Iodine biofortification in tomato
Martina Landini1, Silvia Gonzali1, and Pierdomenico Perata*

1 PlantLab, Scuola Superiore Sant’Anna, Via Mariscoglio 34, 56124, Pisa, Italy

Abstract
Iodine is an essential element in the human diet, and iodine deficiency is a significant health problem. No attempts to increase iodine content in plant-derived food (biofortification) have so far been particularly effective. We studied iodine uptake in tomato (Solanum lycopersicum L.) to evaluate whether it is possible to increase the iodine concentration in its fruits. Iodine translocation and storage inside tomato tissues were studied using radioactive iodine. Potassium iodide was also supplied at different concentrations to tomato plants to evaluate the resulting iodide concentration both in the vegetative tissues and the fruits. The results indicate that iodine was taken up better when supplied to the roots using hydroponically grown plants. However, a considerable amount of iodine was also stored after leaf treatment, suggesting that iodine transport through phloem also occurred. We found that tomato plants can tolerate high levels of iodine, stored both in the vegetative tissues and fruits at concentrations that are more than sufficient for the human diet. We conclude that tomato is an excellent crop for iodine-biofortification programs.

Key words: biofortification / iodine deficiency / phytotoxicity / Solanum lycopersicum

Accepted March 14, 2011

1 Introduction
Iodine is an essential element for human physiology (Andersson et al., 2005), being involved in the synthesis of thyroid hormones. The recommended dietary allowance (RDA) for adults amounts to 150 μg iodine per day (Pearce et al., 2004). Chronic iodine deficiency, called hypothyroidism, can trigger goiter, growth impairment, reproductive failure, hearing loss, cretinism, and several kinds of brain damage (Andersson et al., 2005; Delange, 2000; Dillon and Milliez, 2000; Haddow et al., 1999). Although iodine deficiency can be treated, it is still a public health problem for almost 35% of the world’s population (Pearce et al., 2004; Winger et al., 2008), and the population at risk is more than one billion (Winger et al., 2008; World Health Organization, 2004).

Iodized salt is the most common approach for dietary iodine supplementation (Andersson et al., 2005; Delange and Lecomte, 2000). However, since iodine supplementation may cause problems during food processing, it is difficult to control its loss during transport, storage, and food cooking (Winger et al., 2008). Therefore, enhancing iodine content in vegetables represents a cost-effective way to control its deficiency, since iodine in food is readily bioavailable (up to 99%) and assimilated (Dai et al., 2004; Weng et al., 2009; White and Broadley, 2009).

Despite its importance for human nutrition, iodine relevance has not yet been established for plants. Vegetables can accumulate iodine, and increasing iodine application to the soil results in enhancing iodine accumulation in plants (Dai et al., 2004; Whitehead, 1973; Zhu et al., 2003), as it was demonstrated for pakchoi, celery, pepper, radish (Hong et al., 2008), cabbage (Weng et al., 2008a), and spinach (Zhu et al., 2003). However, results are largely affected by the iodine concentration and the chemical form supplied (Blasco et al., 2008; Dai et al., 2004; Mackowiak and Grossl, 1999) and by the growth substrate used (Weng et al., 2008c). Iodide, rather than iodate, has the greatest bioavailability for plants (Umaly and Poel, 1971; Whitehead, 1973), and very low concentrations of iodine, regardless to the form, are beneficial to several crops (Borst-Pauwels, 1961). In tomato plants, a very low amount of iodide can stimulate the tangential growth and, to some extent, improve the yield (Lehr et al., 1958). However, at higher concentrations, iodine can be toxic, leading to leaf damages, stunted growth, and death (Lehr et al., 1958). Once inside the plant, a xylem flux of iodine seems to be largely predominant (Lewis and Powers, 1941; Shinonaga et al., 2001; Weng et al., 2008c, 2009; Whitehead, 1973), but, generally, the absorbed iodine is not uniformly distributed among plant tissues, ranking as follows: root > leaf > stem (Weng et al., 2008a). Finally, a further aspect, which needs explanation, is whether iodine uptake could be affected by other nutrients. A negative correlation between nitrate and halogens has been speculated for radish, lettuce, and cabbage (Roorda van Eysinga and Spann, 1985; Sheppard and Ender, 1992; Weng et al., 2008b).

