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REVIEW

Agronomic biofortification as a means of enriching plant foodstuffs with iodine

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Abstract

Iodine is indispensable in the diet of humans and other mammals and iodine deficiencies cause serious illnesses. The content of iodine in food (with the exception of marine foodstuffs) does not meet the nutritional needs of humans, and for this reason prophylactic iodination of salt is currently carried out in many countries. Biofortification of plants with iodine can become a widespread, alternative means of supplying iodine-rich foods. In the present study, we discuss the main issues related to the cultivation of plants enriched with iodine. We describe the effect of various forms of iodine fertilizer on crops, such as natural iodine sources, organic iodine, iodate and iodide salts, as well as ways of biofortifying crops: fertigation, foliar and soil application, and by hydroponics. Effective biofortification of plants with iodine increases its concentration to levels corresponding to human nutritional requirements whilst preserving the desirable eating qualities of the plants. Because each species reacts in a specific manner to a particular chemical form of iodine application, fertilization and cultivation methods, and other conditions, the development of proper cultivation technologies is essential to bring about widespread biofortification with iodine.

Keywords

agronomic biofortification; iodized fertilizer; quality of plants

Iodine – an essential micronutrient

Iodine is a micronutrient essential for the proper functioning of the physiology of humans and animals (mainly mammals) [1]. As a component of thyroid hormones (triiodothyronine and thyroxine), iodine participates in the regulation of key metabolic processes at the cellular level, such as protein biosynthesis and metabolism of sugars, fats and nucleic acids, and contributes to the growth and proliferation of cells [2]. It is also necessary for the development and functioning of the nervous system and other organs [2]. The recommended daily dose of iodine depends on the stage of life and age. Requirements for various age groups are: young children (1 to 8 years) – 90 µg d⁻¹, older children (9 to 13 years) – 120 µg d⁻¹, adults > 14 years – 150 µg d⁻¹, and for women during pregnancy and breastfeeding – 220 and 270 µg d⁻¹, respectively [3]. Iodine deficiencies are particularly harmful in the diet of pregnant women, because they contribute to permanent foetal developmental disorders and to delayed physical and mental development in children [4]. Inadequate iodine intake most often causes

thyroid gland enlargement (goitre), and also increases the risk of thyroid and stomach cancer [4]. Iodine deficiency may contribute to the development of autism in maturing boys; statistically significant lower levels of urinary iodine were found in an autistic group when compared with the control group [5]. The World Health Organization has indicated iodine deficiency as one of the main factors influencing human health [6]. In Poland, owing to prophylactic iodination of salt, iodine intake at the population level is satisfactory. However, iodine supplementation in people with higher requirements for this nutrient, such as pregnant women, is insufficient in terms of compliance with recommendations and the quality of supplementation [7]. A similar problem applies to supplementation for infants [8]. The main source of iodine at the population level is iodinated table salt. However, excessive salt intake may increase the risk of hypertension [9]. To prevent cardiovascular disease, it is recommended that salt intake should be limited, which poses the risk of also reducing iodine in the diet. Therefore, there is a need for other means of introducing iodine into the diet. The highest content of iodine is found in marine food, such as seaweeds, seafood, and fish. In many countries, however, including Poland, the consumption of fish and other marine products does not satisfy the body's need for iodine [9]. In industrialized countries, the main source of iodine is from milk and dairy products, but both the level of consumption of these products and the iodine content in milk vary widely, which makes them an unpredictable source of the element [10]. Foods of plant origin such as fruit and vegetables, with the exception of seaweed, are a relatively poor source of iodine [11]. The typical iodine content in cultivated plants is 0.1–0.15 mg kg⁻¹ fresh weight (FW) (e.g., 0.12 mg kg⁻¹ in spinach, 0.15 mg kg⁻¹ in broccoli, and 0.14 mg kg⁻¹ in pea seeds) [12]. Animal studies have confirmed that the iodine contained in plants is better absorbed and much more effective and safer than inorganic iodine in salt. Mice fed with iodine-enriched feed in inorganic form have shown weight loss and goitre; those taking large doses of organic iodine from kelp algae did not show signs that the iodine is in any way toxic. [13]. In our opinion, tests conducted on animals suggest that the consumption of plant products rich in iodine may be safer for the consumer, and the risk of reaching toxic doses is much lower than with iodine salts. The same findings apply to humans. Properly carried out biofortification should increase the concentration of iodine in a plant without any negative impact on its growth. Iodine doses should be strictly optimized. A high dose of iodine can disturb the normal growth of plants, especially those grown in a soil-less system and pose a threat to human health [14]. Therefore, it is important to find ways to enrich plants with iodine so that they will become a widely consumed and accessible source of iodine in food.

