https://doi.org/10.17221/175/2020-HORTSCI

Effects of selenomethionine on the antioxidative enzymes, water physiology and fruit quality of strawberry plants under drought stress

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Citation: Wang B., Yang W.L., Shan C.J. (2022): Effects of selenomethionine on the antioxidative enzymes, water physiology and fruit quality of strawberry plants under drought stress. Hort. Sci. (Prague), 49: 10–18.

Abstract: To investigate the role of selenomethionine (SeMet) in regulating the drought tolerance of strawberry plants, we explored the effects of SeMet on the antioxidative enzymes, water physiology and fruit quality of the strawberry plants under drought stress (DS). In this study, we used the strawberry variety 'Sweet Charlie' as the material and investigated the effects of SeMet on the drought tolerance of the strawberry plants through foliar spraying and pot experiments. The results showed that the DS obviously enhanced the activities of the antioxidant enzymes (ascorbate peroxidase, superoxide dismutase, peroxidase and catalase) and increased the malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents in the leaves of the strawberry plants, compared with the control. Meanwhile, the DS markedly improved the fruit quality parameters of the soluble solids (SS), soluble sugar, vitamin C (Vc) and sugar-acid ratio. Compared with the DS alone, SeMet obviously enhanced the activities of the above antioxidant enzymes in the leaves and fruit quality parameters of SS, sugar-acid ratio and the soluble sugar and Vc contents, but decreased the MDA and H_2O_2 levels in the strawberry leaves under the DS. However, the DS markedly decreased the photosynthetic pigment chlorophyll (Chl) and carotenoid (Car) contents, net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), relative water content (RWC), fruit weight and plant height and biomass. Compared with the DS alone, SeMet significantly increased the Chl and Car contents, Pn, Tr, Gs, RWC, fruit weight and plant height and biomass. Meanwhile, SeMet al.ne decreased the MDA and H_2O_2 levels and improved the other indicators except for the RWC compared with the control. Our present results concluded that SeMet relieved the adverse impacts of DS on the strawberry growth by enhancing the antioxidant enzymes, photosynthesis and water conditions of the leaves, which promoted the fruit weight and quality. Thus, SeMet can be considered as a regulator to improve the drought tolerance and fruit weight and quality of strawberries.

Keywords: water deficit; SeMet; fruit quality traits; water physiological traits; *Fragaria* × *ananassa* Duch.

The strawberry is an important perennial plant. The fruits of strawberry plants contain a variety of nutrients, such as vitamin C (Vc) and phenolic compounds. Thus, strawberry fruits have a high nutritional value and health function. It has been documented that drought stress (DS) negatively affects the growth and development of strawberry plants (Merlaen et al. 2019). Therefore, it will be important to improve the drought tolerance of strawberry plants by using exogenous chemicals. Selenium (Se) is an important beneficial element for plants. It has been reported that Se plays important roles in regulating the plant growth and development, yield and quality of grains and fruits, as well as in the absorption of mineral elements (Malheiros et al. 2019; Hajiboland et al. 2020; Kaur,

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Supported by the Key Scientific and Technological Research Projects in Henan Province (No. 212102110131).

Sharma 2021; Xie et al. 2021). Meanwhile, many studies have shown that Se played an important role in fighting against stresses, including salt, drought, heat and cadmium stresses (Sattar et al. 2019; Saleem et al. 2020; Sharifi et al. 2021; Xie et al. 2021). It has been documented that DS induced the overproduction of reactive oxygen species (ROS), which caused peroxidation injuries to the plants (Bashir et al. 2021). Plants could fight against DS by increasing the antioxidant potential of themselves through the antioxidant defence system, including the superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), etc. antioxidant enzymes (Mozafari et al. 2019; Mustafa et al. 2021). In this way, plants can alleviate the peroxidation injury induced by ROS under DS. Therefore, it is important to increase the antioxidant potential of plants in stressful situations. Previous studies elucidated that Se enhanced the function of the antioxidant defence system of many plants, such as tomatoes and olives (Proietti et al. 2013; Rady et al. 2020). However, the effects of Se on the antioxidant defence system of strawberries under DS is still unclear. It has been reported that organic Se had a higher absorption efficiency and lower toxicity than inorganic Se (Schiavon et al. 2020). Thus, it will be interesting to investigate the effects of organic Se on the antioxidant defence system of strawberries under DS, which can provide a theorical basis for the use of organic Se in enhancing drought tolerance in the production of strawberries.

