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ORIGINAL PAPER

Modification of soybean and lupine sprouting conditions: influence on yield, ROS generation, and antioxidative systems

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Abstract
Formation of free radicals' and antioxidants' biosynthesis during seeds sprouting strongly depends on plant growth conditions, especially while sprouts are fortified in iron. The influence of watering with $FeSO_4$ solution on soybean and lupine sprouting, yield of iron accumulated in plant tissue, as well as free radical (reactive oxygen and nitrogen species) synthesis and antioxidant activity (capacity) were the objective of this study. Optimal iron accumulation and biomass efficiency were obtained for 3 day watering with water, followed by abiotic stress application (Fe^{2+} ions). For lupine sprouts, maximal radical formation (up to ~ 5.7 a.u.) took place on the fifth day of culturing in these conditions, while, for soybean sprouts, continuous increase in their activity was observed until the seventh day (up to ~ 5.8 a.u.). Total antioxidant activity in lupine sprouts increased definitely, even more than three times during 7 days of lupine growing, but, for soybean, it was almost steady. The same trend was observed in phenolic compounds and flavonoids contents. For soybean sprouts, these antioxidants were almost steady, while, for lupine sprouts in these conditions, the intensive synthesis of these antioxidants was observed. In response to high iron content applied from the fourth day in the cultivation media, lupine sprouts synthesize low molecular antioxidants effectively. For soybean sprouts, another mechanism of defense against oxidative stress should be expected.

Keywords Abiotic stress · Flavonoids · Fortified sprouts · Free radicals · Phenolic compounds · Total antioxidant capacity

Introduction

There is a growing interest in the global functional food market. This food is defined as designed for lowering the risk of various illnesses and/or having health promoting effects. One of the most important groups of functional food is food enriched in vitamins and minerals, since food enrichment and especially food biofortification is the most effective, long-term method of preventing malnutrition from these compounds deficiency [1, 2].

Seeds sprouting may bring many dietary benefits into food. Sprouts are sources of numerous valuable nutrient components, such as vitamins, trace elements, amino acids, and antioxidants. Seeds' sprouting increases the seeds' nutritive value, and this increase depends on the sprouting conditions used—thus, it may be controlled. For example, high concentration of iron has been applied during legume sprouting to simulate overexpression of ferritin formation in soybean and lupine. Obtained sprouts accumulated 70 times more of iron [3], mainly enclosed in form of plant ferritin, which is an antioxidant compound synthesized by the plant defense system as a response to the high iron content [4, 5]. Hereby, the sprouts may become a good source of dietary iron with high bioavailability [3, 6]. Iron is an important microelement for all living organism, because it is a co-factor of many proteins.

It determines the activity of numerous antioxidant enzymes involved in the reaction of transfer of electrons, but it also generates reactive species formation. Thus, it is obvious that the application of these specific sprouting conditions (i.e., excessive concentration of iron ions in the culturing media) disturbs the balance between the content of free radicals and antioxidant system. The high content of iron in the plant environment stimulates expression of ferritin, which sequesters these ions, preventing the formation of highly toxic radical HO· via Fenton and Haber–Weiss reactions. However, simultaneously, many radical scavengers are synthesized simultaneously [7].

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Reactive species are involved in many metabolic processes. Recognized exogenous sources of reactive oxygen species (ROS) for human cells are pollutants, tobacco smoke, drugs, xenobiotics, radiation, ethanol, some hormones (estrogen, noradrenaline), heavy metal ions, and other mediators [8]. Under steady conditions, reactive species are scavenged by various defense mechanisms, but it is possible that the molecules may appear in the human diet. Even if ROS and RNS (reactive nitrogen species) present in food are usually not indicated as a reactive species inductor in human cells, it is worth controlling the food in terms of their presence, because they cause lipid, protein, and nucleic acid peroxidation, reducing the nutritional value of food. From the nutritional point of view, the most undesirable is lipid peroxidation, especially polyunsaturated fatty acids, which are phospholipids components. That is why, one of the critical parameters characterising the sprouts enriched with Fe (apart from the concentration of this element) should be the relationship between the level of ROS and activity of antioxidants [9].

