https://doi.org/10.17221/45/2023-HORTSCI

Effects of sodium selenate and selenomethionine on the plant growth, fruit quality, and 5-hydroxytryptophan metabolism of 'Qingcui' plums

Xie Ping Sun[1]*, *Chen Yang*[1], *Lin Ling Kou*[2], *Chun Xia Lu*[1]*, *Guo Qiang Han*[1]

[1]*School of Advanced Agriculture and Bioengineering, Yangtze Normal University, Fuling, P. R. China*
[2]*Agricultural Technology Station of Chongqing, Yubei, P. R. China*

**Corresponding authors: 451529639@qq.com; xieping444@163.com*

Citation: Sun X.P., Yang C., Kou L.L., Lu C., Han G.Q. (2024): Effects of sodium selenate and selenomethionine on plant growth, fruit quality, and 5-hydroxytryptophan metabolism of 'Qingcui' plum. Hort. Sci. (Prague), 51: 141–151.

Abstract: Selenium (Se) is a beneficial element for plant growth and development. In this study, three-year-old potted 'Qingcui' plums were treated with Na_2SeO_4 (Se^{6+}) or selenomethionine (SeMet Se^{2-}) to explore the effect of Se on the plant growth, fruit quality, and 5-hydroxytryptophan (5-HTP) metabolism. Flower and fruit numbers, fruit quality and yield, Se content, and 5-HTP metabolites and enzymes were detected. The results showed that the flower and fruit numbers, and yield were significantly increased by the Se application. There were no significant differences in the fruit diameters, fruit mass, edible part ratio, titratable acids, water content, and solid acid ratio among the treatments. The total soluble solids, soluble protein, and malondialdehyde contents under the Se^{6+} treatment showed no significant difference compared to the Se^{2-} treatment, but they were significantly higher than these under control by 16.71%, 39.13%, and 36.27%, respectively. The Se application markedly increased plant the Se content, and Se contents in the roots and leaves, or the fruits were significantly larger by the Se^{6+} treatments than the Se^{2-} treatment. The leaves' pigment contents under the Se^{2-} treatments were significantly larger than those under the control or Se^{6+} treatment. Tryptophan was not significantly influenced, the 5-HTP and 5-methoxytryptophan contents were reduced in the roots, and increased in the leaves, and the serotonin content was only significantly increased in the roots by the Se treatments. The tryptophan hydroxylase and hydroxyindole-O-methyltransferase levels were slightly influenced, and the tryptophan decarboxylase level in the roots or fruits was significantly increased by the Se treatments. The Se application had beneficial effects on the plant growth, fruit quality, and Se content, especially in the Se^{6+} treatment, and influenced the 5-HTP metabolism.

Keywords: number of flowers and fruits; yield; serotonin; selenium

Selenium (Se) is a beneficial element for higher plants, while the intake of fruits and vegetables enriched in Se is beneficial for people to prevent diseases (Schiavon et al. 2020; Mehraban et al. 2023). However, Se deficiency in the diet is a global problem, and researchers have reported that a low dietary Se intake in many countries (even below 0.2 mg/kg) led to a lower content in the human body (Delesalle et al. 2017). For example, 39−61% of Chinese residents had lower daily Se intakes

Supported by Youth Project of Science and Technology Research Program of Chongqing Education Commission of China (Grant No. KJQN202101408).

https://doi.org/10.17221/45/2023-HORTSCI

than the World Health Organization/ Food and Agriculture Organization of the United Nations (WHO/FAO) recommended value (26−34 μg/day) (FAO/WHO 2001; Dinh et al. 2018). The bio-fortification of edible plants with Se is an excellent way to increase the dietary Se intake of humans (Puccineli et al. 2020). The standard Se content of fruits in China is 10–50 μg/kg (fresh mass (f.m.)). Thus, the movement of Se across the soil, crop, and environmental interfaces is crucial for improving the Se status of humans. Se in the agro-ecosystem in both inorganic and organic forms, and the capacity of wild peaches for Se enrichment was found to be more assertive with Se^{6+} (Na_2SeO_4) or Se^{2-} (Selenomethionine (SeMet)) than with Se^0 or Se^{4+} (Sun et al. 2020a). As higher woody plants, fruit trees show higher selectivity for different Se forms than herbaceous crops. At the same time, an appropriate application of Se can increase the fruit yield and quality (Wang et al. 2013; Jing et al. 2017; Puccineli et al. 2020), and ameliorate oxidative damage under abiotic stress (Kaur, Nayyar 2015; Zahedi et al. 2019a).

The plum (*Prunus salicina* L.) is a critical temperate deciduous fruit tree widely planted throughout the world (Rampackova et al. 2021; Halase et al. 2023). The fruit contains high nutritional values in terms of carbohydrates, minerals, and vitamins, having various medicinal values (Usenik et al. 2008; Jiang et al. 2019; Fajardo et al. 2020; Lara et al. 2020). A previous study showed that the plum fruit contains serotonin (Gonzalez-Flores et al. 2011), and a Se application can increase the serotonin content of the 'Qingcui' plum (Sun et al. 2020b). The 'Qingcui' plum, yellow or green in colour, crispy and sweet, and a freestone, was originated in China and is widely planted in southwest China including the Chongqing area (Sun et al. 2023).

The biosynthesis pathway from tryptophan to serotonin includes two possible ways: the first one is the tryptophan synthesis to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TPH), and then the 5-HTP synthesis to serotonin by tryptophan decarboxylase (TDC); the other is that first, the tryptophan synthesis to tryptamine by TDC, and then the tryptamine synthesis to serotonin by tryptophan hydroxylase (T5H). The plum 5-HTP content was significantly increased by Se applications (Sun et al. 2020b), which maybe indicate that Se induces 5-HTP synthesis.

Although 5-HTP is a critical signalling molecule in the nervous system of animals, it was first identified in the medicinal herb cowhage (*Mucuna pruriens*) in 1954 (Bell, Fellows 1996). Similarly, there are large amounts of 5-HTP in the harvested seeds of *Griffonia simplcifolia*, which has attracted commercialisation. This plant has beneficial effects on young subjects with high levels of acute stress (Emanuele et al. 2010; Carnevale et al. 2011), as well various diseases, such as depression, insomnia, chronic headaches, and obesity and binge eating (Xu et al. 2020). More importantly, 5-HTP has overwhelming advantages over synthetic anti-depressants and tryptophan in alleviating the numerous impacts of serotonin deficiency syndrome (Carnevale et al. 2011). In contrast to its effects on humans, 5-HTP inhibited plant growth (Hagin 1989). 5-HTP converts to serotonin by aromatic acid decarboxylase (AADC) in animals (Bajwa et al. 2015) or TDC in plants (Park et al. 2008).

There are few reports on the interactions between Se and 5-HTP. The Se treatment significantly increased the 5-HTP content, but showed no significant effects on the serotonin levels in plums (Sun et al. 2020b). Additionally, hydroxyindole-O-methyltransferase (HIOMT) can convert 5-HTP into 5-methoxytryptophan (5-MTP) (Harumi, Matsushina 2000), which was found to have anticancer activity and protect against vascular injury as well as oxidative injury in cardiomyocytes (Chou, Chan 2014; Wu et al. 2014; Ho et al. 2016). In this study, we measured the content of the intermediates and the enzymes levels in the 5-HTP synthesis pathway to assess the effect of different Se forms on the metabolism of 5-HTP in 'Qingcui' plums.

