**EFFECTS OF DIFFERENT SELENIUM FORMS ON SELENIUM ACCUMULATION, PLANT GROWTH, AND PHYSIOLOGICAL PARAMETERS OF WILD PEACH**

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**Abstract:** Different forms of selenium (Se) were added to detect the mechanism of Se transportation, distribution in seedling of wild peach (*Amygdalus persica* L.), and the effects on plant growth and some physiology parameters. 1.0 mg/kg of Se2–, Se0, Se4+ and Se6+ (from selenomethionine, Se powders, sodium selenite, sodium selenate respectively) were added in 4.1L plastic pots planting 15 cm–high wild peach seedlings. After 45 days treatments, seedling growth parameters, Se concentrations in different parts of the plants, and leaf sugar contents were determined. We found that all 4 forms of Se treatments increased shoot length, stem diameter, dry weight, especially Se4+ and Se6+ significantly promoting on plant dry weight. Wild peach showed different Se enrichment ability, via Se bioconcentration factors (BCF) in the order of Se2– > Se6+ > Se4+ > Se0. But it showed the same trend in the four Se forms distribution, as the largest Se content was in roots, followed by in leaves and stems. There was no significantly difference on leaf pigments concentration of the Se forms treatment except Se0. Four forms of Se treatments significantly reduced leaf malondialdehyde (MDA) concentration, and obviously increased reducing power, invisibly increased the content of reducing sugar, and decreased the content of total sugar on different levels. All in all, different Se forms effected wild peach growth and physiological parameters on different level, and wide peach in Se in Se6+ and Se2– forms owed the highest Se compared to other two forms. It may lead to better understanding for the transformation and distribution of different Se forms in wild peach.

**Keywords:** Dry weight, *Amygdalus persica*, selenium transportation, oxidation resistance

**Introduction**

Selenium (Se) is a beneficial element for plants, affect in seed germination, plant growth ([Sun X. et al. 2018b](#_ENREF_24)), fruit production and quality ([Pezzarossa et al. 2012](#_ENREF_16)), and heavy metal uptake and translocation ([Zhu et al. 2017](#_ENREF_28)). The effect of Se is dependent on combined forms, related to the plant species. Se exists in the soil mainly as selenate (Se6+), selenite (Se4+), and organic forms (Se2-), while elemental Se (Se0) and selenide-Se also exist. Different Se forms show different biological activity; several studies have investigated the two main inorganic Se forms, Se4+ (from selenite) and Se6+ (from selenate), in the soil ([Guerrero et al. 2014](#_ENREF_6)), while little attention has been focused on the effect of Se2-and Se0 on on plant growth and development. Plant absorbed inorganic Se, and changed into organic Se in the ecological chain of Se. Biofortification with Se during fertilization of food crops provides an effective approach to increase human Se intake. The organic Se in crop plant was mainly as SeMet, γ-glutamyl-Se-methylselenocysteine, selenocysteinethose have potent cancer chemopriventive activity ([Rayman 2012](#_ENREF_18)).

Se-enriched fruits is becoming more and more popularity, Se fertilizer increases nutrition quality, including soluble sugar, Vitamin C, soluble protein, soluble solid. Sprayed Se4+ and Se6+ were both substantially metabolished into SeMet, and up to 85% of Se was in the form of SeMet in *brassica* seeds and meal ([Seppänen et al. 2010](#_ENREF_20)). However, root application of Se remarkably increased Se accumulation compared to foliar application, and may ensure the safe intake of Se through Se-fortified rice and the largest Se-amino acids in Se2- form, followed by Se4+ and Se6+ treatments ([Yin et al. 2019](#_ENREF_26)). Se is not the essential element for higher plants, at low doses is generally beneficial to plant growth and development, but at high does it becomes toxic to plants. Se has been demonstrated to regulate plant growth by strengthening the stress tolerance mechanisms like antioxidant and secondary metabolite metabolism ([Diao et al. 2014](#_ENREF_4); [Elkelish et al. 2019](#_ENREF_5)). Finding the best Se forms to produce Se-enriched fruit is of increasing importance.

Rootstock is very important plant component, which acquire water and nutrient from the soil and transport to all other organs of the plants for their growth and development. Wild peach germplasm is the mainly rootstock of peach, pluot, apricot, and plum, and could offer many useful genes for plant improvement ([Cao et al. 2017](#_ENREF_3)). Previous studies have reported effects of different forms of Se (mainly on Se4+ and Se6+) on crop growth and production. However, to the best of our knowledge, this is the first report on the effect of four forms of Se on wild peach growth and physiology parameters, and the mechanism of Se accumulation and the capacity of leaf antioxidant.