The plant growth was closely related with the photosynthetic performance and water conditions (Zhang et al. 2019). More and more evidence has proven that Se plays a vital role in improving the photosynthetic performance and water conditions of plants, such as radishes and bread wheat (Sattar et al. 2019; Auobi Amirabad et al. 2020). Besides, Liu et al. (2001) clarified that deficit irrigation promoted the qualities of strawberry fruits, including the sugar-acid ratio and the soluble solid (SS), vitamin C (Vc) and soluble sugar contents, etc. Some studies have shown that Se could also improve the above qualities of strawberry fruits (Zhang et al. 2020). However, the effects of organic Se on the photosynthetic performance, water conditions and fruit quality of strawberry plants under DS is still unclear. Therefore, it will be important to investigate the effects of organic Se on the photosynthetic performance, water conditions and fruit quality of strawberry plants under DS, which can further provide a theorical basis for its use in strawberry production under water deficit.

In the current experiment, we explored the effects of one type of organic Se, named selenomethionine (SeMet), on the ascorbate peroxidase (APX), SOD, POD and CAT antioxidant enzyme activities, the photosynthetic performance parameters of the net photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Gs), the photosynthetic pigment chlorophyll (Chl) and carotenoid (Car) contents, the relative water content (RWC), as well as the malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents in the leaves, and the fruit average weight and fruit quality of the strawberry, as well as the plant height and biomass under DS. The aim of this study was to elucidate upon the roles of SeMet in regulating the growth and fruit quality under DS, which will provide useful information for its use in strawberry production under water deficit.

MATERIAL AND METHODS

Plant material, growth conditions and treatments. The strawberry plant variety 'Sweet Charlie' was used as the experimental material. Plants with six expanded leaves were planted in 1.5 gallons plastic pots containing 3.5 kg of culture soil which was composed of 30% peat and 70% garden soil. In the soil, the available nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn) contents were 141.2, 110.8, 180.2, 25.0, 38.6, 0.86, 17.0 and 10.2 mg/kg, respectively. In the soil, the total Se content was 0.08 mg/kg. The pots were then placed in artificial chambers. In the artificial chambers, the day/night temperature, photosynthetic active radiation, relative humidity and the length of daylight were set at 25/15 °C, 500 μmol/m^2/s, 60% and 10 h, respectively. Plants with similar growth condition were picked out for the whole experiment. We designed three levels of water treatments, including a normal water treatment, a mild DS treatment and a moderate DS treatment. The water content in the culture soil for the normal water treatment, mild DS treatment and moderate DS treatment was 75%, 60% and 40% field water-holding capacity (FC), respectively. The time the drought stress begun was seven days after planting. During the whole experiment, we used an 0.01 g electronic balance (HZY-B6000 Xinling, China) to weigh and then add water into the culture soil of the different water treatments, in order to control the water content in the culture soil at 75%, 60% and 40% FC, respectively. To explore the effects of SeMet, the leaves of the strawberry plants were sprayed by 30 mL of 30 μM SeMet [≥ 98% (TLC), Sigma, USA], which was selected from 10, 30 and 90 μM SeMet under different water treatments. The control plants were only sprayed with 30 mL of distilled water. We sprayed the strawberry plants every five days from the beginning of the different water treatments and the spraying frequency was three times. Each treatment had two strawberry plants and was repeated four times. After 15 days of the SeMet application to the plants under the different water treatments, the upper leaves of the strawberry plants under the different treatments were sampled and frozen by liquid nitrogen. The above leaves were then kept at –80 °C until the antioxidant enzyme analyses were performed. Fresh upper leaves of the strawberry plants under the different treatments were used to determine the RWC, chlorophyll (Chl) and carotenoid (Car) contents, MDA and H_2O_2 contents. Meanwhile, we determined the net photosynthetic rate (Pn), transpiration rate (Tr) and, stomatal conductance (Gs) of the plants under the different treatments by a LI-COR6400 (LI-COR Inc, USA) photosynthetic instrument. At the mature period of the strawberry fruit, the plant height, biomass, fruit average weight and quality were determined.