MATERIAL AND METHODS

Plant material and growth conditions. The experiments were conducted in a greenhouse in Fuling, Chongqing, China (29°45'N, 107°15'E). The climate belongs to the humid monsoon climate of the middle subtropic zone. The annual average temperature is 18.1 °C, the annual average precipitation is 1 072 mm, there is 1 248 hours of sunlight per year, and the altitude is about 230 m. Three-year-old 'Qingcui' plums were planted in growth boxes (39 × 39 × 35 cm) with garden soil (loam, pH 6.3; content of organic matter, 10.0 g/kg; total N, P, and K, 0.84, 0.23, 16.2 g/kg, respectively) on January 7, 2019. The plum trees were maintained under normal management until July 13, 2019. Then 12 trees were selected, where 0.337 mmol Na_2SeO_4 or

https://doi.org/10.17221/45/2023-HORTSCI

SeMet was dissolved in 2 L water before being added into each plant (n = 4) (the soil Se content was about 0.5 mg/L), and the control (n = 4) treatment used 2 L of water only. The plant height was about 200–270 cm, the stem diameters was about 20 mm, and the branch numbers was about 25.

Plant growth. In 2020, the number of flowers was counted on March 10, 15, 20, 25, and 30, and the number of young fruits was counted on April 5, 10, 15, and 20. Finally, the number of mature fruits was courted on July 25.

Selenium content. The edible part of the fruits, mature leaves, and roots were collected on 15 July 2020, and overdyed at 60 °C and stored until further analysis. The Se content was determined as follows: 0.400 g of dry material was added to 5 mL of nitric acid, and soaked overnight. Then, the material was ground at 160 °C for 4 hours. After cooling to room temperature, the acid digests were diluted to 25 mL with 1% nitric acid. The total Se content was measured by inductively coupled plasma mass spectrometry on an X series instrument (ICP-MS, Thermo Fisher Scientific, USA), provided by Sci-Tech innovation Co, Ltd (Qingdao, China).

Leaves' pigment contents. The leaves' pigment contents were determined spectrophotometrically by the method described by Lichtenthaler and Welburn (1983). The fresh round leaf disks from the same area were abtained from the leaves using a perforator, and soaked in 8 mL of 95% ethyl alcohol, and kept in the dark at 4 °C until the leaves fade green. Then, the solution was diluted 4-fold with 95% ethyl alcohol, and the absorbance was measured at 665 nm, 649 nm, and 470 nm using a UV-spectrophotometer (Shimadzu UV-16A, Shimadzu, Corporation Kyoto, Japan) to detect the contents of chlorophyll a, chlorophyll b, chlorophyll $a + b$, and the carotenoids.

Levels of tryptophan hydroxylase (TPH), tryptophan decarboxylase (TDC), and hydroxyindole-O-methyltransferase (HIOMT), and 5-methoxytryptophan. The roots, leaves, and fruit enzyme levels of the TPH, TDC, and HIOMT, and 5-methoxytryptophan contents were measured by double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kits customised by Sci-Tech innovation Co., Ltd (Qingdao, China), according to manufacturer's instructions (Jiangsu Meimian Industrial Co. Ltd. (Jiangsu, China) (Mohamed et al. 2020). The detection range of TPH, TDC, HIOMT, and 5-methoxytryptophan were 6–260 U/L, 16–650 U/L , 2.5–85 U/L , and 5–180 ng/L, respectively. The TPH, TDC, and HIOMT enzyme levels was described as U/g and the content of 5-methoxytryptophan was expressed as ng/g.

Tryptophan, 5-hydroxy tryptophan (5-HTP), and serotonin content. Frist, the sample was crushed and mixed, and then 1.00 g was accurately weighted before added 5mL of 0.4 mol/L perchloric acid to perchloric acid. This was followed by mixing, then ultrasonic extraction for 30 minutes, and centrifugation at 6 000 g for 5 minutes. The first supernatant was removed and the sample was extracted again. Then, the two supernatants were mixed and filtered through a 0.22 μm pore-size membrane before the High-performance liquid chromatography (HPLC) analysis. The compounds were separated on a Syncromis^{TM} C18 column (250 mm × 4.6 mm, 5 μm) with isocratic elution at a flow rate of 1.0 mL/min, the column was 35 °C, and the sample load was 10 μL. For tryptophan, the mobile phase was water: methanol (80 : 20, v/v), while for 5-hydroxy tryptophan and serotonin, it was 0.05 mol/L KH_2PO_4 (pH 2.68): methanol (90 : 10, v/v). The elution of compounds was monitored at 280 nm.

Fruit diameters, edible part ratio, and yield. The transverse and longitudinal diameters were measured using Vernier callipers (0.01 cm), the fruit and kernel mass was measured using a balance (0.01 g), and the edible part ratio was calculated as $(W_{fruit} - W_{kernel})/W_{fruit} \times 100\%$. The yield was calculated as the total fruit harvested mass per plant.

Total soluble solids and titratable acid contents. The total soluble solids were measured using a digital refractometer (Pocket Refractometer Pal^{TM}, Atago, Japan). After calibrating to zero with distilled water, and then use gauze clean the prism surface. After 0.3 mL fruit juice was added on the prism, the brix (%) was measured by 3 seconds. The titratable acids were titrated with 0.1 mol/L NaOH, using phenolphthalein as a pH indicator. The endpoint was determined by titration until the reddish colour does not dissipate over 30 s. The titratable acid content was calculated from the consumed NaOH, and the conversion factor was 0.067 (malic acid). The solid acid ratio was calculated as the soluble solids divided by the titratable acids.

Soluble protein content. The soluble protein content was detected using Coomassie brilliant blue G-250, form 5 g fruit was ground, and centrifuged, after which 0.3 mL of the supernatant was added to 1.5 mL of Coomassie brilliant blue

https://doi.org/10.17221/45/2023-HORTSCI

G-250 and mixed for 2 minutes before being measured at 595 nm (Zahedi et al. 2019b). The soluble protein content was calculated using a bovine serum albumin standard curve.

Malondialdehyde (MDA) content. Fresh leaves were cut with a hole puncher (diameter 1.5 cm), then, 6 round blades were selected and ground with quartz sand in 4 mL of 10% trichloroacetic acid. The resulting homogenate was centrifuged at 4 000 ×*g* for 10 min. Then, 1 mL of the resulting supernatant was combined with 1 mL of 0.6% thiobarbituric acid, mixed and boiled in a water bath for 10 minutes. After rapid cooling in water and centrifugation at 4 000 ×*g* for 10 minutes, the absorbance (A) was measured at 450 nm, 532 nm, and 600 nm. The malondialdehyde (MDA) content content was calculated as follows: MDA (mol/L) = $(6.45 \times A_{532} - 0.56 \times A_{450}) \times 10^{-6}$ (Bai et al. 2010).

Statistical analysis. Origin 8.5 (OriginLab Corporation, USA) was used to evaluate the variation in the parametric data among the treatments. The differences between the means were tested for significance with Fisher's LSD ($P < 0.05$) level using an analysis of variance (ANOVA).