Recent studies demonstrated that leafy vegetables such as spinach (Zhu et al., 2003; Dai et al., 2006) or lettuce (Blasco et al., 2008; Voogt et al., 2010) can store iodine in their edible tissues, making them good candidates for iodine biofortification programs. The aim of the present work is to evaluate the ability of tomato plants to absorb and store iodine in vegetative tissues and fruits. Due to its widespread distribution and...
its easy growth on a commercial scale, tomato would represent a preferred crop in biofortification programs. Moreover, the possibility of fresh consumption of its fruits prevents the risk of iodine loss with certain cooking methods. To achieve our goals, radioactive iodine (\(^{125}\text{I}\) as NaI) was used for visualizing iodine distribution and storage inside plant tissues, while potassium iodide (KI) was supplied to study iodine toxicity and the overall amount stored in tomato fruits. Different kinds of treatments as well as different growth substrates were compared to set up the best experimental conditions for enhancing iodine concentration in fruits of this species.

2 Materials and methods

2.1 Plant material

All the experiments were carried out using tomato (Solanum lycopersicum L. cv. MicroTom). Tomato seeds were sown on wet filter paper under continuous light until germination. When the cotyledons were well expanded, the plants were transplanted into soil or transferred into a hydroponic system. This latter was based on thick gravel (3–5 mm in diameter) and a nutrient solution whose composition was as follows (in mM): NO\(_3\)-N 12; NH\(_4\)-N 0.5; P 1.30; K 8; Ca 4; Mg 1.19; Na 9; SO\(_4\)-S 1.59; Cl 9.87; (in \(\mu\)M): Fe 19.5; B 28.6; Cu 3.6; Zn 4.5; Mn 10.9; Mo 0.2. Electrical conductivity (EC) was 2.84 dS m\(^{-1}\), and pH 5.8. Fresh solution was added weekly. Plants grown in soil (Hawita Flor, Vechta, Germany) were watered twice a week. All plants were grown in plastic pots (diameter 5 cm) in a growth chamber, with 80 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) PAR (Gro-Lux, Sylvania, OH, USA), 12 h light photoperiod, 24°C temperature, 55% relative humidity.

2.2 Reagents and chemicals

Potassium iodide was purchased from Sigma-Aldrich (St Louis, MO, USA), while the radioactive iodine (\(^{125}\text{I}\) as NaI) from Perkin Elmer (Waltham, MA, USA).

2.3 Iodine-uptake experiments

Tomato plants, grown in hydroponics or soil, were treated with radioactive iodine starting at the age of: (1) 2 weeks, (2) 1 month, or (3) 2 months after germination. Iodine was supplied as root or leaf treatment, and in both the experiments, a total of three iodine-feeding treatments were performed, at 2-day intervals. In the root-treatment experiments, the iodine stock solution (Na\(^{125}\text{I}\), 10 Ci mL\(^{-1}\)) was diluted to the final activity of 0.25 Ci mL\(^{-1}\), and 1 mL was supplied directly to the gravel of the hydroponic system or to the soil of each treated plant. At the end of the experiment, after the three feeding treatments, a total amount of 0.75 \(\mu\)Ci iodine was supplied per plant. In the leaf-treatment experiments, 25 \(\mu\)L of radioactive iodine (Na\(^{125}\text{I}\), 10 Ci mL\(^{-1}\)) were spotted on a leaf blade. The first true leaf or a well expanded leaf at the second branch was treated in 2-week-old or 1-/2-month-old plants, respectively. Also in this case, three iodine-feeding treatments were performed, for a total amount of iodine supplied per plant equal to 0.75 \(\mu\)Ci. Each experiment was replicated three times.