Agronomic biofortification as the key to enrichment of plants with iodine

Biofortification is the process of increasing the nutrient content of edible plants and has gained widespread recognition as a cost-effective way of providing micronutrients to the population. Deficiencies of vitamins and micronutrients such as zinc, iron, magnesium, selenium, and iodine occur in one-third to one-half of the world's population [15]. Amongst the commonly available methods of correcting iodine deficiency in the diet, we can distinguish supplementation, fortification of livestock feed and biofortification of plants by agronomic or biotechnological methods [15]. Among the methods of counteracting iodine deficiency, apart from salt iodination, agronomic biofortification methods are the most promising and cost-effective.

Agronomic biofortification of plants with iodine has numerous advantages. It focuses on the production of plants containing this valuable nutrient in higher concentrations than in traditionally grown plants. Iodine is not counted among nutrients essential for higher plants, but plants can take it up from the soil [5]. Using agronomic measures, the iodine supplied to plants undergoes natural processes of incorporation into tissues, where it is present in the form of chemical compounds typical for those tissues, bound to proteins, or from a fiber, and thus it is absorbed more easily [16]. In food fortified with iodine, this micronutrient has been introduced during a technological process, often in the form of chemical compounds not found naturally in plants. Moreover, some of it may be lost during the production processes of fortified foodstuffs. The reactions

of iodine and its salts in food matrices are however not fully understood. Iodate is a strong oxidant and iodide is a reducing agent and so their use in foodstuffs can initiate redox reactions. These, in turn, can affect the properties of foodstuffs, its durability and stability [17]. There is also a risk that the concentrations of the substance introduced into the fortified foodstuff will be too high or even potentially harmful [14].

Agronomic biofortification, unlike biotechnological fortification, can be widely used. Genetic manipulation associated with the creation of biotechnologically improved plant cultivars may be opposed by consumers, many of whom still fear GMOs [18]. A fundamental question in the case for biofortification of plants with iodine is the selection of suitable concentrations of fertilizer for a specific crop plant. The final concentration of this element in the plant depends on the species, growing conditions, type of fertilizer used, soil composition, moisture, pH, and prevailing redox conditions [13,16].

Chemical forms of iodine used for biofortification of crop plants

The key question in the biofortification of plants with iodine is the form of iodine used. In agronomic biofortification of plants, natural sources of iodine, organic iodine, iodate and iodide salts have all been used.

One natural source of iodine is from marine algae, especially kelp (*Laminaria japonica* Aresch.), a brown seaweed that can accumulate iodine and other minerals (potassium, magnesium, and iron) in high concentrations [19]. The concentration of iodine in *L. japonica* may reach 734 mg kg⁻¹ FW [20]. Kelp can be used as an excellent exogenous source of iodine. Iodic fertilizer composed of kelp and diatomaceous earth has been used in the cultivation of radish, spinach, and Chinese cabbage [21]. Due to its porous structure, diatomaceous earth has a high capacity to absorb minerals, and thus fertilizer prepared in this manner can prevent iodine losses from the soil and facilitate the plant's absorption of greater amounts of nutrients from the surrounding soil. Vegetables may absorb exogenous iodine from soil. The uptake amounts increase with the application intensity of algal organic iodized fertilizer [21]. Unfortunately, the use of algae as the main component of fertilizer for biofortification with iodine has a number of limitations. It can only be justified economically in a coastal zone due to the cost of transporting the biomass. Furthermore there is a risk of uncontrolled inadvertent introduction of heavy metals into the environment from contaminated algae.