**Materials and methods**

The experiment was conducted in a greenhouse of the School of Advanced Agriculture and Bioengineering, Yangtze Normal University. The seeds of wide peach (*Amygdalus persica* L.) were sterilized with 15% H2O2 for10 min before refrigerated at 4 degrees in a ziplock bag. Most of the seeds were germinated and planted in non–woven bags (8.3 cm in diameter and 13.8 cm in height) with a substrate (vermiculite: peat soil: perlite: 1:1.2:0.4). On March 5, 2018. The seedling were maintained under normal management condition, and Hoagland’solution was added once. On April 21, seedlings were transplanted into 4.1L plastic pots (20 cm in height) with fine sand and vermiculite (2:1 in volume) as the substrate. On May 6, 2018, Se2– (from selenomethionine), Se0 (Se powders), Se4+ (sodium selenite), and Se6+ (sodium selenate) were added to 15 cm–high seedlings to maintain roots at a 1.0 Se mg/kg1 condition for 50 days, and no Se–added seedlings as the control.

 At the end of the treatment with different Se forms, wild peach seedlings (8 biological replicates per treatment) were harvested and plant height, root length, stem diameter, and dry weights of leaves, stems and roots (determined after 48 h at 60 °C in a drying oven) were measured for four biological replicates per treatment. The root shoot ratio was calculated as the root dry weight divided by the total dry weight of shoot and stem. A sample of the fresh leaf tissue (which was maintained at –80 °C before measuring) was collected for the measurement of photosynthetic pigments, and another for the remaining assays, which was maintained at –80 °C before measuring.

 Leaf pigments were extracted using 95% ethyl alcohol in the dark for 24 h. The contents were determined spectrophotometrically in accordance with that described by Lichtenhaler and Wellburn ([Lichtenhaler and Wellburn 1983](#_ENREF_11)). Ten round blades punched from leaves were soaked in 8 mL 95% ethyl and kept in the dark at 4 °C until the color faded to white. Then, the solution was measured at 665 nm, 649 nm, 470 nm before 4 times dilution by a UV–spectrophotometer (Shimadzu UV–16A, Shimadzu, Corporation Kyoto, Japan). Leaf pigments were quantified as follows:

Chlorophyll a (mg/L) = 13.95 × OD665 – 6.88 × OD649

Chlorophyll b (mg/L) = 24.96 × OD649－7.32 × OD665

Total Chlorophyll (a + b) (mg/L) = Chlorophyll a + Chlorophyll b

Carotenoids (mg/L) = (1000 × OD470 – 3.27 × Chlorophyll a–104 × Chlorophyll b)/OD229

Se concentrations in leaf, stem, and root samples were detected by the method used by Naeem et al. (2013). Plant samples (each 0.2000 g) were digested using 5 mL of concentrated nitric acid for 2 h at 60 °C, and then the temperature of each was increased to 100 °C for 3 min, 140 °C for 3 min, 160 °C for 3 min, 180 °C for 3 min, and 190 °C for 15 min. The acid digests were then diluted to 50 mL with double distilled water and analyzed for Se contents by inductively coupled plasma-atomic emission spectroscopy (PE ICP-OES Optima 8000, Perkin Elmer Inc., Ewing, New Jersey, USA). The total Se uptake was measured as the follow:

The total Se uptake(mg/plant) = leaf dry weight × Se concentration of leaf + stem dry weight × Se concentration of stem + root dry weight × Se concentration of root

Se transaction factors (TFs) and Se bioconcentration factors (BCF) were quantified by Seneviratne et al. (2015) as follows:

TFstem/root = Se concentration of stem/Se concentration of root

TFleaf/root = Se concentration of leaf/Se concentration of root

BCF = Se concentrations of whole plant/Se concentrations of soil

20 round blades were obtained using a punch, and then weighed after oven-drying. LMA was calculated as leaf dry mass divided by the area of the round blades.

Sample of 0.20 g of wild peach leaves were fully ground into slurry with 10 mL trichloroacetic acid and quartz sand. This was centrifuged at 4000 r/min for 10 min, and then 2 mL of the extract was added to 1 mL of 0.6% thiobarbituric acid and mixed. After heating at 100 °C for 30 min, the mixture was centrifuged. Thereafter, the absorbance of the supernatant was read at 532 and 450 nm.

The MDA concentration was calculated as y (mol/L) =（6.45 × A532 - 0.56 × A450) × 10-6.

