stress-related metabolic responses in wheat plants. Similar results were also obtained with the use of other inhibitors, such as 2-aminoindan-2-phosphonic acid (AIP) (Mauch-Mani and Slusarenko, 1996; Peiser *et al.*, 1998; Solecka and Kacperska, 2003; Klejdus *et al.*, 2013), *α*-aminooxyacetic acid (AOA) (Hammerschmidt, 1984; Carver *et al.*, 1991; Peiser *et al.*, 1998), *α*-aminooxy-*β*-phenylpropionic acid (AOPP) (Moerschbacher *et al.*, 1990; Carver *et al.*, 1992; Peiser *et al.*, 1998; Taheri and Tarighi, 2011), and O-benzylhydroxylamine (OBHA) (Hoagland, 1985; Feduraev *et al.*, 2021). The observed shift in the wavelength at maximum fluorescence emission further suggests qualitative alterations in the phenolic profile, likely reflecting reduced accumulation of phenylpropanoids, which are UV-absorbing compounds in plant tissues (Cerovic *et al.*, 2002).

In parallel, the pronounced increase in total soluble carbohydrates (TSC) (Table 1) accompanied by an increase in osmotic potential (Ψ_O) (Table 1) suggests a reallocation of carbon toward carbohydrate metabolism and osmotic regulation (Hura *et al.*, 2016). Enhanced carbohydrate accumulation under reduced phenolic biosynthesis has been documented as a metabolic trade-off, in which carbon is less

utilized in secondary pathways to support osmotic adjustment and energy supply during plant development (Caretto *et al.*, 2015). However, despite carbohydrate accumulation, the overall reduction in phenolic compound levels may lead to a decrease the seedlings' defence capacity, potentially explaining the inhibitory effects of HBH on coleoptile and seedlings elongation (Fig. 1A-B), chlorophyll content (Table 3), the photosynthetic apparatus activity (Table 4) and stomata conductance (Fig. 2). Modulation of PAL activity likely triggers a cascade of metabolic changes, as inhibition of a key enzyme in the phenylpropanoid pathway can affect multiple interconnected pathways, including primary and secondary metabolism (Rohde *et al.*, 2004; Urban and Hura, 2023). The preferential accumulation of soluble carbohydrates in coleoptiles may reflect both altered growth dynamics, as the developing shoot reallocates resources under modified metabolic conditions, and stress response mechanisms induced by inhibitor (Planchais *et al.*, 2000).

In our opinion, the observed increase in soluble carbohydrate content, suggests that sugars are preferentially accumulated in coleoptiles rather than utilized for growth. Moreover, at early developmental stages, coleoptiles and seedlings can still utilize seed reserves, including carbohydrates (Aguirre *et al.*, 2018) and phenolic compounds (Xu *et al.*, 2020). Therefore, at BBCH 10 and BBCH 11 stages the inhibition of PAL is not the only factor that may directly affect phenolic content and indirectly influence the level of soluble carbohydrates. Cereal grains are a rich in phenolic compounds such as benzoic and cinnamic acids, anthocyanidins, quinones, flavonols, chalcones, flavonones, and amino-phenols (Liu, 2007; Okarter and Liu, 2010). Jańczak-Pieniążek *et al.* (2023) demonstrated the presence of phenolic compounds in triticale seeds, including p-hydroxybenzoic acid in flour, syringic acid in whole grain and bran, and ferulic acid and sinapic acid in bran.

