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Selenium reduces cadmium accumulation and alleviates cadmium-induced quality degradation in tobacco

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ABSTRACT

A greenhouse pot experiment was conducted using two contrasting tobacco cultivars Guiyan1 (Cd-sensitive) and Yunyan2 (Cd-tolerant) to evaluate the effect of external selenium (Se) on Cd-induced degradation of tobacco quality. Results showed that 3 mg/kg Cd reduced K, Mg, Mn, and Zn contents, but increased Cd, P, Ca, Fe contents in both cultivars. Addition of Se in Cd solution (Cd + Se) dramatically alleviated Cd-deteriorate effects on tobacco quality, markedly reduced Cd concentration, but increased Se and K contents in tobacco leaves compared with Cd alone treatment. Exogenous Se counteracted Cd-induced alterations in carbohydrate, e.g. it significantly depressed Cd-induced increase in total/reducing sugar, and sucrose contents, but elevated the reduced starch content. Furthermore, Se significantly depressed the elevated ratios in reducing sugar/total nitrogen, total sugar/total protein and reducing sugar/nicotine, but elevated total nitrogen/nicotine, which returned near to their control levels.

Keywords: toxic pollutant; metal; micronutrient; cigarette ash; *Nicotiana tabacum* L.

Cadmium (Cd) is one of the most toxic pollutants due to its high toxicity to both plants and animals (Satarug et al. 2003). In soils, Cd can easily enter tobacco plants through the roots and become enriched in leaves, and about 10% of Cd in a cigarette is absorbed in the course of smoking (Willers et al. 2005). Accordingly, Cd contamination in tobacco has caused wide public concern. Considering the large-scale of the medium/slightly contaminated farmlands, approaches such as the application of chemical regulators might offer a cost-effective and practically acceptable strategy for full utilization of natural resource and safe tobacco production.

Cadmium stress significantly degraded the yield and quality of many crops, through increasing Cd content and reducing protein, starch and crude grease content (Guo et al. 2011). Nicotine, protein and carbohydrate, the main chemical components of tobacco leaves, have played an important role in the formation of tobacco quality. Generally, tobacco with balanced nitrogen, nicotine and carbohydrate contents tastes better than that with lower or higher contents (Mendell et al. 1984). Although many studies have been reported on different adverse effects of Cd, none has focused on the association between Cd stress and tobacco quality. Therefore, a question arises as to whether the tobacco quality is affected by Cd stress.

Selenium (Se) is considered to be of importance for human health, through synthesizing selenoproteins, preventing cells from oxidative damage (El-Demerdash and Nasr 2014). Although Se seems unnecessary to higher plants, many researchers have reported that Se could increase the tolerance of plants to many biotic and abiotic stresses (Feng et al. 2013). Our previous study demonstrated that Se is able to decrease Cd uptake in rice and tobacco seedlings (Lin et al. 2012, Liu et al. 2015). However, little information is available about the effects of Se on the quality of tobacco grown in Cd contaminated soil. Thus, the main objective of the present study was to determine the function of exogenous Se in alleviating Cd-induced degradation of tobacco quality in 2 cultivars differing in Cd tolerance. We aimed at finding a feasible way

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to reduce risks associated with Cd toxicity for sustainable production of tobacco and to produce Se-enriched tobacco especially grown in slightly or moderately Cd contaminated soils.

MATERIAL AND METHODS

Plant material and experimental design. A greenhouse pot experiment was conducted on Zijingang Campus at Zhejiang University in Hangzhou, China. Air-dried soil was sieved and 4.5 kg of air-dried soil were filled in plastic pots (5 L, 20 cm height). The soil used in this investigation had a pH of 6.9 and total N and available P and K contents of 2.4%, 38.2 mg/kg and 31.5 mg/kg, respectively. There were 4 treatments: control; Se (2.5 mg/kg Se); Cd (3 mg/kg $CdCl_2$), and Cd + Se (3 mg/kg $CdCl_2$ + 2.5 mg/kg Se). Cd and Se were applied as solutions containing Na_2SeO_3 and/or $CdCl_2$, respectively, to achieve a total soil concentration of 3 mg/kg Cd and 2.5 mg/kg Se. After addition of Cd/Se solutions, the soil was allowed to equilibrate for 30 days in a greenhouse.

