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Iodine biofortification improves yield and bioactive compounds in melon fruits

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ABSTRACT

Iodine (I) is a crucial micronutrient for human health, as its insufficient intake can lead to various health problems, such as thyroid dysfunction. Although not essential for terrestrial plants, I can act as a biostimulant at appropriate concentrations, promoting good crop productivity and metabolism changes. This study aimed to investigate the effects of foliar spray of I on melon yield, antioxidant compounds, and their accumulation in fruits. The experiment involved applying different doses of I (0, 5, 10, 15, and 20 µM/L) every 15 days after transplanting. Results showed that low doses of I (5 µM/L) improved melon yield and commercial quality, while high doses (20 µM/L) decreased yield and commercial quality, but increased the biosynthesis of bioactive compounds and I on the fruits. Therefore, plant biofortification is an important technique to increase I concentrations in crops and produce functional foods with potential health benefits.

Keywords: *Cucumis melo*, antioxidants, biostimulation, crop productivity, foliar fertilization.

RESUMO

Biofortificação com iodo melhora rendimento e compostos bioativos em frutos de melão

O iodo (I) é um micronutriente crucial para a saúde humana, pois sua ingestão insuficiente pode levar a diversos problemas de saúde, como disfunção da tireoide. Embora não seja essencial para plantas terrestres, I pode atuar como bioestimulante em concentrações adequadas, promovendo boa produtividade das culturas e alterações no metabolismo. Este estudo teve como objetivo investigar os efeitos da pulverização foliar de I na produtividade do melão, compostos antioxidantes e seu acúmulo nos frutos. O experimento envolveu a aplicação de diferentes doses de I (0, 5, 10, 15 e 20 µM/L) a cada 15 dias após o transplante. Os resultados mostraram que baixas doses de I (5 µM/L) melhoraram o rendimento e a qualidade comercial do melão, enquanto altas doses (20 µM/L) diminuíram o rendimento e a qualidade comercial, mas aumentou a biossíntese de compostos bioativos e I nos frutos. Portanto, a biofortificação de plantas é uma técnica importante para aumentar as concentrações de I nas culturas e produzir alimentos funcionais com potenciais benefícios à saúde.

Palavras-chave: *Cucumis melo*, antioxidantes, bioestimulação, produtividade das culturas, fertilização foliar.

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Iodine is an indispensable micronutrient in the human diet, constituting a crucial element of thyroid hormones, thyroxine (T4), and triiodothyronine (T3). It plays a pivotal role in normal growth, development, and metabolism (Jha & Warkentin, 2020). Consequently, iodine is vital for the typical physical, neurological, and intellectual development of infants and children, as well as for metabolism and function in adults (Andersson & Braegger, 2022). Insufficient iodine intake often leads to health issues, including thyroid dysfunction, which regulates basal metabolism, development, and growth (Chesney & Lieberman, 2022). Conversely, iodine functions as a free radical antioxidant, inducing the expression of free radicals in the thyroid gland (Aceves *et al*., 2021). The population acquires iodine through food consumption, dietary supplements, or iodized table salt (Chotivichien *et al.*, 2021). However, iodine content in salt may significantly diminish during storage, transportation, and cooking due to its volatility (Consentino *et al*., 2023).

Moreover, cultivated plants often contain low levels of this trace element, reflecting its presence in the soil (Jaiswal *et al.*, 2022). An alternative to increase its content in cultivated plants and combat human malnutrition is crop biofortification, which intrinsically enhances the nutritional content and trace elements in the edible parts of the plants (Consentino *et al*., 2023). Various crops have undergone successful agronomic biofortification, enhancing micronutrient content, production, quality, and bioactive compound levels in foods (Rodríguez-Salinas *et al*., 2022). This strategy has the potential to swiftly benefit marginalized communities with high vulnerability, as they may face challenges in accessing nutritional supplements to meet trace element intake (Olson *et al*., 2021). The socially accepted biofortified foods are particularly well-received due to their superior organoleptic characteristics, including taste, smell, and color (Thakur *et al*., 2022).

The melon (*Cucumis melo*) is recognized internationally as one of the fruits with the highest levels of consumption, as its pulp is an essential source of bioactive compounds, vitamins, minerals, and quickly absorbed sugars; therefore, its consumption has beneficial effects on health (Melgoza *et al*., 2022). These characteristics render it a suitable candidate for biofortification, offering the potential to enhance the quality of life concerning health. The objective of this study is to assess the impact of foliar iodine (I) spray on melon crops, investigating its effects on yield, the biosynthesis of bioactive compounds, and their subsequent bioaccumulation in the fruit.