The presence of phenolic antioxidants helps to maintain an antioxidative–prooxidative balance in the plant organism. On the other hand, consumption of phenolic compounds is associated with many health benefits including reduced risk of cancer, cardiovascular disease, neurodegeneration, diabetes, and osteoporosis. It has been observed before [9] that the applied system of seeds' sprouting induced of ROS formation in the sprouts. However, ROS seeds' concentration always increases while sprouting and simultaneously induce an activation of defense system against the radical formation, i.e., large overproduction of antioxidants valuable to human health. Thus, the objective of this study was to evaluate the influence of soybean and lupine sprouting conditions on yield of iron accumulated in plant tissue, free radical (reactive oxygen and nitrogen species) synthesis, as well as antioxidant activity (capacity).

Materials and methods

Experimental material and its preparation

As an experimental material, dried soybean and lupine seeds were chosen. Soybean seeds (*Glycine max*), Nawiko and Augusta varieties were provided by the Department of Genetics and Plant Breeding, the Poznań University of Life Sciences, Poland, while lupine seeds, i.e., yellow lupine (*Lupinus luteus*) Lord variety and blue lupine (*Lupinus angustifolius*) Baron variety, were obtained from the Smolice Plant Breeding in Przebędowo, Poland.

The raw seeds were disinfected (15 min with 70% ethanol). The ethanol was washed away, and experimental materials were divided and classified as 'control seeds' (CS) and 'experimental seeds' (ES) and sprouted in assumed experimental conditions for 7 days. CS were swelled for 6 h at first day, and following day watered with distilled water once a day during the rest 6 days of the experiment, while ES from the 4th day of the experiment were watered once a day with the 25 mM $FeSO_4$ (lupine seeds) and 20 mM $FeSO_4$ (soybean). The remaining germination conditions were controlled and the same for all group of sprouted seeds. Sprouting was carried in special germination dishes placed in climatic chambers (Adaptis, Conviron, Germany), in the same temperature (24 °C), relative humidity 90%, 6 days in dark and with access to natural light imitation in the last 24 h.

Samples for analysis were collected each day. If it was desirable, analysis was performed for the fresh material (free radical determination); the rest were dried in a stream of air until 8–10% of moisture was obtained and stored in the 4 °C for the rest of the analysis.

Biomass efficiency determination

Efficiency of sprouting was calculated as a dry mass of sprouts obtained from 100 g of dry seeds and expressed as a percentage.

Total iron determination

The samples were mineralized at 450 °C to obtain carbon-free white ash. Afterwards, the ash dissolved in 1 N nitric acid was filtered and analyzed on iron content using flame atomic absorption spectrometry with deuterium (Zeiss AAS-3, Jena) ($\lambda = 248.3$, slit width of 0.15 nm) [10].

Measurement of reactive species generation

Total activity of reactive oxygen (ROS) and nitrogen (RNS) species was measured with the probe DCFH (2′,7′-dichlorodihydrofluorescein). DCFH is able to detect hydroxyl, peroxyl, and other reactive species produced by cells.

To prepare the probe, 0.00122 g of DCFH-DA (2′,7′-dichlorodihydrofluorescein diacetate, Sigma-Aldrich) in 1.5 mL of DMSO (Sigma-Aldrich) was dissolved. Next, the hydrolysis took place by addition 0.5 mL 0.25 M NaOH (in the dark, during 30 min. at room temperature). After that, the pH of the solution was decreased down to 7.4 by 0.25 M HCl to stop the process and the volume was adjusted to 25 mL with 0.1 M phosphate buffer pH 7.4. The obtained 0.01 M solution of DCFH was kept at 4 °C and used in next 4 days.

Samples for free radical determination were collected twice each day of sprouting (18 and 24 h after watering). 2.5 g of the material was shredded in ten times diluted PBS and 30 min shaken. Centrifuged extract made up to 50 mL.

Subsequently, the 1.97 mL of the phosphate buffer (with temperature 37 °C), 0.235 mL of the analyzed sample, and 0.100 mL of the prepared DCFH solution were mixed in temperature 37 °C, and the fluorescence intensity was measured (Spectrofluorimeter RF-5001 PC, Shimadzu). Measurement was done in the temp. 37 °C each 1 min during 10 min, at excitation wavelength $\lambda = 480$ nm and emission wavelength $\lambda = 520$ nm.