The two tobacco cvs. Guiyan1 and Yunyan2 identified as Cd-sensitive and tolerant (Liu et al. 2015), respectively, were used in this study. Seeds were germinated in sterilized moist vermiculite in a growth room at 22–25°C. Seedlings in 4-leaf stage were transplanted to the above mentioned pots (1 plant/each pot). The experiment was laid in a split-plot design with treatment as the main plot and genotype as sub-plot, and there were fourteen replicates for each treatment. At 90 days after transplant, middle leaves of mature plants were harvested for quality analysis.

Metal analysis. Dried plant materials were powdered and then ashed at 550°C and extracted with 30% HNO_3 for 24 h (Chen et al. 2008). Cd and metal concentrations were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (SPS 1200 VR, Seiko Co., Ltd., Chiba, Japan). Total N content was analysed by Kjeldahl TM K-306 (BUCHI Labortechnik).

Determination of nicotine, carbohydrate and total protein contents. Determination of nicotine content was conducted according to Willits et al. (1950). The total protein content was determined by a total protein quantitative assay kit (Nanjing Jiancheng Bioengineering Institute). DNS colorimetry (3,5 dinitrosalicylic acid) was used to determine reducing sugar content in tobacco (Miller 1959). The sucrose content was measured according to Li et al. (2003). Total soluble sugar content was measured by spectrophotometric method (Hewitt 1958), and then the leftovers were used to measure starch content according to Dubois et al. (1956).

RESULTS

Effect of exogenous Se on Cd and element concentrations. In contrast to Cd alone treatment, addition of Se significantly ($P < 0.05$) decreased leaf Cd levels by 30.4% and 18.5% in cvs. Guiyan1 and Yunyan2, respectively. On the other hand, addition of Cd caused a dramatic reduction of leaf Se concentration in cv. Guiyan1 (Cd + Se vs Se alone treatment). However, this reduction was not significant in cv. Yunyan2 (Figure 1).

Cd stress significantly decreased K, N and Mg, but increased P and Ca contents in leaves of both cultivars (Tables 1 and 2). Exogenous Se markedly suppressed Cd-induced increase in leaf P and Ca contents. The P and Ca concentrations in Cd + Se treatments were 23.0% and 22.6% in cv. Guiyan1,

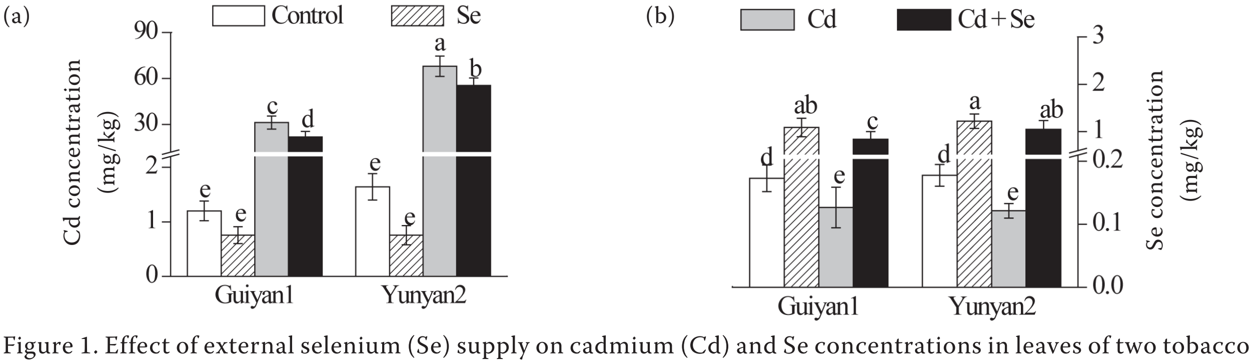

Figure 1. Effect of external selenium (Se) supply on cadmium (Cd) and Se concentrations in leaves of two tobacco cvs. Guiyan1 and Yunyan2 under Cd stress. Error bars represent standard deviation values (n = 5), different letters indicate significant differences ($P < 0.05$) among the 4 treatments and within the two cultivars

