# Selenium Biofortification in Lentil

# (*Lens culinaris* Medikus subsp. *culinaris*)


THE UNIVERSITY OF
WESTERN AUSTRALIA

**Md. Mahmudur Rahman**
B.Sc. in Agriculture
M.S. in Genetics and Plant Breeding
Bangladesh Agricultural University, Bangladesh

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## Statement of candidate contribution

The work presented in this thesis does not contain any material that has been accepted for award of any other degree or diploma at any university.

I declare that this thesis is my own work and was not written by another person except where acknowledgement is made in the text. This thesis contains material previously published in the journal of "Food Research International" 2013, volume 54, issue 2 and "The Journal of Agricultural Science", Cambridge (in press).

The above publication pertaining this thesis was written by me and the co-authors were involved in the experimental design and management, discussion of results, structure of the papers and editorial comments to finalise them. All contributions made by other individuals have been duly acknowledged.

Md. Mahmudur Rahman

## Abstract

A low concentration of micronutrients in the diet causes micronutrient malnutrition globally, increases mortality and morbidity rates and reduces the quality of life. Dietary diversification, nutrient supplementation and food fortification are effective against micronutrient malnutrition but have limited success in rural regions of developing countries due to poverty and food habits. Biofortification, an agriculture-based approach, can reduce micronutrient malnutrition especially in rural areas of developing countries.

Biofortification is the production of micronutrient-dense crops by means of agronomic management and/or plant breeding. Recent biofortification programs have focused on iron, zinc and vitamin-A. Yet there are other important elements that need attention such as selenium (Se). Selenium is a constituent of selenoproteins, enzymes and anti-oxidants. Globally over one billion people suffer from Se deficiency. Lentil may be an effective vehicle to supply dietary Se to affected populations. This study to design a biofortification strategy for lentil used a baseline survey in farmers' fields in Bangladesh and designed field experiments in Bangladesh and Australia to unravel genotypic and environmental effects on seed Se concentration and to evaluate foliar Se application.

A farmers' field survey and genotypic evaluation experiment of seven advanced breeding lines at four locations were conducted in Bangladesh during 2010–11 to determine Se concentration in lentil. Total Se concentration was measured in soil and lentil seeds. Mean of soil and lentil seed Se concentration in farmers' fields was 163 µg/kg and 312 µg/kg, respectively, with the highest being 173 µg/kg and 370 µg/kg in Rajshahi division, a major lentil growing area in Bangladesh. There were significant genotype and location differences observed for seed Se, Se yield, and seed yield.

However, the genotype × location interaction was not significant for seed Se concentration.

Australian lentil is grown during wet-winter and a relatively longer growing period compared to the shorter growing warmer environment in Bangladesh. Australian lentil is also exported to many countries in South Asia, the Middle East and Europe with populations having low dietary Se concentration. A Se foliar application experiment at two locations and genotypic evaluation experiment of 12 genotypes at seven locations were conducted from April to December 2011 in South Australia and Victoria, Australia. Preliminary screening of 12 diverse germplasm accessions having five common genotypes with the Se foliar application and the genotypic evaluation experiment for response to Se foliar application was also conducted during July to December 2012 in the glasshouse at The University of Western Australia. Foliar application of a total of 40 g/ha of Se as potassium selenate ($K_2SeO_4$) - 10 g/ha during full bloom and 30 g/ha during the flat pod stage - increased seed Se concentration from 201 to 2772 µg/kg, but had no effect on seed size or seed yield. With water and Se foliar application cultivars PBA Herald (238 vs 3327 µg/kg) and PBA Ace (239 vs 2957 µg/kg) had high seed Se concentrations. In the genotypic evaluation experiment, a significant genotype and location effect was observed for seed Se concentration, but the interaction effect was non-significant as found in Bangladesh. In the germplasm screening experiment in glasshouse, seed Se concentration increased significantly with Se application over water application and there was also significant genotypic variation for Se uptake. The results from the glasshouse experiment were consistent with Se foliar application experiment in the field in Australia.

In summary, foliar application of Se is an efficient approach to improve seed Se concentration in lentil. Consumption of 20 g of lentil from Bangladesh will supply 11%

of the recommended daily allowance (RDA) of Se, whereas 20 g of biofortified Australian lentil will supply 100% of the RDA of Se. With clear genetic differences in Se uptake in lentil exhibited, there is scope to breed the crop for an improved Se seed content.

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## Publications arising from this thesis

### Journal Articles

Chapter 3

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Chapter 4

**Rahman, M.M.**, Erskine, W., Materne, M.A., McMurray, L.M., Thavarajah, P., Thavarajah, D. and Siddique, K.H.M. (2014). Enhancing selenium concentration in lentil (*Lens culinaris* subsp. *culinaris*) through foliar application. *The Journal of Agricultural Science*, *Cambridge* (Accepted).