The reducing power was evaluated by the method described by Oyaizu ([1986](#_ENREF_12)); 0.20 g of wild peach leaves was grounded with 3 mL 80% ethanol, and centrifuged at 5000 r/min for 10 min. One milliliter of supernatant was mixed with a phosphate buffer (2.5 mL, 0.2M, PH = 6.6) and then 1 mL potassium ferricyanide (1%). The mixture was rapid cooling after incubation at 50 °C for 20 min. The mixture was added to 2.5 mL of 10% trichloroacetic acid before centrifuged at 3500 r for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL distilled water, 0.5 mL of 0.1% FeCl3 and the absorbance was measured at 700 nm after standing for 10 min.

For reducing sugar assay, 0.20 g of wild peach leaves was ground with distilled water; after heating at 50 °C for 5 min, the mixture was filtered and determined with Fehling reagent titration. The total sugar content was measured as 10 mL of 6 mol/L hydrochloric acid and 15 mL distilled water was added to the ground wild peach leaves, and was heated 30 min for 100 °C. The mixture was neutralized with 6 mol/L sodium hydroxide before adding the Fehling reagents.

Values were expressed as the mean ± SD with at least 4 replications. One– or two–way ANOVA comparisons among means were conducted using Origin 8.0 followed by the Fisher LSD test at P < 0.05.

**Results**

**Growth and biomass:** The growth of wild peach was affected by different Se forms at different levels (Fig. 1, 2, 3). Compared to the control, the shoot length was significantly increased by 12.6%, 14.3%, 18.5% in Se2-, Se4+, Se6+ forms. respectively, and stem diameter remarkably increased by 18.7% in Se0 and 20.1% in Se6+ forms. Root length in the Se0 form was longest, increased by 29.1%, compared to the control, while root length in the Se6+ form was significantly reduced by 17%, compared to the control. Dry weight of stems significantly increased by 67.83% in the Se4+ form and 65.15% in the Se6+ form treatments. The largest leaf dry weight was in the Se4+ form treatment, and reached a significant level compared to other treatments. There were no significant differences in root dry weight among all Se form treatments. The dry weight of the whole plant in Se4+ form treatment, significantly increased by 87.9%, compared to the control, Se2-, and Se0+ treatments, followed by Se6+ form treatment; there was no significant difference between Se6+ form treatment and other treatments. The root shoot ratio was significantly increased by different Se of form treatments, especially under Se0 treatment by 96.00%, and under Se4+ treatment by 60.00%.

**Leaf pigments concentration:** Leaf pigments of wild peach were significantly reduced in the Se0 form treatment by 26.26% in chlorophyll a, 20.99% in chlorophyll b, 24.75% in chlorophyll a+b, and 16.50% in carotenoid (Fig. 4). There was no significant difference in the concentration of leaf pigments among the other four treatments, but in Se2- and Se6+ forms, leaf pigment concentration reduced compared to the control. In Se4+ form treatment leaf pigments increased.

**Se concentration in plant:** All four Se forms significantly increased plant Se concentrations in leaf, stem, and root (Fig. 5). Compared to the control, the largest Se concentration of leaf and stem was approximately 11.28 mg/kg and 4.23 mg/kg in Se6+ form treatment, followed by Se2-, Se4+, and Se0. On the other hand, highest root Se concentration was observed under Se2- treatment (17.62 mg/kg), significantly increased compared to other Se form treatments, followed by Se6+, Se4+, Se0, and control treatments. Under Se2-, Se0, and Se4+ treatments, Se concentration was in the order root > leaf > stem in wild peach.

**Se transaction factors (TFs) and Se bio concentration factors (BCF):** Under the control condition, the TF stem/root of wild peach was significantly higher than in the four forms of Se treatments, while the Se TF leaf/root of wild peach showed no significant difference compared to the four Se form treatments (Table 1), but under Se6+ treatments, wild peach showed largest TF leaf/root compared to the other treatments. BCF of Se was significantly affected by different Se form treatments. Wild peach in Se2- and Se6+ treatments showed the largest BCF, followed by Se4+, control, and Se0.

**Specific leaf weight, malondialdehyde (MDA) concentration, and reducing power (FRAP):** There was no significant difference in specific leaf weight (SLW) in the four forms of Se treatment (Fig.6). Compared to the control, wild peach in Se2- and Se4+ reduced by 6.37% and 2.38%, respectively. In contrast, the SLW of wild peach in Se0 and Se6+ was increased by 3.10% and 4.30%, respectively, compared to the control. Leaf malondialdehyde (MDA) concentration was significantly reduced in Se2-, Se0, Se4+, Se6+forms by 43.43%, 45.08%, 39.00%, 37.26%, respectively, while the reducing power was significantly increased in the different Se form treatments in the order of Se0 (79.41%) > Se6+(53.53%) > Se4+(43.83%) >Se2-(21.51%) (Fig.7).