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Table 1. Effect of external selenium (Se) supply on macroelement concentration (mg/kg dry weight) in leaves of two tobacco cultivars under cadmium (Cd) stress

Treatment		P	S	K	Ca	Mg
Guiyan1	control	2460.4[d]	8981.1[a]	20 780.5[b]	8664.4[de]	2387.1[c]
	Se	2583.3[cd]	8904.8[a]	21 130.5[ab]	8510.1[e]	2325.2[c]
	Cd	3507.8[a]	6582.5[d]	14 504.1[f]	12 263.3[a]	1767.1[e]
	Cd + Se	2699.4[c]	7963.6[b]	17 755.5[d]	9497.7[c]	2049.2[d]
Yunyan2	control	2411.1[d]	8847.2[a]	21 408.3[ab]	8510.3[e]	3038.3[a]
	Se	2556.6[cd]	8859.5[a]	21 566.6[a]	8510.2[e]	2991.1[a]
	Cd	3405.5[a]	7571.7[c]	17 030.5[e]	11 298.8[b]	2354.8[c]
	Cd + Se	2925.2[b]	8176.5[b]	19 962.9[c]	9125.5[cd]	2673.1[b]

Different letters indicate significant differences ($P < 0.05$) among the 4 treatments and within the two cultivars

14.1% and 19.2% in cv. Yunyan2, respectively, lower than those of Cd alone treatment. Meanwhile, Se addition evidently alleviated the Cd-induced decrease: K, N and Mg concentrations in Cd + Se treatment being 22.4, 44.8 and 15.9% in cv. Guiyan1 and 17.2, 31.2 and 13.5% in cv. Yunyan2, respectively, higher than Cd-alone treatment (Figure 2, Tables 1 and 2).

As to microelements, Cd stress significantly decreased Mn and Zn but increased Fe and Cu contents (Table 3). Exogenous Se significantly alleviated the impact of Cd stress. For example, Mn and Zn concentrations increased by 35.3% and 44.7% in cv. Guiyan1 and 21.3% and 12.0% in cv. Yunyan2, respectively (Table 3). Moreover, plants grown in Se and Cd + Se treatments showed higher Se contention compared with control and Cd-alone treatments (Figure 1b).

Table 2. Effect of external selenium (Se) supply on major chemicals contents (%) in dry tobacco leaves of two tobacco cultivars under cadmium (Cd) stress

Treatment	TS	RS	NIC	NIT	Pro
Guiyan1					
Control	22.69[c]	19.14[c]	2.32[a]	2.24[a]	9.11[a]
Se	21.79[c]	18.76[c]	2.27[a]	2.16[ab]	9.16[a]
Cd	31.27[a]	25.30[a]	1.73[e]	1.34[e]	6.44[c]
Cd + Se	25.71[b]	21.55[b]	2.07[c]	1.94[cd]	8.94[b]
Yunyan2					
Control	22.86[c]	19.13[c]	2.12[bc]	2.08[bc]	9.22[a]
Se	21.38[c]	18.32[c]	2.07[c]	2.03[bc]	9.37[a]
Cd	29.57[a]	24.43[a]	1.62[e]	1.38[e]	6.79[c]
Cd + Se	25.92[b]	21.72[b]	1.92[d]	1.81[d]	8.64[b]

Different letters indicate significant differences ($P < 0.05$) among the 4 treatments and within the two cultivars. TS – total sugar; RS – reducing sugar; NIC – nicotine; NIT – total nitrogen; Pro – total protein