Assays of APX, SOD, POD and CAT. Each fresh sample of leaves (0.5 g) was homogenised in 10 mL of an ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.1% (w/v) *n*-octyl-β-ᴅ-galactopyranoside (CHAPSO). The homogenates were then centrifugated at 12 000 × *g* for 10 minutes. The supernatant was used for the further antioxidant enzyme assays. The APX (EC 1.11.1.11), SOD (EC 1.15.1.1), POD (EC 1.11.1.7) and CAT (EC 1.11.1.6) activities were measured according to Nakano and Asada (1981), Giannopolitis and Ries (1977), Scebba et al. (2001) and Kato and Shimizu (1987), respectively. One unit of APX activity was defined as a decrease of 0.01 per minute in the absorbance at 290 nm. One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition in the nitro blue tetrazolium chloride monohydrate (NBT) reduction. One unit of POD activity was defined as an increase of 0.01 per minute in the absorbance at 470 nm. One unit of CAT activity was defined as a decrease of 0.001 per minute in the absorbance at 240 nm. Their specific activities were expressed as units mg protein. The protein concentration was determined according to Bradford (1976).

Assays of Chl and Car. The Chl and Car contents were determined according to Lichtenthaler and Wellburn (1983). Each fresh sample of leaves (0.2 g) was homogenised in a mortar with the addition of quartz sand and 80% acetone. After filtration, the solution was filled up to 20 mL. Then the absorbance was determined at 665, 649, and 470 nm by a TU-1810 UV-Vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China).

Assays of RWC, Pn, Tr and Gs. The RWC was determined according to Barrs and Weatherley (1962). Then the RWC value was calculated by using the following equation: RWC = [(fresh weight (FW) – dry weight (DW))/(saturated weight (TW) – DW)] × 100. The parameters of the leaf gas exchange, including the Pn, Tr and Gs, were determined by an LI-COR6400 photosynthesis system (LI-COR Inc, USA) from 10:00 am to 12:00 pm. After start up and preheating, the LI-COR6400 photosynthetic apparatus was calibrated. The conditions in the leaf chamber were set as a leaf temperature of 25.0 °C, a photosynthetic active radiation of 1 000 $\mu mol/m^2/s$ and a CO_2 concentration of 400 ppm. To measure the Pn, Tr and Gs under all the treatments, the top recently expanded leaves were first equilibrated and then their steady-state gas exchange values were recorded.

Assays of MDA and H_2O_2. The MDA and H_2O_2 contents were analysed according to Hodges et al. (1999) and Brennan and Frenkel (1977), respectively. The MDA content was measured according to the thiobarbituric acid (TBA) reaction. The H_2O_2 content was determined by measuring the absorption of the titanium-hydroperoxide and calculated from the standardised H_2O_2 curve.

Assays of plant height and biomass. The plant height was determined by a ruler. Fresh samples of the whole strawberry plants were weighed and dried in an oven at 80 °C for 72 h. Then, the dry weights were recorded.

Assays of fruit average weight and quality. At the mature period of the strawberry fruit, five mature fruits were randomly selected from each treatment and weighed. The fruit average weight is the average value of the above fruits under the different treatment. The SS content was determined by a handheld refractometer. The Vc content was determined according to Shan et al. (2018). The titratable acid (TA) content was measured according to Li (1994).

https://doi.org/10.17221/175/2020-HORTSCI

The soluble sugar content was determined according to Wei (2009). The sugar-acid ratio was calculated as the ratio of the soluble sugar content to the TA content.

Determination of Se content. To analyse the Se contents, 0.5 g of fine powder from each dry sample of the leaves and fruits were digested and then the concentrations of the Se in the extracts were determined according to Dai et al. (2013). The Se standard curve was prepared by using a series of diluted solutions with a concentration range from 0 to 50 μg/L of its commercially available standards. The Se GBW(E)080215 (100 μg/mL) standard solution was purchased from the National Institute of Metrology, China. The dilutions were prepared by diluting the Se standard solution using ultrapure water.

