https://doi.org/10.17221/337/2020-PSE

Key factors affect selenite absorption in wheat leaf blades: pH, temperature, light intensity and leaf position

*Sinan Liu[1], Feiyan Yu[1], Zihao Fu[1], Jinyong Yang[1], Menglin Chen[1], Yihan Fu[3], Yajuan Li[1,2], Huiqing Chang[1,2], Wenli Zhou[1,2], Xugang Wang[1,2], Lianhe Zhang[1,2,4]**

[1]Agricultural College, Henan University of Science and Technology, Luoyang, Henan, P.R. China
[2]Luoyang Key Laboratory of Plant Nutrition and Environmental Ecology, Luoyang, Henan, P.R. China
[3]Agricultural College, Henan Agricultural University, Zhengzhou, Henan, P.R. China
[4]Henan Jinxiwang Agricultural Science and Technology Company Limited, Luoyang, Henan, P.R. China
**Corresponding author: lhzhang2007@126.com*

Citation: Liu S.N., Yu F.Y., Fu Z.H., Yang J.Y., Chen M.L., Fu Y.H., Li Y.J., Chang H.Q., Zhou W.L., Wang X.G., Zhang L.H. (2020): Key factors affect selenite absorption in wheat leaf blades: pH, temperature, light intensity and leaf position. Plant Soil Environ., 66: 431–436.

Abstract: Foliage-spraying selenite solution is an effective measure to enhance selenium (Se) concentration in wheat grains. However, how pH, temperature, light intensity, and leaf position affects selenite absorption in wheat leaf blades is not fully understood. In this study, the effects of pH, temperature, light intensity, and leaf position on selenite absorption in wheat leaf blades were investigated. The results indicated that the selenite absorption rate dramatically decreased with increasing pH. Further study revealed that aquaporin inhibitors such as $HgCl_2$ and $AgNO_3$ strongly inhibited selenite absorption at pH 3.0. Light and higher temperatures significantly promoted selenite absorption. Newly expanded leaf blades had higher rates of selenite absorption than younger and older leaf blades. Thus, higher rates of selenite absorption in leaf blades should attribute to the entrance of selenite into mesophyll cells *via* aquaporins in the form of H_2SeO_3 at low pH values. Foliage-spraying selenite solution on upper leaf blades at lower pH values benefited to increase the selenite absorption rate in wheat leaf blades.

Keywords: Se fortification; foliar fertilisation; selenium species; environment factor; efficiency of selenite absorption

Selenium (Se) is an essential trace element for humans and other animals. It can play crucial roles in eliminating free radicals and peroxides and maintaining cell membrane integrity by forming the active site as SeCys in glutathione peroxidase. Endemic diseases such as Keshan disease and Kashin-Beck disease have been associated with Se deficiency (Tan et al. 2002). The average daily Se intake of 50–60 μg has been recommended for humans (Institute of Medicine 2000). However, the Se intake is actually lower in the majority of European countries and some parts of China than the recommended dietary allowance of Se and does not meet the requirement for protection against cancer, cardiovascular diseases, and other severe infectious diseases, including HIV disease (Rayman 2004). The human body acquires mainly Se from plant foods, especially cereals in their diet. Wheat (*Triticum aestivum* L.) is one of the staple foods around the world. The planted area reached 24.19 million hectares in 2016, producing 12.88 billion tonnes of wheat grains in China. However, low Se concentration in wheat grains decreases the nutritional quality and cannot meet human health requirements for Se. Se occurs mainly in the form of selenomethionine (SeMet) in wheat grains (Cubadda et al. 2010). SeMet is readily incorporated into selenoproteins and offers much stronger bioavailability for human health compared with inorganic Se. Therefore,

Supported by the National Natural Science Foundation of China, Grant No. 31470108; by the NSFC-Henan Joint Fund, Project No. U1904114, and by research foundation from the Henan University of Science and Technology, Grant No. 13560036.

https://doi.org/10.17221/337/2020-PSE

the production of large quantities of Se-enriched wheat is an effective way for humans to satisfy the dietary Se requirement.

Se fertilisers can be applied to increase Se concentration in the edible parts of plants through different measures including seed soaking, seed coating, foliar spraying, and soil application. Foliar spraying with Se or application of Se in the soil is an efficient measure to increase the grain Se concentration (Wang et al. 2013, Ekanayake et al. 2015). It was reported that the spraying of foliage with fertilisers had potential benefits such as reducing fixation and leaching residues into the soil compared with applying fertilisers to the soil (Miao et al. 2015, Wang et al. 2015, 2016). Since Se is readily adsorbed by oxides of iron after it is applied to the soil, spraying the foliage with Se has been widely performed to increase the grain Se concentration (Wang et al. 2013, Ekanayake et al. 2015).

