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BIOLOGICAL QUALITY OF TRITICALE GREEN PLANTS BIOFORTIFIED WITH IODINE

JAKOŚĆ BIOLOGICZNA ZIELONYCH ROŚLIN PSZENŻYTA BIOFORTYFIKOWANYCH JODEM

Abstract: Plant biofortified with iodine may be an alternative source of this element in human diet. The purpose of the research was to determine the effect of fertilization on quality of green plant biological iodine. Iodine in the form of KIO_3 or KI was applied to the soil or sprayed on the plants. Iodine application (2.50 or 5 kg · ha^{-1}) was split, with the first half applied together with spring nitrogen fertilization and the other half applied one month before sampling for laboratory tests. In all the tested combinations of fertilization iodine increases iodine content in green plants compared to control. The application rates and means of fertilization with iodine salts did not adversely affect the biological quality of the plants.

Keywords: triticale, iodine, biological quality

Introduction

Iodine is an essential element in a proper, balanced diet for humans and animals (mainly mammals). It is necessary for the production of thyroid hormones, which through their influence on protein biosynthesis regulate the metabolism of sugars, fats and nucleic acids and contribute to cell growth and proliferation [1]. The development and functioning of the nervous system and other organs depends on the availability of iodine in the diet. Iodine deficits during pregnancy and childhood lead to a number of

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illnesses, such as impaired foetal development, cretinism, and delayed physical and mental development, while in adults they cause thyroid enlargement (goitre) and increase the risk of thyroid and stomach cancer [2]. In many countries, despite the implementation of table salt iodization programmes and supplementation of infant formulas and preparations for pregnant women, the problem of health disorders associated with iodine deficiency remains [3]. Owing to prophylactic salt iodization in Poland, iodine intake at the population level is adequate [4], but excessive consumption of table salt may increase the risk of another disease of civilization, hypertension. Prevention of cardiovascular disease involves limiting intake of salt and thus of iodine, which creates the risk of reducing iodine in the diet. Other food sources of iodine, such as milk and dairy products, eggs, and marine fish [5], can largely satisfy the need for iodine. Daily intake of iodine, depending on a person's physiological state and age, should be between 90 and 250 μg, and for an adult 150 μg [4]. Iodine is also essential in animal nutrition. Cows' requirement for iodine is 0.5 mg/kg of dry weight of diet, which corresponds to 9–11 mg of I per day. It increases to 36–40 mg I per day in the presence of goitrogens and nitrates [6], and during peak lactation, e.g. in Ireland, the standard is 60 mg/day. Brzozka [6] reports that the iodine deficiency in cows in Poland is about 10–55 mg/day and requires supplementation, as iodine affects fertility as well as the content of iodine in milk. Supplementation may be in the form of iodine-enriched salt licks, but high sodium intake is associated with the risk of electrolyte imbalances. The importance of iodine in metabolism and the complexity of factors that cause iodine deficiency are the main reasons for the need to find rich and readily available natural sources. The solution proposed in this paper is to introduce more iodine to the food chain through biofortification of triticale plants. Green triticale plants can be used to feed animals, e.g. as silage, and extracts from them can be used to produce dietary supplements for humans.

Materials and methods

In the years 2013–2014, a field experiment was conducted at the Experimental Station in Zamosc belonging to the University of Life Sciences in Lublin (50°42′ N, 23°12′ E) to fortify winter triticale with iodine. Triticale was grown on brown soil of loess origin, classified as good wheat complex, valuation class 2, with the following grain-size composition: 47 % silt, 40 % clay, 11 % sand and 1.92–2.04 % humus. Chemical analysis of the soil showed that it was very rich in phosphorus and potassium, with average content of magnesium, and its pH was slightly acidic (pH in KCl = 6.7). In accordance with agricultural research standards, nitrogen, phosphorus and potassium fertilizers were applied before sowing at the rates recommended for the triticale variety and the content of these elements in the soil. The second portion of nitrogen was applied in the spring. Iodine in the form of KIO_3 or KI was applied to the soil and sprayed on the plants. At the base of the control (variant I), which was without iodine fertilizing, we set up four other variants in which we used mineral fertilizing by iodine : variant II – 2.5 $kg \cdot ha^{-1}$ KI soil application, III – 5 $kg \cdot ha^{-1}$ KI soil application, IV – 2.5 $kg \cdot ha^{-1}$ KI foliar application, V – 5 $kg \cdot ha^{-1}$ KI foliar application, VI – 2.5 $kg \cdot ha^{-1}$ KIO_3 soil application, VII – 5 kg · ha^{-1} KIO_3 soil application, VIII – 2.5 kg · ha^{-1} KIO_3 foliar application, IX – 5 kg · ha^{-1} KIO_3 foliar application. Iodine application (2.50 or 5 kg · ha^{-1}) was split, with the first half applied together with spring nitrogen fertilization and the other half applied one month before sampling for laboratory tests.

