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SELENIUM CONCENTRATIONS IN LENTIL SEEDS FROM BENGAL - GANGETIC PLAINS (INDIA) INCLUDING ARSENIC-CONTAMINATED ZONES

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ABSTRACT

Seed selenium (Se) concentrations of 22 genotypes (21 cultivars and one local check) of lentil (*Lens culinaris* Medik.), grown in different parts of West Bengal (India) including arsenic-affected areas, were estimated. Se content varied significantly in genotypes with highest (167 µg Se kg^{-1}) and lowest (9 µg Se kg^{-1}) content in cv. 'Pusa 4' and 'VL Massor 44', respectively. The 9 genotypes grown in arsenic-prone areas showed deficient Se levels. However, 'Pusa 1', 'Pusa 6', 'JLS 1', 'Sehore 74-3', 'VL Masoor 44' and 'VL Masoor 103' exhibited increased Se levels when grown outside the As-contaminated zones, indicating possible inhibition of Se uptake by As. Marginal variations in Se content in genotypes 'B 17', 'PL 406' and 'S 256' between As-contaminated and non-contaminated areas suggested genotype-dependent accumulation of Se.

Key words: Arsenic, lentil, nutritional improvement, seed, selenium.

INTRODUCTION

Selenium (Se) is vital in global public health systems as it is involved in intra- and extra-cellular glutathione peroxidases, iodothyronine deiodinase, thioredoxin reductase and in many seleno-proteins. In all these cases Se is present as amino acid selenocysteine which forms the part of catalytic active site of enzyme. Se-containing compounds have been recognized as key components of enzymes, antioxidant compounds and hormones (Rayman, 2002). Se has 3 levels of biological activity viz., trace amounts required for normal growth and development, moderate concentrations stored to maintain homeostatic functions and elevated concentrations inciting toxic effects (Hamilton, 2004). Se plays role in antioxidative mechanisms of plants (Ekelund and Danilov, 2001). Se at level of 0.1 mg kg^{-1} plant weight delayed senescence and promoted the growth of ageing in lettuce (*Lactuca sativa*) and ryegrass *Lolium perenne* seedlings (Xue *et al*., 2001; Germ *et al*., 2007). Daily intake of <55 µg Se has serious health implications such as juvenile cardiomyopathy (Keshan disease) as reported from China and arsenicosis in Bangladesh and West Bengal (India) (Combs, 2001; Spallholz *et al*., 2004).

Lentil (*Lens culinaris* Medik), second only to chickpea among the food legumes grown in India, is predominantly consumed as *dhal*, a thick soup made from whole grain or split pulse. Worldwide most lentil production occurs under Mediterranean winter conditions (Middle East, Mediterranean region, Australia, etc.), during dry season in north Indian sub-tropical savannah and under temperate summer climates in North America. The eastern half of Indo-Gangetic plain of South Asia including India, Nepal and Bangladesh share 32% lentil production (FAOSTAT, 2008). In India, lentil is mostly grown in Madhya Pradesh, Uttar Pradesh, Bihar and West Bengal and as winter annual crop in Assam, Uttarakhand, Jammu and Kashmir, Chhattisgarh, Maharashtra, Punjab, Rajasthan and Haryana (Kumar *et al*., 2009). India which was leading lentil producing country till 2008-09 has slipped to 2nd position (FAO STAT, 2008). Bioaccumulation and transport of arsenic (As) in various genotypes of lentil grown in Bengal-Gangetic plains has recently been reported (Talukdar, 2013). Se biofortification in lentil has been recognized as a measure to prevent arsenicosis in Bangladesh and some parts of West Bengal (Spallholz *et al*., 2004). The protective effect of Se is due to the formation of a novel arsenic-selenium compound, selenobis (*S*-glutathionyl) arsinium ion in erythrocytes which is then excreted in bile. The As-Se species is presumed to be a mechanism by which body system eliminates As that necessarily involves the loss of one atom of essential Se for every atom of As excreted (Thavarajah *et al*., 2008). A global survey on lentil genotypes has revealed large-scale variations in Se contents in seeds (Thavarajah *et al*., 2011). Kumar and Krishnaswamy (1997) observed locational differences in Se content of green gram. However, no information is available on Se concentrations in seeds of Indian lentil genotypes/cultivars grown in Gangetic plain of West Bengal. Considering the importance of Se in mineral biofortification of lentil crops particularly in As-contaminated regions of West Bengal, the present investigation was carried out to analyze Se content in lentil seeds in As-contaminated districts of West Bengal (India).