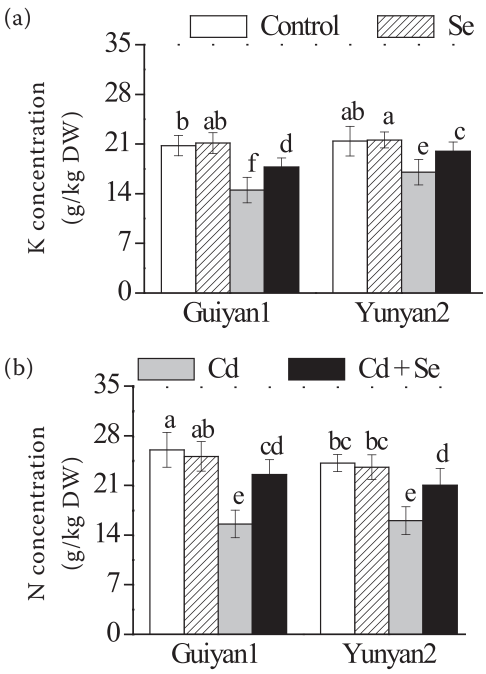

Figure 2. Effect of external selenium (Se) supply on potassium (K) and nitrogen (N) concentrations in leaves of two tobacco cvs. Guiyan1 and Yunyan2 under cadmium (Cd) stress. Error bars represent standard deviation values ($n = 5$), different letters indicate significant differences ($P < 0.05$) among the 4 treatments and within the two cultivars. DW – dry weight

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Table 3. Effect of external selenium (Se) supply on microelement concentration (mg/kg dry weight) in leaves of two tobacco cultivars under cadmium (Cd) stress

Treatment	Cd	Se	Fe	Mn	Zn	Cu
Guiyan1						
Control	1.21^{e}	0.17^{d}	113.82^{ef}	36.63^{c}	14.47^{a}	3.41^{bc}
Se	0.75^{e}	1.16^{ab}	115.51^{e}	38.46^{abc}	13.26^{ab}	3.22^{bcd}
Cd	31.37^{c}	0.13^{e}	160.04^{a}	22.86^{e}	8.60^{d}	4.41^{a}
Cd + Se	21.82^{d}	0.92^{c}	139.20^{c}	30.93^{d}	12.44^{bc}	3.72^{b}
Yunyan2						
Control	1.64^{e}	0.18^{d}	108.58^{f}	38.72^{ab}	13.78^{ab}	2.83^{d}
Se	1.13^{e}	1.28^{a}	113.46^{ef}	40.28^{a}	12.87^{ab}	3.06^{cd}
Cd	68.01^{a}	0.14^{e}	147.73^{b}	31.23^{d}	10.83^{c}	3.53^{b}
Cd + Se	55.42^{b}	1.12^{ab}	132.22^{d}	37.86^{bc}	12.13^{bc}	3.17^{bcd}

Different letters indicate significant differences ($P < 0.05$) among the 4 treatments and within the two cultivars

Effect of exogenous Se on nicotine and total protein content. The nicotine content showed a significant difference between the two cultivars, with 8.6% more in Guiyan1 than in Yunyan2 in the control. This might be due to their genotypic difference but need further verification. Cadmium stress significantly decreased the nicotine and total protein contents in both cultivars, with reduction of 25.4% and 29.3% in cv. Guiyan1 and 23.6% and 26.4% in cv. Yunyan2, respectively, compared with control. However, nicotine and total protein content under Cd + Se treatment were notably increased by 19.6% and 38.8% in cv. Guiyan1, and 18.5% and 27.2% in cv. Yunyan2, respectively, compared with Cd-alone treatment (Figure 3).

Effect of exogenous Se on carbohydrate content. A significant increase of total sugar, reducing sugar and sucrose content was observed in leaves of both cultivars under Cd-alone treatment (c.f. increased by 37.7, 32.2 and 67.8% in cv. Guiyan1 and 29.3, 27.7 and 37.6% in cv. Yunyan2, respectively, compared with control). Se addition significantly diminished Cd-induced increase in these 3 types of carbohydrates. For example, compared with Cd alone treatment, total sugar, reducing sugar and sucrose contents were decreased by 17.8, 14.8 and 30.2% in cv. Guiyan1 and 12.3, 11.1 and 18.2% in cv. Yunyan2, respectively, under Cd + Se treatment (Figures 4a–c). However, an opposite tendency was detected in starch content of two tobacco cultivars (c.f. 49.2% and 20.4% lower than control in Guiyan1 and Yunyan2, respectively, under Cd-alone treatment). Exogenous Se markedly alleviated Cd-induced inhibition of starch accumulation (c.f. 35.8% in cv. Guiyan1 and 12.3% in cv. Yunyan2 higher than Cd-alone treatment) (Figure 4d).