Following the application of algal iodine fertilizer at a rate of 12 mg m⁻², the average iodine concentration in the edible parts of cabbage, spinach, potherb mustard, Chinese cabbage, coriander and celery was 9.1, 1.8, 5.8, 4.2, 19.3, and 9.4 mg kg⁻¹, respectively [13]. When this fertilizer was applied at a rate of 75 mg m⁻², the average concentration of iodine in the edible parts of eggplant, hot pepper, cucumber, tomato, and long cowpea was 15.56, 21.30, 10.48, 7.74, and 8.42 mg kg⁻¹, respectively [13]. Both leafy vegetables and those with edible fruits may accumulate iodine from the organic sources. Since the concentrations of iodine also increased in the other parts of the plants, stems, and leaves of these plants that are inedible for humans can be added to feed for poultry and livestock. In this way, the amount of iodine in the food chain can be increased without additional costs, which in turn will affect the intake of iodine in the human diet.

Amongst the organic forms of iodine compounds available, attempts have been made to use iodoacetic acid. In hydroponic spinach, a positive biofortification effect was obtained using this form of iodine [22]. When the fertilizer was applied at rates from 0.05 to 0.1 mg dm⁻³, the iodine concentration in spinach leaves was higher than following application of inorganic forms of iodine. Moreover, the plants grew well, and the concentration of vitamin C increased. Iodine uptake depended here on the iodoacetic acid concentration in the medium. When CH₂ICOO⁻ was applied at rates <0.05 mg dm⁻³, the iodine concentration was lower than when supplied at the same concentration in the iodate form. At concentrations of CH₂ICOO⁻ fertilizer >0.05 mg dm⁻³, iodine uptake by plants was much higher than in the case of application of IO₃⁻.

In agronomic iodine biofortification, the inorganic forms I⁻ and IO₃⁻ usually supplied in potash are the most widely used. Plants can take up and accumulate mainly inorganic forms of iodine. The IO₃⁻ form is reduced by the plant enzyme iodate reductase [23]. In addition, nitrate reductases in plants can use iodate as an alternative electron acceptor

in the enzymatic reaction [24]. Nitrate reductase transforms NO_3^- into NO_2^- using the reduced form of NAD as an electron donor. It has been demonstrated that IO_3^- can be converted to I^- in this reaction [24]. Owing to these enzymatic reactions, IO_3^- is less toxic to plant roots than I^- . The effectiveness of biofortification may also be influenced by the ability of plant roots to reduce IO_3^- to I^- [24].

Under field conditions, iodide from the soil is more susceptible to losses caused by leaching, due to its greater solubility as compared to the iodate form [25]. In soil, IO_3^- is more efficiently absorbed by plants than is I^- , and in soil-less cultures it is less toxic than iodide. Amongst the inorganic forms, iodate is more suitable for biofortification [26]. Inorganic iodine is taken up by the roots through specific transport systems and then moves mainly through the xylem to other parts of the plant. Phloem transport routes have also been detected, e.g., in lettuce and tomatoes [23,27]. The effect of biofortification with iodine may also be influenced by the ability of higher plants to metabolize the iodide ion to methyl iodide, which as a volatile compound, can escape into the atmosphere through the stomata. Methyl iodide is formed as a result of a methyltransferase-catalyzed reaction. The substrates in this reaction are S-adenosyl-L-methionine and iodide ion, and the products are methyl iodide and S-adenosyl-L-homocysteine [28]. These enzymes also utilize thiocyanate as a substrate. Methyltransferases have been described in plants such as *Batis maritima* L., *Brassica oleracea* L., and *Arabidopsis thaliana* (L.) Heynh. They show considerable homology to methyltransferases involved in responses to salinity or glucosinolate metabolism, suggesting that methyl iodide production may be a defence mechanism in plants [28]. Literature research indicates that there is no single pattern according to which chemical forms of iodine affect plants [12–27]. Each species will react in a different way, depending on the method of cultivation and application of iodine. The appropriate chemical form of iodine and the agrotechnical treatments used for iodine fertilization should be adapted for each plant and cultivation method.