Statistical analysis. The data in the tables are the mean of four replicates. We used SPSS statistics (version 25.0) to perform the statistical analyses. To perform the analysis of variance (ANOVA), we tested the assumptions of normality on the data sample by the Kolmogorov-Smirnov test. We tested the heteroscedasticity on the data sample by both the Graphical test method and the Goldfeld-Quandt test. The means were compared by one-way ANOVA and Duncan's multiple range test at a 5% level of significance.

RESULTS

Effect of SeMet on the antioxidant capacity of the strawberry plants under DS. The mild and moderate DS both enhanced the APX, SOD, CAT and POD activities, and increased the MDA and H_2O_2 levels compared to the control (Table 1). The SeMet application to the drought-stressed plants significantly enhanced the above antioxidant enzyme activities in the strawberry leaves even further. In comparison with the mild DS alone, the SeMet application increased the APX, CAT, SOD and POD activities by 49.4%, 28.6%, 26.4% and 23.8% under the mild DS, respectively. In comparison with the moderate DS alone, the SeMet application increased the APX, CAT, POD and SOD activities by 31.3%, 28.9%, 25.6% and 22.0% under the moderate DS, respectively. Meanwhile, the SeMet application to the drought-stressed plants significantly reduced the MDA and H_2O_2 levels. Compared with the mild DS alone, the SeMet application decreased the MDA and H_2O_2 levels by 31.7% and 23.3% under the mild DS, respectively. Compared with the moderate DS alone, the SeMet application decreased the MDA and H_2O_2 levels by 34.6% and 19.5% under the moderate DS, respectively. Compared to the control, the SeMet application alone also significantly increased the above antioxidant enzyme activities and reduced the MDA and H_2O_2 levels.

Effects of SeMet on Pn, Chl and Car of the strawberry plants under DS. The mild and moderate DS both reduced the Pn and the photosynthetic pigment Chl and Car contents compared with the control (Table 2). The SeMet application to the drought-stressed plants significantly increased the above indicators. In comparison with the mild DS alone, the SeMet application increased the Pn, Chl and Car

Table 1. Effects of SeMet on the antioxidant enzyme activities and the MDA and H_2O_2 contents in the leaves of strawberry plants under drought stress

Treatment	APX (IU/mg protein)	SOD (IU/mg protein)	CAT (IU/mg protein)	POD (IU/mg protein)	MDA (nmol/g FW)	H_2O_2 (μmol/g FW)
Control	1.10 ± 0.13^d	23.0 ± 2.61^d	2.25 ± 0.25^d	2.57 ± 0.29^e	4.5 ± 0.38^c	0.46 ± 0.05^c
SeMet	1.50 ± 0.15^c	30.0 ± 3.10^c	2.97 ± 0.30^c	3.33 ± 0.27^d	3.3 ± 0.40^d	0.32 ± 0.04^d
Mild DS	1.60 ± 0.19^c	28.0 ± 2.44^c	2.90 ± 0.26^c	3.07 ± 0.34^d	6.0 ± 0.55^b	0.60 ± 0.06^b
SeMet + mild DS	2.39 ± 0.25^b	35.4 ± 2.80^b	3.73 ± 0.37^b	3.80 ± 0.43^c	4.1 ± 0.40^c	0.46 ± 0.05^c

SeMet – selenomethionine; DS – drought stress; APX – ascorbate peroxidase; SOD – superoxide dismutase; CAT – catalase; POD – peroxidase; MDA – malondialdehyde; H_2O_2 – hydrogen peroxide; FW – fresh weight

$^{a–e}$Different letters indicate a statistical difference at $P < 0.05$

Values represent mean ± standard deviations ($n = 4$)

The plants were treated as follows: Control, 75% field water-holding capacity; SeMet, spray application of 30 μM SeMet to the strawberry plants under a 75% field water-holding capacity; Mild DS, 60% field water-holding capacity; SeMet + Mild DS, spray application of 30 μM SeMet to the strawberry plants under a 60% field water-holding capacity; Moderate DS, 40% field water-holding capacity; SeMet + Moderate DS, spray application of 30 μM SeMet to the strawberry plants under a 40% field water-holding capacity