Tobacco quality index. Cadmium stress clearly increased the ratios of total sugar/total protein, reducing sugar/total nitrogen, reducing sugar/nicotine, being 94.7, 121.1 and 7.72% in cv. Guiyan1 and 75.4, 92.4 and 66.9% in cv. Yunyan2 higher than control, respectively (Table 4). However, an opposite tendency was observed in total nitrogen/nicotine, with a reduction of 20.6% and 13.3% in cvs. Guiyan1 and Yunyan2, respectively, compared with control. Addition of Se evidently diminished the influence of Cd. In Cd + Se treatment, the values

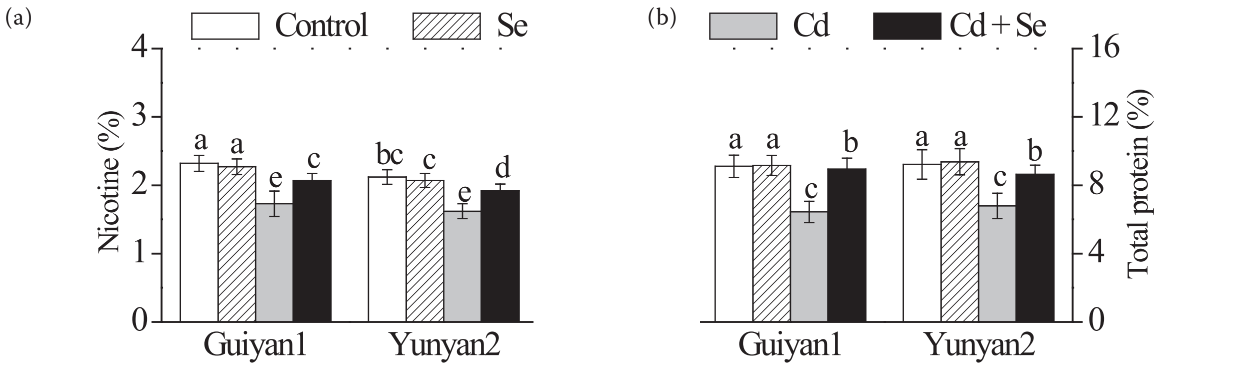

Figure 3. Effect of external selenium (Se) supply on nicotine and total protein contents in dry leaves of two tobacco cvs. Guiyan1 and Yunyan2 under cadmium (Cd) stress. Error bars represent standard deviation values (n = 5), different letters indicate significant differences ($P < 0.05$) among the 4 treatments and within the two cultivars

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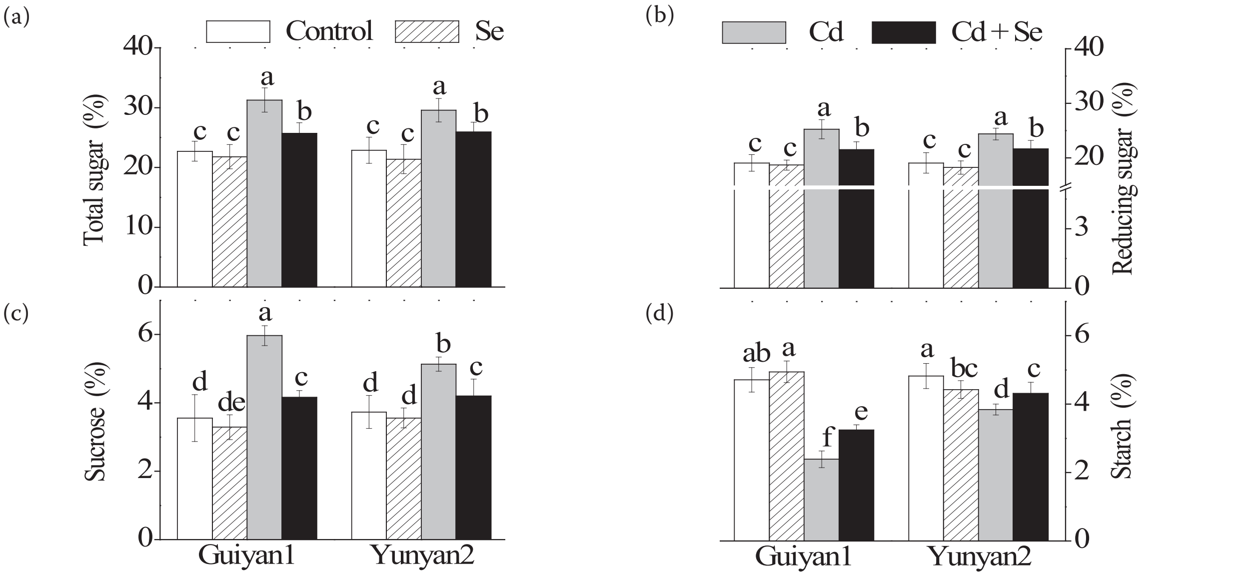