The relative fluorescent intensity (RFI) was compared to the results obtained for DCFH solution in ten times diluted PBS. Changes in RFI values were used for comparison in free radical activity [expressed in arbitrary units (a.u.)].

Methanol extract preparation

Dried and milled sprouts were hexane defatted by Soxhlet extraction (Büchi Labortechnik AG, Flawil, Switzerland). Following, each sample was extracted three times with 80% methanol. The samples were mixed with the solvent (1:10), shaken for 30 min, and centrifuged. The extraction was twice repeated (with the fresh solvent volume − 25 mL). Mixed supernatants were evaporated under reduced pressure using an R-215 rotavapor (Büchi Labortechnik AG, Flawil, Switzerland). The residue was quantitatively dissolved in 50 mL 80% methanol [11].

Total antioxidant capacity determination

To determine the total antioxidant capacity, the TRAP method was chosen, based on the antioxidants ability to stop oxidation of the same probe as for free radical activity measurements: DCFH (2′,7′-dichlorodihydrofluorescein) as a result of peroxide radical scavenging [12].

Subsequently, the 1.97 mL of the phosphate buffer (with temperature 37 °C), 0.235 mL of the analyzed sample (methanol extract), 0.100 mL of the prepared DCFH solution, and 0.2 M solution of 2,2′-azobis (2-amidynopropan) (AAPH, free radical generator) were mixed in temperature 37 °C, and the fluorescence intensity was measured (Spectrofluorimeter RF-5001 PC, Shimadzu). Measurement was done in the temp. 37 °C each 1 min during 10 min, at excitation wavelength $\lambda = 480$ nm and emission wavelength $\lambda = 520$ nm. After determination Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) concentration was read from calibration curve (time of the tenfold increase in fluorescence intensity versus Trolox concentration). Total antioxidant activity of the analyzed extracts was calculated according to the formula:

$$\mathrm{TRAP} = C_t \cdot \frac{V_c}{V_p} = C_t \cdot \frac{2.405}{0.235},$$

where C_t is the Trolox concentration (μM), V_c is the total solution volume, and V_p is the volume of the sample added.

TRAP is expressed as Trolox equivalent/100 g of dry matter (TE/100 g d.m.)

Total phenolic compound content

The total phenolic content in methanolic extract was determined by the Folin–Ciocalteu method [13]. An aliquot (0.2 mL) of the methanol extract was diluted with Folin–Ciocalteu reagent (0.5 mL). After 3 min, saturated solution of sodium carbonate (0.1 mL) was added. The flask was filled with water up to 10 mL and allowed to stand at room temperature. After 1 h, absorbance at $\lambda_{max} = 725$ nm against a reagent blank was measured using a UV–Vis spectrophotometer SP 8001 (Metertech Inc., Taipei, Taiwan). Gallic acid (0–70 μg/10 mL) was used to produce standard calibration curve $y = 0.0109x$ ($R^2 = 0.9959$). The concentration of phenolic compounds in the extracts was expressed in milligrams of gallic acid equivalents (GAE) per 100 g of dry plant material.

Total flavonoid content

The total flavonoid content was determined by a colorimetric method [14]. Obtained methanol extract was mixed with the same volume of 2% $AlCl_2$, and the mixture was allowed to stand for another 10 min. The absorbance was measured against the prepared blank at 430 nm. Quercetin standard solutions (0.002–1.118 M) were used to prepare a standard curve ($y = 40{,}133x$; $R^2 = 0.9996$). The concentration of flavonoids in the extracts is expressed as milligrams of quercetin equivalent (QE) per 100 g of dry plant material.

Statistical analysis

All extracts were prepared in two repetitions, while their analysis three times. All data were presented as mean ± SD. The statistical significance of the difference between the control and the treated sample was assessed by one-way ANOVA and post hoc Tukey's tests using Statistica 10.0 (StatSoft). Results were considered statistically significant at $P < 0.05$.

Results and discussion

Abiotic stress resulted from the increased content of iron in time of legume seeds sprouting leads to the metal accumulation by ferritin in growing plants. Thus, the sprouts, even if strongly degenerated, may become a source of iron in the diet of people suffering from its deficiency [5]. Moreover, it was found that the sprouts still are source of valuable antioxidant species [11], but also an information about the free radicals increased formation in these sprouts appeared [9].