Table 2. Effects of SeMet on the Pn, Tr, Gs, RWC and the of Chl and Car contents in the leaves of the strawberry plants under drought stress. The plants were treated as in Table 1

Treatment	Chl (mg/g FW)	Car (mg/g FW)	Pn (μmol/m^2/s)	Tr (mmol/m^2/s)	Gs (mmol/m^2/s)	RWC (%)
Control	2.30 ± 0.25^{b}	0.46 ± 0.06^{b}	8.0 ± 0.77^{b}	4.7 ± 0.50^{b}	0.15 ± 0.02^{b}	92.7 ± 9.50^{a}
SeMet	2.59 ± 0.29^{a}	0.65 ± 0.07^{a}	9.7 ± 1.10^{a}	6.0 ± 0.66^{a}	0.18 ± 0.02^{a}	94.6 ± 9.50^{a}
Mild DS	2.00 ± 0.23^{c}	0.31 ± 0.04^{c}	7.0 ± 0.80^{c}	4.1 ± 0.44^{c}	0.12 ± 0.02^{c}	88.5 ± 8.44^{b}
SeMet + mild DS	2.25 ± 0.20^{b}	0.44 ± 0.05^{b}	8.5 ± 0.85^{b}	5.0 ± 0.60^{b}	0.15 ± 0.02^{b}	91.9 ± 9.10^{a}
Moderate DS	1.75 ± 0.21^{d}	0.33 ± 0.04^{c}	5.0 ± 0.47^{e}	3.0 ± 0.44^{e}	0.07 ± 0.01^{e}	81.0 ± 7.88^{c}
SeMet + moderate DS	2.10 ± 0.24b^{c}	0.46 ± 0.05^{b}	6.0 ± 0.56^{d}	3.6 ± 0.39^{d}	0.10 ± 0.01^{d}	86.4 ± 8.83^{b}

SeMet – selenomethionine; DS – drought stress; Chl – chlorophyll; Car – carotenoids; Pn – net photosynthetic rate; Tr – transpiration rate; Gs – stomatal conductance; RWC – relative water content; FW – fresh weight

$^{a–e}$Different letters indicate a statistical difference at $P < 0.05$

Values represent mean ± standard deviations ($n = 4$)

contents by 21.4%, 12.5% and 41.9% under the mild DS, respectively. In comparison with the moderate DS alone, the SeMet application increased the Pn, Chl and Car contents by 20.0%, 20.0% and 39.3% under the moderate DS, respectively. Compared to the control, the SeMet application alone significantly increased the Pn, Chl and Car contents by 21.2%, 12.6% and 41.3%, respectively.

Effects of SeMet on Tr, Gs and RWC of the strawberry plants under DS. The mild and moderate DS both reduced the Tr, Gs and RWC compared with control (Table 2). The SeMet application to the drought-stressed plants significantly increased the above indicators. In comparison with the mild DS alone, the SeMet application increased the Tr, Gs and RWC by 21.9%, 25.0% and 3.8% under the mild DS, respectively. In comparison with the moderate DS alone, the SeMet application increased the Tr, Gs and RWC by 20.0%, 28.5% and 6.7% under the moderate DS, respectively. Compared to the control, the SeMet application alone significantly increased the Tr and Gs by 27.7% and 20.0%, respectively. However, the SeMet application alone had no obvious effect on the RWC compared with control.