RESULTS

The total number of flowers of plums treated with the two forms of Se application on March 15–25th was significantly higher than in the control treatment. The effect of the Se^{2-} treatment was slightly better than the Se^{6+} treatment (Figure 1A). The number of fruits per plant was also significantly increased by the two forms of Se, and the treatment with Se^{6+} had a slightly better effect than the treatment with Se^{2-} (Figure 1B). The number of mature fruits per plant under the Se^{6+} treatment was the highest, followed by Se^{2-}, and the plums treated with either form of Se had significantly higher fruit numbers than the control (Figure 1C).

Compared to the control, the two forms of Se application showed no significant difference in the fruit transverse or longitudinal diameter, fruit mass, edible part ratio, and water content (Table 1). The yield per plant was significantly increased by the Se^{6+} treatment, followed by the Se^{2-} treatment, and the yield of the control was significantly lower than that of the two forms of Se.

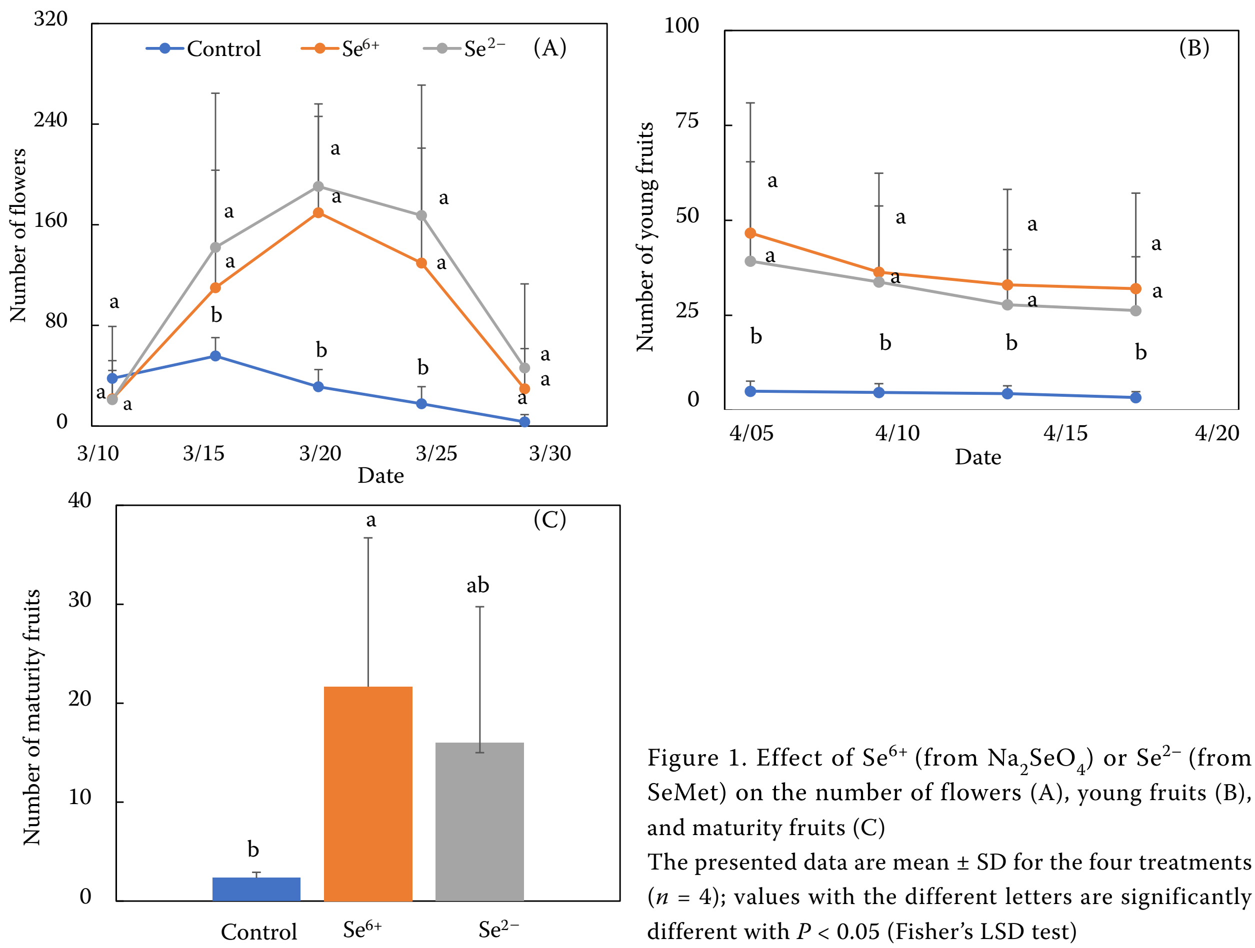

Figure 1. Effect of Se^{6+} (from Na_2SeO_4) or Se^{2-} (from SeMet) on the number of flowers (A), young fruits (B), and maturity fruits (C)

The presented data are mean ± SD for the four treatments (n = 4); values with the different letters are significantly different with $P < 0.05$ (Fisher's LSD test)

https://doi.org/10.17221/45/2023-HORTSCI

Table 1. Effect of Se^{6+} (from Na2SeO4) or Se^{2-} (from SeMet) on the fruit transverse or longitudinal diameter, fruit mass, edible part ratio, water content, and yield

Treatment	Transverse diameter (mm)	Longitudinal diameter (mm)	Fruit mass (g)	Edible part ratio (%)	Water content (%)	Yield (g/plant)
Control	24.00 ± 1.86^{a}	22.16 ± 1.5^{a}	6.62 ± 1.42^{a}	95.30 ± 0.77^{a}	86.61 ± 1.26^{a}	15.44 ± 3.82^{b}
Se^{6+}	23.49 ± 1.30^{a}	22.17 ± 1.30^{a}	6.37 ± 1.01^{a}	94.82 ± 1.17^{a}	87.62 ± 1.79^{a}	137.92 ± 95.76^{a}
Se^{2-}	25.15 ± 1.32^{a}	23.18 ± 1.29^{a}	7.52 ± 0.94^{a}	96.0 ± 0.005^{a}	85.29 ± 1.15^{a}	103.45 ± 12.85^{a}

^{a,b}Values in the columns marked with a different lowercase letter differ significantly at $P < 0.05$ (Fisher's LSD significant test).

Sweet and acid makes a major contribution to the flavour value of plum fruit. The treatment with the two forms of Se significantly increased the total soluble solids content of the fruits, but there was no significant difference between the Se^{2-} and Se^{6+} treatments (Table 2). The range of titratable acid and the solid acid ratio was also increased by the treatments with the two forms of Se, but the difference was insignificant. The soluble protein content was significantly increased in the Se^{6+} treatment, and the Se^{2-} treatment also increased the level, but the difference was insignificant. The fruit MDA content was induced by the Se treatment, and the content was significantly increased with the Se^{6+} treatment.

In the treated plants, the roots' Se content was the highest, followed by the leaves, and the fruits' Se content was the lowest (Table 3). The Se content of each organ of the plums treated with Se^{6+} was significantly higher than in the Se^{2-} treatment. At the same time, the Se content of the Se^{2-} treated plants was significantly higher than that of the control plants.

