

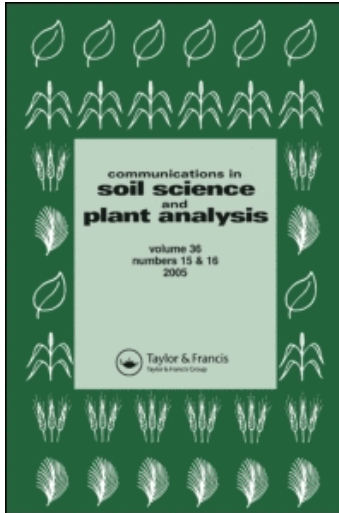
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## Iodine Fortification Plant Screening Process and Accumulation in Tomato Fruits and Potato Tubers

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*Iodine is an essential microelement for human health, and the recommended daily allowance (RDA) of such element should range from 40 to 200  $\mu\text{g day}^{-1}$ . Because of the low iodine contents in vegetables, cereals, and many other foods, iodine deficiency disorder (IDD) is one of the most widespread nutrient-deficiency diseases in the world. Therefore, investigations of I uptake in plants with the aim of fortifying them can help reach the important health and social objective of IDD elimination. This study was conducted to determine the effects of the absorption of iodine from two different chemical forms—potassium iodide ( $\text{I}^-$ ) and potassium iodate ( $\text{IO}_3^-$ )—in a wide range of wild and cultivated plant species. Pot plants were irrigated with different concentrations of  $\text{I}^-$  or  $\text{IO}_3^-$ , namely 0.05% and 0.1% (w/v)  $\text{I}^-$  and 0.05%, 0.1%, 0.2%, and 0.5% (w/v)  $\text{IO}_3^-$ . Inhibiting effects on plant growth were observed after adding these amounts of iodine to the irrigation water. Plants were able to tolerate high levels of iodine as  $\text{IO}_3^-$  better than  $\text{I}^-$  in the root environment. Among cultivated species, barley (*Hordeum vulgare* L.) showed the lowest biomass reductions due to iodine toxicity and maize (*Zea mays* L.) together with tobacco (*Nicotiana tabacum* L.) showed the greatest. After the screening, cultivated tomato and potato were shown to be good targets for a fortification-rate study among the species screened. When fed with 0.05% iodine salts, potato (*Solanum tuberosum* L.) tubers and tomato (*Solanum lycopersicum* L.) fruits absorbed iodine up to 272 and 527  $\mu\text{g}/100$  g fresh weight (FW) from  $\text{IO}_3^-$  and 1,875 and 3,900  $\mu\text{g}/100$  g FW from  $\text{I}^-$ . These uptake levels were well more than the RDA of 150  $\mu\text{g day}^{-1}$  for adults. Moreover, the agronomic efficiency of iodine accumulation of potato tubers and tomato fruits was calculated. Both plant organs showed greater accumulation efficiency for given units of iodine from iodide than from iodate. This accumulation efficiency decreased in both potato tubers and tomato fruits at iodine concentrations greater than 0.05% for iodide and at respectively 0.2% and 0.1% for iodate. On the basis of the uptake curve, it was finally possible to calculate the doses of supply in the irrigation water of iodine as iodate (0.028% for potato and 0.014% for tomato) as well as of iodide (0.004% for potato and 0.002% for tomato) to reach the 150  $\mu\text{g day}^{-1}$  RDA for adults in 100 g of such vegetables, to efficiently control IDD, although these results still need to be validated.*

**Keywords** Fortification, iodine, phytotoxicity, *Solanum lycopersicum*, *Solanum tuberosum*

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## Introduction

Iodine is an essential microelement for human health (Welch and Graham 1999; Zhu et al. 2003). The physiological role played by iodine in the human body is the synthesis of thyroid hormones, thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>), by the thyroid gland. In turn, the roles of the thyroid hormones can be summarized as (1) growth and development and (2) control of metabolic processes in the body (Anonymous 2002). Therefore, serious iodine deficiency leads also to functional and developmental abnormalities such as hypothyroidism. In neonates, iodine deficiency causes mortality and low birth weight; severe iodine deficiency in the fetal and neonatal periods can also lead to cretinism, which is characterized by stunted growth, mental and other neurological retardation, and delay in the development of secondary sex characteristics. In adults, iodine deficiency causes a reduction in mental function and lethargy (Anonymous 2002).

The dietary allowance of iodine (as recommended by the National Research Council of the U.S. National Academy of Sciences in 1989) is 40  $\mu\text{g day}^{-1}$  for young infants (0–6 months), 50  $\mu\text{g day}^{-1}$  for older infants (6–12 months), 60–100  $\mu\text{g day}^{-1}$  for children (1–10 YEARS), 150  $\mu\text{g day}^{-1}$  for adolescents and adults, and 200  $\mu\text{g day}^{-1}$  for pregnant and lactating women. These amounts are thought to allow normal hormone production without stressing the thyroid iodide-trapping mechanism. Excess iodine intake is more difficult to define. Many people regularly ingest huge amounts of iodine—in the range of about 10 to about 200  $\text{mg day}^{-1}$ —without apparent adverse effects (Anonymous 2002).

