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RESEARCH ARTICLE

Evaluation of selenium and carotenoid concentrations of 200 foxtail millet accessions from China and their correlations with agronomic performance



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Abstract

As selenium and carotenoids are essential micronutrients, the determination of their concentrations in different varieties is important in the breeding of foxtail millet (*Setaria italica* L. P. Beauv.). To identify selenium- and carotenoid-enriched foxtail millet varieties and to analyze correlations between trace elements and agronomic traits, we measured the selenium and carotenoid concentrations of 200 Chinese accessions by high-performance liquid chromatography and atomic fluorescence spectrometry. Our analysis revealed that lutein concentration in 200 foxtail millet accessions followed normal distribution and average was $3.1 \mu\text{g g}^{-1}$. The mean value of zeaxanthin concentration in 200 accessions was $8.6 \mu\text{g g}^{-1}$. Lutein and zeaxanthin concentrations were higher in the foxtail millet from Liaoning than in varieties from other locations, with averages of 10.0 and $3.5 \mu\text{g g}^{-1}$, respectively. The average measured selenium concentration was $100.3 \mu\text{g kg}^{-1}$. The highest average selenium concentration, $110.3 \mu\text{g kg}^{-1}$, was found in varieties from Shanxi. Varieties from Inner Mongolia had the lowest average selenium concentration, $84.7 \mu\text{g kg}^{-1}$, which was significantly lower ($P < 0.05$) than that of Shanxi. Selenium concentrations of 23 varieties were higher than $117.9 \mu\text{g kg}^{-1}$, accounting for 11.5% of the total, thereby were considered to be enriched in selenium. In addition, we identified 29 lutein-enriched varieties ($>4.27 \mu\text{g g}^{-1}$) and 30 zeaxanthin-enriched ones ($>12.63 \mu\text{g g}^{-1}$), which corresponded to 14.5 and 15% of tested accessions, respectively. Correlation analysis revealed that selenium concentration was significantly positively correlated with spikelet length ($P < 0.01$), while zeaxanthin concentration was significantly correlated with grass weight ($P < 0.05$) and spikelet length ($P < 0.01$). No correlation was found between lutein concentration and agronomic characters, selenium content or zeaxanthin content. Our results should contribute substantially to the selection of suitable varieties for the development of plants with desired levels of these nutritionally important elements. These results will significantly contribute towards selection of the most suitable varieties for obtaining plants with desired levels of these nutritionally important elements.

Keywords: foxtail millet varieties, selenium, carotenoids, HPLC, correlation analysis

1. Introduction

Foxtail millet (*Setaria italica* L. P. Beauv.) is an ancient crop originated in China (Crawford 2009) and distributed widely in warm and temperate regions of Asia, Europe, North America, Australia, and North Africa, used for grain and

Received 14 May, 2015 Accepted 14 August, 2015
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doi: 10.1016/S2095-3119(15)61160-1

forage. It was domesticated 4 100 years ago and formed abundant germplasm resources through long-time natural and artificial selection (Dong and Zheng 2006). Now more than 27 000 foxtail millet accessions were conserved in the National Center for Crop Germplasm Conservation of China (Lu 2006; Li *et al.* 2009a). After decortication, millet is rich in protein and crude fat and is used as a staple food in China. Millet has seven essential amino acids whose contents are higher than those in other crops, this is particularly true for methionine and tryptophan, which are important for the prevention of atherosclerosis and for soft of blood vessel (Mao *et al.* 1997; Wang *et al.* 2001). Trace elements and vitamin are also abundant in millet, for example, mean vitamin E and carotenoid contents can reach up to 31.36 and 0.19 mg 100 g⁻¹, respectively (Shi *et al.* 2007). Recent advances in *Setaria* genomics appear promising for genetic improvement of cereals and biofuel crops towards providing multiple securities to the steadily increasing global population (Muthamilarasan and Prasad 2015).

