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Natural variation of selenium concentration in diverse tea plant (*Camellia sinensis*) accessions at seedling stage

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ABSTRACT

Selenium (Se) is a dietary essential trace element for humans and animals. Owing to Se being obtained from food or other source of supplementation, Se-enriched tea is a possible source of supplemental Se and thus has been drawing much attention. This study aimed to identify the variation of the Se concentrations among 14 elite *Camellia sinensis* cultivars against two-Se-levels supplementation at seedling stage. In the present study, the genetic diversity was assessed by 93 alleles revealed by 12 SSR primers in the *C. sinensis* accessions. Among the scored 93 alleles, 81 (87.10%) were polymorphic, and the average Nei's gene diversity (*He*) was 0.253 and average shannon information index (*I*) was 0.394, indicating a relatively high genetic variation. Significant variations were observed for the Se concentration among the accessions in both treatments ($P < 0.01$), ranging from 0.300–0.445 mg kg^{-1} and 0.357–0.537 mg kg^{-1} in control (0) and Se-supplied (4 mg kg^{-1}) treatments respectively. The quality components content, tea polyphenols and amino acid, were sensitive to Se-supplement in a few of accessions, whereas soluble sugar and aqueous extract content appeared unchanged between the contrast treatments. In addition, the genetic variation among the tea plant cultivars was not well correlated with the Se concentration. Even then, the genetic variation in Se concentration across the 14 accessions indicated the possibility of utilizing tea plant accessions to develop optimized cultivars for Se-enriched tea. For the relative high Se concentration in the tea, tea plant is supposed to be a Se-enriched species. These results may enhance our understanding the genetic variation in tea plant.

1. Introduction

Selenium (Se) is a trace element and essential to human beings and animals for its function of anti-oxidation, anti-radiation and anti-cancer etc (Martinez and Charlet, 2009). Se deficiency occurs when the intake is below a critical level and thus suffers from a biogeochemical endemic, such as Keshan disease, Kashin-Beck disease and white Myopathy (Peng et al., 1999; Zagrodzki et al., 2000). The modern medical studies showed that more than 40 kinds of human diseases are probably caused by low level of Se in blood (Rayman, 2000). Fortunately, the disease can be recovered after treatment with Se-supplement. The organic Se supplement with diet is significantly better and safer than inorganic Se supplement with drug (Navarro-Alarcon and Cabrera-Vique, 2008). Se is a key constituent of Se-dependent enzymes with antioxidant function, such as glutathione peroxidase and other selenoproteins (Rayman, 2000; Schiavon et al., 2013). Increasing the daily dietary Se intake by implementing the biofortification strategies could substantially increase Se contents in food products through plant breeding, genetic engineering, or use of Se fertilizers (Wu et al., 2015). Se-enriched green tea has higher antioxidant and prebiotic activities than regular green tea (Molan et al., 2009). According to an experiment in vitro, Wang et al. suggested that Se-containing polysaccharide should be applied as a novel selenium source in dietary supplements, with potent antioxidant properties (Wang et al., 2012). Therefore, discovering Se-containing compound is critically important for improving human health.

Clinical and preclinical studies suggested that Se intake and green tea consumption enable to decrease the risk of certain cancers, indicating Se and green tea can be used as chemopreventive agents for various cancers and epidemiological. Furthermore, the combination of Se-enriched milk protein and 0.5% green tea extraction was more effective in suppressing colorectal oncogenesis than

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either agent alone (Clark et al., 1996; Yang, 1997). The particularly benefits that Se and green tea as a combination are perhaps due to their co-administration and potential complementary mechanisms in the diet (Hu et al., 2013). Despite of this, Novotny et al. (2010) doubted the effective of Se or green tea as the agent for chemoprevention of cancer in humans based on the current clinical trial data. They indicated that it is not practicable to make selenium-enriched tea to supplement Se in human diet. The Se dispersibility of tea leaves in tea infusion correlates with that of protein and both were greatly decreased during tea processing, distinct lower Se in green tea (8.3%) and black tea (10.1%) compared with that 93.8% in fresh tea leaves (Wu and Long, 2011).

With the increasing of health care consciousness, Se-enriched tea has become a possible source of supplemental Se. In recent years, much attention has been paid to the development of Se-enriched tea by planting tea plant in the three seleniferous regions in China, Enshi Autonomous Prefecture of Hubei, Ziyang County of Shanxi and Kaiyang County of Guizhou (China environmental monitoring station, 1990). Tea polysaccharides extracted from a Se-enriched Ziyang green tea displayed noteworthy scavenging effects against $OH^{\bullet}$ and $O_2^{\bullet -}$, and high antioxidant effects in vitro, and the effects were furtherly verified by suppressing CCl_4-induced oxidative liver damage in mice at 100, 200 and 400 mg/kg BW (Wang et al., 2014). Hu et al. (2003) found that Se concentration was significantly increased, the numbers of sprouts and the yield were significantly increased, the sweetness and aroma of green tea leaves were also significantly enhanced, and bitterness was significantly decreased with the application of selenium. Additionally, the antioxidant activity of Se-enriched green tea harvested during the early spring is enhanced compared to regular tea, indicating the positive relationship between Se content and the antioxidant activity of tea extract (Xu et al., 2003).