Effects of SeMet on the growth and fruit average weight of the strawberry plants under DS. The mild and moderate DS both reduced the plant height, plant biomass and fruit average weight compared with control (Table 3). The SeMet application to the drought-stressed plants significantly increased the above indicators. Compared to the mild DS alone, the SeMet application increased the plant height, plant biomass and fruit average weight by 15.0%, 16.9% and 19.0% under the mild DS, respectively. In comparison with the moderate DS alone, the SeMet application increased the plant height, plant biomass and fruit average weight by 20.6%, 20.0% and 21.3% under the moderate DS, respectively. Compared with the control, the SeMet application alone significantly increased the plant

Table 3. Effects of SeMet on the plant height and biomass, fruit weight and the contents Se in the fruits and leaves of the strawberry plants under drought stress. The plants were treated as in Table 1

Treatment	Plant height (cm)	Plant biomass (g FW/plant)	Fruit average weight (g)	Se content in fruit (mg/kg FW)	Se content in leaves (mg/kg FW)
Control	22.3 ± 1.84^{b}	125.0 ± 10.35^{b}	26.0 ± 2.84^{b}	0.005 ± 0.00^{d}	0.050 ± 0.01^{d}
SeMet	25.0 ± 2.83^{a}	140.0 ± 14.30^{a}	31.0 ± 3.10^{a}	0.085 ± 0.01^{a}	0.960 ± 0.10^{a}
Mild DS	18.7 ± 1.66^{c}	103.5 ± 11.00^{c}	21.0 ± 2.22^{c}	0.003 ± 0.00^{d}	0.042 ± 0.01^{d}
SeMet + mild DS	21.5 ± 2.40^{b}	121.0 ± 12.42^{b}	25.0 ± 2.50^{b}	0.070 ± 0.07^{b}	0.800 ± 0.55^{b}
Moderate DS	16.5 ± 1.70^{d}	90.0 ± 9.54^{d}	18.3 ± 2.00^{d}	0.002 ± 0.00^{d}	0.030 ± 0.00^{d}
SeMet + moderate DS	19.9 ± 2.12^{c}	108.0 ± 9.65^{c}	22.2 ± 2.40^{c}	0.056 ± 0.05^{c}	0.660 ± 0.45^{c}

SeMet – selenomethionine; DS – drought stress; Se – selenium; FW – fresh weight

$^{a–d}$Different letters indicate a statistical difference at $P < 0.05$

Values represent mean ± standard deviations ($n = 4$)

height, plant biomass and fruit average weight by 13.6%, 12.0% and 19.2%, respectively.

Effects of SeMet on Se contents in leaves and fruits of the strawberry plants under DS. The mild and moderate DS did not significantly affect the Se contents in the leaves and fruits of the strawberry plants compared with control (Table 3). The SeMet application to the drought-stressed plants significantly increased the Se contents in the leaves and fruits of the strawberry plants. In comparison with the mild DS alone, the SeMet application increased the Se contents in the leaves and fruits by 19.0- and 23.3-fold under the mild DS, respectively. In comparison with the moderate DS alone, the SeMet application increased the Se contents in the leaves and fruits by 22.0- and 28.0-fold under the moderate DS, respectively. Compared to the control, the SeMet application alone significantly increased the Se contents in the leaves and fruits by 19.2- and 17.0-fold, respectively.

Effects of SeMet on the fruit quality of the strawberry under DS. Compared with the control, the mild and moderate DS increased the fruit quality parameters, including the sugar-acid ratio and the SS, Vc and soluble sugar contents (Table 4). The SeMet application to the drought-stressed plants significantly increased the above fruit quality parameters even further. In comparison with the mild DS alone, the SeMet application increased the sugar-acid ratio and the SS, Vc and soluble sugar contents by 25.1%, 22.5%, 23.3% and 23.9% under the mild DS, respectively. In comparison with the moderate DS alone, the SeMet application increased the sugar-acid ratio and the SS, Vc and soluble sugar contents by 29.6%, 14.3%, 18.6% and 29.3% under the moderate DS, respectively. Compared to the control, the SeMet application alone also significantly increased the sugar-acid ratio and the SS, Vc and soluble sugar contents of the strawberry fruits by 35.6%, 20.0%, 19.3% and 14.7%, respectively..