The best sources for diet are algae and animal sea life, such as shellfish, white deep-water fish, and the brown seaweed kelp; these organisms absorb iodine from the water. The iodine content of terrestrial foods is generally much lower, and the concentration varies with geographical location, ranging widely from 10 to maximum 200  $\mu\text{g kg}^{-1}$  fresh weight (FW) (Anonymous 2002).

Volatilization from the oceans and precipitation of ocean water to the soil is the source of most iodine entering the terrestrial food chain (Fuge and Johnson 1986); the geological origin of soil has also a great influence on the iodine content of plants and other organisms (Anke, Groppe, and Bauch 1993). Moreover, because of eluviation, the content of iodine in soil is relatively low in inland and mountainous areas. In these regions, this fact leads to iodine deficiency in vegetables and forage crops, the source of iodine for domestic animals, and to the prevalence of iodine deficiency disorder (IDD) (Zhu and Tan 1989).

In 1994, approximately 225 million people in the world were estimated to be affected by IDD (Anonymous 1994). At that time, the World Health Assembly adopted universal salt iodization (USI), the iodization of salt for both human and livestock consumption, as the method of choice to eliminate IDD (Anonymous 2007). However, the effectiveness of this measure largely depends on the social, economic, and cultural situation of the considered population. At present, IDD still remains a significant public health problem in 47 countries, as compared to 54 in 2004 and 126 in 1993 (Anonymous 2007). Therefore, alternative iodine supplementation is needed to complement the traditional USI approach (Dai et al. 2004).

The approach of *voluntary fortification* could be also pursued. This strategy in fact is believed to be a cost-effective way to improve human nutrition, by means of supplementation of trace elements in the food chain through plant uptake (Jopke et al. 1996; Dai et al. 2006; Blasco et al. 2008).

A bottleneck in reaching the goal of biofortification is the increase in plant iodine accumulation without causing adverse effects on growth. Unlike mammals, higher plants do not require iodine, a nonessential element for higher plants, although plants can take up

iodine from soil. However, plants can be adversely affected even by low concentrations. The degree of phytotoxicity is dependent on the chemical form of iodine existing in the soil solution. Some findings indicate iodide is more phytotoxic than iodate (Umaly and Poel 1971), which may be due to the greater ability of plant roots to absorb the reduced element (Böszörményi and Cseh 1960). Whitehead (1975) suggested that iodate is reduced to iodide before its uptake by plant roots, and later Zhu et al. (2003) deduced that the slow uptake rate of iodate is likely caused by this reduction process.

Iodine supplementation in the food chain by plant uptake was attempted by some researchers, who have studied the capacity of accumulation of iodine mostly in (leaf) vegetables (Weng et al. 2003, 2008). Weng et al. (2003) applied iodine fertilizer made up of kelp and diatomaceous earth to agricultural plants such as radish (*Raphanus sativus* L.), spinach (*Spinacia oleracea* L.), and Chinese cabbage (*Brassica chinensis* L.). Iodine concentrations were greater in the leaf blades of the vegetables than in other tissues, suggesting differences in iodine accumulation between species and between tissues (Weng et al. 2003). In a hydroponic experiment with spinach, Zhu et al. (2003) showed that iodate ( $\text{IO}_3^-$ ) had a less detrimental effect on biomass production of spinach plants than iodide ( $\text{I}^-$ ). In a greenhouse pot experiment performed to select vegetables for iodine uptake and to investigate the residual effect of iodate fertilization on the growth and on iodine uptake of spinach plants, Dai et al. (2004) showed that plant species responded differently to increasing iodine dose in soil and that, although the loss and reduction in bioavailability of iodine in soils were unavoidable, the development of controlled-release iodine-containing fertilizers could be a way to further improve the efficiency of iodine supply. An experiment (Blasco et al. 2008), in which lettuce (*Lactuca sativa* L.) in hydroponic culture was used, showed that the application rates were  $\leq 40 \mu\text{M}$  or lower as  $\text{I}^-$  avoided reduced plant biomass while causing foliar accumulation. Foliar iodine accumulation was adequate to meet the recommended dietary allowance for adults ( $150 \mu\text{g day}^{-1}$ ). Furthermore, the treated plants showed a significant increase in antioxidant compounds after the application of iodine. Hong et al. (2008) applied both inorganic iodine (KI) and organic seaweed iodine to agricultural plants such as Chinese cabbage (*Brassica chinensis* L.), lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.), and carrot (*Daucus carota* L.). Iodine levels in vegetables increased with the increasing addition of iodine, and iodine concentrations were greater in the root and in the leaf blades of the vegetables than in other tissues, in agreement with the results described by Weng et al. (2003). Moreover, Hong et al. (2008) showed that vegetable growth was inhibited when the iodine concentration added was greater than  $50 \text{ mg kg}^{-1}$ , and tolerance against iodine toxicity was greater in carrot than in other species. Finally, seaweed composite iodine fertilizer was demonstrated to have a lower but more durable decreasing effect on biomass than potassium iodide (KI).