Methods of application of iodine compounds to biofortify crops

Common methods of iodine application include fertigation, leaf spraying, amendment of a nutrient solution, or direct application of iodine compounds to the soil.

Fertigation, which combines fertilization with irrigation, is very useful for applying iodine salts because they are fully soluble. The benefits of fertigation have been confirmed in leafy vegetables. Application of potassium iodate at a high rate of $40 \mu\text{g dm}^{-3}$ at 2-day intervals for 4 weeks significantly increased the content of iodine in vegetables in the fourth week [29]. The concentration of iodine in fluted pumpkin (*Telfairia occidentalis* Hook.) ranged from 89 to $140 \mu\text{g } 100 \text{ g}^{-1}$ FW, in vegetable marrow (*Cucurbita pepo*) from 60 to $76 \mu\text{g } 100 \text{ g}^{-1}$ FW, and in water leaf [*Talinum triangulare* (Jacq.) Willd.] from 61 to $73 \mu\text{g } 100 \text{ g}^{-1}$ FW [25]. Biofortification by means of fertigation becomes even more effective when iodine salts are used together with humic substances or organic acids, as confirmed in the cultivation of spinach, where satisfactory biofortification effects were achieved when KIO_3 and humic acids were applied simultaneously [30].

Iodination of irrigation water is a beneficial and cost-effective delivery method for iodine because the technology required is simple. In the southern part of Xinjiang Province, Northwest China, where goitre is endemic, attempts have been made to use iodinated water for irrigation on a large scale [31]. During a 7-year experiment, the iodine concentration in the soils significantly increased, and significant amounts of iodine were also introduced into the food chain, as evidenced by an increase in iodine concentration in human urine [31]. The long-term persistence of an increased concentration of iodate in the soil is an argument for the effectiveness and cost-effectiveness of this method of iodine supplementation. However, this method requires the use of a large quantity of iodine, and also poses the risk of uncontrolled release of iodine into the environment.

Spraying leaves with a solution containing iodine salts is an effective biofortification method, which has been confirmed in numerous experiments. In lettuce cultivation, foliar spraying contributed more effectively to accumulation of iodine than did soil application, and furthermore it had no negative effect on crop quality or biomass [25]. However, foliar application of iodine was found to be less effective than root treatment in

tomato cultivation [28]. A higher concentration of iodine in tomato fruits was obtained when KI fertilizer was supplied to the roots of plants cultivated hydroponically [32]. In radish cultivation, spraying the leaves with iodine fertilizer (KI) increased the iodine concentration to 735.5 mg I kg⁻¹ dry weight in the edible parts of the plant [33].

This method has been used for the biofortification of vegetables such as tomatoes, carrots and leafy vegetables. It should also be used for the enrichment of cereals. Spraying the leaves of maize, rice and wheat causes them to absorb iodine at a high rate, and phloem transport is activated in the plant evidenced by the fact that the amount of iodine in the grains increases [26]. During an extensive trials on the biofortification of rice, wheat, and maize, field experiments were carried out in various parts of the world, including Thailand, Brazil, Pakistan, and Turkey. Plants were sprayed twice, first at heading and then at the early milk stages. KI was applied at concentrations in a range of 0.010–0.10%, and KIO₃ at 0.013–0.129% w/v salt in a spray solution [26]. The iodine fertilizers applied at those rates had no negative impact on biomass or grain yield. Large amounts of the fertilizers inhibited plant growth, particularly when supplied as KI. The concentrations of iodine in the grain increased significantly, especially when higher levels of fertilizer were applied [26].