MATERIAL AND METHODS

Study area

The investigation took place during the spring-summer cycle of 2021 within a protected shade house structure at the Torreón Technological Institute (ITT) in Torreón, Coahuila, Mexico (26°30′15″N, 103°22′07″W, altitude 1120 m). The shade house is constructed of a 2 mm thick galvanized steel support structure and 1.25" and 1.5" square profiles and insect screen with 25 x 25" polyethylene wire, 720-gauge, UV treatment, diffused light, and 30% shade (crystal color) composition.

Plant material and growing conditions

Melon seedlings Crusier hybrid (Harris Moran®), with six true leaves, were transplanted in black polypropylene troughs measuring 5.0 x 0.30 x 0.30 m in length, width, and height with a capacity of 0.45 m^3. The plants were transplanted into a growing medium consisting of river sand and perlite (v/v, 80:20), which had been pre-sterilized with 5% NaCl. Seedlings are placed in separate rows at a distance of 30 cm from each other (3 seedlings per linear meter). Water and nutrient requirements for the crop were determined based on the nutrient solution outlined by Steiner (1984). The solution was composed of the following elements (me/L): NO_3 12.0; H_2PO_4 1.0; SO_4 7.0; K 7.0; Ca 9.0 and Mg 4.0 along with the following micronutrients (in mg/L): Mn 0.2; Cu 0.06; B 0.05; Zn 0.02; Mo 0.05; and Fe 2.0. Maintaining a pH of 5.5 and an electrical conductivity of 2 dS/m, this nutrient solution was applied at different phenological stages (35, 50, 75, and 100%, corresponding to flowering, fruit set, fruit growth, and ripening stages, respectively) using an automated irrigation system. The plants received three daily waterings via the irrigation system, with each pot receiving 0.85 L from transplanting to the beginning of flowering and 3.5 L from flowering to harvest. This system used 8000-gauge irrigation lines, and emitters placed every 15 cm (T-tape®). A pressure gauge, Soon-Hua model 1434700 (Three-way meter®), was employed to keep the pressure steady at 15 lb. For determining when to apply irrigation, substrate moisture sensors were used. These sensors measure the volumetric content of water in the substrate. It trained the plants to grow as a single stem and supported them with agricultural raffia, which was affixed to the peak of the shade net structure. Bees were used to pollinate flowers, introducing them to the shade home during the flowering stage. Throughout the growing season, the temperature inside the shade house exhibited fluctuations between 27 and 37°C for both minimum and maximum values. Concurrently, the relative humidity fluctuated between 40% and 45% for both minimum and maximum levels.

Experimental design and treatments

The experimental design was completely randomized. The treatments consisted of four concentrations of I, applied foliar: 5, 10, 15, and 20 μM/L, with a control treatment of distilled water. Potassium iodide (KI, 99% purity, Jalmek®) served as the source of I, and commercial surfactant (INEX-A®, 0.02% v/v) was used along with distilled water. The preparation of the various doses involved using a stock solution of I. Subsequently, the four doses of iodine were created in individual one-liter volumetric flasks. Each solution was then divided into distinct concentrations, with each concentration being mixed separately and adjusted with distilled water. The finalized solutions were then transferred into manual sprayers with a capacity of 1000 mL. Each treatment was applied to six plants, representing one experimental unit (EU). Foliar sprays were made every 15 days for seven applications during the crop cycle (80 days). These applications were conducted in the morning, specifically between 8:00 and 10:00, utilizing a manual sprayer.

Evaluated variables

Yield and fruit quality

Fruits were harvested 90 days after transplanting once they reached marketable maturity (characterized by a well-formed net and an easily detachable peduncle from the main branch). For fruit weight (FP), all harvested fruits were weighed on a digital scale (Adir®) with a capacity of 5 kg. Fruit size was quantified by measuring the polar and equatorial diameters using a digital vernier caliper (500-192-30 Mitutoyo). Total soluble solids (TSS) were measured with a manual refractometer (Atago Master 53M).

Bioactive compounds in fruit

Sample processing

Procedure for extract preparation: In the process of identifying bioactive substances, 2 g of melon fruit was amalgamated with 10 mL of 80% ethanol. The mixture was kept in agitation for a day using a "Stuart" shaker. Subsequently, the tubes were subject to centrifugation at 120x g for the same duration. The supernatant, referred to as the ethanolic extract (EE), was separated for subsequent analytical procedures.

Phenolic content

Total phenolic content was quantified by the Folin-Ciocalteu method (Sariñana-Navarrete *et al.*, 2021). Samples were quantified with an ultraviolet (UV)-Vis spectrophotometer at 760 nm (GENESYS 10S UV-Vis), and the outcomes were articulated as mg GAE/100 g fresh weight (FW).