**The concentration of reducing sugar and total sugar:** There was no significant difference in the concentration of reducing sugar among the four forms of Se treatments, as Se2-, Se0, and Se4+ treatments increased by 11.35%, 8.49%, and 8.76%, respectively, while Se6+ reduced by 4.07% compared to the control. The total sugar concentration was significantly reduced by different Se form treatments as in Se6+ by 38.01%, in Se4+ by 24.82, in Se2- by 22.05%, in Se0 by 10.13% (Fig. 8). Meanwhile, leaf total sugar concentration showed a significantly negative linear relationship with shoot length (*p* = 0.0084 < 0.001) (Fig 9).

**Discussion**

A positive effect of 1.0 Se6+ mg/kg treatment on two peach rootstocks plant growth as higher total leaf area values, dry weight compared to the control, 2.5, and 5.0 Se6+ mg/kg concentration treatments ([Pezzarossa et al. 2010](#_ENREF_17)). In citrus seedlings, 1.0 Se6+ mg/L application also can increase plant shoot length and fresh/dry weight compared to the control, but the most appropriate concentration was 2.0 Se6+ mg/L ([Sun X. et al. 2018b](#_ENREF_24)). And the dry weight of shoot and root of maize was promoted at the dose of 1.3 Se4+ mg/kg ([Sali; et al. 2018](#_ENREF_19)). In our study, supplied four form Se in 1.0 mg/kg greatly influenced growth parameter; the largest the root length and stem diameter was in Se0 form, the longest shoot length was in Se6+ form, and the largest dry weight was in Se4+ and Se6+treatments. Among all growth parameters, plant dry weight change as a direct indication of the status of vegetation, which indicate that wild peach under Se4+ and Se6+ form Se showed the best growth. While, there was no significantly promotion on total dry weight in the treatments of Se2- and Se0 forms, but root shoot ratio was significantly increased compared to control. No significant difference on the dry weight of root, but the stem dry weight were remarkably improved by Se4+ and Se6+ form treatments.

Different Se forms also influenced the ability of plants to accumulate and translocate Se. Se translocation from root to shoot (TFleaf/root) was described as Se6+ > Control > Se4+ > Se2- >Se0, while Se accumulation from soil to plant (BCF) was described as Se6+ > Se2- > Se4+ > Se0 > Control, which the same as the order in the total Se uptake parameters. Zayed et al ([1998](#_ENREF_27)) found that 4 types crop absorb different Se forms in this order of Se6+ >Se2- >Se4+. Bioavailability of Se6+ is considerably higher in soil than Se4+ increasing uptake of Se by plants ([Peng et al. 2017](#_ENREF_15)). Meanwhile Se6+ is maintained in inorganic state, while Se4+ maintained organic state in plant ([Hu et al. 2018](#_ENREF_9)). However, the growth difference not only came from Se forms, but also on the different plant species sensitivities to Se concentration. Concentrations of Se6+ < 80 μM, Se4+ < 20 μM could increase cucumber growth ([Hawrylaknowak et al. 2015](#_ENREF_8)), and Se tended to exhibit a positive effect on tomatoes plant growth, as selenite and selenate were supplied at less than 0.05 mg/L and 0.5 mg/L, respectively ([Wang et al. 2019](#_ENREF_25)).

In our study, 4 forms Se treatments resulted in no significant decrease in chlorophyll a, chlorophyll b, chlorophyll a+b, and carotenoid, except for Se0 treatment. Variation in leaf pigment concentration of plants grown with different forms of Se have been reported by earlier researchers ([Sharma et al. 2010](#_ENREF_22); [Sun X. et al. 2018a](#_ENREF_23)). Sun et al. ([2018a](#_ENREF_23)) reported there was no significant different on leaf pigments concentration in citrus receiving Se4+ and Se2- treatment. In contrast, higher chlorophyll concentration in *brassica napus* was observed in plants with Se4+ and Se6+ treatment ([Sharma et al. 2010](#_ENREF_22)). A 1.3 mg/kg dose of Se had a positive effect on dry weight of shoot and root, as well as on the contents of chlorophyll b, total chlorophyll, and carotenoids, except for chlorophyll a ([Sali; et al. 2018](#_ENREF_19)).