Selenium is an essential trace element for humans. Medical research has confirmed that long-term inadequate intake of selenium may be primarily responsible for many human diseases (Ray *et al.* 2006; Navarro-Alarcon and Cabrera-Vique 2008). In China, Keshan and Kashin-Beck diseases have been confirmed as selenium deficiency diseases. In addition, selenium deficiency is associated with cancer, cardiovascular and cerebrovascular diseases, acquired immune deficiency syndrome (AIDS), diabetes, and 40 other diseases (Donaldson 2004; Silvera and Rohan 2007; Zhou and Diamond 2009). Because sufficient intake of selenium can enhance human resistance to these diseases, interest is increasingly focusing on the breeding of foxtail millet varieties and lines with high selenium content.

Carotenoids are bioactive substances found in fruits and vegetables. These compounds, function as vitamin A precursor in humans (Krinsky 1989; Matsuno 1991) and may supply 70 and 30% of vitamin A demands in developing and developed countries, respectively (Olson 1987; Ge *et al.* 1995). Numerous studies have demonstrated that all carotenoid components have antioxidant properties; they are able to quench free radicals and prevent the oxidation of low-density lipoprotein (Oshima *et al.* 1996) and further can reduce the cancer incidence (Peto *et al.* 1981; Rao and Rao 2007). Lycopene has been shown to lower blood pressure and to reduce the incidence of coronary heart disease (Iribarren *et al.* 1997; Dwyer *et al.* 2001; Sesso *et al.* 2004). Lutein and zeaxanthin are the main pigment components of Bozzi's foramina, lutein can be used for the cataract prevention (Handelman 2001) and zeaxanthin can prevent eye injury (Sperling *et al.* 1980; Ma and Lin 2008). With the discovery of their human health benefits,

the demand and consumption of carotenoids are rising (Sun *et al.* 2007). An increasing number of studies are focusing on the composition of crop germplasm, such as in rice, wheat and maize (Wang F M *et al.* 2009; Wang F *et al.* 2012; Zhou *et al.* 2009). Researchers are also seeking to identify varieties with high carotenoids content for quality breeding. Carotenoids are abundant in foxtail millet and can be safely used to color candy, food and beverages (Wang *et al.* 2001). Measurement of yellow pigment content of 102 foxtail millet varieties (lines) from north Chinese cultivation areas revealed that millet quality was positively correlated with the content of that pigment (Wang *et al.* 2001). In the current study, we selected 200 foxtail millet accessions from 10 Chinese provinces representing major cultivation areas. The seeds were planted and grown under identical cultivation conditions. The seeds were used to test the concentrations of selenium, lutein and zeaxanthin, and thereby to explore the genetic variation among different varieties of foxtail millet resources, to provide a theoretical basis for the breeding of new varieties with high selenium levels and the development of functionally healthy food.

2. Materials and methods

2.1. Plant materials

200 accessions, including important varieties and new breeding lines, were supplied by the National Center for Crop Germplasm Conservation of China. Their origins are listed in Table 1.

Seeds of these accessions were grown side by side during the rainy season of 2010 at the experimental station of the Institute of Crop Science, Chinese Academy of Agricultural, Beijing, China. Mature grains (>60 d after anthesis) were harvested, surface-sterilized, morphologically characterized and then stored at -30°C for later analysis.

2.2. Detection of selenium concentration in varieties

Selenium concentration was detected according to the GB/T 5009.93-2003 standard protocol. Dried samples of foxtail millet grains were ground with a grinding mill. Approximately 0.5 g of each ground sample was then digested in 10 mL of 4:1 HNO₃:HClO₄ at 150°C for 2 h. Once the digested solution has colorized, and cooled, 5 mL of 50% aqueous HCl was added to the digested solution to reduce Se⁶⁺ to Se⁴⁺. After 30 min, when the sample was completely mineralized, the solution was transferred quantitatively to a 50-mL volumetric flask. The selenium concentration was determined by atomic fluorescence spectrometry (AFS-2202a; Beijing Jitian, Beijing, China) under the following conditions: 300 V,

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