Flavonoid content

The colorimetry method quantified the flavonoid content (Sariñana-Navarrete *et al.*, 2021). The samples were quantified with an ultraviolet (UV)-Vis spectrophotometer at 510 nm (GENESYS 10S UV-Vis), and the outcomes were articulated as mg QE/100 g FW.

Antioxidant capacity

Total antioxidant capacity was measured by the $DPPH^+$ method (Sariñana-Navarrete *et al.*, 2021). The samples were quantified with an ultraviolet (UV)-Vis spectrophotometer at 517 nm (GENESYS 10S UV-Vis), and outcomes are presented in the form of µM equivalent of Trolox/100 g FW.

Vitamin C

Was determined by titration according to the method described in Hernandez-Hernandez *et al.*, 2019). The absorbance of the samples was then measured at 515 nm using a GENESYS 10S UV-Vis spectrophotometer). Results are reported as mg vitamin C/100 g FW.

Iodine content in fruit

Iodine content was determined using the alkaline digestion technique (Medrano-Macias *et al.*, 2021). All reagents were prepared, and all materials were cleaned using deionized water. The iodine concentration was measured using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES). The results were expressed in µg/kg dry weight (DW).

Statistical data analysis

The data underwent evaluation via one-way analysis of variance and comparison of means with Tukey's test ($p \leq 0.05$) using STATISTICA software (version 10.0; StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Yield and fruit quality

The findings indicate that foliar spraying with I has a notable effect on both yield and commercial quality. Fruits from plants treated with the lowest iodine doses exhibited the highest values in terms of yield, weight, size, and total soluble solids (TSS). However, an inverse relationship was observed as the iodine dosage increased, leading to a decline in both yield and commercial quality (Table 1). These results imply that at low concentrations, iodine acts as a biostimulant, effectively boosting both crop productivity and quality (Riyazuddin *et al.*, 2022). This positive effect spans from the regulation of gene expression to integration into diverse proteins (Kiferle *et al.*, 2021), including iodate reductase. Iodate reductase contributes to various cellular functions, inducing beneficial effects in plants (Mynet & Wain, 1973). However, with an escalating dose, the outcome turns negative due to the phytotoxicity of iodine, a consequence of its oxidation within the cells. This leads to the inhibition of CO_2 assimilation, stomatal conductance, and photosynthesis (Kiferle *et al.*, 2021), along with the initiation of chlorosis, necrosis, reduction in size, and abscission of leaves (Mynet & Wain, 1973). In the case of melon fruits, TSS exhibited the highest values at low I dose but decreased with increasing amounts. Past research has reported changes in sugar content resulting from iodine application; low doses increase TSS in *Daucus carota* (Lelek *et al.*, 2021), *Malus domestica* (Budke *et al.*, 2020), and *Cucumis melo* (Melgoza *et al.*, 2022). Conversely, high doses induce adverse effects due to their accumulation and subsequent intercellular oxidation, which decrease photosynthetic activity (Pawel, 2023) and thereby limit the supply of photoassimilates to the fruits (Budke *et al.*, 2020). This study demonstrates that with doses of 5 and 10 µM/L of I, the melon plant effectively conducts its biochemical processes, significantly enhancing the quality of the fruits. However, exceeding this dosage may lead to toxicity symptoms, adversely affecting fruit quality and reducing yield. Therefore the beneficial effects of I fertilization are influenced by factors such as concentration, chemical form, duration of exposure, application method, and plant species (Li *et al.*, 2018).

Table 1. Yield and fruit quality of melon fruits with foliar application of I. México, Instituto Tecnológico de Torreón, 2021.

I (µM/L)	Yield (kg/m²)	Fruit weight (kg)	Diameter (mm)		TSS (ºBrix)
			Equatorial	Polar	
0	3.72± 0.16c*	0.845 ± 0.06b	10.94 ± 0.61a	13.46 ± 1.11b	11.83± 1.40b
5	5.13 ± 0.15a	1.115 ± 0.09a	11.40 ±1.12a	15.75 ±0.68a	13.50 ± 0.54a
10	4.50 ± 0.35b	0.800 ± 0.10b	9.72 ± 8.0a	13.24±7.83b	11.33 ± 0.81b
15	4.39 ±0.03b	0.750 ± 0.05b	9.11 ± 7.2a	13.25±6.98b	11.16±0.75b
20	4.03 ± 0.06b	0.738 ± 0.07b	10.29 ± 7.8a	13.00±7.82b	10.93±.0.75b

I= iodine; TSS= total soluble solids; *Values in each column followed by the same letter(s) are not significantly different at ($p \leq 0.05$) (Tukey HSD). *Data are shown as the means ± standard deviation (SD, n = 6).