Selenite absorption in wheat leaf blades is affected by some factors such as pH, temperature, light intensity, and leaf position. Previous studies revealed that pH greatly affected selenite uptake by roots (Zhang et al. 2006). However, whether pH affects selenite uptake in leaf blades is not clear. H_2SeO_3, $HSeO_3^-$, and SeO_3^{2-} occur in selenite solutions at equilibrium in proportions that vary with solution pH (Zhang et al. 2006). H_2SeO_3 represents approximately 27% and $HSeO_3^-$ 72% of total Se at pH 3.0. At pH 5.0, $HSeO_3^-$ accounts for 97.2%, SeO_3^{2-} accounts for 2.4%, and H_2SeO_3 accounts for only 0.4% (Zhang et al. 2006). A recent study revealed that selenite entered mesophyll cells *via* Pi transporters at pH 5.0 (Yang et al. 2019), but how H_2SeO_3 is taken up by mesophyll cells is not fully understood. In addition, how temperature, light intensity, and leaf position affects selenite absorption is also unclear. Here, the effects of aquaporin inhibitors, pH, temperature, and light intensity on selenite absorption in wheat leaf blades were investigated in order to improve the efficiency of Se absorption in leaf blades and increase Se concentration in grains by taking efficient measures.

MATERIAL AND METHODS

Plant materials and growth conditions. Wheat (winter wheat cv. Aikang 58 was planted in the fields in the Kaiyuan campus of Henan University of Science and Technology and harvested in 2017) seeds were surface-sterilised and germinated on moist filter paper in an incubator at 35 °C. Wheat seedlings were cultured in full-strength Hoagland solution in a growth chamber. The light temperature was maintained at 24 °C for 14 h, and the dark temperature was 18 °C for 10 h. The relative humidity was controlled at 67%, and the light intensity at the top of plants was approximately 300 μmol/m^2/s photosynthetic photon flux. The nutrient solutions were aerated every 4 h with an air compressor and renewed every 3 days. The pH was adjusted to 5.5 every day. Leaf-blades were excised at the base for selenite absorption after 40 days of growth in the full-strength nutrient solutions (Yang et al. 2019).

Selenite absorption experiments at different pH. Newly excised leaf blades were transferred to absorption solutions containing 100 μmol/L $CaCl_2$, 5 mmol/L MES (morpholinoethanesulfonic acid), and 2 μmol/L Na_2SeO_3 of varying pH (3.0, 4.0, 5.0, 6.0, 7.0, and 8.0) for 3 h. After the termination of selenite absorption, the leaf blades were rinsed, blotted, and oven-dried at 80 °C for Se analysis.

Assay of selenite absorption affected by $HgCl_2$ and $AgNO_3$. Newly excised leaf blades were transferred to absorption solutions containing 5 mmol/L MES, 0.5 mmol/L $Ca(NO_3)_2$ and 2.0 μmol/L Na_2SeO_3, with 20 μmol/L $HgCl_2$ or 50 μmol/L $AgNO_3$ at pH 3.0 or pH 5.0, respectively. After the termination of selenite absorption, the leaf blades were rinsed, blotted, and oven-dried at 80 °C for Se analysis.

Assay of selenite absorption affected by temperature and light intensity. Newly excised leaf blades were placed in absorption solutions containing 5 mmol/L MES, 0.5 mmol/L $Ca(NO_3)_2$, and 2 μmol/L Na_2SeO_3 for 3 h. Replicates of this absorption solution were maintained in three separate temperature treatments: 4, 25, and 35 °C. Similarly, the selenite absorption experiment was performed under well-lit (300 μmol/m^2/s photosynthetic photon flux) and dark conditions. After the termination of selenite absorption, the leaf blades were rinsed, blotted, and oven-dried at 80 °C for Se analysis.

Assay of selenite absorption affected by leaf position. Newly excised leaf blades from different leaf positions were placed in an absorption solution containing 5 mmol/L MES, 0.5 mmol/L $Ca(NO_3)_2$, and 2 μmol/L Na_2SeO_3 for 3 h. After the termination of selenite uptake, the leaf blades were rinsed for Se concentration analysis.