The pigment content of the leaves from the plants treated with Se^{2-} was higher than in the Se^{6+} and control treatments, and the difference was statistically significant. The Se^{6+} treatment did not differ from the control treatment in chlorophyll *a*, chlorophyll *b*, chlorophyll *a* + *b*, and carotenoids (Table 4).

The TPH level showed similar trends in the roots and leaves, and there was no significant change under the treatments with the two forms of Se (Figure 2). However, the TPH level in the fruits was significantly higher under the Se^{6+} treatment than the control. The Se^{2-} treatment had no significant impact. The TDC level was significantly increased in the roots, reaching the highest level in the Se^{2-} group, followed by Se^{6+} and the control. In leaves, the Se^{6+} treatment increased the TDC level, while Se^{2-} reduced the level. In the fruits, the TDC level was significantly decreased by the treatments with the two forms of Se. There was no significant difference in the HIOMT levels in the roots and fruits, but the level was reduced in the leaves following the Se^{6+} treatment.

Table 2. Effect of Se^{6+} (from Na_2SeO_4) or Se^{2-} (from SeMet) on the fruit total soluble solids, titratable acids, solid acid ratio, soluble protein content, and malondialdehyde (MDA) content

Treatment	Total soluble solids (%)	Titratable acids (%)	Solid acid ratio	Soluble protein content (%)	MDA content [μmol/m^2 (f.m.)]
Control	11.01 ± 0.26^{b}	0.92 ± 0.15^{a}	12.15 ± 1.36^{a}	0.23 ± 0.024^{b}	52.63 ± 10.81^{b}
Se^{6+}	12.85 ± 0.40^{a}	0.98 ± 0.12^{a}	12.71 ± 1.48^{a}	0.32 ± 0.041^{a}	71.72 ± 6.84^{a}
Se^{2-}	13.41 ± 0.49^{a}	0.97 ± 0.03^{a}	13.80 ± 0.45^{a}	0.27 ± 0.030^{ab}	61.02 ± 7.13^{ab}

^{a,b}Marked with a different lowercase letter differ significantly at $P < 0.05$ (Fisher's LSD significant test); f.m. – fresh mass

Table 3. Effect of Se^{6+} (from Na_2SeO_4) or Se^{2-} (from SeMet) o'on the selenium (Se) content of the roots,leaves, and fruits

Treatment	Roots Se content [mg/kg (d.m.)]	Leaves Se content [mg/kg (d.m.)]	Fruits Se content [mg/kg (d.m.)]
Control	0.72 ± 0.25$^{c(a)}$	0.14 ± 0.03$^{c(b)}$	0.00 ± 0.00$^{c(c)}$
Se^{6+}	2.52 ± 0.17$^{a(a)}$	1.92 ± 0.46$^{a(b)}$	0.30 ± 0.07$^{a(c)}$
Se^{2-}	1.56 ± 0.20$^{b(a)}$	0.29 ± 0.05$^{b(b)}$	0.05 ± 0.02b$^{(c)}$

The lower-case letters represent column comparisons, and the lower-case letters in brackets represent horizontal comparisons that differ significantly at $P < 0.05$ (Fisher's LSD significant test); d.m. – dry mass

https://doi.org/10.17221/45/2023-HORTSCI

Table 4. Effect of Se^{6+} (from Na_2SeO_4) or Se^{2-} (from SeMet) on the plum leaf contents of chlorophyll *a*, chlorophyll *b*, chlorophyll *a* + *b*, and carotenoids

Treatment	Chlorophyll *a* [g/m^2 (f.m.)]	Chlorophyll *b* [g/m^2 (f.m.)]	Chlorophyll *a* + *b* [g/m^2 (f.m.)]	Carotenoids [g/m^2 (f.m.)]
Control	0.74 ± 0.11^b	0.34 ± 0.07^b	1.11 ± 0.19^b	0.19 ± 0.01^b
Se^{6+}	0.83 ± 0.03^b	0.23 ± 0.02^b	1.12 ± 0.05^b	0.21 ± 0.02^b
Se^{2-}	1.04 ± 0.07^a	0.40 ± 0.04^a	1.44 ± 0.11^a	0.22 ± 0.02^a

^{a,b}Values in the columns marked with a different lowercase letter differ significantly at $P < 0.05$ (Fisher's LSD significant test). f.m. – fresh mass

The content of tryptophan in the leaves was the highest, followed by the roots, but was not detected in the fruit (Figure 3A). There was no significant difference between the two forms of Se treatments and the control. The content of 5-HTP was slightly reduced by the two forms of Se in the roots, while it was significantly increased in the leaves under the Se^{6+} treatment (Figure 3B). The treatment with Se^{2-} also increased the content, but the difference was not significant. No tryptophan or 5-HTP was detectable in the fruits, indicating a content of less than 1.299 μg/g f.m. and 2.593 μg/g f.m., respectively. The range of 5-methoxytryptophan in the roots was reduced by the Se treatment, especially in the Se^{6+} group, and the difference was significant. Conversely, the 5-methoxytryptophan content in the leaves was significantly increased by the two treatments, with the Se^{6+} increasing it the most, followed by Se^{2-} (Figure 3C). At the same time, the fruit content of 5-methoxytryptophan was also significantly increased in the groups treated with the two forms of Se compared to the control. The Se treatment also significantly increased the roots' serotonin content (Figure 3D). The content was also increased in the leaves, but the difference was not significant. The serotonin content of the fruits was similar between the groups.

DISCUSSION

Se is a beneficial elemental for higher plants, and has been reported to increase the fruit production, fruit quality, antioxidant capacity, and other valuable traits (Chu et al. 2013; Wang et al. 2013; Kaur, Nayyar 2015; Jing et al. 2017; Zahedi et al. 2019b; Puccineli et al. 2020). However, the effects of Se on the plant flowering and fruiting have been less