Previous studies had shown that iodine does not seem to be phloem mobile (Herrett et al. 1962); therefore, iodine accumulation in cereal grains could be very low. In fact, Mackowiak and Grossl (1999) showed that even with  $100 \mu\text{M}$  (approximately  $10 \text{ mg kg}^{-1}$ ) growth solution, the iodine concentration in rice grains was still not adequate to meet the RDA.

The strategy of plant biofortification is still open for research and application, and the accumulation efficiency at high levels of iodine supply should be investigated through a screening in a wider range of species, including cereals, the wild gene pools of crops, and the fruit- and tuber-producing cultivated plants.

The aims of this study were (1) to investigate the effects of different iodine concentrations (in both chemical forms as iodide and iodate) on the growth of three wild relatives of barley and six cultivated plant species; (2) to perform a rate study to seek out the tolerance

to the two iodine sources added to potato and tomato; and (3) to identify maximum iodine concentration in selected plant parts. This information was then used to indicate iodine uptake efficiency by comparing the rate added to the soil and the concentration found in the selected plant part.

## Materials and Methods

### *Plant Materials and Growth Conditions*

Six cultivated plant species were used—wheat (*Triticum aestivum*, cv. Chinese Spring), maize (*Zea mays*, hybrid Belgrano), barley (*Hordeum vulgare*, cv. Tremois), potato (*Solanum tuberosum*, cv. Primura), and tomato (*Solanum lycopersicum*, cv. UC82 kindly provided by Fulvia Rizza, Agricultural Research Council (CRA) of Fiorenzuola d'Arda, Italy)—as they are highly cultivated crops in Italy. Tobacco (*Nicotiana tabacum*, genotype SR1, also provided by Fulvia Rizza), also cultivated in Italy, was chosen as a good model species for accumulation in the leaves. Moreover, three wild species of genus *Hordeum*, kindly provided by Roland von Bothmer—*Hordeum marinum* ssp. *marinum* H524 (seaside barley), *Hordeum marinum* ssp. *gussoneanum* H 819 (Mediterranean barley), and *Hordeum murinum* ssp. *leporinum* H 509 (leporinum barley)—were used because they are barley's wild relatives and, because they evolved in seaside habitats, are expected to be more tolerant of iodine.

Plants were grown at C.R.A. of Fiorenzuola d'Arda, in greenhouse conditions, in 12-cm-diameter pots filled with commercial peat-based compost (pH 6) (Vigorplant s.r.l., Italy), one plant per pot, each representing a replicate. The experiment was arranged in a split-plot design, with iodine forms as the main plot and concentrations as the subplot. All treatments were replicated twice.

Plants, at the third-leaf stage, were treated with two iodine pure salts, potassium iodide and potassium iodate, dissolved in water and supplied in the irrigation water. Four concentrations (0.05, 0.1, 0.2, and 0.5%) plus a control (0.0) (w/v) (0.0, 2.34, 4.67, 9.35, and 23.36 mM, respectively) were used for iodate [saline solution of potassium iodate ( $\text{KIO}_3$ ), MW = 214] and two concentrations (0.05 and 0.1%) plus control (0.0) (w/v) (0.0, 3.01, and 6.02 mM, respectively) for iodide [saline solution of potassium iodide (KI), MW = 166]. Iodine treatments were applied weekly for 1 month, and for each treatment 28 mL of saline solution were supplied. Considering iodine molecular weight of 127, the distributed amounts of the element were: 0, 33, 66, 132, and 330 mg  $\text{pot}^{-1}$  from iodate and 0, 43, and 86 mg  $\text{pot}^{-1}$  from iodide. One week after the last supply, individual plants were weighed to evaluate biomass accumulation and samples collected. When not differently specified, the measured aboveground biomass consisted of leaves and shoots.

### *Iodine Content Analysis*

Iodine concentration in potato tubers and tomato fruits was measured using the U.S. Environmental Protection Agency method 3052 [nitric acid ( $\text{HNO}_3$ )–hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), microwave digestion] and an inductively coupled plasma mass spectrometry (ICP-MS) analysis procedure.