Thus, the objective of this study was to evaluate the influence of soybean and lupine sprouting conditions on yield of iron accumulated in plant tissue (YFe), free radical (reactive oxygen and nitrogen species) synthesis, as well as antioxidant activity (capacity). Thanks to this, it is possible to optimize conditions of the sprouts enriched in iron growth to obtain optimal biomass yield and increased antioxidants content to eliminate the effects of free radical formation.

Due to the confirmed in the literature stability of native ferritin mRNA in germinating seeds, up to the third day of sprouting [15], to initiate the intensive biomass growth, the germination in water in the first days was applied followed by the watering with the $FeSO_4$ in the next days. The most limited ability of iron accumulation was noted for the sprouts watered with water up to the cultivation day 4, which resulted probably both from short time of iron ion application, and from hydrolysis of present in the seeds mRNA coding the ferritin expression [15]; this hydrolysis limited the defense mechanism capacity against the stress resulted from high iron concentration. But also the stress conditions applied in the first day of seeds growth proved to be not desired (Fig. 1). To maximize the iron accumulation, the best was to apply for the first 3 days watering with water and then use the stress conditions. The duration of watering with distilled water also strongly influenced on biomass growth. Even if the biomass growth was the largest of course during the sprouting 7 days in water, the decrease in biomass, when the sprouts were 3 days watered with water and continued with the $FeSO_4$ solution, was not strongly decreased (Table 1). Thus, these conditions were considered as an optimal to maximize iron accumulation by growing sprouts. It should be noted that the introduction of 3 days of sprouts watering with water significantly reduces environmental pollution and the consumption of chemical agents to obtain the sprouts enriched in iron. Moreover, it was observed that maximal iron accumulation took place for soybean growing in 20 mM of $FeSO_4$ and increasing of this concentration did not influence that accumulation statistically, while, for lupine, the most favorable was 25 mM $FeSO_4$.

Thus, these conditions (3 days of growth with pre-watering with distilled water before applying the stress) were

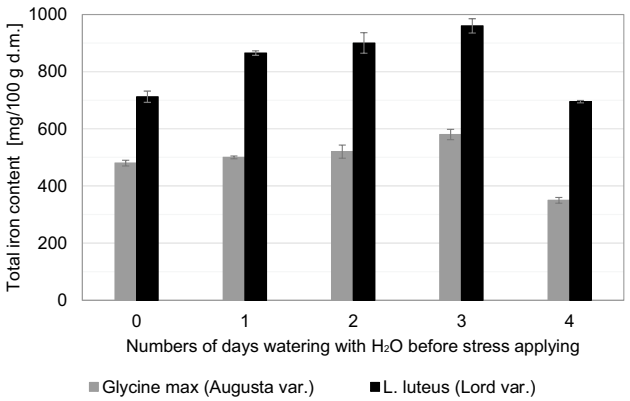

Fig. 1 Influence of sprouts watering with water before application of iron ion stress (20 mM for soybean and 25 mM for lupine seeds) on total iron content in the final material (sprouts dry matter)

Table 1 Efficiency of biomass obtaining at the end of the process calculated as a dry mass of sprouts obtained from 100 g of dry seeds (%) after watering with water (from 0 to 3 days) and cultivation in successive days with different concentrations of iron (15–25 mM $FeSO_4$)