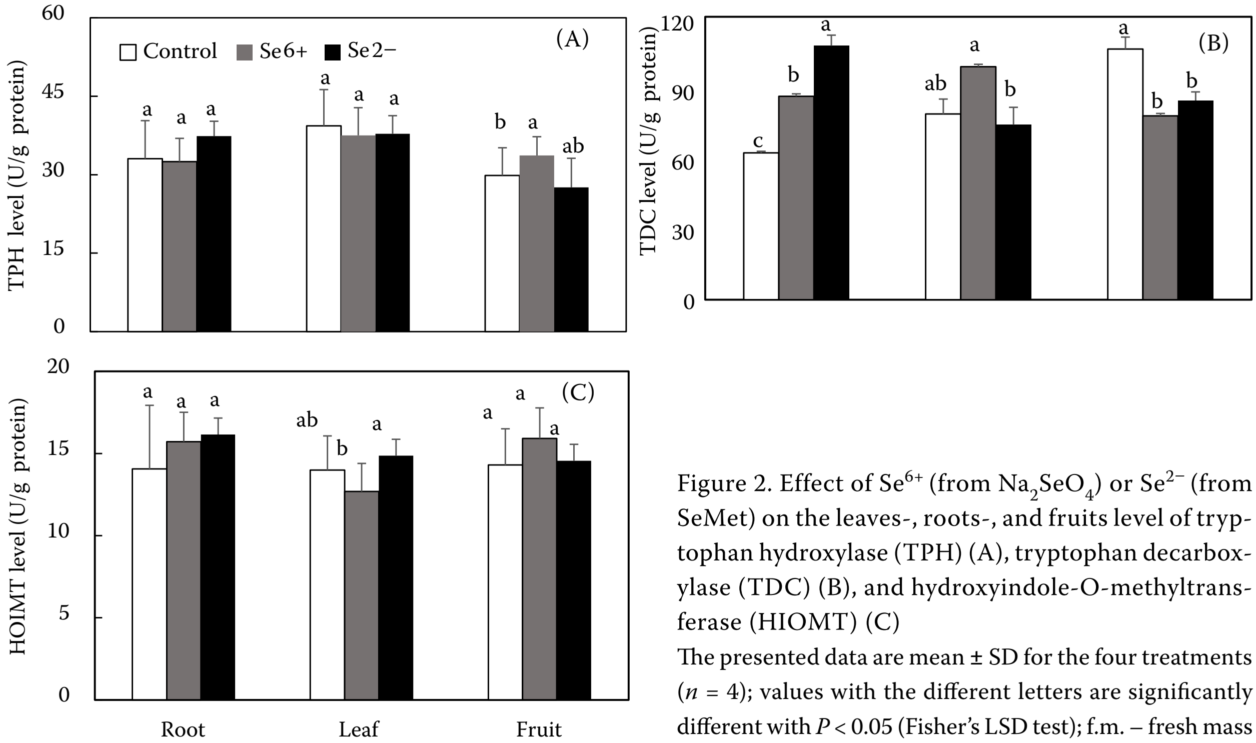

Figure 2. Effect of Se^{6+} (from Na_2SeO_4) or Se^{2-} (from SeMet) on the leaves-, roots-, and fruits level of tryptophan hydroxylase (TPH) (A), tryptophan decarboxylase (TDC) (B), and hydroxyindole-O-methyltransferase (HIOMT) (C)

The presented data are mean ± SD for the four treatments (n = 4); values with the different letters are significantly different with $P < 0.05$ (Fisher's LSD test); f.m. – fresh mass

https://doi.org/10.17221/45/2023-HORTSCI

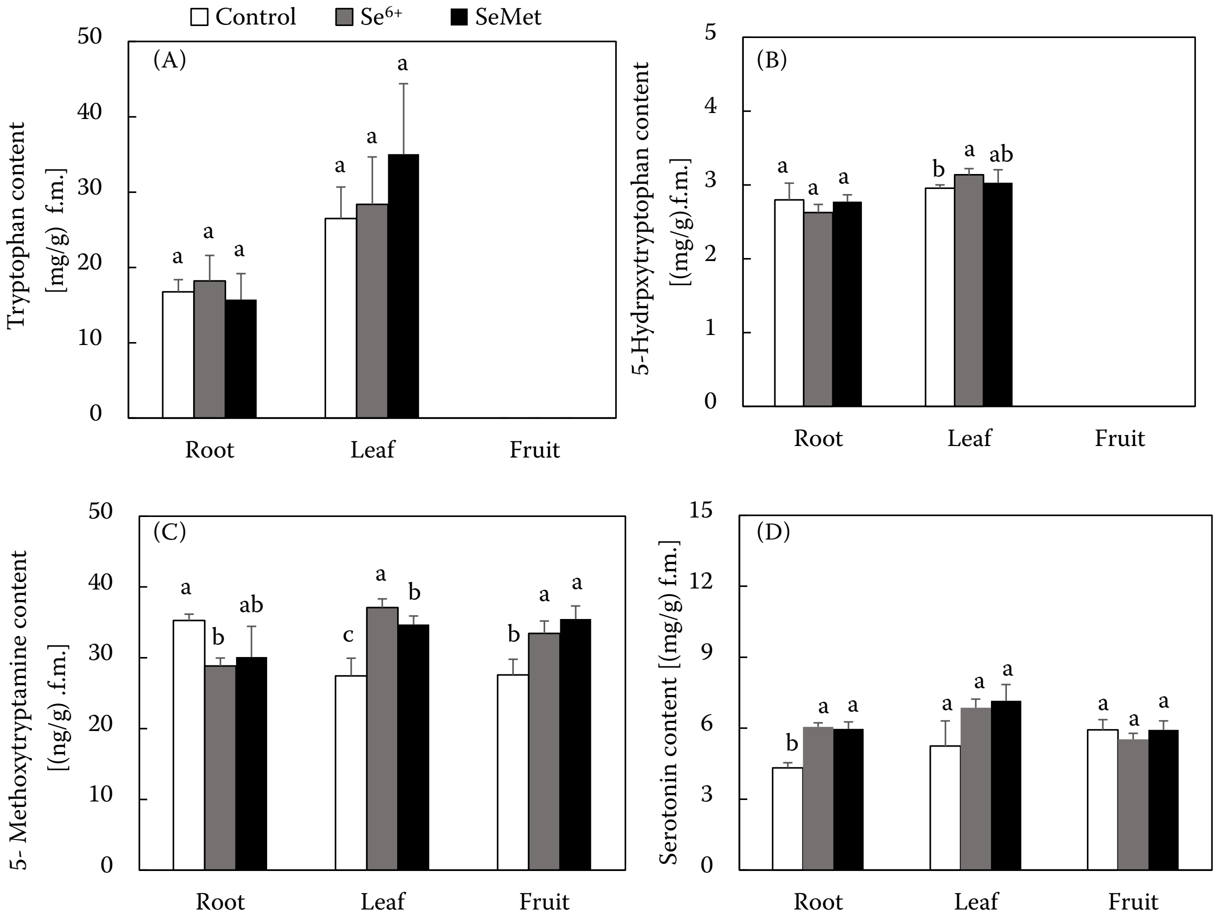

Figure 3. Effect of Se^{6+} (from Na_2SeO_4) or Se^{2-} (from SeMet) on the leaves-, roots-, and fruits content of tryptophan (A), 5-hydroxytryptophan (B), 5-methoxytryptophan (C), and serotonin (D)

The presented data are mean ± SD for the four treatments (n = 4); valuaes with the different letters are significantly different with $P < 0.05$ (Fisher's LSD test). f.m. — fresh mass

studied. Our previous studies showed that Se^{6+} and Se^{2-} had a beneficial impact on plum growth via the plant height and stem diameter with the same material (Sun et al. 2020a). In the following year, plants with a Se application promoted the plum flower bud initiation, increasing the numbers of flowers and fruits, and the yield per plant (Figure 1, Table 1). The two forms of Se had no significant influence on the fruits' appearance quality and some intrinsic qualities (Tables 1 and 2). A Se application at different doses increased the yield per plant in tomatoes (Gaucin-Delgado et al. 2020), and a Se application increased the number of fruits per plant and yield of okra (Ali et al. 2020). While little study has been undertaken on woody fruit trees, our research showed that applying Se via the roots had beneficial effects on the flowering and fruiting of plum trees by the higher numbers of flowers and fruits, but the mechanism is still unclear.