Bioactive compounds

A diet abundant in foods containing bioactive compounds is vital for human health, as these compounds play a crucial role in preventing chronic degenerative diseases (Zaremba *et al.*, 2022). This observation underscores the importance of boosting the levels of antioxidant compounds in consumable fruits. This study suggests that biofortification with I significantly enhances the content of bioactive compounds in melon fruits (Table 2). The highest I dose (20 µM/L) markedly increased the levels of bioactive compounds, including total phenols, flavonoids, antioxidant capacity, and vitamin C, surpassing the control by 88.73, 66.68, 76.35, and 87.43%, respectively. This observed enhancement is likely due to the oxidative stress induced by the foliar application of I on the plants. In response to this stress, plants undergo metabolic adaptation, leading to the synthesis of secondary metabolites. This adaptive mechanism improves their survival rate, showcasing an evolutionary process in stress response (Kiferle *et al*., 2021). Comparable findings have been documented in other research endeavors where high doses of I boosted the biosynthesis and accumulation of bioactive compounds (Maglionie *et al*., 2022). Reducing reactive oxygen species production is often perceived as a defense mechanism (Nephali *et al*., 2020), and enhances plant tolerance under stress conditions. Maintaining vital functions such as photosynthesis and transpiration in the leaves is essential for the plant to sustain its health and survival (Mynet & Wain, 1973). Additionally, I impact redox metabolism (Zhang *et al.,* 2023) by acting as a moderate prooxidant, promoting biosynthesis, and accumulating low molecular weight bioactive compounds (Kiferle *et al.*, 2021). These include phenolic compounds, flavonoids, amino acids, carotenoids, glutathione (GSH), and ascorbic acid, which all play critical roles in detoxifying reactive oxygen species (Zhang *et al.,* 2023). The response of the crop to iodine application depends on the concentration used, and its effects range from promoting development and productivity to inducing stress or toxicity (Riyazuddin *et al.,* 2022). In this instance, there is a presumption that a stress condition was induced, given the observed decrease in yield (Table 1) and the concurrent increase in non-enzymatic antioxidants. This increase is likely aimed at preventing oxidative damage to the plant (Kiferle, 2019).

Table 2. Effect of foliar spray on non-enzymatic antioxidants and I concentration in melon fruit. México, Instituto Tecnológico de Torreón, 2021.

I (µM/L)	Total phenolic (mg AGE/100 g FW)	Flavonoids (mg QE/100 g FW)	Capacity AOX (µM equiv. Trolox/100 g FW)	Vitamin C (g/100 g FW)	I (µg/kg DW)
0	161.37 ±10.7e	110.62 ± 24.44e	72.15 ± 11.67e	3.2± 1.47b	1.13±1.1b
5	168.83 ±3.2d	123.80 ±5.52d	77.78 ±6.04d	3.64±0.73ab	2.71±0.55a
10	173.30 ±1.2c	129.54 ±11.26c	84.38 ±0.55c	3.64± 1.3ab	2.59±0.95a
15	176.11 ± 4.0b	145.48 ±10.41b	90.34 ±6.51b	3.46± 0.6ab	2.88±0.5a
20	180.72 ± 8.6a	165.88 ±30.81a	94.49 ±10.65a	3.66± 1.21a	2.89±1.2a

I= iodine; FW= fresh weight; *Different letters indicate differences between treatments. Tukey test ($p \leq 0.05$). *Data are shown as the means ± standard deviation (SD, n = 6).

Iodine content in fruit

Agronomic biofortification is a practical approach to increasing the content of trace elements in plant-based foods and mitigating the health problems associated with their deficiency in human populations. In this study, the accumulation of I in the edible parts of the fruits was dose-dependent (Table 2). The foliar application of I resulted in a notably increased accumulation of this element in the edible parts of the fruit, exceeding the control by 39%. These findings align with previous studies (Budke *et al*., 2020), which reported that foliar spraying with iodine significantly increases its content in the edible parts of the plant. Although the results obtained in this study are based on dry weight, they may still indicate iodine mobility to high-demand sites (US, 2023). Biofortification with iodine through foliar fertilization increases yield, bioactive compounds, and their concentration in the edible part of the melon plant. A low dose can improve the yield and commercial quality of melon fruit. At the same time, higher amounts increased the biosynthesis of bioactive compounds and iodine concentration in the edible part. The incorporation of iodine through foliar application offers an alternative to enhance both the yield and nutritional value of melon fruits. This has the potential to address dietary health concerns among individuals with iodine deficiencies. While these findings could have a significant positive impact on society if the consumption of melons were periodic, it is worth noting that melons are typically consumed during a specific season each year. As a result, it might be more feasible to apply these treatments to horticultural crops that are consumed throughout the majority of the year.

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