It should be underlined, that the observed responses may also be partially linked to the direct toxic effects of the inhibitor on the photosynthetic apparatus activity, photosynthetic pigments and stomata conductance of triticale seedlings (Xiao *et al.*, 2020). The values of chlorophyll a fluorescence parameters, including F_v/F_m, ABS/CS_m, TR_O/CS_m, Et_0/CS_m, DI_O/CS_m, RC/CS_m, PI, ψ_O, and φ_{Eo}, significantly changed relative to the control, indicating reduced PSII photochemical efficiency (Table 4). The simultaneous decrease in values of F_v/F_m, overall performance index of PSII photochemistry (PI), and stomata conductance (g_S) (Fig. 2), indicates that HBH impairs both diffusive CO_2 supply into the mesophyll and the photosynthetic apparatus activity. These results are in agreement with studies demonstrating that toxic/chemical stress can negatively influence photosynthesis (Mano *et al.*, 2009) and PS II activity (Kalaji *et al.*, 2016). Additionally, organic compounds, especially those that do not participate in the natural plant metabolism, may exert phytotoxic effects (Tomar *et al.*, 2019). Such compounds can disturb photosynthesis by decreasing chlorophyll biosynthesis (Singh and Prasad, 2018), damaging photosystem II (Kummerová *et al.*, 2008), altering electron transport (Zeb *et al.*, 2022), or inducing oxidative stress (Liu *et al.*, 2009).

The DPPH-based antioxidant potential varied considerably among tissues in response to HBH treatment indicating a substantial reduction in radical-scavenging capacity (Table 2). In coleoptiles antioxidant potential ($DPPH_C$) decreased from 50.45% (control) to 37.63% (HBH), and in roots ($DPPH_R$) dropped from 32.91 to 18.92%. Such declines are often associated with decreased level of phenolic compounds, which are key contributors to non-enzymatic antioxidants in plants (Fadda *et al.*, 2014; Tsimogiannis *et al.*, 2017). In contrast, the observed increase in the antioxidant potential of germinating seeds ($DPPH_{GS}$) resulted from a higher accumulation of HBH in seeds compared with roots and coleoptiles. One possible explanation may be the increased accumulation of the inhibitor in germinating seeds. HBH itself may contribute to the antioxidant capacity due to its chemical structure, which includes a benzene ring and conjugated double bonds capable of stabilizing reactive oxygen species (Urban and Hura, 2023). Miyazawa *et al.* (1998) showed that the activity of cell wall bound peroxidase in tomato hypocotyls was enhanced by treatment of roots with 4-hydroxybenzoic acid hydrazide. Moreover, the antioxidant activity of 4-hydroxybenzhydrazide derivatives has been demonstrated, which is consistent with their broad pharmacological actions and potential relevance in mitigating oxidative stress-related processes (Mateev *et al.*, 2025).

In summary, the present study demonstrates that HBH treatment exerts negative effects on photosynthetic apparatus activity, antioxidant capacity, and stomatal conductance. Chlorophyll *a* fluorescence analysis revealed pronounced declines in PSII photochemical efficiency, energy absorption, electron transport, and the number of active reaction centers, ultimately resulting in a significant reduction in seedling growth.

5. CONCLUSIONS

Our study clearly indicates that phenylalanine ammonia-lyase (PAL) activity is essential for the early growth and development of winter triticale. The application of 4-hydroxybenzoic acid hydrazide (HBH) caused the expected metabolic effect, namely a reduction in phenolic compound content accompanied by an increase in soluble carbohydrate levels. However, it cannot be unequivocally concluded whether this response results from altered carbon allocation under inhibited phenylpropanoid metabolism or from seed reserve mobilization during early developmental stage of winter triticale.

The results obtained in this study indicate a predominantly negative impact of 4-hydroxybenzoic acid hydrazide (HBH) on germination and early growth of winter triti-

cale seedlings, manifested by reduced growth dynamics and limited photosynthetic activity. The findings suggest a toxic effect of the inhibitor at the applied concentration of 10^{-3} M, which may be associated with the low biomass and restricted metabolism of heterotrophic seedlings at very early developmental stage. Therefore, future research should focus on the autotrophic phase of seedling growth, when a fully active metabolism is established, in order to better evaluate the long-term physiological/molecular consequences of HBH treatment. Moreover, subsequent studies should be extended to gas exchange measurements, analyses of non-stomatal limitations of photosynthesis, as well as assessments of yield-related traits.

Conflict of interests. The authors do not declare any conflict of interest.

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