The intrinsic quality of the fruits, in terms of the total soluble solids, titratable acids, solid acid ratio, and soluble protein was increased by the two forms of the Se application. Similar results were also reported in tomatoes (Gaucin-Delgado et al. 2020), pomegranates (Zahedi et al. 2019b), grapes (Zhu et al. 2017), strawberries (Zahedi et al. 2019a), peaches and pears (Pezzarossa et al. 2012). With the intrinsic fruit quality increased, the MDA content was also increased by the Se treatment, which was a direct indicator of the cell membrane integrity. Although, the MDA content kept low levels to damage the cells, the Se^{6+} application showed higher MDA contents compared to the Se^{2-} application and significantly higher than the control. Se can induce oxidative stress to cause toxicity in plants (Hoewyk 2013). Ferreira et al. (2020) reported that an increased Se^{6+} concentration raised the MDA content and reached significant levels of more than

https://doi.org/10.17221/45/2023-HORTSCI

5 μM. Moreover, the plants under 5 μm Se^{2-} (SeMet) significantly increased the MDA content (Malheiros et al. 2020). Organic and inorganic Se treatments had different reported influences on the antioxidant levels on broccoli florets (Gui et al. 2022). Our study also indicated that the two forms of Se applications changed the antioxidant level, but the different distinct types of stress could not be made clear.

Plum fruits that are bio-fortified with Se can be considered a functional food that promotes optimal human health and helps reduce the risk of various diseases. The daily dietary intake of Se supplied by fruits and vegetables is estimated to be 1.21 μg per person per day (Diaz-Alarcon et al. 1994). Bratakos et al. (2010) reported that the total daily dietary intake of Se amounts was an average of 87 μg. In recent recommendations, the daily dose of Se was set at 55–220 μg, and the upper limit of Se intake should be limited to 400 μg for adults (USDA-ARS 2012; Vinceti et al. 2018). Similarly, the Chinese Nutrition Society recommends that the minimum daily intake of Se for adults reach at least 50 μg. Se-enriched fruit bio-fortified through soil fertilisation has rarely been studied compared to foliar fertilisation, especially in woody fruit trees. Our study showed that supplying plum roots with 0.337 mmol Se^{6+} for one year quickly achieved the suggested standards in the edible parts (0.01—0.05 mg/kg f.m. in China), but supplying 0.337 mmol Se^{2-} for one year did not achieve the suggested standard. In this respect, soil fertilisation with Se^{6+} was better than Se^{2-} for selenium fortification in plums.

Usually, fruits and vegetables show lower levels of Se enrichment (Bratakos et al. 2012; Garousi 2017), which makes a speciation analysis difficult or even impossible. However, plants applied with Se fertilisers can reach the appropriate concentrations for the analysis (Thiry et al. 2012). Moreover, the biological Se enrichment in crops can have a positive impact on human nutrition and health. In our experiments, the fruits of plums treated with either form of Se were more nutritious than the control treatment, whereby Se^{6+} was better than the Se^{2-} treatment. The water content in the edible part of the plum fruit was approximately 85—87% (Table 3). The treatments with the two forms of Se significantly increased the Se content in the edible part of the fruit. The upper limit of the daily dietary intake of Se is equivalent to 16.29 kg of fortified plums, thus making it impossible to reach a Se overdose.

The Se content of the underground plant parts and leaves was significantly higher than in the fruits, which is consistent with the results of Salkić et al. (2019). Leaves, which are the critical organ of photosynthesis, are richer in minerals than buds and fruits. A selenium application can improve the photosynthesis and protect the photosystem II in fruit tree leaves (Feng et al. 2015). In agreement with these findings, both forms of Se increased the leaves' pigment contents in our study, particularly under the Se^{2-} treatment. Compared to the leaves, the roots' Se content was higher. Se fertiliser recovery (SeFR) is low, and most Se taken up accumulates in the non-harvested plant parts (Ebrahimi et al. 2019). Our study also showed the same trend, and the SeFR of Se^{6+} appeared to be higher than Se^{2-}. Moreover, also similar to Wu et al. (2020), where they reported on a novel Se hyperaccumulation plant (*Cardamine violifolia*), the Se accumulation efficiency was $Se^{6+} > Se^{2-}$.

5-HTP has been reported in many plants (Bell and Fellows 1966; Emanuele et al. 2010; Sun et al. 2020b), and is used to prevent and treat some diseases (Meloni et al. 2020; Xu et al. 2020). For example, broilers' dietary supplementation with 5-HTP can reduce the accumulation of abdominal fat and be beneficial to the intestinal immune function (Wang et al. 2020). In the Se-treated plums, the levels of the 5-HTP synthesis and metabolism were influenced by both forms of Se, but the effects were different in the roots, leaves, and fruits in our study.

Tryptophan, a precursor for serotonin (Murch et al. 2000) and 5-HTP biosynthesis, was not significantly influenced by the Se application in the roots and leaves in our study, similar to that reported by Li et al. (2016). Moreover, Se had no significant influence on the 5-HTP content in the roots, but significantly increased the serotonin content, while reducing the 5-methoxytryptophan content. In tomatoes, the leaves' serotonin content was induced considerably by 3 μmol/L of different Se forms (Na_2SeO_4, Na_2SeO_3, Se-Cys) of treatments by application to the roots for three days (Li et al. 2016). By contrast, the 5-HTP range of the leaves was increased by the Se treatment, the 5-methoxytryptophan content was considerably increased, and there was no significant change in the serotonin content. Our previous study also showed that the two forms of Se did not significantly influence the serotonin content, and induced the 5-HTP content in the leaves (Sun et al. 2020b). Tryptophan, tryptamine, and 5-HTP are

https://doi.org/10.17221/45/2023-HORTSCI

precursors of serotonin, and the TDC and TPH enzymes are involved in the serotonin synthesis (Murch et al. 2000; Park et al. 2008; Sun et al. 2020b). TPH is converted into 5-methoxytryptophan by HIOMT (Harumi and Matsushima 2000). There was a different trend in the relationship between the serotonin and 5-methoxytryptophan in the leaves and roots. The contents of tryptophan and 5-HTP in the fruits were below the detection limit, which precluded any analysis of the influence of Se on these metabolites. However, the 5-methoxytryptophan content was increased, and the serotonin content showed no significant influence on the fruits while the TPH increased, the TDC decreased, and the HIOMT did not significantly change by the treatments with the two forms of Se. These results may indicate that the 5-HTP synthesis and metabolism were similar in the fruits and leaves, and different from that in the roots. Furthermore, the Se fertilisation does not appear to be an effective way to obtain a higher serotonin content in 'Qingcui' plum fruits, but can be obtained with a higher 5-methoxytryptophan content.

CONCLUSION

The present study showed that root fertilisation with Se^{6+} and Se^{2-} was beneficial for the plum fruit yield and quality. The Se contents in the roots, leaves, and fruits under the Se^{6+} treatment were significantly higher than those under the Se^{2-} treatment. The leaves' pigment contents under the Se^{2-} treatment were significantly higher than those under the Se^{6+} treatment. However, either form of Se application influenced the 5-HTP synthesis in similar ways, with the underground organs mainly upregulating the tryptophan–›5-HTP–›serotonin pathway, and the aboveground organs mainly upregulating the tryptophan–›5-HTP–›5-methoxytryptophan pathway.