Lipid peroxidation was determined in the terms of MDA in leaf tissues of both Se forms treated and control plant. It was increased in Se concentrations from 2 to 15μM by Se4+ or Se6+ form treatment on lettuce ([Hawrylak-Nowak 2013](#_ENREF_7)), similar results were also reported by Jain et al. ([2015](#_ENREF_10)), where sugarcane was exposed to high does of Se. However, we found that the leaf MDA concentration was significantly reduced in different Se forms at the same concentration. Se could promote enzymatic activity, regulate the reactive oxidative species ([Patel et al. 2018](#_ENREF_14)). Furthermore, a significant increase was observed on FRAP for all four forms of Se treatments in the present study. The FRAP assay depicts electron donating capacity of bioactive molecules thereby allow the determination of their reducing power ([P. Siddhuraju and Becker 2003](#_ENREF_13)). The leaf in Se0 form showed the highest reducing power and the lowest dry weight and pigment concentration. Total sugar, reducing sugar have mainly been reported at seed generation, fruit ripening, and storage ([A.B.J. Lakho et al. 2017](#_ENREF_1); [Bogevska et al. 2017](#_ENREF_2); [Sett 2016](#_ENREF_21)), we found that reducing sugar for the four forms of Se treatment increased marginally and the total sugar was significantly reduced compared to the control. In general, glycol-metabolism changed under environmental stress, and the plant exhibited highest total sugar concentration and lowest shoot length under Se0 form stress.

**Conclusion**

We investigated the effect of four forms of Se via root application on Se accumulation and growth parameters in wild peach as cultivar peach, plum, apricot rootstocks. The results showed that root application at 1.0mg/kg concentration in Se6+ and Se2- forms had the best effect on Se accumulation, and Se6+ and Se4+ forms had largest dry weight. Plant owned the lowest Se accumulation and dry weight under Se0 forms treatment compared to other three of Se forms, but also had the largest stem diameter, longest root length, and the largest root shoot ratio. Moreover, application of Se increased reducing power, and reducing sugar, but reduced the total sugar concentration. This study expands our understanding of the Se forms in Se-enrich fruit and suggest that SeMet and Se6+ under very lower concentration treatment may easily obtain Se-enriched fruits.

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Table 1 The effects of different Se forms on transaction factors (TFS) and bioconcentration factors (BCF)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment  | TF stem/root | TF leaf/root | BCF | Total Se uptake(mg plant–1) |
| Control | 0.67±0.00a | 0.91±0.44ab | 0.16±0.05c | 0.37±0.06d |
| Se2- | 0.16±0.04c | 0.35±0.04b | 7.74±0.84 a | 31.63±7.47b |
| Se0 | 0.10±0.02d | 0.41±0.01b | 0.77±0.02bc | 3.36±0.57d |
| Se4+ | 0.16±0.01c | 0.65±0.35ab | 1.99±0.47b | 11.96±0.79c |
| Se6+ | 0.48±0.04b | 1.34±0.64a | 7.85±1.31a | 40.94±3.68a |

Different letters in the same row indicate significant differences between treatments by LSD test (p<0.05)

Fig.1

Fig. 1 Effects of different Se forms on wild peach shoot length and stem diameter. Different letters indicated significant differences beween treatments by LSD test at *p* < 0.05

Fig.2



Fig. 2 Effects of different Se forms on wild peach root length. Different letters indicated significant differences beween treatments by LSD test at *p* < 0.05

Fig.3



Fig. 3 Effects of different Se forms on wild peach leaf, stem, root dry weight and total dry weight. Different letters indicated significant differences beween treatments by LSD test at *p* < 0.05. Capital letters indicate total dry weight and lowercase letters indicate organs dry weight

Fig. 4

Fig. 4 Effects of different Se forms on wild peach pigment concentration. Different letters indicated significant differences beween treatments by LSD test at *p* < 0.05

Fig.5



Fig. 5 Effects of different Se forms on wild peach Se concentration of leaf, stem and root. Different letters indicated significant differences beween treatments by LSD test at *p* < 0.05

Fig.6



Fig. 6 Effects of different Se forms on wild peach leaf mass per area (LMA). Different letters indicated significant differences beween treatments by LSD test at *p* < 0.05

Fig. 7



Fig. 7 Effects of different Se forms on wild peach malondialdehyde (MDA) concentration and reducing power. Different letters indicated significant differences beween treatments by LSD test at *p* < 0.05

Fig 8



Fig. 8 Effects of different Se forms on wild peach concentration of reducing sugar and total sugar. Different letters indicated significant differences beween treatments by LSD test at *p* < 0.05

Fig 9



Fig. 9 The linear correlation analysis of sugar concentration and stem diameter under 4 forms of Se treatments.