The closed digestion was performed by placing the sample in a polytetrafluoroethylene (PTFE) vial (or bomb). After adding the digestion reagents (10 mL  $\text{HNO}_3$  65% and 2 mL  $\text{H}_2\text{O}_2$  30%), the bomb was hermetically sealed and placed in a microwave oven for irradiation. The determination of iodine by ICP-MS was performed by using isotope dilutions of

$I^{127}$ , and iodine concentrations in the samples were determined by means of a calibration curve obtained with the method of standard additions (Larsen and Ludwigsen 1997).

To assess the iodine accumulation efficiency (AE) of the two plant organs analyzed, their agronomic efficiency of accumulation was calculated using the following simple equation:

$$AE = \frac{\text{total amount } (\mu\text{g}) \text{ of iodine in tomato fruits or potato tubers harvested}}{\text{total amount } (\mu\text{g}) \text{ of iodine applied}} \times 100$$

### Statistical Analysis

Fresh weight data and weight reduction data were used for the analysis of variance (ANOVA) with SYSTAT 9 (SPSS Inc. 1999). Weight reductions were used as dependent variable, while salt (iodide/iodate), its concentration, and plant species were used as independent variables. To test for iodine accumulation, the content of the element ( $\text{mg kg}^{-1}$ ) in plant tissue was used as a dependent variable, while salt (iodide/iodate), its concentration, and plant species were independent factors. For all analyses, probability values of  $\leq 0.05$  were considered as statistically significant. Multiple comparisons for iodine contents, where the ANOVA result was statistically significant, were carried out using the Tukey's honestly significant difference test (0.05).

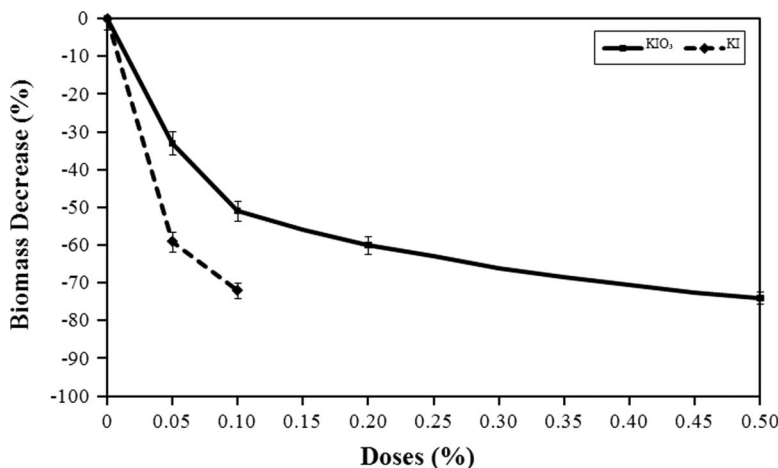
## Results

### Effects of Iodine on Plant Growth

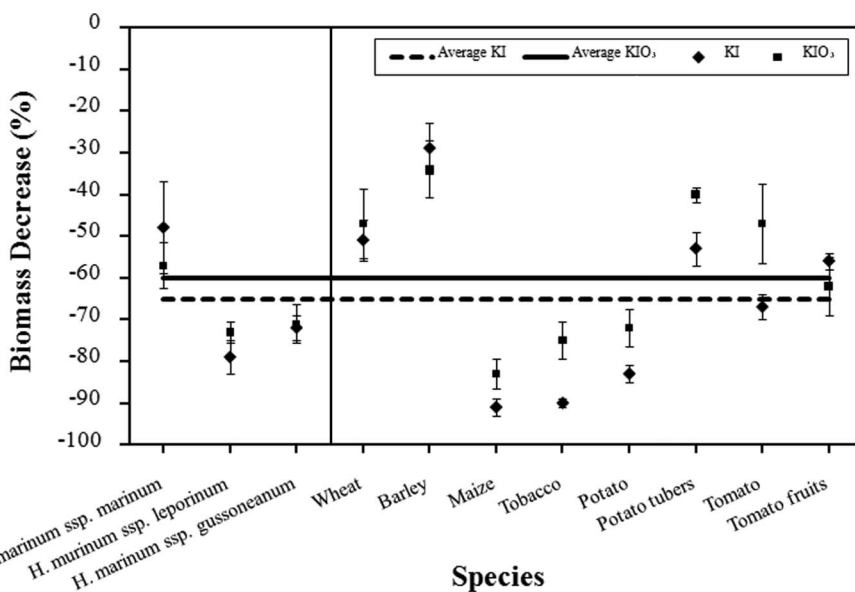
Iodine application with irrigation water had a detrimental effect on biomass yields, even at the lowest concentrations ( $0.05\% = 43 \text{ mg iodine pot}^{-1}$  for KI and  $33 \text{ mg iodine pot}^{-1}$  for  $\text{KIO}_3$ ). Phenotypic effects of phytotoxicity on leaves (not shown) increased with the increase of either iodine supply source, being milder, as a general observation, for iodate additions. In Figure 1, the effect of high dosage of iodine on the biomass decrease is presented as averages for all the nine plant species. The greatest biomass decrease rate was observed at the lowest iodine concentration. Slower progressive biomass decrease was observed with greater iodine concentration (Figure 1). On average, the iodate treatment had much less effect on plant biomass than the iodide for all the species considered (Figure 1).