Laboratory tests

Iodine in the triticale plants was determined by the ICP-MS method (inductively coupled plasma mass spectrometry) after incubation with TMAH (tetramethylammonium hydroxide) [7].

Extracts of the green triticale plants for determination of protein, reducing sugars and antioxidant properties were prepared from their above-ground parts. The plants were chopped, 100 g of the sample was rinsed in sterile water, and 100 cm^3 of sterile distilled water at 4 ^{o}C was added. After cooling in an ice bath, the samples were homogenized for 2 min and centrifuged for 5 min at 3500 rpm, and then the extract was collected.

The protein content was determined according to the Lowry method [8].

The content of reducing sugars was determined by the Miller method using 3,5-dinitrosalicylic acid (DNS) [9] with reference to a glucose standard curve. The absorbance was measured at 575 nm.

Total antioxidant capacity was determined by the DPPH method. When the synthetic DPPH (2,2-diphenyl-1-picrylhydrazyl) radical reacts with antioxidants, it takes on electrons from these compounds and undergoes a colour change [10]. A methanol solution of DPPH was added to an extract from the green parts of the triticale. The reaction mixture was incubated for 30 min in the dark. The decrease in absorbance was measured at 517 nm [11]. The capacity of the antioxidants in the seedling extract to counteract the oxidation reaction was calculated according to the following equation [10]:

$$\%\ \text{inhibition} = \frac{A_0 - A_c}{A_0} \cdot 100$$

where: A_0 – initial absorbance of DPPH radical solution;
A_c – mean absorbance for the added sample at concentration c.

Total antioxidant capacity was expressed as Trolox equivalent (a synthetic antioxidant) per g of fresh weight of the aerial parts of the triticale plants.

Total content of carotenoids and chlorophyll a and b was determined by spectrophotometry [12]. The aerial parts of the plant were chopped and 100 g samples were extracted with 80 % acetone. Absorbance was measured at 663 nm, 646 nm and 470 nm. The content of chlorophyll and carotenoids in the test sample was expressed as mg · g^{-1} fresh weight (f.w.).

Results and discussion

Iodine is an element that can be taken up by both the roots and the above-ground parts of plants, where it is absorbed by the stomata and reaches a high degree of saturation in the epidermal cuticle layer [13]. Various forms of iodine are used for biofortification: $CH_2ICOONa$, NaI, $NaIO_3$, KI, KIO_3, or organic iodine from algae [14–16]. The KI and KIO_3 used in the present study for biofortification of triticale plants and the means of application – foliar or soil – resulted in an increase in iodine concentration in the plants. The iodine content in the control triticale plants was 0.131 mg I · kg^{-1} f.w, which is within the typical range of iodine content in crop plants (e.g. broccoli contains 0.15 mg · kg^{-1} iodine, spinach 0.12 mg · kg^{-1}, and pea seeds 0.14 mg · kg^{-1} [17]. The iodine-fertilized triticale accumulated this element Fig. 1. For the same KI concentrations, soil fertilization resulted in higher accumulation of iodine in the plants than foliar fertilization. Similarly, higher content of iodine in the plant was found after soil application of KIO_3 than after foliar application of the same concentrations. Application of iodine in the form of KI resulted in a higher content in the plants than the same amount in the form of KIO_3. Following iodine biofortification, e.g. 2.5 kg · ha^{-1} applied to the soil in the form of KI, triticale plants satisfy cows' requirement for iodine (for a dose of 40 mg I/day) [6] in the amount of about 12 kg of fresh weight.

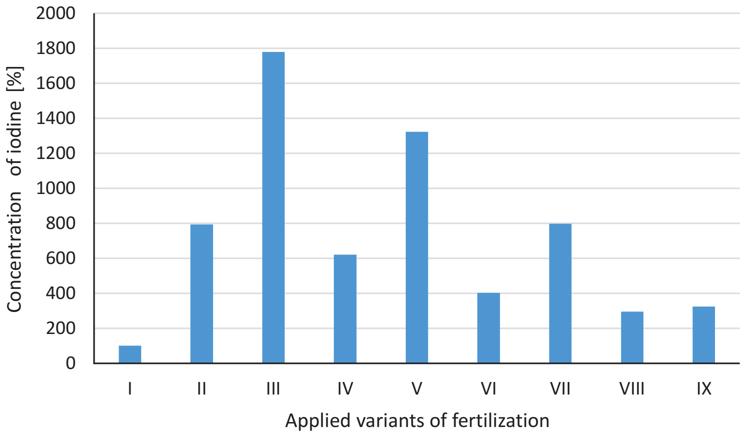

Fig. 1. Iodine content in green plants of triticale

Biofortified plants can be added to silage and thus enrich cows' diets with iodine, thereby increasing its intake in the human diet through milk. Many farmers grow triticale for silage from whole plants. After the triticale harvest, maize can be sown for silage on the same field.