2.4 Nitrate experiment

Two groups of 2-week- and 2-month-old tomato plants grown in hydroponics were treated with radioactive iodine (Na\(^{125}\text{I}\), 0.25 \(\mu\)Ci mL\(^{-1}\)). Starting from the beginning of the iodine treatment, one set of both 2-week- and 2-month-old plants was maintained in the usual complete hydroponic solution, while another identical set was grown in a modified nutrient solution in which the nitrate concentration was diluted 1:10 (reaching a final concentration of 1.2 mM nitrate). Three root iodine feeding treatments (1 mL for each treatment) were carried out, at 2-day intervals, for a total amount of iodine supplied per plant equal to 0.75 \(\mu\)Ci. Each experiment was replicated three times.

2.5 Visualization of radioactive-iodine uptake

At the end of each experiment, both leaf- and root-treated plants were cut at the hypocotyl level, gently washed, blotted onto filter paper, and then exposed for 2 d to a multipurpose phosphor storage screen (Cyclone Storage Phosphor System, Packard, CT, USA) in order to obtain a digital image of the radioactivity distribution. For the leaf treatment, each Na\(^{125}\text{I}\)-treated leaf was removed from the original plant and exposed on a separate screen. When possible, sepals and fruits from each treatment were collected at the end of the experiment. Fruits were cut into several longitudinal sections in order to display where iodine was stored. All the digital images were obtained and analyzed using a phosphoimager (Cyclone Storage Phosphor System, Packard). Data are presented as DLUs (digital light units), and the scanned images are shown using false colors, where red and blue indicate a high or low level of radioactivity, respectively.

2.6 Iodine toxicity

To investigate the effects of iodine on tomato plant physiology and fruit production, a dose-response experiment was carried out. Plants at the flowering stage (about 45 d old) and grown in hydroponics were treated. Different concentrations of KI (0, 5, 10, 20 mM), added to the hydroponic solution, were tested, and three treatments (once a week) were carried out. During this period, the plants were observed and photographed. Twenty-four days after the beginning of the treatment, all the plants were collected to analyze the iodine content. Fruits were sampled at the mature green stage for all the iodine treatments and also at the red ripe stage for the 5 mM KI treatment, and analyzed separately. Three replicates were analyzed for each treatment.

2.7 Iodine measurements

The iodine content inside plant vegetative tissues and fruits was measured using the inductively coupled plasma–mass spectrometry (ICP-MS) technique (Yoshida et al., 2007). Iodine was determined using an isotope dilution with \(^{127}\text{I}\). The iodine concentration in the samples was evaluated using a calibration curve obtained with the standard additions method. Three replicates were analyzed for each treatment. Analyses were carried out by Neutron Spa (Modena, Italy).
3 Results

3.1 Iodine uptake in tomato plants: storage and distribution

Plants at different developmental stages were root- or leaf-treated with radioactive iodine. After the treatment, iodine was clearly detectable in all the treated plants (Fig. 1a). Regardless of the plant age, the amount of iodine accumulated after the root treatment was higher than that stored in leaf-treated plants (Fig. 1). In the youngest plants, especially in the root-treated ones, iodine was widely distributed in the aerial parts, being strongly accumulated both in the stem and in the leaves (Fig. 1a). In adult plants, iodine was prevalently stored in the stem and in the main veins of the leaves (Fig. 1a). In 2-month-old plants, iodine was accumulated not only in vegetative organs but also, although at lower levels, in fruits (Fig. 1a).

When plants were grown in soil, the amount of radioactive iodine accumulated after a root treatment was dramatically lower, both in young and adult plants (Fig. 2a, b).

3.2 Iodine uptake and nitrate

Nitrate may interfere with iodine uptake (Roorda van Eysinga and Spaan, 1985). For this reason, an experiment was performed to compare iodine uptake and accumulation in plants in the presence of different nitrate concentrations. Young and adult tomato plants, previously grown in a full-strength hydroponic solution, were treated with iodine in the presence of a high (12 mM) or low (1.2 mM) nitrate concentration. The latter was modified only at the beginning of the iodine treatment to avoid that plant root architecture and growth were influenced by different nitrate levels. The results show that when plants were maintained in the presence of low nitrate, iodine uptake and accumulation were negatively affected (Fig. 3a–c). This was particularly evident in 2-week-old plants (Fig. 3a, c), but also in plants at the reproductive stage the final iodine levels detected in the presence of low nitrate were lower (Fig. 3b, c). The reduction in iodine accumulation of plants treated with low nitrate concentration was more evident in the vegetative rather than reproductive organs (Fig. 3a, b).