### Conference Proceedings

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### Poster presentation

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# Chapter 1

# General Introduction

Micronutrient malnutrition affects more than half of the world's population (Mayer *et al.* 2008) reducing livelihoods and the quality of life. The main cause of micronutrient malnutrition is a poor quality diet with low concentrations of essential human nutrients. Dietary diversification, nutritional supplementation and food fortification are effective in reducing micronutrient malnutrition. However, in rural areas of developing countries, these methods are of limited effectiveness due to poverty and food habits. Biofortification is a new agriculture-based approach to help reduce micronutrient malnutrition especially in rural areas at minimal cost.

Recent biofortification has mainly focussed on iron (Fe), zinc (Zn) and vitamin A deficiency. Some of the developments include a Fe-rich common bean (*Phaseolus vulgaris* L.) variety in Congo and Rwanda (HarvestPlus 2013), Fe-rich rice (*Oryza sativa* L.) in Myanmar (Aung *et al.* 2013), Zn-rich rice in Bangladesh (The Daily Star 2013) and vitamin-A-rich cassava (*Manihot esculenta* Crantz) in Congo and Nigeria (HarvestPlus 2013). There are however other essential nutrients that can be deficient in humans. For example, selenium (Se) is essential to humans and more than one billion people suffer from Se deficiency (Lyons *et al.* 2003). Se deficiency occurs from low Se concentrations in food caused by low soil Se. Soil Se deficiency is reported in New Zealand, Australia, UK, Thailand, Denmark, Finland, central Siberia, northeast to south central China, Turkey, parts of India, Nepal and Bangladesh (Fordyce 2005; Lyons *et al.* 2005; Spallholz *et al.* 2004; Spallholz *et al.* 2008b), which is reflected in low Se levels in local diets. Agronomic biofortification by soil application of Se-enriched fertilizer in Finland significantly increased soil Se, food Se and human plasma Se (Varo *et al.* 1988). In field experiments, after foliar Se application, seed Se concentration significantly increased in wheat (*Triticum aestivum* L.) (Broadley *et al.* 2010; Ducsay *et al.* 2007), maize (*Zea mays* L.) (Cary and Rutzke 1981), barley (*Hordeum vulgare* L.) (Sima and Gissel-Nielsen 1985), rice (Fang *et al.* 2008; Hu *et al.* 2002), soybean

(*Glycine max* L.) (Djanaguiraman *et al.* 2005; Yang *et al.* 2002a), pea (*Pisum sativum* L.) (Smrkolj *et al.* 2005a), common bean (Smrkolj *et al.* 2007), buckwheat (*Fagopyrum esculentum* Moench) (Smrkolj *et al.* 2006; Stibilj *et al.* 2004) and pumpkin (*Cucurbita pepo* L.) (Smrkolj *et al.* 2005b; Stibilj *et al.* 2004).

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is consumed widely in many Se-deficient countries. The crop is rich in protein and several micronutrients making it a potential vehicle for Se biofortification. The objectives of this thesis are to:

1. Study the variation of Se concentration in soil and lentil seed grown under Bangladeshi and Australian environments.
2. Study the genotype and environment interaction of seed Se concentration.
3. Study the effect of Se foliar application on seed Se concentration.
4. Study the genetic variation in lentil germplasm for seed Se concentration.

The thesis has five chapters:

**Chapter 1:** General introduction

**Chapter 2:** The Literature review critically looks at the state of knowledge and research gap in the field of biofortification especially on Se.

**Chapter 3: ‘**Selenium biofortification in lentil (*Lens culinaris* Medikus subsp. *culinaris*): Farmers’ field survey and genotype × environment effect’; investigated the variation of total Se concentration in lentil seeds grown in Bangladeshi farmers’ fields. The chapter also explored genotype × environment interaction of Se concentration in lentil seeds and the variation of Se concentration in lentil-growing soils in Bangladesh. (The manuscript from this chapter was published on 2013 in *Food Research International, 54*(2), 1596–1604).

**Chapter 4:** 'Enhancing selenium concentration in lentil (*Lens culinaris* subsp. *culinaris*) through foliar application'; evaluated the effect of Se foliar application on lentil seed size, seed yield and seed Se concentration at two contrasting locations in Australia. It also assessed the genotypic variability among 12 lentil germplasm lines in a glasshouse experiment and reports the genotype × environment interaction for seed Se concentration at seven locations. (Part of this chapter has been accepted in *The Journal of Agricultural Science*, *Cambridge*).

**Chapter 5:** 'General discussion' deals with the key findings from the research in this thesis and their implications for future research.