No symptoms of chlorosis or necrosis were observed in the crop, which suggests that the levels of iodine applied have no toxic effect on triticale plants.

Lawson et al. [16] found that iodine in field cultivation of vegetables, applied at 15 kg · ha^{-1} in the form of KIO_3 or KI, caused symptoms of chlorosis and necrosis, but at lower application rates KI proved more effective in biofortification. The literature provides data on the biofortification of various species of plants with iodine. Many data pertain to leafy vegetables, because they contain more iodine than root plants. Attempts have been made at biofortification of various plants with iodine: cabbage [14], lettuce [18], celery [19] and radish [20]. A positive effect of fertilization with iodine compounds, increasing this element while maintaining the high eating quality of the edible parts of plants, has been found for spinach [21], cabbage [22], celery [23] tomatoes [24], and zucchini [25]. Enriching plants with iodine poses the risk of reducing their biological quality, which has been reported for lettuce [26], carrots [27], tomato and potato [28], and Chinese cabbage [14]. The iodine-biofortified plants obtained were to be consumed directly by humans. The main problem in these studies, however, was that the level of iodine applied was not optimized so as not to exceed the standards for human intake, while maintaining the high biological and commercial quality of plants intended for eating.

Our assessment of the biological quality of the triticale plants focused on determining the content of photosynthetic pigments, soluble sugars, soluble proteins and the total content of substances with antioxidant properties, and thus important substances in the diet of humans and animals.

Only soil application of KI caused an increase in the content of soluble sugars in the triticale plants. Plant material from the remaining experimental treatments did not differ statistically significantly from the control in the content of soluble sugars (Table 1). Soluble sugars are an energy substrate, which in plant extracts occur mainly as monosaccharides, disaccharides and oligosaccharides [29]. The content of soluble sugars in plants fluctuates significantly depending on weather conditions and the stage of growth. In fresh and stored biofortified carrot, a reduction in glucose, fructose, sucrose, and total soluble sugars was observed, with stronger effects induced by fertilization with KI than KIO_3 [27].

Table 1

The content of same biological quality parameters in green plants of triticale fertilized with iodine

Variant of fertilization	I	II	III	IV	V	VI	VII	VIII	IX	*LSD*
Soluble protein content [$mg \cdot g^{-1}$ f.w.]	1.73	2.10*	2.17	1.98*	2.08*	2.16*	1.89	2.14*	2.27*	0.189
Reducing sugars [$mg \cdot g^{-1}$ f.w.]	0.34	0.40 *	0.45 *	0.34	0.35	0.32	0.34	0.35	0.35	0.049
Total antioxidant capacity [mmol Trolox $\cdot g^{-1}$ f.w.]	48.44	55.34*	56.32*	58.07*	45.95	64.10*	54.60*	66.32*	60.23*	4.78

Means designated with the same row are different significantly at $p < 0.05$ from the control; *LSD* – Least significant difference.

The concentration of soluble proteins in the extract of the triticale plants increased in all samples fertilized with iodine (Table 1). Compared to other elements, research on the effects of iodine fertilization on structural components such as proteins is conducted on a small scale. The concentration of free amino acids has been found to increase in radish after foliar application of KI. The same effect was obtained with fertilization with KIO_3, but biofortification with this form of iodine was carried out together with nitrogen fertilization [20]. Proteins play a key role in growth and metabolism in the cell. Studies on biofortification with iodine often determine parameters such as growth intensity and biomass production, and thus processes in which the participation of proteins is crucial. In maize cultivation, biofortification with iodine has been shown to cause a decrease in biomass production [28] and in yield [22]. A reduction in biomass was noted following biofortification of rice (*Oryza sativa* L.) with KI and KIO_3, while application of a KI solution at concentrations of 0.5 % and 0.75 % was found to reduce the plant size, panicle length, grain number and yield of rice [29]. A negative impact of KI on rice growth was also reported by Kato et al. [30]. Similarly, a decrease in biomass was observed in cultivation of wheat (*Triticum aestivum* L.) fertilized with iodine [28].

The content of carotenoid pigments did not change significantly in plants fertilized with iodine (Table 2). Carotenoids in the human diet perform important functions. They are precursors of vitamin A, an antioxidant present in the lipophilic environment of the cell, and may also affect the expression of some genes [31].