Statistically significant ( $P \leq 0.05$ ) differences have been observed in terms of biomass decrease between the treatments, the iodine forms, and the plant species. Only the interaction dose  $\times$  iodine form was highly significant, and therefore only main effects are discussed.

In Figure 2, the biomass decrease of each plant species, plus tomato fruit and potato tuber weight decreases, is represented for both iodine salts, as an average effect for all the applied concentrations. In general, the biomass decrease was greater due to iodide than to iodate. However, in several cases the difference between the effects of the two salts was not significant, as in the case of wheat, barley, *H. marinum* ssp. *marinum*, *H. murinum* ssp. *leporinum*, *H. marinum* ssp. *gussoneanum*, as well as for the tomato fruits (Figure 2). Barley and wheat plants, together with the potato tubers, showed the least detrimental effects on biomass, as far as the treatment effects for both salts were considered. In particular, the best result in terms of tolerance to high iodine supplies was obtained for barley. On the



**Figure 1.** Effects of KI and KIO<sub>3</sub> supply on the biomass decrease of all treated species. For each dose, the reported value refers to the average of values obtain for all the species. Bars indicate standard errors.



**Figure 2.** Effects of iodine supply, as both iodide and iodate, on the biomass decrease of each plant species, plus fruit and tuber weight, decrease for tomato and potato respectively. The vertical line within the figure divides the wild (on the left) from the cultivated (on the right) tested species. For each sample, the reported values refer to average doses applied. Bars indicate standard errors; the horizontal lines indicate the average for each salt for the experiment.

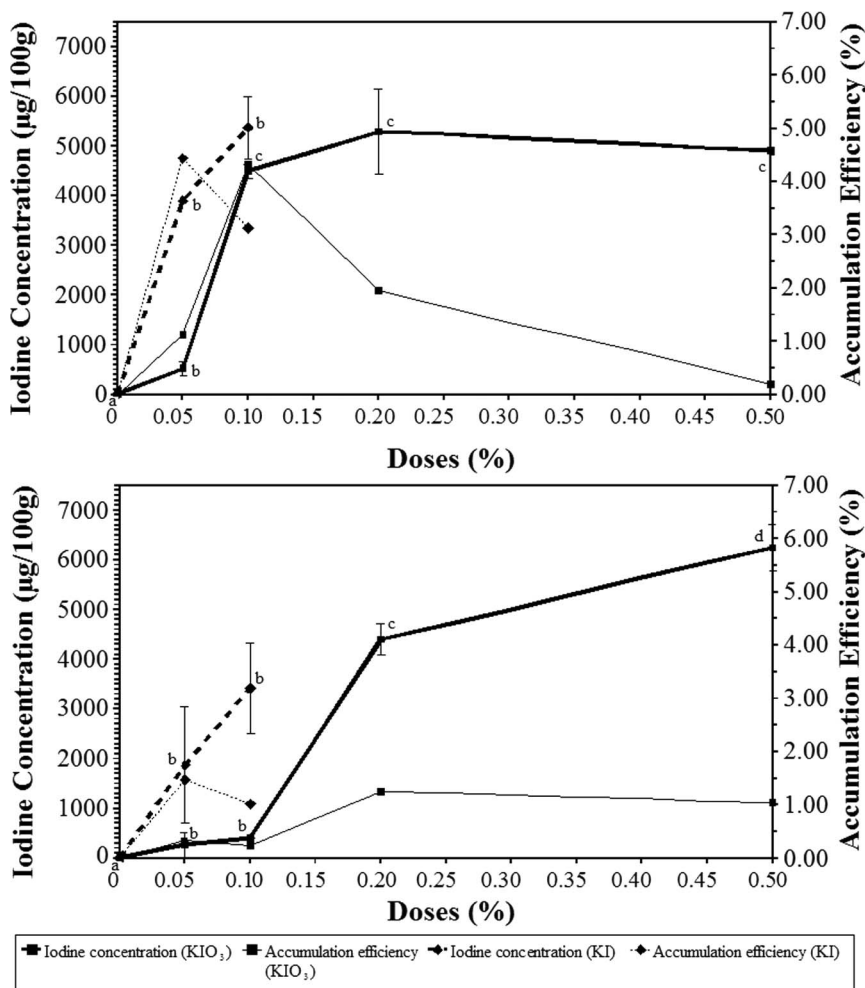
other hand, the investigated wild barley relatives did not show a better performance at high iodine supply than the cultivated barley species.