The cultivated tea plants consist of *Camellia sinensis* with small leaf as China type, *Camellia assamica* with big leaf as Assam type and *C. sinensis* subspecies *lasiocalyx* Wight with intermediate leaf as Cambod type (Roy and Chakraborty, 2009; Mondal, 2002). For the highly heterogeneous characteristic and intercrossable without reproduction barrier, the existed natural population is a mixture of the three taxa types (Banerjee, 1992). The hybrid tea plant has been selected as cultivated cultivars by artificial selection or domesticated based on yield, quality and adaptability etc. Understanding the relationship between genetic variations and target traits for the tea plant accessions is necessary. Over the past years, intensive studies mainly focused on the genetic diversity based on the molecular markers. Currently, the widely cultivated tea plant shows rich morphological variability. The tea plant was supposed to accumulate a broad range of genetic variation for its adaptation to diverse environmental conditions in the cultivated region. A range of 0.25–4.0 mg kg^{-1} Se is a recommended level for Se-enriched tea standard and excessively intake is harmful to human beings (NY/T600-2002). However, there is no available information regarding the genetic variation of Se concentration among the main tea plant varieties. Therefore, we characterized the diversity of Se concentration in the tea leaves among a list of elite tea plant cultivars at seedling stage using a hydroponics system.

2. Materials and methods

2.1. Plant materials

A total of 14 *C. sinensis* accessions were used in the present study (Table 1). The accessions are the elite cultivars widely planted in China currently. They are locally or nationally registered cultivars with distinctive elite features of quality, yield, tolerance and distinctive phonological phase. The cutting vegetative tea seedlings were used for hydroponics experiment.

2.2. DNA Extraction

Genomic DNA was extracted from about 0.5 g young leaves of each cultivar using the CTAB method following the Huang's protocol (Huang et al., 2003). The antioxidant of $Na_2S_2O_5$ is an essential component during the extraction.

2.3. Hydroponic experiment

The uniform 10-month-old cutting seedlings of the 14 accessions were selected for the experiment on 30th march, 2013. In our previously preliminary hydroponics experiment, tea plants were cultured in a list of Se concentration, from 0 to 32 mg kg^{-1} (Figs. S1 and S2). Obviously, the plants presented Se-toxic symptoms once the Se level is up to 16 mg kg^{-1}, and slight spots were also found in the plant cultured under the 8 mg kg^{-1} Se. The plants showed best growth performance in the 4 mg kg^{-1} Se. Therefore, Se was supplied as Na_2SeO_3 at the Se concentrations of 4 mg kg^{-1}, with the treatment without Se as control. Three replicates were performed with 12 seedlings per pot containing 7 L of half strength Hoagland nutrient solution and full strength Arnon solution for each concentration and each accession. The pH of nutrient solution ranged from 4.5 to 5.0. The solution was refreshed once a week. The experiment was conducted for 8 weeks in a greenhouse with a 450–600 μEm^{-2} s^{-1} photon density flux for 12 h per day, 80% relative humidity and day/night temperature of 32/24 °C. After 8 weeks, the young and old leaves were harvested as mixture and were steamed for 1 min followed by drying at 80 °C to constant weight.

2.4. Se concentration determination

The Se concentrations in the leaf were determined after being ground to a uniform fine consistency by a plant mill. About 2.0 ± 0.001 g of fine powder was thoroughly digested with 10 mL mixture of concentrated HNO_3 and $HClO_4$ (9:1, v:v). According to the national food safety standards and the protocol for Se determination, the Se concentrations in the solution were analyzed using an atomic fluorescence spectrometer analysis instrument (AFS-610B). The parameters of the instrument were as follows: voltage being 230 V, the HCL main and auxiliary cathodic currents being 60 and 0 mA, the flow rate of carrier gas being 700 mL min^{-1}, the atomizer being room temperature and atomization style being flame atomic absorption, both the sampling and injection pump speed being 100 r min^{-1}, sampling and injection time being 8 and 26 s, respectively.

2.5. SSR loci genotyping

A total of 12 SSR primers were listed in Table 2. PCR amplifications were performed in 20 μL reaction mixture, containing 2.0 μL 10 × PCR buffer ($+Mg^{2+}$), 0.4 μL dNTP (10 mM in total), 1.0 μL each primer (10 mM), 0.5 U Taq polymerase and 50 ng genomic DNA as PCR template. The thermocycling conditions were touchdown PCR and was as follows: initial denaturation at 94 °C for 3 min; 10 cycles of 30 s at 94 °C, 30 s for the unique annealing temperature with −1 °C/cycle, and 30 s at 72 °C; 26 cycles of 30 s at 94 °C, 30 s for the unique annealing temperature, and 30 s at 72 °C; final extension at 72 °C for 7 min to polish the ends of the PCR products. For the first 10 cycles, the initial annealing temperature should be 2–5 °C above the estimated Tm of the primers. The annealing temperature was then gradually decreased until it reached the temperature below the calculated annealing temperature of the primers.

PCR products were resolved by 8% polyacrylamide gels and visualized by silver staining. Each polymorphic band was scored as '1' for presence and '0' for absence to generate a binary data matrix. The binary data was used to calculate the genetic similarity