DISCUSSION

DS usually causes oxidative damage which is indicated by the level of lipid peroxidation to the plants, such as wheat, the commend reed and strawberry, etc. (Mozafari et al. 2019; Zhang et al. 2019; Mustafa et al. 2021). In this study, we observed an enhanced level of lipid peroxidation indicated by the MDA and H_2O_2 levels in the strawberry leaves under DS, which indicated that the DS also induced oxidative damage to the strawberry plants. Yin et al. (2010) and Mozafari et al. (2019) reported that DS enhanced lipid peroxidation of strawberry plants indicated by the higher MDA and H_2O_2 levels, which is in line with our current results. Thus, previous as well as our current results clearly indicate that DS induced oxidative damage to strawberry plants. It has been documented that plants, such as wheat, the common reed and sunflower, could fight against DS through antioxidant enzymes (Zhang et al. 2019; Nasirzadeh et al. 2021; Sher et al. 2021). Zhang et al. (2019) reported that the common reed fought against DS by enhancing SOD, POD and CAT antioxidant enzymes. In this study, we showed that the DS enhanced the APX, SOD, POD and CAT antioxidant enzymes in the strawberry leaves, which was identical with the results of previous studies in other plants. Some studies have shown that inorganic Se relieved oxidative damage in plants through antioxidant enzymes under many stresses, such as salt, drought, heat and cadmium stresses (Sattar et al. 2019; Saleem et al. 2020; Sharifi et al. 2021; Xie et al. 2021). For drought

Table 4. Effects of SeMet on the fruit qualities of the strawberry plants under drought stress. The plants were treated as in Table 1

Treatment	Soluble solid content (%)	Vitamin C (mg/100 g FW)	Soluble sugar content (%)	Sugar-acid ratio
Control	7.0 ± 0.81^c	44.0 ± 3.82^c	6.10 ± 0.55^d	6.34 ± 0.57^d
SeMet	8.4 ± 0.87^b	52.5 ± 5.55^b	7.00 ± 0.64^c	8.60 ± 0.80^b
Mild DS	8.0 ± 0.83^b	55.0 ± 6.20^b	7.10 ± 0.77^c	7.10 ± 0.74^c
SeMet + mild DS	9.8 ± 0.90^a	67.8 ± 7.24^a	8.80 ± 0.85^b	8.88 ± 0.94^b
Moderate DS	8.4 ± 0.90^b	59.0 ± 5.70^b	8.12 ± 0.90^b	7.60 ± 0.85^c
SeMet + moderate DS	9.6 ± 0.83^a	70.0 ± 6.35^a	10.50 ± 0.90^a	9.85 ± 0.90^a

SeMet – selenomethionine; DS – drought stress; FW – fresh weight

$^{a-d}$Different letters indicate a statistical difference at $P < 0.05$

Values represent mean ± standard deviations ($n = 4$)

stress, Proietti et al. (2013) showed that inorganic Se alleviated the oxidative damage in olive plants by enhancing the APX and CAT activities. Auobi Amirabad et al. (2020) also reported that inorganic Se improved the APX and CAT activities in radishes under drought stress. For the strawberry, it has been reported that inorganic Se enhanced the APX, SOD and CAT activities under cadmium stress (Zhang et al. 2020). The above studies indicated that inorganic Se had something in common with regulating the APX and CAT activities. However, there is still no knowledge about the role of SeMet in regulating the antioxidant enzymes of strawberry plants under DS. Our findings showed that SeMet increased the SOD, APX, CAT and POD activities in strawberry leaves under both mild DS and moderate DS. In this way, the SeMet application further reduced the level of lipid peroxidation indicated by the MDA and H_2O_2 levels under the mild and moderate DS. Compared with inorganic Se, our findings showed that SeMet had something in common with regulating the APX, SOD and CAT activities in strawberry plants. However, the effects of both forms of Se on the POD activity were different in the strawberry plants. This difference may be due to the different types of stress. Our current results indicated that SeMet could be considered as a regulator to enhance the drought tolerance of strawberry plants by improving the antioxidant enzyme activities.