Assay of Se concentration. Dried samples were weighed and placed into 100 mL digestion tubes, and a 5-mL acid mixture (HNO_3:$HClO_4$; 4:1, *v/v*) was added. The samples were digested at 25 °C overnight and then completely digested at 150–165 °C in a digestion oven. After cooling, a 2.5-mL 6 mol/L

https://doi.org/10.17221/337/2020-PSE

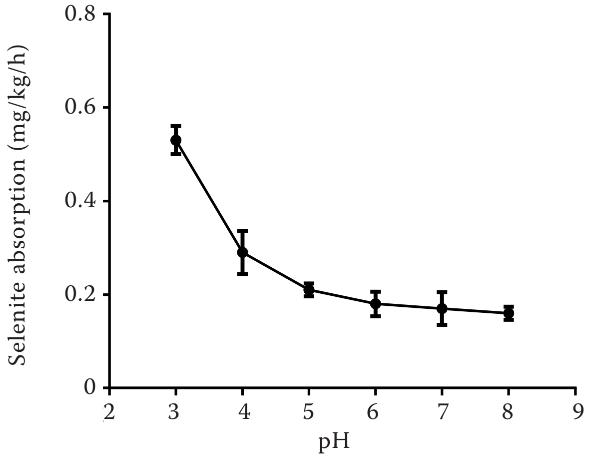

Figure 1. Effects of pH on selenite absorption rate in leaf blades. Values are the means of three replicates

HCl was added to reduce SeO_4^{6+} to SeO_4^{4+} at 100 °C. The digests were diluted with millipore water to a final volume of 25 mL. Se concentrations were determined by atomic fluorescence spectrometry (Beijing Purkinje General Instrument Co., Ltd., PF32, Beijing 2017). Standard tea material (GSV-4, 0.072 mg Se /kg, GBW07605) and a blank were simultaneously digested with the test samples for quality control (Zhang et al. 2006).

Statistical analysis. One-way analysis of variance (ANOVA) was performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, USA) to determine the significant differences ($P < 0.05$) between control and treatments.

RESULTS

Selenite absorption decreases with increasing pH values. To test whether pH values affect selenite absorption in leaf blades, selenite absorption experiments at different pH were performed. The results indicated that the rate of selenite absorption in leaf blades varied with pH values in the absorption solution (Figure 1). Within the pH range of 3.0 to 8.0, leaf blades had the highest rate of selenite absorption at pH 3.0, followed by pH 4.0, with the lowest absorption rate at pH 8.0. The rate of selenite absorption declined sharply as pH increased from 3.0 to 5.0, but it did not vary markedly with changes in pH from 5.0 to 8.0.

Aquaporin inhibitors decrease selenite absorption. H_2SeO_3 is taken up by plant roots *via* aquaporins. To test whether H_2SeO_3 is also taken up by mesophyll cells *via* aquaporins, the effects of aquaporin inhibitors such as $HgCl_2$ and $AgNO_3$ on selenite absorption were investigated in leaf blades. The results revealed that $HgCl_2$ and $AgNO_3$ inhibited selenite absorption by 56% and 66% at pH 3.0 (Figure 2A), respectively, and by 33% and 38% at pH 5.0, respectively (Figure 2B). The extent to which $HgCl_2$ and $AgNO_3$ inhibited selenite absorption differed as pH increased from 3.0 to 5.0.

Effects of light intensity and temperature on selenite absorption. Both light intensity and temperature affect stomatal opening. It was postulated that light intensity and temperature could affect selenite uptake. Thus, the effects of light intensity and temperature on selenite absorption were investigated. The results indicated that the selenite uptake rate in wheat leaf blades was significantly higher under well-lit conditions compared with the dark condition (Figure 3A). In addition, the selenite absorption rate at 35 °C was significantly higher compared with that at 4 °C and 25 °C, respectively. The temperature did not significantly affect absorption rates in the range of 4–25 °C (Figure 3B). These results suggested that higher light intensity and a higher temperature (35 °C) could enhance the rate of selenite absorption in leaf blades.