3.3 Phytotoxic effects of iodine

In order to detect possible phytotoxic effects and consequences for fruit production, an iodine dose–response experiment was carried out supplying KI at increasing concentrations (0, 5, 10, 20 mM) to plants at the flowering stage. After 3 weeks of iodine treatments, all the plants survived and produced fruits (data not shown), but those treated with iodine showed symptoms of phytotoxicity (Fig. 4). The main physiological effects observed were leaf chlorosis and burns, mainly located at the tips of the leaves and becoming more evident when the iodine concentration increased (Fig. 4a). Toxicity symptoms initially appeared on the lower leaves and moved gradually towards the upper parts of the plant. Generally, the lowest branches were those mostly injured and discolored quickly, becoming brownish and, finally, turning necrotic. At the highest iodine concentrations (10–20 mM), tomato branches showed a strong epinasty in comparison to the control plants and the leaf edges were down-curved (Fig. 4b). Another effect was the presence of small white spots on the adaxial leaf surface (Fig. 4c). This was also observed for the lowest iodide concentration applied (5 mM) and generally increased with the iodide concentration, gradually moving...
towards the upper leaves. A difference in color was observed between the iodide-treated plants and the control ones, the former leaves being darker green (Fig. 4d). This effect was evident particularly for the lowest concentrations used (5–10 mM). A slight anthocyanin accumulation was observed in the stem and along the main veins of the iodine-treated leaves (data not shown). However, no phytotoxic effects were observed in fruits and flowers (Fig. 4e, f), apart from some white spots on a few fruits treated with 10 mM KI, similar to those observed on leaves (Fig. 4e). Regardless to the iodine concentration supplied, flowers and fruits grew and developed normally (Fig. 4e, f).

3.4 Iodide concentration in tomato vegetative tissues and fruits

Plants treated with KI in the dose-response experiment were collected at the end of the treatment and analyzed for their iodine concentration in vegetative tissues and fruits. High concentrations of iodine were found in leaves and stems (Fig. 5a). The iodine concentration increased in proportion to its concentration in the hydroponic medium (Fig. 5a). The highest value (approximately 9000 mg [kg FW]–1) was observed in plants treated with 20 mM iodide (Fig. 5a). However, also at concentrations of 5 and 10 mM, the iodine concentration was very high, approximately 3000 and 5000 mg (kg FW)–1, respectively (Fig. 5a). Green fruits contained lower levels of iodine, reaching the maximum concentration (30 mg [kg FW]–1) in plants treated with 20 mM KI (Fig. 5b). At the lowest concentration supplied (5 mM), the iodine content in fruits was still high enough (10 mg [kg FW]–1) to fulfill the goal of a biofortification program (Fig. 5b). Tomato fruits treated with 5 mM KI were also collected at the red ripe stage and analyzed. Red fruits showed the same iodine concentration as green fruits (Fig. 5b).

4 Discussion

Most of the attempts to develop iodine biofortification of crops have failed, particularly those aimed at increasing iodine con-
concentration in fruits (Mackowiak and Grossl, 1999). Our results obtained for tomato showed that when iodine was supplied in the growth medium, root uptake and xylem transport were highly efficient, as the element was found widely distributed in the shoot. Iodine was accumulated in the stem and in the main veins of the leaves, but in many cases, particularly in young plants, it also spread to cover the entire leaf blade (Fig. 1a). Previous results obtained in cabbage indicated that also in this species iodine distribution in the shoot was not uniform and iodine was stored predominantly in the main leaf veins (Weng et al., 2008a). Hydroponics was more effective than soil in promoting iodine absorption (Fig. 2a, b), probably because in soil iodine can be retained by organic matter, being therefore less available for plant uptake. The overall amount of iodine taken up by tomato plants was generally higher when iodine was supplied to the roots rather than onto the leaf blade (Fig. 1a, b). These results imply that root treatment is most effective in terms of iodine uptake and storage. Hydroponic culture, with iodine added to the nutrient solution, thus, gives excellent possibility for tomato biofortification.