The most sensitive plants were in turn maize, tobacco, and the two wild species *H. murinum* ssp. *leporinum* and *H. murinum* ssp. *gussoneanum*, together with the potato

aboveground biomass. The weight decreases of tomato fruits and potato tubers were the same or lower respectively than their aerial parts, thus showing them to be good targets for iodine accumulation (Figure 2).

### Iodine Accumulation Efficiency

Statistically significant ( $P \leq 0.05$ ) differences have been observed in terms of iodine concentration among the treatments, iodine forms, and plant tissue (Figure 3). The interaction dose  $\times$  iodine form was highly significant, while other interactions were not significant ( $P \leq 0.05$ ).



**Figure 3.** Iodine uptake and iodine accumulation efficiency in tomato fruits (A) and potato tubers (B) treated with the different doses (0.00%, 0.05%, 0.1%, and 0.5%) and different salts (KI and KIO<sub>3</sub>) of iodine. Iodine concentration values on a fresh weight basis are expressed as replicate means; bars indicate standard errors; letters indicate statistically different values according to Tukey's test ( $P \leq 0.05$ ). Iodine accumulation efficiency is expressed as percentage.



Potato and tomato successfully accumulated large amounts of iodine in their edible tissues, from 272 to 6,245 and from 527 to 5,375  $\mu\text{g } 100 \text{ g}^{-1}$ , respectively. With such levels of iodine uptake, at the lowest dose of 0.05% as potassium iodate in irrigation water, tomato and potato overtook significantly the RDA for adults of 150  $\mu\text{g day}^{-1}$ , on a 100 g FW basis. The quantities of iodine accumulated at the lowest dose of supply of potassium iodide resulted in more than 10 times the RDA in potato and more than 20 times the RDA for adults in tomato, on the same basis of daily consumption (Figure 3 and Table 1). As a general trend, with increasing dose of iodine applied, increasing content of the element in plant tissues was observed. However, increasing application rates of iodine as  $\text{KIO}_3$  to tomato formed a plateau as the application rate approached 0.2% (Figure 3).

The two species showed a similar behavior with respect to the accumulation of iodine even if different tissues were examined.

The iodine uptake of plants treated with control doses (0.0%) (w/v) were similar between the two species, and the maximum levels of accumulation were highly comparable, apart from the fact that tomato reached a plateau content of 4,000–6,000  $\mu\text{g } 100 \text{ g}^{-1}$  at lower application rate than the potato tuber (Figure 3).

The iodine uptake and the iodine AE of the plants treated with iodide were greater than in those treated with iodate (Figure 3 and Table 1). The iodine concentration in the tomato fruits of plants treated with 0.05% iodide was about seven-fold greater than that in the fruits of plants treated with 0.05% iodate (3,900 and 527  $\mu\text{g } 100 \text{ g}^{-1}$ , respectively) and similar to the iodine content reached at 0.1% iodate (4,500  $\mu\text{g } 100 \text{ g}^{-1}$ ). Moreover, the iodine AE in the fruits of tomato plants treated with 0.05% iodide was 4.44%, which was comparable with that of fruits treated with 0.1% iodate (Figure 3A). The iodine concentration, in tubers of potato plants treated with 0.05% iodide, reached 1,875  $\mu\text{g } 100 \text{ g}^{-1}$ , which was approximately seven-fold greater than in the tubers of plants treated with 0.05% iodate (272  $\mu\text{g } 100 \text{ g}^{-1}$ ) and still almost five-fold greater than in the tubers of plants treated with 0.1% iodate (403  $\mu\text{g } 100 \text{ g}^{-1}$ ). The iodine AE in the tubers of potato plants treated with 0.05% iodide was 1.47%, which was comparable with the AE of potato plants treated with 0.2% iodate (Figure 3B).

While the iodine content accumulated in tissues showed an increasing trend until a plateau, a decrease in the AE was observed for both plant species and chemical types. The AE started to decrease when the iodine tissue content approached the plateau level (Figure 3). For iodide, the limit concentration was 0.05% for both species. The limiting iodate concentration was greater for potato, 0.2% (Figure 3B), and lower, 0.1%, for tomato (Figure 3A).

Finally, the iodine AE in the fruits of tomato plants was generally greater than that in the tubers of potato plants.