The effectiveness of foliar biofortification with iodine can be increased by adding some commonly used foliar fertilizers or other agrochemicals to the spray. When a surfactant was added to the spray, absorption of iodine through the leaves increased, as did its content in the grain [26]. Similarly, adding KNO₃ to KI increased iodine absorption, but there was no increase in iodine content in the case of Ca(NO₃)₂ [26]. The leaves of butter lettuce absorbed iodate at a higher rate when CaCl₂ was added to the solution [25]. The positive effect of CaCl₂ on iodine absorption by the leaves was explained as being a result of the humectant effect.

The capacity of soil to accumulate iodine is limited. Exogenous iodine added to the soil can be leached into the groundwater or undergo oxidation processes and pass into the atmosphere [13]. Experiments in which iodine compounds have been applied to the soil have usually been carried out under controlled conditions, in greenhouses or plastic tunnels. The iodine content in soils varies, and the average global soil iodine concentration is 2.6 mg kg⁻¹ (worldwide mean) [34]. Iodine application of up to 10 mg kg⁻¹ of soil does not adversely affect plant growth [35]. For the purpose of biofortification, however, higher concentrations of iodine salts are used, so it is crucial to determine the range of beneficial and toxic iodine concentrations. To significantly increase the iodine concentration in plant tissues, concentrations of iodine salts <50 mg kg⁻¹ of soil are used, and depending on the species, varying results of biofortification have been obtained [16]. In some species, such as Napa cabbage, the use of >25 mg kg⁻¹ of soil reduces the plant biomass [36]. Positive results of this means of biofortification have been obtained in the case of soybean seeds (*Glycine max* L.). Furthermore, iodine fertilization has been shown to reduce the effects of stress induced by the presence of cadmium ions [37].

After soil fertilization of Napa cabbage, lettuce, carrot, or tomato, the highest iodine concentration in plant tissues was found in the roots, lower concentrations in the leaves and stems, and the lowest in the fruit, with the exception of carrots where the iodine content in the root was half of that in the stem. The highest iodine content was obtained in Napa cabbage, followed by lettuce, carrot, and tomato, in that order [36]. Field trials have been conducted on calcareous loam soils in China during cultivation of maize, soybean, potato, cabbage, canola, and winter wheat. The soil was sprayed with a fertilizer in the form of a potassium iodate solution at 0.59 kg ha⁻¹ kg and scarified to a depth of 20 cm. In this experiment, effective biofortification was obtained only in the case of cabbage, where there was a twofold increase in the iodine concentration in the leaves. This method was not effective in the case of wheat, maize, soybean, rapeseed, or potato, as there was no increase in iodine in the grain or tubers [38].

The quality of plants biofortified with iodine

The literature provides many examples of effective biofortification of plants with iodine. Studies have been conducted on numerous species of common vegetables such as lettuce,

celery, cabbage, courgette, radish, spinach, tomato, and others [22,26,29,30,33,39,40]. In most of the experiments, it has been possible to select a concentration of iodine for fertilization to increase its concentration in the plant whilst maintaining the high eating quality of the edible plant parts. A positive effect of biofortification is easier to obtain in leafy vegetables than in root vegetables due to the nature of iodine transport in the plant. The use of iodine salts in hydroponics usually results in increased biomass of leafy vegetables such as Napa cabbage, spinach, and lettuce [16,39,40]. In the cultivation of strawberries, an increase in iodine concentration in the fruits, an increase in plant biomass and improved fruit quality have all been obtained [41]. Due to their common consumption and their health benefits, tomatoes can be a key plant for iodine biofortification, especially as high accumulation of iodine in the fruit, up to 10 mg kg⁻¹ of FW, has been obtained [26]. As the author notes, the concentration of iodine in tomato fruit was more than sufficient to cover the daily human consumption of 150 µg. Other researchers also stress the need for further research to adapt iodine concentrations for biofortification and to avoid levels that can be harmful to plants and human health [14].