Genus	Variety	Watering with water (number of days)	Efficiency of biomass obtaining at the end of the process (%) after successive days of cultivation in		
			15 mM $FeSO_4$	20 mM $FeSO_4$	25 mM $FeSO_4$
Soybean	Augusta	0	86.4 ± 2.0 a*	89.0 ± 2.8 a	94.7 ± 0.6 a
		1	85.7 ± 3.0 a	89.3 ± 1.1 a	85.6 ± 2.0 b
		2	84.7 ± 0.5 a,b	88.1 ± 0.1 a	82.2 ± 1.9 c
		3	82.8 ± 0.4 c	84.4 ± 0.4 b	85.0 ± 4.5 b
	Nawiko	0	86.0 ± 0.8 b	87.4 ± 0.5 a	91.9 ± 3.1 a
		1	87.8 ± 1.1 a	87.7 ± 3.6 a	89.0 ± 2.4 a
		2	85.6 ± 0.2 b	87.0 ± 0.3 a	82.9 ± 3.2 b
		3	87.2 ± 3.0 a	86.7 ± 1.1 a	89.1 ± 2.4 a
Lupinus	Baron	0	85.9 ± 1.1 a	86.6 ± 0.5 a	80.8 ± 1.7 b
		1	83.6 ± 2.2 b	85.9 ± 1.1 a	71.2 ± 2.1 c
		2	85.9 ± 2.7 a	85. 8 ± 2.7 a	83.1 ± 0.5 a
		3	84.0 ± 1.1 a,b	84.3 ± 1.4 a,b	81.6 ± 2.4 a,b
	Lord	0	84.3 ± 0.5 a	86.8 ± 4.2 a	82.8 ± 3.4 a,b
		1	84.1 ± 0.9 a	86.9 ± 3.1 a	86.0 ± 1.9 a
		2	82.4 ± 0.4 b	85.2 ± 0.3 a	85.0 ± 2.0 a
		3	82.9 ± 0.7 b	83.6 ± 1.6 b	86.8 ± 1.8 a

*Values denoted by different letters in a table cell differ statistically significantly at the significance level $\alpha = 0.05$

chosen for the sprouts preparation. Because it was suggested that radical formation is an important factor affecting the nutrient value of iron-enriched sprouts Przybysz et al. [9], following the free radical (FR) activity in these materials was studied and compared to antioxidant formation.

The FR activity (reactive oxygen and nitrogen forms) in sprouts cultured in abiotic stress was always higher than for controlled sprouts (i.e., cultured 7 days on distilled water). However, different trends were noted for soy and lupine sprouts. Abiotic stress strongly influenced on FR formation in lupine just after applying. On the fifth day of lupine sprouting, when the 25 mM of iron was applied in the culture, relative fluorescence intensity (RFI) strongly increased reaching a maximum value 5.5–6.0 a.u. (Fig. 2a). This day was critical for FR formation also for sprouts cultured in distilled water, for which the RFI 1.6–1.8 a.u. was achieved in fifth day. Then, the free radical activity decreased, and achieved in the last day of the sprouts culturing the value ~ 2.6 times higher than for sprouts cultured in distilled water. While, for soybean (Fig. 2b), continuous increase in FR activity was noted, both for sprouts cultured in water as for sprouts cultured in increased iron content. The fluorescence intensity achieved in the seventh day of culturing the value ~ 6.0 a.u. just after watering them, and during next 5 h, it was decreased down to 3.7 a.u. for Augusta and 5.1 a.u. for Nawiko sprouts, but it was still 2.2–3 times higher value than in controlled sprouts (cultured in water). It was observed, during the whole experiment, that radical activity usually decreased when the time which has elapsed, since watering was longer (compared the effect 18 and 24 h after watering), especially in soybean sprouts (Fig. 2).

Free radical formation was significantly higher than in controlled sprouts. However, due to the observed intensive biomass growth under the proposed conditions, we may expect efficient action of the defense mechanism against the formed free radical activity, i.e., natural antioxidant formation.

Total antioxidant capacity (TAC), which reflects the antioxidants formation in the examined material, was almost stable for soybean sprouts (Fig. 3). However, for lupine sprouts, it was strongly increased, and in the 7th day, it was even 3–3.5 higher than for the sprouts from the first day of the experiment (increase from 3.93 ± 0.93 TE/100 g d.m. up to 11.98 ± 0.8 for Lord variety, and from 3.09 ± 0.12 up to 11.08 ± 1.05 for Baron variety), and significantly higher than for obtained soybean sprouts (Fig. 4) (increase from

