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Enhancing iodine content and fruit quality of pepper (*Capsicum annuum* L.) through biofortification

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ABSTRACT

Iodine is an essential trace element for human health. Its deficiency in biogeochemical environment affects about two billion people worldwide. Besides universal salt iodization, the biofortification of crops with iodine has been proposed as a strategy for improving human nutrition. This study aims at exploring the effects of iodine biofortification on the fruit quality of the pepper plants. The contents of iodine, ascorbic acid, soluble sugar and total acidity of the pepper fruits grown in solution at various iodide concentration levels were measured. Furthermore, in order to reveal the mechanism of fruit quality change, the variations in Chl-a, malondialdehyde (MDA), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) of the pepper leaves at various growth periods in response to various iodine treatments were determined. The results indicated that the iodine content of the pepper fruits grown in 0.25–5.0 mg L^{-1} KI solutions can amount to 350–1330 μg kg^{-1} FW, matching the 150 μg d^{-1} dietary iodine allowance recommended by WHO. Thus, the pepper can be used as a candidate crop for iodine biofortification. In addition, low-moderate levels (0.25–1.0 mg L^{-1}) of iodine application improved the fruit quality by enhancing the ascorbic acid and soluble sugar contents, and by reducing the total acidity of pepper fruits as well. Generally, after iodine treatments, the Chl-a concentration, and CAT, POD, SOD activities of the pepper leaves increased, while the MDA concentration decreased. The changes in photosynthetic and antioxidant capacities of the plants promoted the improvement of the pepper fruit quality.

1. Introduction

Iodine deficiency is one of the most serious public health issues worldwide, and nearly one-third of the human population still has an insufficient iodine intake (Mina et al., 2011; Andersson et al., 2012). If the iodine intake is insufficient, iodine deficiency disorders (IDD), such as goiter, cretinism, reduced IQ, miscarriages, birth defects, and higher neonatal mortality, may occur (Hetzel, 1983, 2005; Laurberg et al., 2010). The iodine deficiency is largely related to the geochemical environment. In many areas of the world, especially in mountainous areas and flood plains, soils and waters contain very low amounts of iodine, which negatively influences on the iodine content of crops, thus increasing the risk of iodine deficiency among people who consume foods primarily produced there (Chandra et al., 1999; Lin et al., 2004; WHO, 2007).

The usually recommended strategy to control iodine deficiency is through universal salt iodization and more recently through iodine fortification of flour (Subbulakshmi and Naik, 1999; WHO, 2007). Nevertheless, this might also transiently increase the proportion of thyroid disorders due to iodine excess (Mina et al., 2011; Zimmermann et al., 2008). As an alternative strategy, iodine biofortification through crops attracts increasing research interest in recent years (Welch and Graham, 2005; Hong et al., 2007; Landini et al., 2011; Smoleń and Sady, 2012; Weng et al., 2013, 2014; Smoleń et al., 2015). Previous studies showed that the iodine contents of the edible tissues of crops increased with increasing exogenous iodine concentration, yet, excess iodine application reduced the vegetable growth and production (Zhu et al., 2003; Gonda et al., 2007; Hong et al., 2009). In addition, there are known reports indicating that the iodine may also act as a beneficial element for plants, *e.g.* it positively influenced on the nitrogen use

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Table 1
Components and concentrations of Hoagland nutrient solution (Lian, 1992).

Macro elements		Trace elements	
Constituents	Concentration/mmol L^{-1}	Constituents	Concentration/μmol L^{-1}
KNO_3	6.00	H_3BO_3	10.00
$Ca(NO_3)_2$	3.50	$MnSO_4{\cdot}H_2O$	0.50
KH_2PO_4	1.33	$ZnSO_4{\cdot}7H_2O$	0.50
$MgSO_4{\cdot}7H_2O$	0.50	$CuSO_4{\cdot}5H_2O$	0.20
NaCl	0.48	$(NH_4)6Mo_7O_{24}$	0.01
		Fe-EDTA	200.00

efficiency of plants (Blasco et al., 2012; Smoleń and Sady, 2012), and improved the productivity and yield (Medrano-Macías et al., 2016). Nevertheless, it is less known about how the iodine biofortification affect the nutrition quality of the edible parts of the target plants.

Pepper (*Capsicum annuum* L.) is one of the most widely grown and commercially important fruit vegetables, with a worldwide cultivation covering more than 480,000 ha (FAOSTAT, 2013). It is cultivated as an annual crop in open fields or under greenhouse conditions for both fresh consumption and industrial processing. The nutritional and nutraceutical properties of pepper are well-known, due to the presence of a mix of bio-molecules such as ascorbic acid, sugars, polyphenols, carotenoids and antioxidants (Lo Scalzo et al., 2014). Hong et al. (2009) found that the pepper fruits grown in iodine fertilized soil may enrich a few mg iodine in per kg dry weight.