DS also has a significant effect on the water physiology of the plants. For strawberry plants, Ghaderi and Siosemardeh (2011) reported that the DS significantly reduced the Chl and Car contents, as well as the water physiological parameters (Pn, Tr, Gs and RWC) of strawberry plants. In this study, our findings showed that the mild and moderate DS both significantly reduced the Chl and Car contents, as well as water physiological parameters (Pn, Tr, Gs and RWC) in the strawberry leaves, which was consistent with the results of Ghaderi and Siosemardeh (2011). Thus, the previous study and our current study both indicated that DS had negative effects on the Chl and Car contents, as well as water physiological parameters (Pn, Tr, Gs and RWC) of strawberry plants. Sattar et al. (2019) reported that inorganic Se increased the Chl content, the Pn, Tr, Gs and water relation parameters of bread wheat under water deficit. Auobi Amirabad et al. (2020) reported that inorganic Se improved the photosynthetic performance of tomatoes under drought stress. However, there is still no report on the role of SeMet in regulating the water physiological parameters of strawberry plants under DS. In this study, we found that SeMet further increased the Chl and Car contents, as well as water physiological parameters (Pn, Tr, Gs and RWC) of strawberry plants under DS compared with DS alone. Compared with the effects of the inorganic Se in other plants, our findings showed that SeMet played the same roles in regulating the Chl and Car contents, as well as the water physiological parameters (Pn, Tr, Gs and RWC) in strawberry plants. The above studies indicated that inorganic Se and SeMet had something in common in regulating the water physiological parameters. For plant growth, Rady et al. (2020) reported that inorganic Se improved the growth of tomatoes under drought stress. Iqbal et al. (2015) showed that inorganic Se improved the growth of spring wheat under heat stress. Ashraf et al. (2018) showed that inorganic Se also improved the growth of maize under salt stress. These studies indicated that inorganic Se could promote plant growth under different stresses. For the strawberry, Zahedi et al. (2019) reported that selenium-nanoparticles (Se-NPs) improved the growth of the strawberry under salt stress. However, the effects of SeMet on the growth of the strawberry is still unknown. In this study, we found that SeMet promoted the growth of strawberry plants indicated by the plant height, plant biomass and fruit average weight under DS. The results of the different studies suggested that different forms of Se can improve the growth of plants under stresses, including the strawberry. Thus, our current results showed that SeMet could be considered as a regulator to enhance the drought tolerance of strawberry plants by playing a positive role in regulating the water physiology.

Liu et al. (2001) reported that deficit irrigation could improve the qualities of strawberry fruits, including the sugar-acid ratio and the SS, Vc and soluble sugar contents, etc. In this study, we also found that DS improved the above indicators of strawberry fruits, which is in line with the results of Liu et al. (2001). Zhu et al. (2018) showed that inorganic Se improved the nutritional quality of tomato fruits, including the soluble sugar and Vc content, etc. Jing et al. (2017) also showed that inorganic Se improved the fruit quality of the winter jujube, including the soluble sugar content, sugar-acid ratio and Vc content, etc. These studies indicated that inorganic Se could improve the fruit quality of plants. For the strawberry, Zhang et al. (2020) reported that inorganic Se could improve the fruit quality of the strawberry, including the Vc, soluble sugar and total SS contents under cadmium stress. However, there is still no knowledge about the role of SeMet in regulating the fruit quality of strawberry plants under DS. In current study, we found that SeMet increased the sugar-acid ratio and the SS, Vc and soluble sugar contents in the strawberry fruits under the mild and moderate DS. Thus, our current results indicated that the SeMet application to the drought-stressed strawberry plants is an effective strategy to improve the fruit quality of strawberry plants.

Perin et al. (2019) reported that mild DS improved the fruit qualities of strawberry plants through the plant hormone abscisic acid (ABA). In this study, we found that SeMet could further improve the fruit qualities of strawberries. However, whether SeMet improve the fruit qualities through ABA is still unclear. Therefore, it will be very interesting to clarify the specific role of ABA in the improvement of the strawberry fruit qualities by SeMet.

According to the National Standard for Contaminant Limit in Food of China (GB 2762-2005), the safe content of Se in fruits is less than 0.1 mg/kg. In this study, we found that the Se contents in the fruits of the SeMet-treated strawberries were all less than 0.1 mg/kg. Therefore, our results clearly show that the application of 30μM SeMet to drought-stressed strawberry plants is a safe and effective strategy to improve the drought tolerance and fruit quality of strawberry plants.

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Recieved: December 14, 2020
Accepted: October 8, 2021