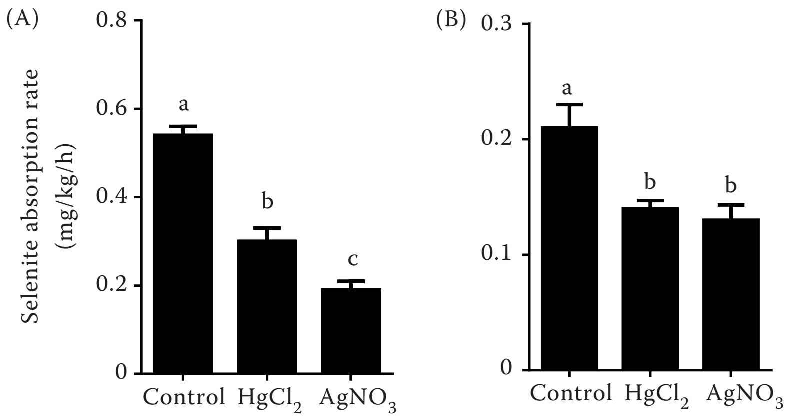

Figure 2. $HgCl_2$ and $AgNO_3$ inhibit selenite absorption at (A) pH 3.0 and (B) pH 5.0. Values are the means of three replicates. Error bars represent standard deviation (n = 3). Different letters of a, b, and c indicate differences among different treatments in the same treatment

https://doi.org/10.17221/337/2020-PSE

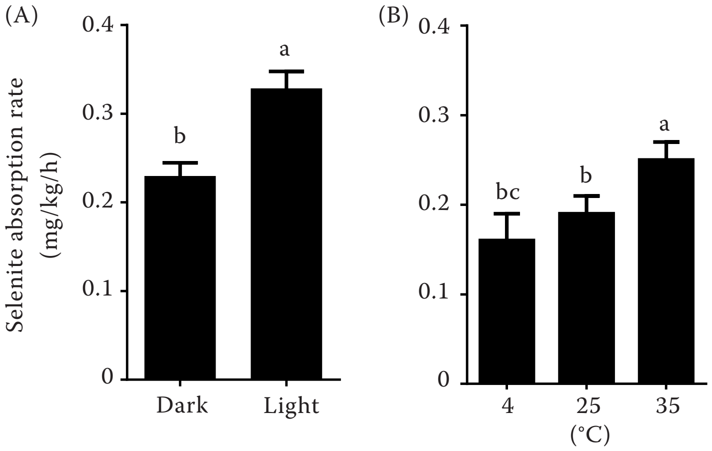

Figure 3. Effects of light intensity and temperature on selenite absorption rate in leaf blades. Values are the means of three replicates. Error bars represent standard deviation (n = 3). Different letters of a, b, and c indicate differences among different treatments in the same treatment

Effects of leaf position on selenite absorption. Leaf-blades from different leaf positions vary greatly in the cuticular wax composition. Thus, selenite absorption rates from different leaf positions were investigated. The results indicated that the newly expanded flag leaf blade had the highest selenite absorption rate, followed by the penultimate leaf blade and the penultimate third leaf blade. The penultimate fourth leaf blade had the lowest selenite absorption rate (Figure 4).

DISCUSSION

Foliar spraying with Se could effectively increase the Se concentration in grains (Wang et al. 2013, Ekanayake et al. 2015). pH, temperature, light intensity, and leaf position are important factors to affect selenite absorption in wheat leaf blades. In this study, it was found that the absorption rate of selenite in leaf blades decreased significantly with increasing pH. At pH 3.0, the absorption rate of selenite in leaf blades reached the highest levels. In selenite solutions, H_2SeO_3, $HSeO_3^-$, and SeO_3^{2-} are in equilibrium in proportions that vary with solution pH (Zhang et al. 2006). H_2SeO_3 represents approximately 27% and $HSeO_3^-$ 72% of total Se at pH 3.0. At pH 5.0, $HSeO_3^-$ accounts for 97.2%, SeO_3^{2-} accounts for 2.4%, and H_2SeO_3 accounts for only 0.4% (Zhang et al. 2006). Thus, the dominant form of selenite in solution varies with pH values. The effects of pH on selenite absorption by leaf blades may result from the differences in Se species between pH values. $HgCl_2$ and $AgNO_3$ are typical aquaporin inhibitors, and both significantly inhibited selenite absorption at pH 3.0, indicating that H_2SeO_3 could enter mesophyll cells *via* aquaporins after it diffuses across cuticles.