MATERIALS AND METHODS

Fresh apparently healthy seeds of 21 lentil cultivars (Pusa 1, Pusa 4, Pusa 6, PL 234, PL 406, PL 639, C-31, C-77-12, JLS 1, C 3, DPL 62, DPL 15, LH 84-8, B 17, B 177, B 235, S 256, S 177, Sehore 74-3, VL Masoor 44 and VL Masoor 103) along with a check (Nadia Local 1) were collected from Pulse and Oilseed Research Station, Berhampore (Murshidabad), West Bengal and local seed nurseries/ farmer's fields in Nadia (Chakdaha and Kalyani), Hooghly, Baruipur, 24 Pargana (N), and South Dinajpur (Gangarampur block) districts in West Bengal (India). The whole Chakdaha block and outskirts of Kalyani, Baruipur and several parts of Murshidabad district including pulse growing areas are severely As-affected with soil As ranging from 10.19 (in Kalyani) to 34.56 mg kg^{-1} (in Baruipur) (Bhattacharyya *et al*., 2010; Talukdar, 2013b). Cultivars 'Pusa 1', 'Pusa 6', 'JLS 1', 'B 17', 'PL 406', 'Sehore 74-3', 'S 256', 'VL Masoor 44' and 'VL Masoor 103' are grown sporadically during winter in these areas. The Gangarampur area of Dinajpur district is not a part of Gangetic West Bengal but represents sub-Himalayan plain of North Bengal. This region and Hooghly district are not generally considered As-prone areas (soil As 0.03-0.18 mg kg^{-1}) (Talukdar, 2013b). Except local check, all genotypes were advanced breeding lines developed by IARI (New Delhi), IIPL (Kanpur) and PORS (West Bengal) released for cultivation in Eastern and Northern India. The local check cultivar is well adapted to the agro-climatic conditions of Gangetic West Bengal (tropical summer monsoon, humid winter with 22/14°C and clay-alluvial soil with pH 7.0). After collection each cultivar was raised in various farmer's fields at above selected study sites following 'national test guidelines of lentil' (Protection of Plant Varieties and Farmer's Rights Authority, Govt. of India, 2007) with little modifications [Test plot design; number of rows, 6; row length, 5 m; plant spacing, 30 x 15 cm; replication, 4 with 200 plants $replication^{-1}$ for each accession] in soil pH 6.8-7.2 (Gangetic Bengal) and 6.2 (South Dinajpur); soil type, clay-alluvial for Gangetic Bengal and sandy-alluvial for South Dinajpur; sowing period, Nov.-Dec. as winter rabi crop, mild irrigation during flowering]. Fresh seed samples were taken from bulked harvest of each cultivar for analysis of Se content.