REFERENCES

Ali J., Jan I.U., Ullah H. (2020): Selenium supplementation affects vegetative and yield attributes to escalate drought tolerance in okra. Sarhad Journal of Agriculture, 36: 120–129.

Bajwa V., Murch S.J., Saxena P.K. (2015): Melatonin rich plants: production, significance in agriculture and human health. In: Paek K.Y., Murthy H., Zhong J.J. (eds): Production of Biomass and Bioactive Compounds Using Bioreactor Technology. Springer, Dordrecht.

Bai T., Li C., Ma F., Feng F., Shu H. (2010): Responses of growth and antioxidant system to root-zone hypoxia stress in two *Malus* species. Plant and Soil, 327: 95–105.

Bell E.A., Fellows L.E. (1966): Occurrence of 5-hydroxy-L-tryptophan as a free plant amino-acid. Nature, 210: 529.

Bratakos M.S., Zafiropoulos T.F., Siskos P.A., Ioannou P.V. (2010): Selenium in foods produced and consumed in greece. Journal of Food Science, 52: 817–822.

Carnevale G., Viesti V.D., Zavatti M., Zanoli P. (2011): Anxiolytic-like effect of *Griffonia Simplicifolia* Baill. seed extract in rats. Phytomedicine, 18: 848–851.

Chou H.C., Chan H.L. (2014): 5-Methoxytryptophan-dependent protection of cardiomyocytes from heart ischemia reperfusion injury. Archives of Biochemistry and Biophysics, 543: 15–22.

Chu J., Yao X., Yue Z, Li J., Zhao J. (2013): The effects of selenium on physiological traits, grain selenium content and yield of winter wheat at different development stages. Biological Trace Element Research, 151: 434–440.

Delesalle C., Bruijn M., Wilmink S., Vandendriessche H., Mol G., Boshuize B., Grinwis G. (2017): White muscle disease in foals: focus on selenium soil content. A case series. BMC Veterinary Research, 13: 121.

Diaz-Alarcon J.P., Navarro-Alarcon M., Herminia L.G.D.L.S., Lopez-Martinez M.C. (1994): Determination of selenium levels in vegetables and fruits by hydride generation atomic absorption spectrometry. Journal of Agricultural and Food Chemistry, 42: 2848–2851.

Dinh Q.T., Cui Z., Huang J., Tran T.A., Wang D., Yang W., Zhou F., Wang M., Yu D., Liang D. (2018): Selenium distribution in the Chinese environment and its relationship with human health: a review. Environment International, 112: 294–309.

Ebrahimi N., Hartikainen H., Hajiboland R., Seppanen M.M. (2019): Uptake and remobilization of selenium in *Brassica napus* L. plants supplied with selenate or selenium-enriched plant residues. Journal of Plant Nutrition and Soil Science, 182: 196–202.

Emanuele E., Bertona M., Minoretti P., Geroldi D. (2010): An open-label trial of L-5–hydroxytryptophan in subjects with romantic stress. Neuroendocrinology Letters, 31: 663–666.

Fajardo J., Gaffen D., Eisner A., Kern M., Hooshmand S. (2020): Effects of dried plum (prunes) on bone density and strength in men. Current Developments in Nutrition, 4: 21–21.

FAO/WHO (2001): Selenium. In: Human Vitamin and Mineral Requirements, Rome, 235–256.

Feng T., Chen S.S., Gao D.Q., Liu G.Q., Bai H.X., Li A., Peng L.X., Ren Z.Y. (2015): Selenium improves photosynthesis

https://doi.org/10.17221/45/2023-HORTSCI

and protects photosystem II in pear (*Pyrus bretschneideri*), grape (*Vitis vinifera*), and peach (*Prunus persica*). Photosynthetica, 53: 609–612.

Ferreira R.L.C., Prado R.M.P., Junior J.P.S., Gratao P.L., Tezotto T., Cruz F.J.R. (2020): Oxidative stress, nutritional disorders, and gas exchange in lettuce plants subjected to two selenium sources. Journal of Plant Nutrition and Soil Science, 20: 1215–1228.

Garousi F. (2017): Selenium in soil-plant-food systems. Acta Universitatis Sapientiae Alimentaria, 10: 91–106.

Gaucin-Delgado J.M., Hernández-Montiel L.G., Sánchez-Chavez E., Ortega-Ortiz H., Fortis-Hernández M., Reyes-Pérez J.J., Preciado-Rangel P. (2020): Agronomic biofortification with selenium improves the yield and nutraceutical quality in tomato under soilless conditions. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48: 1–12.

Gonzalez-Flores D., Velardo B., Garrido M., Gonzalez-Gomez D., Lozano M., Ayuso M.C., Barriga C., Paredes S.D., Rodriguez A.B. (2011): Ingestion of Japanese plums (*Prunus salicina* Lindl. cv. Crimson Globe) increases the urinary 6-sulfatoxymelatonin and total antioxidant capacity levels in young, middle-aged and elderly humans: nutritional and functional characterization of their content. Journal of Food and Nutrition Research, 50: 229–236.

Gui J.Y., Rao S., Gou Y., Xu F., Chen S.Y. (2022): Comparative study of the effects of selenium yeast and sodium selenite on selenium content and nutrient quality in broccoli florets (*Brassica oleracea* L. var. Italica). Journal of the Science of Food and Agriculture, 102: 1707–1718.

Hagin R.D. (1989): Isolation and identification of 5-hydroxyindole-3-acetic acid and 5-hydroxytryptophan, major allelopathic aglycons in quackgrass (*Agropyron repens* L. Beauv.). Journal of Agricultural and Food Chemistry, 37: 1143–1149.

Halasz J., Szendy G., Ivanovska B. (2023): The self-inconceivability locus and chloroplast DNA regions of *Prunus domestica* reflect the origin and genetic diversity of traditional cultivar. Journal of the American Society for Horticultural Science, 148: 230–239.

Harumi T., Matsushima S. (2000): Separation and assay methods for melatonin and its precursors. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 747: 95–110.

Ho Y.C., Wu M.L., Su C.H., Chen C.H., Ho H.H., Lee G. L., Lin W.Y., Hsu Y.J., Kou C.C., Wu K. K., Yet S.F. (2016): A novel protective function of 5-methoxytryptophan in vascular injury. Scientific Reports, 6: 25374.

Hoewyk D.V. (2013): A tale of two toxicities: malformed selenoproteins and oxidative stress both contribute to selenium stress in plants. Annals of Botany, 112: 965–972.

Jiang C., Fang Z., Zhou D., Pan S., Ye X. (2019): Changes in secondary metabolites, organic acids and soluble sugars during the development of plum fruit cv. 'Furongli' (*Prunus salicina* Lindl). Journal of Agriculture and Food Sciences, 99: 1010–1019.

Jing D.W., Du Z.Y., Ma H.L., Ma B.Y., Liu F.C., Song Y.G., Xu Y.F., Li L. (2017): Selenium enrichment, fruit quality and yield of winter jujube as affected by addition of sodium selenite. Scientia Horticulturae, 225: 1–5.