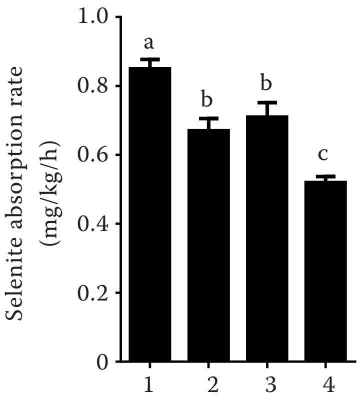

Figure 4. Effects of leaf position on selenite absorption rate in leaf blades. Values are the means of three replicates. Error bars represent standard deviation (n = 3). Different letters of a, b, and c indicate differences among different leaf positions. 1, 2, 3, and 4 – flag leaf blade, penultimate leaf blade, penultimate third leaf blade, and penultimate fourth leaf blade, respectively

Leaf temperatures vary substantially depending on seasons, light, wind, and plant species (Feller 2006). The temperature of leaf blades in the shadow was generally below the air temperature, while the temperature of fully sun-exposed leaf blades was often considerably higher than the air temperature (Feller 2006). Diffusion within plant cuticles is extremely temperature-dependent (Baur and Schönherr 1995). The temperature has a pronounced effect on diffusion and the permeation of membranes by water (Schönherr et al. 1979, Schönherr and Mérida 1981), as was observed with isolated plant cuticular membranes and leaf discs (Schreiber 2001). With increasing temperature, the solute mobility in cuticles increases greatly, which is attributed to the enhanced fluidity of the amorphous wax fraction

https://doi.org/10.17221/337/2020-PSE

(Schreiber and Schönherr 1993, Baur and Schönherr 1995). Thus, it was postulated that enhanced selenite absorption with increasing temperature was closely related to enhanced fluidity of the amorphous wax fraction. In addition, elevated temperature stimulates a stomatal opening (Feller 2006). The tendency of stomata to open at a higher temperature probably reflects an increase in H^+ pump activity (Rogers et al. 1979, 1981, Ilan et al. 1995). Thus, the observed increase in selenite absorption with elevated temperature should partly be attributed to stimulate stomatal opening.

Leaf-blades of different leaf positions varied greatly in development time, resulting in the difference in the composition of wax. Since the cuticle waxes act as a transport barrier limiting nutrient penetration across the cuticle (Riederer and Schreiber 2001, Schreiber 2005), it was postulated that leaf position could affect selenite absorption. Here, the newly expanded flag leaf blade had higher rates of selenite absorption than older leaf blades. Thus, selenite absorption was postulated to relate to differences in cuticular wax composition at different developmental stages (Riederer and Schreiber 2001, Schreiber 2005). Cuticular waxes predominantly contain long-chain fatty acids, aldehydes, alkanes, branched alkanes, primary alcohols, secondary alcohols, unsaturated fatty alcohols, ketones, wax esters, triterpenoids, and sterols. The most common components are n-alkanes and primary alcohols, both of which range from C16 to C36 (Buchholz 2006). Gas chromatography-mass spectrometry analysis revealed that primary alcohols and triterpenoids dominated in the tender leaf blades and the fully expanded ones, respectively (Zhu et al. 2018). Alcohols and β-diketones were the major components of cuticular wax in leaf blades and leaf sheaths of wheat. The major component of the wax changes from octacosanol to β-diketone during the development process of flag leaf sheaths (Tulloch 1973). Thus, differences in selenite uptake should be attributed to the differences in primary alcohol, triterpenoid, and β-diketone composition between newly expanded flag leaf blades and fully expanded leaf blades of wheat. In addition, the newly expanded flag leaf blade has strong metabolism compared with older leaf blades. The mesophyll cells are provided enough energy for high-flux nutrient supply to guarantee vigorous growth. Thus, the newly expanded flag leaf blade has higher ability to absorb selenite than older leaf blades.

REFERENCES

Baur P., Schönherr J. (1995): Temperature dependence of the diffusion of organic compounds across plant cuticles. Chemosphere, 30: 1331–1340.

Buchholz A. (2006): Characterization of the diffusion of non-electrolytes across plant cuticles: properties of the lipophilic pathway. Journal of Experimental Botany, 57: 2501–2513.

Cubadda F., Aureli F., Ciardullo S., D'Amato M., Raggi A., Acharya R., Reddy R.A.V., Prakash N.T. (2010): Changes in selenium speciation associated with increasing tissue concentrations of selenium in wheat grain. Journal of Agricultural and Food Chemistry, 58: 2295–2301.