Figure 4. Effect of external selenium (Se) supply on four carbohydrates contents in dry leaves of two tobacco cvs. Guiyan1 and Yunyan2 under cadmium (Cd) stress. Error bars represent standard deviation values (n = 5), different letters indicate significant differences (P < 0.05) among the 4 treatments and within the two cultivars

of total sugar/total protein, reducing sugar/total nitrogen, reducing sugar/nicotine were prominently recovered, being 40.8, 41.2 and 28.8% higher in cv. Guiyan1 and 30.8, 32.2 and 25% in cv. Yunyan2, respectively, than those in Cd-alone treatment, and the value of total nitrogen/nicotine were recovered to the level close to control (Table 4).

DISCUSSION

Exogenous Se decreases Cd accumulation and counteracts Cd-induced changes in nutrient uptake. Se is an essential element for animals including humans. Similar to our previous study (Liu et al. 2015), Cd concentration in Cd + Se treatments was decreased compared with Cd alone treatments (Table 3), while biomass increased significantly (data not shown). As was reported by Schützendübel et al. (2001), Se and Cd could compete for the specific binding sites in carrier proteins. This can partly account for the lower Cd retention under Cd + Se treatment. The present study indicates the pronounced role of Se in protection against Cd toxicity and its potential use for reducing Cd concentration in tobacco plants.

The effect of Cd on essential elements varied with elements, which is consistent with our previous studies (Liu et al. 2015). Specifically, we found Cd stress increased Cd and reduced K, S and Mg contention in tobacco leaves (Figures 1 and 2,

Table 4. Effect of external selenium (Se) supply on comprehensive indicators of tobacco quality of two tobacco cultivars under cadmium (Cd) stress

Treatment	TS/Pro	RS/NIT	RS/NIC	NIT/NIC
Guiyan1				
Control	2.49^{d}	8.57^{d}	8.23^{e}	0.96^{a}
Se	2.37^{d}	8.71^{d}	8.14^{e}	0.93^{ab}
Cd	4.85^{a}	19.07^{a}	14.91^{a}	0.79^{c}
Cd + Se	2.76^{c}	11.12^{bc}	10.43^{c}	0.93^{ab}
Yunyan2				
Control	2.48^{d}	9.20^{cd}	9.03^{d}	0.98^{a}
Se	2.32^{d}	9.05^{cd}	8.73^{de}	0.96^{a}
Cd	4.36^{b}	17.72^{a}	14.77^{a}	0.83^{bc}
Cd + Se	3.01^{c}	12.47^{b}	11.43^{b}	0.92^{ab}
Normal level*	2~2.5	8~10	8~10	≈1

Data are mass ratios of corresponding substances. Different letters indicate significant differences (P < 0.05) among the 4 treatments and within the two cultivars. TS/Pro – total sugar/total protein; RS/NIT – reducing sugar/total nitrogen; RS/NIC – reducing sugar/nicotine; NIT/NIC – total nitrogen/nicotine. *Normal levels were quoted from Liu (2003)