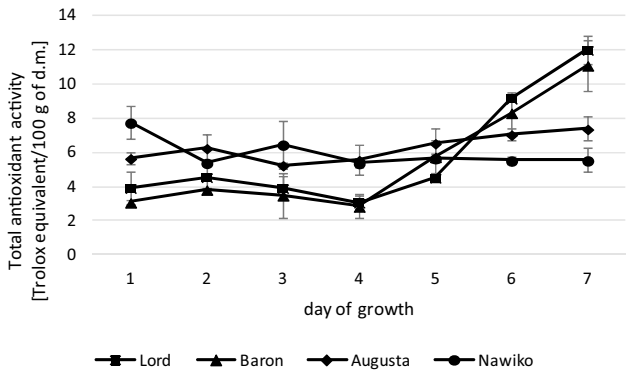

Fig. 3 Total antioxidant capacity (activity) (TAC) (Trolox equivalent/100 g of dry matter) determined with TRAP method for soybean (varieties Augusta and Nawiko) and lupine (varieties Lord and Baron) sprouts enriched in iron

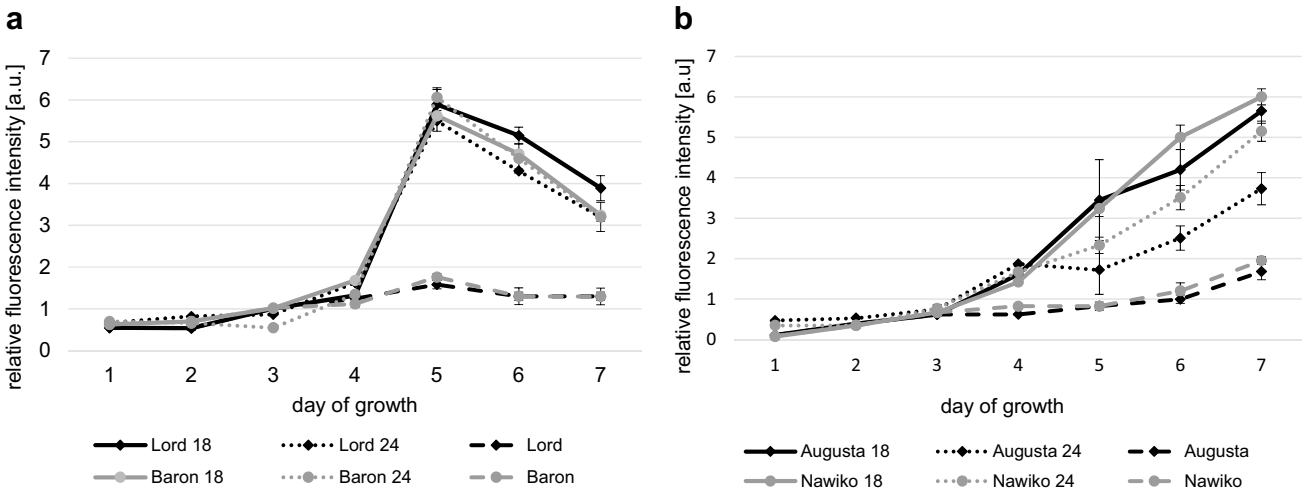

Fig. 2 Changes in the activity of reactive oxygen and nitrogen species (relative fluorescence intensity of DCFH probe) in the 7-day germinating in the discussed conditions (3 days of growth with pre-watering with distilled water before applying the stress): **a** lupine seeds (varieties Lord and Baron) and **b** soybean seeds (varieties Augusta and Nawiko). Samples were collected 18 and 24 h after watering with proper $FeSO_4$ solution. Superscripts: 18–18 h after watering and 24–24 h after watering

5.66 ± 0.31 TE/100 g d.m. up to 7.37 ± 0.69 for Augusta variety, and slight decrease from 7.74 ± 0.98 down to 5.54 ± 0.68 for Nawiko variety). Usually, in time of sprouting antioxidants formation is observed, especially in last days of the process, but it can be disrupted in abiotic stress conditions [11, 16]. For lupine sprouts, it was observed, how strongly the sprouting conditions influence on the antioxidants formations (Fig. 3). The results showed that in the conditions chosen for producing of seed enriched in iron not only iron accumulation and biomass increase was the highest, but it was also connected to the significantly higher antioxidants formation in the material. In that condition, fivefold increase in TAC was observed compare to dry seeds.

Thus, it can be expected that, in prepared in proposed way material, activity of antioxidant to a large degree will eliminate the undesirable effects of free radical formation.