## Discussion

In this study, we applied high doses of iodine to a wide range of herbaceous plant species, setting up a plant screening process to investigate differences in the toxicity effects and accumulation responses. The treatments led to similar phytotoxicity symptoms in all nine monocot and dicot plant species investigated, including chlorosis of older leaves and necrosis of leaf tips expanding to larger areas of the leaf blade, quite similar to those observed in rice after 10 and 100  $\mu\text{M}$  iodide treatments (Mackowiak and Grossl 1999). We found that even when used at 0.05% in irrigation water (2.34 mM for  $\text{KIO}_3$  and 3.01 mM for KI), although allowing a great accumulation of the element (Figure 3), iodine inhibited the

**Table 1**  
Values of iodine uptake and iodine accumulation efficiency in tomato fruits and potato tubers for each level of iodine applied

I concentration (%)	I concentration (mg pot <sup>-1</sup> )	Tomato iodine uptake (µg 100g <sup>-1</sup> )	Tomato accumulation efficiency (%)	Potato iodine uptake (µg 100g <sup>-1</sup> )	Potato accumulation efficiency (%)
<b>Iodate treatments</b>					
0	0	10 ± 3 a	0	16 ± 0 a	0
0.05	33	527 ± 138 b	1.12	272 ± 248 b	0.33
0.10	66	4,500 ± 140 c	4.32	403 ± 7 b	0.24
0.20	132	5,295 ± 855 c	1.95	4,405 ± 305 c	1.25
0.50	330	4,920 ± 0 c	0.20	6,245 ± 465 d	1.04
<b>Iodide treatments</b>					
0	0	10 ± 3 a	0	16 ± 0 a	0
0.05	43	3,900 ± 0 b	4.44	1,875 ± 1,175 b	1.47
0.10	86	5,375 ± 625 b	3.13	3,420 ± 910 b	1.02

*Note.* Letters indicate statistically different values according to Tukey's test ( $P \leq 0.05$ ).

growth of the plants, leading to extended biomass decrease (Figure 1). This finding is in agreement with the results described in spinach by Zhu et al. (2003).

Our results showed that the threshold for both phytotoxicity and biomass decrease should be set less than  $43 \text{ mg I pot}^{-1}$  (3.01 mM) for iodide and  $33 \text{ mg I pot}^{-1}$  (2.34 mM) for iodate (0.05%). Therefore, we should concentrate future research for biofortification with iodine supply at doses within the range of 0 to 0.05%.

On the other hand, treatment of plants with greater iodine doses could be interesting for the investigation of the capacity of plant tissues to absorb iodine even when the plants show phytotoxicity effects. In fact, when we compare the data of iodine content to those of biomass decrease, potato tubers continue to accumulate iodine in form of iodate even when treated with a 0.2% iodine solution (Figure 3), although plants show consistent and increasing biomass reduction (Figure 1). Such a shock treatment could be even more interesting in potato than in tomato, on the basis of the different behavior of tomato and potato species following iodine supply (Figure 2). In fact, the aboveground part of the potato plant is much more sensitive to the detrimental effects of iodine, leading to desiccation, with respect to the tubers (Figure 2). The reason for this different behavior might be that iodine is transported in the xylem rather than in the phloem (Herrett et al. 1962).

The effect of iodide on plant growth was more detrimental than the effect of iodate (Figure 1). This observation is in agreement with Mackowiak and Grossl (1999), Zhu et al. (2003), and Blasco et al. (2008). The iodide effect is probably due to greater uptake of  $\text{I}^-$  over  $\text{IO}_3^-$  (Mackowiak and Grossl 1999). It has been suggested that iodate is reduced to iodide before its uptake by plant roots (Boszormenyi and Cseh 1960; Whitehead 1975; Zhu et al. 2003); therefore the slower uptake rate of iodate is likely to be limited by the reduction process. In fact, our results showed not only the greater phytotoxicity of iodide, but also its greater accumulation efficiency as compared with iodate (Figure 3).

Wheat and barley showed lower biomass decreases than other species and did not highlight any significant differences in phytotoxicity for either of the salts applied (Figure 2). Wild barley relatives were tested as far as we know for the first time for iodine supply. These species were inferior or comparable to the cultivated *Hordeum vulgare* in terms of the effects of the high iodine treatment rates, although this finding is contrary to what we might expect. In fact, the Mediterranean annual grass species *Hordeum marinum* Huds. is a major inbreeder that occurs in western Eurasian saline meadows along the coast, where greater concentrations of iodine in the soil are supposed to be present (Bothmer et al. 1995). The species consists of two morphologically distinct taxa. *Hordeum marinum* ssp. *marinum*, mainly distributed in the western Mediterranean, and *Hordeum marinum* ssp. *gussoneanum* (Parl.) Thell. are di- ( $2\times$ ) and a tetraploid ( $4\times$ ) cytotypes, which are morphologically indistinguishable from each other (Bothmer et al. 1989, 1995). From the results obtained using *Hordeum murinum*, we can conclude that the wild species used in the present study do not carry favorable alleles for the accumulation of iodine.