Table 2

The content of photosynthesis pigments in green plants of triticale fertilized with iodine

Variant of fertilization	I	II	III	IV	V	VI	VII	VIII	IX	*LSD*
Total carotenoid [$mg \cdot g^{-1}$ f.w.]	4.37	4.77	4.24	4.23	4.13	4.19	4.15	4.31	4.12	—
Chlorophyll a [$mg \cdot g^{-1}$ f.w.]	22.58	23.54	20.00^*	16.82^*	19.38^*	18.98^*	18.08^*	19.02^*	20.30^*	2.095
Chlorophyll b [$mg \cdot g^{-1}$ f.w.]	10.33	12.21	9.89	8.41	8.96^*	9.40	8.74	8.89	9.63	1.778

Means designated with the same row are different significantly at $p < 0.05$ from the control; *LSD* – Least significant difference.

Chlorophyll a concentrations in the plants fertilized with iodine were lower than in the control, except in the case of soil fertilization with 2.5 KI. In that sample, the concentration of chlorophyll b increased as well, whereas it decreased only in the plants in which 2.5 KI was applied to the leaves. Based on the chlorophyll content in the leaves we can diagnose plants' response to biotic and abiotic stresses [32]. Although no symptoms of chlorosis were observed on the triticale plants, the changes in chlorophyll content suggest that the plants' response to iodine fertilization is similar to their response to stress. Potassium iodide may cause a loss of chlorophyll in bean leaves (*Phaseolus vulgaris*) [33] and reduce the photosynthetic efficiency of lettuce plants [34]. Chlorophylls affect human health by stimulating the immune system, influencing hepatic detoxification processes, and normalizing blood pressure [35].

The antioxidant activity of aqueous extracts was measured by the DPPH method. The total antioxidant content increased in the triticale plants fertilized with iodine, except in the case of foliar application of 5 kg $\cdot$ ha^{-1} KI (Table 2). No effect of iodine biofortification on the total antioxidant capacity of radish was demonstrated [20], but it was found to be higher in lettuce plants than in controls [18]. Biofortification with iodine has a varied effect on antioxidant properties depending on the plant species, chemical form and iodine concentration. In spinach leaves, fertilization with $CH_2ICOONa$ caused a decrease in the concentration of ascorbic acid, while $NaIO_3$ increased the concentration of this antioxidant [14]. In *Opuntia ficus-indica*, KI caused an increase in the concentration of vitamin C [36].

Extracts of green triticale plants may become, like the leaves of young green barley, a source of natural health-promoting substances [37]. Green barley has many proponents because it provides vitamins, minerals, amino acids, enzymes and antioxidants [38]. Cultivation of iodine-biofortified triticale will make it possible to obtain green plants with good biological properties and high iodine content. Biofortified triticale plants can be used as an additive in fodder and silage and thus increase the supply of iodine in the food chain. Another possible application of biofortified triticale plants is the production of iodine-rich supplements. A product that has become popular with consumers is green barley leaves, which are used to produce a variety of supplements and food additives. Iodine-biofortified green triticale plants could perform a similar function. The results presented here confirm that they contain antioxidants, soluble proteins and soluble sugars, and an additional advantage is their enrichment with iodine, which is essential for health.

Conclusions

1. Agronomic biofortification of triticale with iodine effectively increases the content of this element in the green parts of the plants.
2. The effects obtained for both iodine accumulation in plants and their biological quality depend on the concentration used, the chemical form of iodine compounds, and the fertilization system
3. The application rates and means of fertilization with iodine salts did not adversely affect the biological quality of the plants.

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JAKOŚĆ BIOLOGICZNA ZIELONYCH ROŚLIN PSZENŻYTA BIOFORTYFIKOWANYCH JODEM

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Abstrakt: Rośliny biofortyfikowane jodem mogą być alternatywnym źródłem tego pierwiastka w diecie człowieka. Celem badań było określenie wpływu nawożenia jodem na jakość biologiczną zielonych roślin pszenżyta. Jod w postaci KIO_3 lub KI (2,50 lub 5 kg $\cdot$ ha^{-1}) był stosowany w glebie lub rozpylany na rośliny. Dawka jodu została podzielona, pierwszą połowę stosowano wraz z wiosennym nawożeniem azotem, a drugą połowę stosowano na jeden miesiąc przed pobraniem próbek do badań laboratoryjnych. We wszystkich badanych kombinacjach nawożenia jodem obserwowano wzrost zawartości jodu w zielonych roślinach pszenżyta w porównaniu do kontroli. Zastosowane dawki i sposoby nawożenia solami jodu nie wpłynęły negatywnie na jakość biologiczną zielonych roślin pszenżyta.

Słowa kluczowe: pszenżyto, jod, jakość biologiczna