Subsequent analyzes were aimed to trace non-enzymatic antioxidants' formation in the material. Figure 5 presents changes in phenolic compounds in time of the sprouts' growing. It has been observed that the trends in these phenolic compounds' formation are comparable to changes noted in TAC. The content of phenolic compounds in soybean sprouts has changed slightly, while sprouting (even if statistical differences were noted) and reached the range 350–400 GE/100 g d.m. However, for lupine sprouts, a significant increase in the content of phenolic compounds was noted, from the fifth day of germination, and in the seventh day, it was three times higher than in the first day of germination (373.12 ± 8.00 GE/100 g d.m compared to 184.54 ± 2.93 GE/100 g d.m for Lord variety, and 354.45 ± 5.11 GE/100 g d.m compared to 233.11 ± 5.08 GE/100 g d.m. for Baron var.). Even if, at the beginning, it was almost twice lower than for soybean sprouts, at the end, the content of phenolic compounds was similar to their content in soybean sprouts. Usually, increase in phenolic compounds was noted in sprouts, especially in last days of germination [17], but it was noted before that in the stress conditions caused by iron ions, the formation of the phenolic compounds in soybean sprouts is inhibited [5]. Decrease of reactive species formation in lupine was probably the result of phenolic compounds' biosynthesis in response to abiotic stress conditions.

The similar trend was observed for flavonoid compounds, presented in Fig. 6. Soybean generally is a good source of flavonoid content belonging to the isoflavone group (mainly genistein and daidzain) [18], while lupine seeds contain mainly flavones, such as apigenin derivatives [12]. The content is strongly dependent on the variety and genus, and growth conditions. In the presented experiment (Fig. 6), reduction of the flavonoids content in sprouting soybean was observed (from 114.35 ± 1.42 down to 87.47 ± 1.37 QE/100 g d.m. for Nawiko variety and from 94.87 ± 3.9 down to 78.82 ± 2.19 QE/100 g d.m. for Augusta variety), while increase in this compound content in Lupin sprouts was very clear (for Lord variety form 69.76 ± 9.07 up to 231.15 ± 3.17 QE/100 g d.m.; while for Baron from 62.94 ± 1.47 up to 212.54 ± 5.82 QE/100 g d.m.).

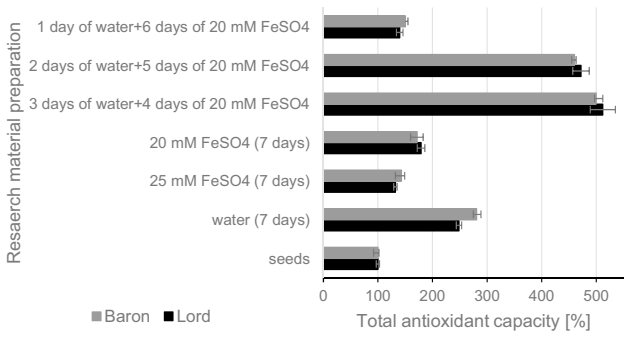

Fig. 4 Influence of sprouting conditions on the total antioxidant capacity (TAC) of lupine sprouts (varieties Lord and Baron) compared to the TAC of lupine seeds (assuming that seeds TAC is 100%)

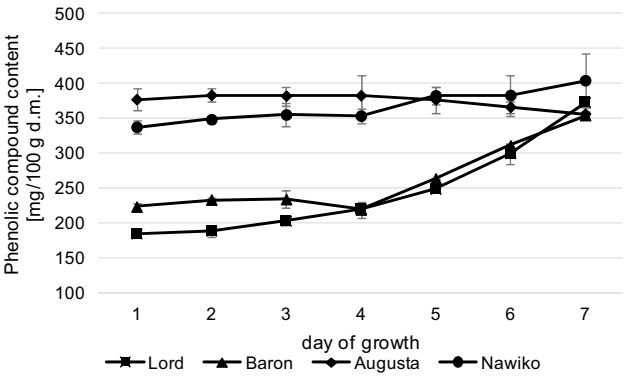

Fig. 5 Changes in the content of phenolic compounds during 7-day germination in the soybean (varieties Augusta and Nawiko) and lupine (varieties Lord and Baron) sprouts enriched in iron