Kaur S., Nayyar H. (2015): Selenium fertilization to salt-stressed mungbean (*Vigna radiata* L. wilczek) plants reduces sodium uptake, improves reproductive function, pod set and seed yield. Scientia Horticulturae, 197: 304–317.

Lara M., Bonghi C., Famiani F., Vizzotto G., Drincovich M.F. (2020): Stone fruit as biofactories of phytochemicals with potential roles in human nutrition and health. Frontiers in Plant Science, 11: 562252.

Li M.Q., Hasan M.K., Li C.X., Ahammed G.J., Xia X.J., Shi K., Zhou Y.H., Reiter R.J., Yu J.Q., Xu M.X. (2016): Melatonin mediates selenium induced tolerance to cadmium stress in tomato plants. Journal of Pineal Research, 61: 291–302.

Lichtenthaler H.K., Wellburn A.R. (1983): Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Analysis, 11: 591–592.

Malheiros R.S.P., Goncalves F.C.M., Brito F.A.L., Zsogon A., Riberio D.M. (2020): Selenomethionine induces oxidative stress and modifies growth in rice (*Oryza sativa* L.) seedlings through effects on hormone biosynthesis and primary metabolism. Ecotoxicology and Environmental Safety, 189: 109942.

Mehrabani L.V., Hassanpouraghdam M.B., Rasouli F., Okcu Z., Marc R.A. (2023): Foliar application of graphene oxide, nano-Fe, and selenium mitigates salinity depression on *Ocimum basilicum*. Turkish Journal of Agriculture and Forestry, 47: 510–528.

Meloni M., Puligheddu M., Sanna F., Cannas A., Carta M. (2020): Efficacy and safety of 5-hydroxytryptophan on levodopa-induced motor complications in parkinson's disease: A preliminary finding. Journal of the Neurological Sciences, 415: 116869.

Mohamed I.A.A., Shalby N., Bai C., Qin M., Agami R.A., Jie K., Wang B., Zhou G. (2020): Stomatal and photosynthetic traints are associated with investigationg soldium chloride tolerance of *Brassica napus* L. cultivars. Plants-BASEL 9: 62

Murch S. J., KrishnaRaj S., Saxena P. (2000): Tryptophan is a precursor for melatonin and serotonin biosynthesis in vitro regenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants. Plant Cell Reports. 19: 698–704.

Park M., Kang K., Park S., Back K. (2008): Conversion of 5-hydroxytryptophan into serotonin by tryptophan decarboxylase in plants, *Escherichia coli*, and yeast. Bioscience, Biotechnology, and Biochemistry 72: 2456–2458.

Pezzarossa B., Remorini D., Gentile M. L., Massai R. (2012): Effects of foliar and fruit addition of sodium selenate on se-

https://doi.org/10.17221/45/2023-HORTSCI

lenium accumulation and fruit quality. Journal of the Science of Food and Agriculture, 92: 781–786.

Puccineli M., Pezzarossa B., Rosellini I., Malorgio F. (2020): Selenium enrichment enhances the quality and shelf life of basil leaves. Plants (Basel), 9: 801.

Rampackova E., Gottingerova M., Kiss T., Ondrasek I., Venuta R., Wolf J., Necas T., Ercisli S. (2021): CIELAB analysis and quantitative correlation of total anthocyanin content in European and Asian plums. European Journal of Horticultural Science, 86: 453–460.

Salkic A., Salkic B., Doric A., Salkic E., Imsirovic E. (2019): The ecophysiological needs of plums and their impact on ecological production of plum in Bosnia and Herzegovina. Journal of Experimental Agriculture International, 38: 1–10.

Schiavon M., Nardi S., Vecchia F.D., Ertani A. (2020): Selenium biofortification in the 21st century: status and challenges for healthy human nutrition. Plant and Soil, 453: 245–270.

Sun X., Wang Y., Han G., Ye S., Zhou X. (2020a): Effects of different selenium forms on selenium accumulation, plant growth, and physiological parameters of wild peach. South African Journal of Botany, 131: 437–442.

Sun X., Han G., Ye S., Luo Y., Zhou X. (2020b): Effects of selenium on serotonin synthesis and the glutathione redox cycle in plum leaves. Journal of Plant Nutrition and Soil Science, 20: 2212–2221.

Sun X., Luo Y., Han G., Zhang W. (2023): Effects of semi-decomposed weeds as substrate on plant growth, antioxidant capacity, and leaf nutrition of plum trees. Journal of Plant Nutrition, 46: 823–834.

Thiry C., Ruttens A., Temmerman L.D., Schneider Y.J., Pussemier L. (2012): Current knowledge in species-related bioavailability of selenium in food. Food Chemistry. 130: 767–784.

USDA-ARS (2012): USDA National Nutrient Data Base for Standard Reference, Release 25. USDA-ARS, Washington, DC:USAD.

Usenik V., Kastelec D., Veberi R., Stampar F. (2008): Quality changes during ripening of plums (*Prunus domestica* L.). Food Chemistry, 111: 830–836.

Vinceti M., Filippini T., Wise L.A. (2018): Environmental selenium and human health: an update. Current Environmental Health Reports, 5: 464–485.

Wang H., Liu S., Li J., Wang L., Lin H. (2020): 5-hydroxytryptophan suppresses the abdominal fat deposit and is beneficial to the intestinal immune function in broilers. Frontiers in Physiology, 11: 655.

Wang Y.D., Wang X., Wong Y.S. (2013): Generation of selenium-enriched rice with enhanced grain yield, selenium content and bioavailability through fertilisation with selenite. Food Chemistry, 141: 2385–2393.

Wu K.K., Cheng H.H., Chang T.C. (2014): 5-methoxyindole metabolites of L-tryptophan: control of COX-2 expression, inflammation and tumorigenesis. Journal of Biomedical Science, 21: 17.

Wu M., Cong X., Li M., Rao S., Yu T. (2020): Effects of different exogenous selenium on se accumulation, nutrition quality, elements uptake, and antioxidant response in the hyperaccumulation plant *Cardamine violifolia*. Ecotoxicology and Environmental Safety, 204: 111045.

Xu D., Fang M., Wang H., Huang L., Xu Z. (2020): Enhanced production of 5-hydroxytryptophan through the regulation of l-tryptophan biosynthetic pathway. Applied Biochemistry and Microbiology, 104: 2481–2488.

Zahedi S.M., Abdelrahman M., Hosseini M.S., Hoveizeh N.F., Tran L.S.P. (2019a): Alleviation of the effect of salinity on growth and yield of strawberry by foliar spray of selenium-nanoparticles. Environmental Pollution, 253: 246–258.

Zahedi S.M., Hosseini M.S., Meybodi N.D.H., Silva J.A. (2019b): Foliar application of selenium and nano–selenium affects pomegranate (*Punicagranatum* cv. malasesaveh) fruit yield and quality. South African Journal of Botany, 124: 350–358.

Zhu S., Liang Y., Gao D., An X., Kong F.(2017): Spraying foliar selenium fertilizer on quality of table grape (*Vitis vinifera* L.) from different source varieties. Scientia Horticulturae, 218: 87–94.

Received: May 3, 2023
Accepted: November 7, 2023