The antioxidants such as SOD, POD and CAT had been proved to be effective in protecting cell membrane from damage by removing excess reactive oxygen free radicals, and reducing hurt induced by overoxidation. Thus, the antioxidant capacity is critical for plant growth (Verma and Dubey, 2003; Zhang et al., 2007).

In this study, the pepper was used as material, a hydroponic experiment is conducted to explore the effects of iodine biofortification on the fruit quality in terms of the contents of ascorbic acid, soluble sugar and total acidity. In addition, the changes in Chl-a, CAT, POD, SOD, and MDA contents of the pepper leaves at different growth periods in response to various iodine treatments were measured to detect the relationship between fruit quality and antioxidant capacity of the pepper plants.

2. Materials and methods

2.1. Iodine biofortification

The pepper seeds were soaked and disinfected in 1% $KMnO_4$ solution for 20 min, then washed with deionized water. They were placed uniformly on clean gauz to germinate at 30 °C in incubator. When seed germination rate reached more than 80%, seedlings were moved to the peat-based commercial seedling substrate to grow, and proper volume of 1/2 Hoagland nutrient solution were supplied (Table 1).

The hydroponic experiment was carried out in a greenhouse with temperature of 25 ± 3 °C during the day and 20 ± 3 °C at night. When the first two true leaves expanded fully, three seedlings with similar size were fixed with sponge and transferred into a PVC bucket (diameter = 30 cm, height = 40 cm) filling with 7L solution. Their roots suspended naturally in the solution, and the greenhouse was well ventilated. They were cultured in tap water in the first three days, then in 1/2 Hoagland nutrient solution for three days, and in complete Hoagland nutrient solution for another three days. Thereafter, the pepper seedlings were grown in iodide (KI) solutions at different concentrations. The treatment iodine concentration gradient was as follows: 0 (CK), 0.25, 0.50, 1.00, 2.50, 5.00 mg L^{-1}. Each treatment had 9 buckets (×3 plants), The nutrient solutions were replaced once every five days. Every day, the pH values of the nutrient solutions were regulated to 5.50 ± 0.10 by adding 0.10 mol L^{-1} NaOH or 0.10 mol L^{-1} HCl. The experiment was repeated for three times.

2.2. Sample preparation

At every growth period, *i.e.* seedling, vegetative, flowering and fruiting period, some newly grown leaves were randomly sampled, then washed with deionized water and dried naturally. They were placed in clean bags and stored at −80 °C for further analysis.

At the stage of commercially ripening, plant organs, including roots, stems, leaves and fruits, were respectively harvested. The collected samples were cleaned with deionized water, and the surface water was soaked up with soft paper. Fresh weights were determined immediately. Sufficient fruit samples were ground for the measurement of ascorbic acid, total soluble sugar, and total acidity. In the meantime, the remaining plant materials were then oven-dried at 70 °C for 72 h to achieve a constant weight. The dried materials were all ground for iodine analysis.

2.3. Chemical analysis

The iodine contents of various plant organs (roots, stems, leaves, and fruits) were measured using an alkaline ashing technique with Sandell-Kolthoff kinetic colorimetry (Lauber, 1975; NHCPRC, 2008). Samples (each approximately 500 mg) were placed in porcelain crucibles, and 2 mL KOH solution (10 mol L^{-1}) was added and fully mixed with each sample. Samples were then dried overnight and incinerated for 3 h at 650 °C, and the dry ash reconstituted with deionized water before colorimetric analysis in the Sandell–Kolthoff reaction.

The ascorbic acid of pepper fruits was measured using titration method of 2,6-dichlorophenol-indophenol (Horwitz, 1975; SAPRC, 1986a). Fresh samples (50.00–100.00 g) were immersed in the same weighted 20.00 g L^{-1} oxalic acid, and mashed rapidly into slurry. Fruit paste (10.00–30.00 g) then was placed into a 100 mL volumetric flask to set a constant volume with 20 g L^{-1} oxalic acid solution. Clear filtrate (5.00–10.00 mL) was drawn into a 50 mL bottle triangle for the analysis of ascorbic acid, and titrated with 2,6-dichlorophenol-indophenol standard solution till it turned red and didn't fade within 15 s.

The total soluble sugar of pepper fruits was measured by the method of anthrone colorimetry (SAPRC, 1986b; Chen and Wang, 2002). Every uniformly mashed fresh sample (1.00 g) was mixed with a small amount of distilled water in a mortar, grounded into homogenate, and then transferred into a 20 mL scale test tube. The mortar was washed with 10 mL distilled water, and the washing liquor was poured into the scale test tube also. The sealed tube was boiled for 10 min in a boiling water bath. After cooling, filtrate was collected in a 100 mL volumetric flask and set to a constant volume for colorimetric analysis.

The total acidity of the pepper fruits was measured by the titration method (SAPRC, 2008). Every uniformly mashed fresh sample