In conclusion, barley, wheat, potato tuber, and to some extent tomato could be interesting crops for a strategy of biofortification, even if the iodine content of cereal grains (not analyzed in the present study) is supposed to be lower than that of their vegetative parts (Mackowiak and Grossl 1999; Shinonaga et al. 2001).

A second goal of this work was to perform a rate study to seek out the tolerance to the two iodine sources added to potato and tomato, as well as to identify iodine concentration in the selected plant products. This information was then used to indicate iodine uptake efficiency by comparing the rate added to the soil and the concentration found in the plant part. The experiment showed successful accumulation of iodine well above the RDA, in all treatments for both plant species, and already at the lowest dose of 0.05% in irrigation

water. The result is achieved considering an RDA for adults of  $150 \mu\text{g day}^{-1}$  and 100 g FW of tomato/potato consumption as references (Figure 3), although this result is obtained with significant biomass detriments, in average of 33% biomass loss with the lowest concentration of iodate and 59% loss with iodide (Figure 1). Lower doses of supply should be therefore investigated and verified experimentally at doses that can be simply extrapolated from the uptake curve of Figure 3 for reaching a content of  $150 \mu\text{g}$  in a 100-g serving of both potato and tomato.

The doses calculated in this way for tomato fruits (Figure 3A) are 0.014% ( $9.24 \text{ mg pot}^{-1}$ ) in the case of iodate and 0.002% ( $1.72 \text{ mg pot}^{-1}$ ) in the case of iodide. For potato tubers (Figure 3B), the doses in the irrigation water should be 0.028% ( $18.48 \text{ mg pot}^{-1}$ ) in the case of iodate and 0.004% ( $3.44 \text{ mg pot}^{-1}$ ) in the case of iodide. Reaching the full RDA for adults for a FW serving of 100 g in some way compensates for the possible cases of lighter servings or loss of the element due to cooking. The use of the new calculated doses of iodine applied should also reduce the average biomass decrease to percentages within the range from 9% to 18% in the case of iodate and within the range from 2% to 5% in the case of iodide, if we extrapolate such data from the biomass decrease curve of Figure 1. At such low biomass detriments, the fortification together with a foreseen increase of food prices is most likely going to compensate growers for the limited yield loss compared to nonfortified fruits and tubers.

The greenhouse experiment indicated that the potential for iodine enrichment in potato and tomato plants was greater with iodide than with iodate, at least in terms of the accumulation efficiency of the element (Figure 3). The lower AE for  $\text{IO}_3^-$  may depend on its lower bioavailability, given the need for reducing the ion by standard reductase before uptake (Blasco et al. 2008).

Results of this work also evidenced that the tomato fruit had greater efficiencies of accumulation than the potato tuber. Considering that fresh tomatoes are commercially cultivated also in hydroponics, this finding would make tomato plants a better target than potato for the supply of iodine into human diets.

The experiments performed where two forms of iodine are supplied to pot plants in the irrigation water could also raise the question about the fate of the supplied iodine in the surrounding environment. A couple of recent publications investigated the movements of iodine in the soil, and the percentages of iodine lost from the soil after an enrichment experiment, by means of a radioactive isotope (Weng et al. 2008, 2009). The authors concluded that the percentages of iodine lost from the soil by leaching and volatilization were a small fraction of the iodine persisting in the soil, respectively, from 4.5 to 6% and from 4.2 to 4.7% in a sandy soil (Weng et al. 2009). Nevertheless, the residual iodine in the soil, particularly fixed in organic forms, as well as the iodine eluviated into surface and groundwater, and the volatilized fractions, could be considered positively to raise the general iodine level in the food chain in the iodine-deficient areas to control IDD (Weng et al. 2008).

## Conclusions

The plant screening process that we performed on six cultivated plus three wild plant species by means of iodine potassium salts supplemented to the irrigation water showed that iodine caused significantly different levels of biomass decrease, depending on the plant species, as well as on the supplied forms. The screening also indicated that contrary to expectations the wild relatives of barley surveyed were not less sensitive to biomass

decrease than the cultivated species and that for a fortification project some plant species and organs can be better targets than others.

An accumulation study done in potato tubers and in tomato fruits made it possible to understand their iodine uptake, at different doses of supply, as well as their iodine AE. On the basis of the uptake curve, it was possible to recommend the doses in the irrigation water of iodine as iodate (0.028% for potato and 0.014% for tomato) as well as of iodide (0.004% for potato and 0.002% for tomato) to reach a full RDA for adults in 100 g of such vegetables and to efficiently control IDD, although these results still need to be validated. More interestingly, such supplies of iodine should make calculated biomass decreases (within the range from 9% to 18% for iodate and within the range from 2% to 5% for iodide) economically sustainable from the growers in view of the greater prices of the fortified foods.

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