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Table 1). Especially K, the main composition of cigarette ash, which determined the flammability and hygroscopicity of tobacco leaves directly. The observed decline in K concentration could partly account for Cd-induced downgrading in tobacco quality. Meanwhile, the decrease of total nitrogen content is possibly due to the inhibition effect of Cd on nitrate reductase (NR), which is the key enzyme of nitrogen metabolism (Weybrew et al. 1983). Se application obviously reversed Cd-induced changes in both micro- and macroelements, especially in the Cd-sensitive cultivar, Guiyan1 (Figure 2, Tables 1 and 3). All these results indicate that Se could alleviate Cd toxicity and enhance tobacco yield and quality through decreasing Cd accumulation and balancing the metabolism of elements.

Exogenous Se counteracts Cd-induced suppression in nicotine and total protein and carbohydrate contents. Nicotine is the source of satisfaction in the course of smoking, which has a great effect on tobacco quality and the health of smokers. The appropriate nicotine content of flue-cured tobacco was suggested to be 1.5–3.5% (Liu 2003). In our results, Cd stress significantly reduced nicotine content in both cultivars, especially in Guiyan1. This reduction might be due to the Cd-induced inhibition on the activity of plant roots, which is the main site of nicotine synthesis (Solt 1957). Meanwhile, the total protein content showed a similar reducing trend to nicotine content under Cd stress (Figure 3b). Possibly, this is the result of Cd-induced disordered nitrogen metabolism. However, compared with Cd-alone treatment, the drastic reduction of nicotine and protein content caused by Cd stress were clearly elevated by Se addition, which recovered to be close to the control levels.

Sugars content was one of the main chemical criteria, which was widely used in cigarette manufacture (Elson et al. 1972). In the current study, Cd stress induced a notable increase in total sugar, reducing sugar and sucrose content. This might be because higher sugar content could enhance the adaptive capacity in maintaining appropriate osmotic potential under Cd stress (Figures 4a–c). Meanwhile, the high sugar content in cigarette tobacco will produce a smoke of acid pH in the course of smoking. The higher sugar content in cigarette tobacco may lead to the increased risk of lung-cancer (Elson et al. 1972). Therefore, Cd-induced increases in sugar contents contributed to a significant decline in the quality of cigarette tobacco. However, all these changes were markedly recovered by the application of Se, especially in Cd-sensitive cv. Guiyan1, indicating that external Se may be beneficial to improve the quality of tobacco under Cd stress.

Exogenous Se counteracts Cd-induced changes in tobacco quality index. The ratios of total sugar/total protein, reducing sugar/total nitrogen, reducing sugar/nicotine and total nitrogen/nicotine were often used as the evaluation criteria of tobacco quality, especially the value of reducing sugar/total nitrogen and reducing sugar/nicotine. In the present study, Cd stress has a negative effect on these indexes, and the ratios of total sugar/total protein, reducing sugar/total nitrogen and reducing sugar/nicotine under Cd stress were elevated over the normal levels (Table 4), indicating that Cd stress could degrade the tobacco quality through disturbing the balance of chemical composition. On the other hand, although the values of reducing sugar/total nitrogen and reducing sugar/nicotine in Cd treatment were significantly higher than normal level, the total nitrogen and nicotine contents were still within the reasonable range. So, we assumed that Cd-induced imbalance in chemical compositions of cigarette tobacco was mainly caused by the disturbance of carbohydrate content (total sugar, reducing sugar, etc.). However, application of Se significantly diminished these adverse effects, indicating that external Se could notably improve the tobacco quality downgraded by Cd stress through balancing carbohydrate content of tobacco plants.

In conclusion, Cd stress degrades tobacco quality through disturbing the dynamic equilibrium of the main components. Supplemental Se significantly alleviates Cd-induced degradation of tobacco quality, reflected by lower Cd accumulation and better-balanced carbon and nitrogen compound contents, and counteracted Cd-induced changes in micro/macronutrients. These results suggest the beneficial effect of external Se in tobacco quality under Cd stress.

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