Total Se content in lentil seeds was measured following a HNO_3-H_2O_2 digestion (Lintschinger *et al*., 2000; Thavarajah *et al*., 2008) with some modifications. The chemicals used were high purity/ analytical grade. Lentil seeds were dried carefully on hot plates and finely powdered (<0.5 mm sieve). Seeds (200 mg) were placed in a digestion tube with 3 ml conc. HNO_3. The tubes were heated to 70-80°C for digestion and 0.5 ml of 30% H_2O_2 added. The resulting solution was vortexed for 3 min. After digestion, the sample solutions were diluted with Millipore water to a known volume and samples reduced with 6 M HCl at 70°C for at least 10-12 min. in a water bath. The total Se content was determined using an atomic absorption spectrophotometer equipped with a flow-injection hydride generator ((FI-HG-AAS, Perkin Elmer Analyst 400). Measurements of total Se were validated using NIST (NIST® USA) standard reference material (tomato leaves; item number 1573a). Total Se content of dried tomato leaves as measured (n = 10) by above method was 0.059 ± 0.009 mg kg^{-1} which showed good correlation (r = 0.886 at $P_{0.05}$) with certified NIST value (0.054 ± 0.003 mg kg^{-1}). The experiment design for total Se concentration was a randomized complete block design with four replicates for each of cultivar. Multiple comparisons of means were carried out by analysis of variance using the SPSS software (SPSS, USA, v 10).

RESULTS AND DISCUSSION

Se content in lentil seeds varied significantly among the 22 genotypes studied which ranged from 9 to 167 $\mu g\ kg^{-1}$ with cv. 'Pusa 4' having highest Se content closely followed by 'Pl 234', 'JLS 3', 'Pl 639', 'C-77-12' and 'DPL 15' (Table 1). The other genotypes had moderate to low Se content. Interestingly, all the 9 genotypes grown in As-contaminated areas exhibited low level of Se content in their seeds. Among these, 'VL Massor 44' possessed less and 'Pusa 1' high Se amounts. Rest 7 genotypes showed a range of 23-58 μg Se kg^{-1}. Remarkably, Se concentrations increased significantly when 'Pusa 1', 'Pusa 6', 'JLS 1', 'Sehore 74-3', 'VL Masoor 44' and 'VL Masoor 103' were grown outside As-contaminated zones. Rest 3 genotypes namely 'B 17', 'PL 406' and 'S 256' showed marginal variations in seed Se content in non-arsenic contaminated areas from that of As-contaminated areas. The study revealed huge variation in seed Se contents not only in non-arsenic prone areas but also in arsenic-contaminated localities, although Se levels reduced in later case. The soil Se content at each location was not analyzed, however earlier studies have shown soil Se significantly affects Se concentration in different food crops (Spallholz *et al.*, 2004; Lyons *et al.*, 2005). Regions such as the Indo-Gangetic plain possess varying Se levels in soils (Thavarajah *et al.*, 2011) which presumably is one of the reasons for variations in seed Se content in 22 genotypes studied. Similar observation was reported in earlier global trials on lentil genotypes grown in soil deficient in Se (Morocco, USA and Turkey) or

Table 1: Selenium concentration in seeds of lentil genotypes including those grown in arsenic-contaminated areas of Gangetic West Bengal

Genotypes	Trial period	Cultivated area	Se content ($\mu g\ kg^{-1}$ seed)[a]	Se content in 9 genotypes grown in As-prone areas[c] ($\mu g\ kg^{-1}$)
Pusa 1[c]	2008-10	Murshidabad, Kalyani	89 ± 0.08	69 ± 0.10[b]
Pusa 4	2008-10	Murshidabad, Kalyani	167 ± 0.09	ns
Pusa 6[c]	2009-10	Murshidabad, Chakdaha	58 ± 0.04	44 ± 0.08[b]
PL 234	2008-10	Nadia	158 ± 0.11	ns
PL 406[c]	2008-10	Nadia, Hooghly	56 ± 0.07	57 ± 0.10
PL 639	2009-10	Nadia	133 ± 0.12	ns
C-31	2008-10	Hooghly	29 ± 0.09	ns
C-77-12	2009-10	Chakdaha, Kalyani	107 ± 0.06	ns
JLS 1[c]	2008-10	Chakdaha, Kalyani	13 ± 0.04	23 ± 0.08[b]
JLS 3	2008-10	Chakdaha, Kalyani	150 ± 0.16	ns
DPL 62	2009-10	Hooghly, Dinajpur (D)	38 ± 0.06	ns
DPL 15	2009-10	Hooghly, Dinajpur (D)	97 ± 0.03	ns
LH 84-8	2008-10	Murshidabad	90 ± 0.05	ns
B 17[c]	2008-10	Beldanga	33 ± 0.02	34 ± 0.08
B 177	2008-09	Beldanga, Bhabta	41 ± 0.08	ns
B 235	2008-10	Nadia, Murshidabad	42 ± 0.10	ns
S 256[c]	2008-09	Nadia, Murshidabad	48 ± 0.03	46 ± 0.05
S 177	2008-09	Nadia, Murshidabad	55 ± 0.10	ns
Sehore 74-3[c]	2008-09	Baruipur, 24 pgs (s)	71 ± 0.21	53 ± 0.17[b]
VL Masoor 44[c]	2009-10	Nadia, Murshidabad	26 ± 0.08	09 ± 0.11[b]
VL Masoor 103[c]	2009-10	Nadia, Murshidabad	65 ± 0.19	39 ± 0.12[b]
Local check	2008-10	Nadia, Murshidabad	67 ± 0.20	38 ± 0.18[b]
$CD_{0.05}$	-	-	6.18	3.57