Ekanayake L.J., Thavarajah D., Vial E., Schatz B., McGee R., Thavarajah P. (2015): Selenium fertilization on lentil (*Lens culinaris* Medikus) grain yield, seed selenium concentration, and antioxidant activity. Field Crops Research, 177: 9–14.

Feller U. (2006): Stomatal opening at elevated temperature: an underestimated regulatory mechanism? General and Applied Plant Physiology, 32: 19–31.

Institute of Medicine (2000): Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, National Academies Press.

Ilan N., Moran N., Schwartz A. (1995): The role of potassium channels in the temperature control of stomatal aperture. Plant Physiology, 108: 1161–1170.

Miao Y.F., Wang Z.H., Li S.X. (2015): Relation of nitrate N accumulation in dryland soil with wheat response to N fertilizer. Field Crops Research, 170: 119–130.

Rayman M.P. (2004): The use of high-selenium yeast to raise selenium status: how does it measure up? British Journal of Nutrition, 92: 557–573.

Riederer M., Schreiber L. (2001): Protecting against water loss: analysis of the barrier properties of plant cuticles. Journal of Experimental Botany, 52: 2023–2032.

Rogers C.A., Sharpe P.J.H., Powell R.D., Spence R.D. (1981): High-temperature disruption of guard cells of *Vicia faba*. Plant Physiology, 67: 193–196.

Rogers C.A., Powell R.D., Sharpe P.J.H. (1979): Relationship of temperature to stomatal aperture and potassium accumulation in guard cells of *Vicia faba*. Plant Physiology, 63: 388–391.

Schönherr J., Eckl K., Gruler H. (1979): Water permeability of plant cuticles: the effect of temperature on diffusion of water. Planta, 147: 21–26.

Schönherr J., Mérida T. (1981): Water permeability of plant cuticular membranes: the effects of humidity and temperature on the permeability of non-isolated cuticles of onion bulb scales. Plant, Cell and Environment, 4: 349–354.

Schreiber L. (2001): Effect of temperature on cuticular transpiration of isolated cuticular membranes and leaf discs. Journal of Experimental Botany, 52: 1893–1900.

Schreiber L. (2005): Polar paths of diffusion across plant cuticles: new evidence for an old hypothesis. Annals of Botany, 95: 1069–1073.

Schreiber L., Schönherr J. (1993): Mobilities of organic compounds in reconstituted cuticular wax of barley leaves: determination of diffusion coefficients. Pesticide Science, 38: 353–361.

Tan J.A., Zhu W.Y., Wang W.Y., Li R.B., Hou S.F., Wang D.C., Yang L.S. (2002): Selenium in soil and endemic diseases in China. Science of The Total Environment, 284: 227–235.

Tulloch A.P. (1973): Composition of leaf surface waxes of *Triticum* species: variation with age and tissue. Phytochemistry, 12: 2225–2232.

Wang J.W., Wang Z.H., Mao H., Zhao H.B., Huang D.L. (2013): Increasing Se concentration in maize grain with soil- or foliar-applied selenite on the Loess Plateau in China. Field Crops Research, 150: 83–90.

Wang Z.H., Miao Y.F., Li S.X. (2015): Effect of ammonium and nitrate nitrogen fertilizers on wheat yield in relation to accumulated nitrate at different depths of soil in drylands of China. Field Crops Research, 183: 211–224.

Wang Z.H., Miao Y.F., Li S.X. (2016): Wheat responses to ammonium and nitrate N applied at different sown and input times. Field Crops Research, 199: 10–20.

Yang J.Y., Yu F.Y., Fu Z.H., Fu Y.H., Liu S.N., Chen M.L., Li Y.J., Sun Q.Z., Chang H.Q., Zhou W.L., Wang X.G., Zhang L.H. (2019): Pathway and driving forces of selenite absorption in wheat leaf blades. Plant, Soil and Environment, 65: 609–614.

Zhang L.H., Shi W.M., Wang X.C. (2006): Difference in selenite absorption between high- and low-selenium rice cultivars and its mechanism. Plant and Soil, 282: 183–193.

Zhu X.F., Zhang Y., Du Z.H., Chen X.B., Zhou X., Kong X.G., Sun W.J., Chen Z.J., Chen C.S., Chen M.J. (2018): Tender leaf and fully-expanded leaf exhibited distinct cuticle structure and wax lipid composition in *Camellia sinensis* cv Fuyun 6. Scientific Reports, 8: 14944.

Received: July 5, 2020
Accepted: August 13, 2020
Published online: September 3, 2020