In addition to the desirable effects of biofortification with iodine, the content of health-promoting bioactive compounds in some plants has also been increased. In biofortified carrot, the concentrations of glucose, fructose, and total sugars also increased [42]. Prickly pear biofortified with iodine contained a higher concentration of vitamin C [43]. Following fertilization of lettuce with 80 µM IO₃⁻ or I⁻, the total content of phenols, flavonoids, anthocyanins, and ascorbic acid increased, and at a higher level of 120 µM of these fertilizers, total antioxidant capacity also increased [39]. Other researchers have found that biofortification changes the activity of enzymes involved in plant response to stress. In lettuce, KI at 40 µM or less have been demonstrated to reduce superoxide dismutase activity but increased the activity of catalase, as well as the concentration of antioxidants such as vitamin C and glutathione [44]. There are far fewer studies reporting successful biofortification of cereal grains, because the rate of phloem transport of iodine is lower than that of xylem transport [26]. Not all biofortification treatments achieve a positive result. The most common negative effect is a decrease in biomass, which has been confirmed in the cultivation of tomatoes and potatoes, carrots, and prickly pear [35,42,43]. A decrease in the biological quality of the crop has been found in tomato, potato, cabbage, lettuce, and carrot fertilized with iodine salts [35,36]. Symptoms of chlorosis and necrosis of lettuce leaves have been observed following soil fertilization at a rate as low as 15 kg ha⁻¹ [25]. Other negative symptoms of the effects of iodine compounds in some plants that may affect the biological quality of the crop include a reduced concentration of bioactive components such as vitamin C, which has been observed in water spinach [22].

Conclusions

Properly conducted agronomic biofortification processes make it possible to obtain plants with an enriched iodine content and good crop quality. Simple treatments, such as fertilization of crops with iodine compounds, will help solve the problem of iodine deficiency on a large scale. Agronomic biofortification should be the basic agricultural strategy in the efforts to eliminate iodine deficiency in the diet, due to its ease of use, the relatively low cost of iodine compounds, economic and public health benefits. Foods of plant origin biofortified with iodine can become common functional foods, which not only ensure the necessary intake of this micronutrient but are also richer in essential bioactive compounds. The key to achieving widespread biofortification with iodine is the development of detailed cultivation technologies for individual species, taking into account the appropriate chemical form and method of iodine delivery to plants. Measures to prevent contamination of the environment, especially soil and surface waters, and toxic effects on living organisms, must be included as part of the strategy.

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Biofortyfikacja agronomiczna sposobem na wzbogacenie żywności pochodzenia roślinnego jodem

Streszczenie

Jod jest niezbędnym składnikiem diety człowieka i innych ssaków, a jego niedobory są przyczyną wielu poważnych schorzeń. Zawartość jodu w żywności (za wyjątkiem żywności pochodzenia morskiego) nie zaspokaja potrzeb żywieniowych człowieka, dlatego w wielu krajach profilaktycznie prowadzi się jodowanie soli kuchennej. Biofortyfikacja roślin w jod może stać się powszechnym, alternatywnym sposobem dostarczenia bogatej w ten składnik żywności. W prezentowanej pracy na podstawie literatury przedmiotu przedstawiono główne problemy związane z uprawą roślin wzbogaconych w jod. Omówiono wpływ nawożenia roślin różnymi formami jodu (jod organiczny, sole jodu – jodan i jodek) stosowanych dolistnie i dokorzeniowo w uprawach polowych i hydroponicznych. Zwrócono uwagę na skuteczność biofortyfikacji roślin w jod, która nie tylko powinna zwiększyć stężenie tego pierwiastka do wartości odpowiadających potrzebom żywieniowym człowieka, ale powinna również zapewnić wysoką jakość konsumpcyjną roślin. Ponieważ każdy gatunek reaguje specyficznie na formę chemiczną jodu i sposoby jego aplikacji, kluczowe dla upowszechnienia biofortyfikacji roślin w jod jest opracowanie szczegółowych technologii uprawy w warunkach biofortyfikacji jodem.