[a]Measurements from non-As contaminated areas of different districts of West Bengal; ns - not studied; [b]Significantly differ from the values taken from plants grown in non-As contaminated soils; [c]9 genotypes grown in both As contaminated and non-contaminated soils. *genotypes differed significantly by ANOVA at $P_{0.05}$.

rich in Se (Canada) (Thavarajah *et al*., 2011). However, the soil Se factor may not be the only reason for significant variations of Se levels as plant uptake system for soil Se might have played an important role in decreased Se level in arsenic-prone zones and its enhancement in low arsenic-contaminated areas. Arsenic is known to inhibit growth of legume plants by inducing oxidative damage (Talukdar, 2011a, 2012c) through conversion of arsenate to highly toxic arsenite within plant body (Mascher *et al*., 2002). Se is known to impede the toxicity of metalloid arsenic by inhibiting oxidative damage and also has the capacity to enhance accumulation of As in hyper-accumulator ferns as a concentration-dependent manner (Srivastava *et al*., 2009). Tolerance of plants to metal toxicity as well as response of antioxidant capacity by exogenous application of Se was reported in other plants also (Hartikainen, 2005; Hajiboland and Amjad, 2007; Hasanuzzaman *et al*., 2012). The present study revealed inhibitory effect of high soil As to acquire soil Se in 6 genotypes grown in As-contaminated areas. By contrast, non-significant changes of seeds Se content of 3 genotypes such as B 17, PL 406 and S 256 grown in both arsenic-prone and low soil As (0.18 mg As kg^{-1} soil) areas strongly indicated genotype-dependent accumulation of Se.

Legumes including lentils are rich source of various types of flavonoids and antioxidants, but at the same time is highly sensitive to diverse types of abiotic stress including heavy metals, salinity and temperature and drought (Chakraborty and Pradhan, 2010; Talukdar, 2011a-c, 2012ab). Among the sixty or so pulse crops consumed by humans, lentil is unique in that as its consumption is increasing 3-4 times faster than growth of human population, likely due to the convenience factors of cooking characteristics and easily digestable protein-rich pulse (Thavarajah *et al*., 2011; Talukdar and Talukdar 2012). This makes lentil an ideal crop to promote improved nutrition through whole food consumption on a global scale. However, in view of importance of Se in human health and nutrition, the declining level of seed Se content in genotypes grown in high arsenic prone regions of Gangetic West Bengal (India) is highly alarming. The results also have high relevance for Bangladesh also, as these regions have large lentil growing lands and high consumption rate. The biofortification of lentil for Se and its genetic improvement may be an important global as well as regional strategy in preventing malnutrition particularly in view of onset of arsenicosis in arsenic-contaminated areas throughout the world.

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