A possible inhibitory effect of nitrate on halide, particularly bromide, uptake, as a consequence of a competition during plant uptake, has been described (Roorda van Eysinga and Spaan, 1985), although results are controversial (Sheppard and Evenden, 1992). Our results show that nitrate did not inhibit iodine uptake (Fig. 3). On the contrary, plants concomitantly fed with iodine and 12 mM nitrate showed a higher iodine accumulation than plants fed with iodine and 1.2 mM nitrate (Fig. 3). The iodine content in fruits was less negatively affected by low nitrate (1.2 mM) than in vegetative organs (Fig. 3a, b). This may suggest that low nitrate could negatively affect the xylem transport of iodine with a limited effect on iodide content of fruits, which largely depends on the phloem transport (Ho et al., 1987).

The possible role of phloem in iodine transport was ruled out by Herrett et al. (1962) and Mackowiak and Grossl (1999). In contrast, we found that a phloem route for iodine transport is present in tomato plants. Spotting radioactive iodide on a single leaf resulted in a widespread distribution of iodine in all the surrounding tissues of the shoot (Fig. 1a, b). Tomato fruits are relatively isolated from the xylem stream, and therefore, they accumulate little amount of mineral elements that are mainly translocated along the transpiration stream (Ho et al., 1987; Mingo et al., 2003). Therefore, an adequate accumulation of iodine inside fruits depends on phloem transport. The fact that in tomato a moderate phloem flux of iodine was ob-
served is particularly noteworthy because, in order to obtain an effective biofortification strategy in this species, it is crucial to ensure an adequate concentration of iodine inside fruits. Iodine treatments to plants at the reproductive stage demonstrated that iodine could be transported into fruits (Fig. 1a).

Plants treated with iodine often show toxicity symptoms, with crop-specific effects. Our data indicate that tomato plants, despite the presence of some phytotoxicity symptoms, tolerate relatively high concentrations of KI (20 mM) without severe injuries (Fig. 4). Plants treated with the lowest iodide concentrations were darker green (Fig. 4d), suggesting a slight production of antioxidant compounds, such as anthocyanins, in response to iodine. In lettuce, iodine can increase the amount of antioxidant compounds, probably interfering with the oxidative state of the plant (Blasco et al., 2008). It is noteworthy, in the framework of iodine-biofortification programs, that iodine application up to 20 mM, starting from the beginning of the reproductive stage, did not importantly affect tomato vegetative and reproductive growth and development.

During tomato ripening, the translocation of nutrients from roots to fruits is higher at the green stage and falls down in later phases (Srivastava and Handa, 2005). It is reasonable to assume that iodine could be more easily translocated during the early fast growth of the fruits. The quantitative data obtained by ICP-MS showed that a huge amount of iodine was stored in both vegetative tissues and in green fruits, reaching the maximum concentration of about 9000 mg kg⁻¹ and 30 mg (kg FW)⁻¹, respectively (Fig. 5a, b). Even at the lowest concentration of KI supplied (5 mM), the iodine concentration in tomato fruits was high (about 10 mg [kg FW]⁻¹). The iodine concentration did not change when fruits reached the mature red stage (Fig. 5b), suggesting that, once inside the fruit, iodine concentration was stable.

5 Conclusion

Our results indicate that iodine uptake and translocation in tomato plants are efficient and lead to considerable iodine accumulation in fruits. The fruit concentration of iodine detected in 5 mM iodide–treated plants was more than enough to cover a daily human intake of 150 μg. Nitrate, at least in the range which is commonly used in hydroponics, had no negative effect on iodine uptake, and thus modifications of the usual nutrient solution used to grow tomato commercial varieties are not required.

Acknowledgments

We thank Prof. A. Pardossi of the Department of Crop Plant Biology of the University of Pisa, for critical reading of the manuscript and useful suggestions, and the staff of the Department of Endocrinology and Metabolism at the University of Pisa, who kindly supplied the radioactive iodine. This research was supported by SQM Europe and by the IODOPLANET project (Italian Ministry of Agriculture and Forestry).

References