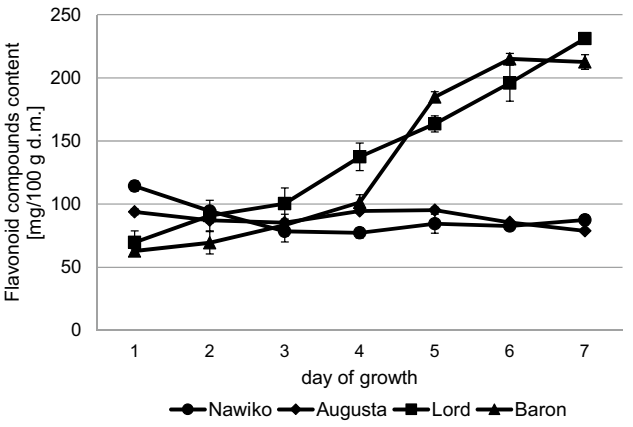

Fig. 6 Changes in the content of flavonoid compounds during 7-day germination in the soybean (varieties Augusta and Nawiko) and lupine (varieties Lord and Baron) sprouts enriched in iron

Result of analysis of phenolic and flavonoid compounds' content is consistent with the designated changes in total antioxidant capacity, which was almost stable in time of soy sprouting but strongly increased, while lupine seeds were germinated in the studied conditions. This intensive formation of antioxidants could strongly inhibit the reactivity of radicals formed in the growing sprouts in response to the applied abiotic stress.

Much more effective system of defense against abiotic stress and as a consequence free radical formation was observed in sprouting lupine than in soybean seeds. In response to high iron content in the fourth day in cultivation media, lupine sprouts not only express ferritin, but also effectively synthesize low molecular antioxidants, while, in soybean sprouts, these small antioxidants' content was stable. Some of this low molecular antioxidants were the same as those found in the seeds, but many of them were not observed before and require further identification, e.g., some lupine isoflavones [19]. Because the tolerance of soybean and lupine seeds to iron content in the culturing media, as well as ferritin expression in the obtained in discussed conditions is comparable [20, 21], it could be expected that, probably, another mechanism of defense against oxidative stress, e.g., associated with the expression of antioxidant enzymes, occurs in sprouting in abiotic stress soybean seeds.

Conclusions

The application of stress from the beginning of the plant growth inhibited its life processes, limiting biomass growth; prolonging watering for more than 3 days when mRNA encoding ferritin in dry seeds was disassembled, inhibited the possibility of its overexpression. On the basis of the studied performed, the optimal conditions for iron accumulation in the sprouts (YFe) were established. The most favorable accumulation of this metal without strong inhibition of biomass growth was reached after 3 days of sprouting in water followed with 4 days of growing in abiotic stress, i.e., watering soybean by 20 mM of $FeSO_4$ and lupine by 25 mM of $FeSO_4$.

Free radical (reactive oxygen and nitrogen species) formation in the proposed conditions was much higher than in controlled sample, i.e., in sprouts cultured for 7 days in water. Different trends in free radical formation were observed for soybean and lupine sprouts. Critical day for FR activity in lupine sprouts fell on the fifth day of sprouting, while, for soybean sprouts, continuous growth in free radical formation was noted. However, it could be observed that synthesis of antioxidant took place simultaneously in the studied material. The total antioxidant capacity for the prepared sprouts was almost steady for soybean sprouts in time of germination, while, in lupine seeds, it increased strongly—more than three times compared to the first day of sprouting. The total antioxidant capacity (activity) for the lupine sprouts prepared in the studied conditions was almost five times higher than that determined in the dried seeds and about twice higher than that determined for sprouts growing in distilled water.

Intensive formation of both phenolics and flavonoid compounds in the lupine sprouts was noted. It can be assumed that their increased synthesis was the response to oxidative stress caused by the presence of iron ions.

The presented experiments confirmed that sprouting conditions strongly influenced on: iron accumulation, biomass growth, free radical formation, and antioxidant activity (capacity). Sprouts, especially lupine sprouts, prepared in the proposed way may be a good source not only of iron, but also antioxidants for human diet.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

Compliance with ethics requirements No human individuals or animals were involved in the presented experiments.

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