This thesis is compiled from individual published/accepted journal articles. I have endeavoured to minimise repetition of text but some repetition between chapters was unavoidable.

# Chapter 2

# Literature Review

## 2.1 Introduction

Micronutrients are required by humans throughout life in small quantities for growth, development and physiological functions. They include dietary trace elements in amounts generally less than 100 mg/day (O'Dell and Sunde 1997). The micro-nutrients or trace elements include iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc and molybdenum. Micronutrients also include vitamins, which are organic compounds required as nutrients by humans (Bender 2009). This chapter outlines the translation of research findings on how to combat with micronutrient malnutrition especially by biofortification, the importance of Se as an essential micronutrient, agronomic biofortification of Se in crops, and how lentil may be used as a potential vehicle for Se biofortification.

## 2.2 Global micronutrient malnutrition

The limited supply of a micronutrient that hampers normal body function is called micronutrient malnutrition ("hidden hunger") and is an important issue globally especially in developing countries. Unfortunately, micronutrient malnutrition affects more than half of the world population (Mayer *et al.* 2008). Micronutrient malnutrition significantly increases mortality and morbidity rates, diminishes cognitive abilities of children and lowers their educational attainment, reduces labour productivity, stagnates national development efforts, and reduces the livelihood and quality of life for all those affected (Combs *et al.* 1996; Combs and Welch 1998; Welch *et al.* 1997; Welch and Graham 1999).

### 2.2.1 Causes of micronutrient malnutrition

The major cause of micronutrient malnutrition is a poor quality diet, characterized by a high intake of staple foods, but low consumption of animal and fish products, fruits, pulses and vegetables, which are rich sources of bioavailable minerals and vitamins

(Bouis *et al.* 2012). Most malnourished people are poor and cannot afford to purchase high-quality, micronutrient-rich foods or cannot grow these foods themselves (Bouis *et al.* 2012).

### 2.2.2 Coping with micronutrient malnutrition

The micronutrients we need come from food. Low concentrations of micronutrients in food and lack of dietary diversity are the main causes of micronutrient malnutrition. Addressing micronutrient malnutrition includes dietary diversification, mineral supplementation and food fortification with micronutrients (White and Broadley 2009). However, due to poverty and food habits, micronutrient-deficient people in the developing world have limited access to the results of these approaches. Biofortification, which uses agricultural techniques to enhance the micronutrient content of staple foods, is a new and complementary approach to address micronutrient malnutrition (Mayer *et al.* 2008).

#### *Dietary diversification*

No single food contains all the necessary vitamins and minerals, and therefore a micronutrient-rich, balanced and varied diet is necessary for adequate intake of micronutrients including fruits, vegetables and meat (Stein *et al.* 2005). Attention needs to be paid to ensuring adequate intakes of oils and fats to enhance the absorption of the limited supplies of micronutrients (Allen *et al.* 2006).

#### *Supplementation*

Supplementation is the intake of relatively high doses of micronutrients, usually in the form of pills, capsules or syrups (Allen *et al.* 2006). Supplementation can supply an optimal amount of a specific nutrient or nutrients in a highly absorbable form, and is often the fastest way to control deficiency in individuals or population groups that have been identified as being deficient (Allen *et al.* 2006). For example, in developing

countries, supplementation programmes have been commonly used to provide Fe and folic acid to pregnant women and vitamin A to infants and children under 5 years of age (Allen *et al.* 2006). Supplementation is a short-term solution to micronutrient malnutrition. It requires an effective distribution system, procurement, and purchasing the micronutrient in a relatively expensive pre-packaged form. It is not readily available in rural areas where the most vulnerable people live. Once supplementation is abandoned, micronutrient malnutrition soon returns (Allen *et al.* 2006).

***Food fortification***

Food fortification is the addition of a micronutrient(s) to foods during the processing of a particular food to increase the intake of micronutrients in order to correct or prevent a deficiency (Allen *et al.* 2006). Fortified food can supply adequate amounts of a nutrient and help maintain body-stored nutrients effectively. As examples, deficiencies of vitamins A and D, some B vitamins (thiamine, riboflavin, and niacin), iodine and Fe have been successfully controlled by food fortification in industrialized countries (Allen *et al.* 2006). Like supplementation, food fortification is not a sustainable approach against micronutrient malnutrition. Food fortification requires food processing equipment and a food distribution network. It is costly and not readily available for people living in rural areas and dependent on locally-produced foods (Allen *et al.* 2006).

Supplementation and food fortification have had limited success in developing countries in rural areas where biofortification can play a vital role to reduce micronutrient malnutrition. Biofortification is now discussed.

### 2.2.3 Biofortification to reduce micronutrient malnutrition

Biofortification is a process to enrich micronutrients in food crops using agronomic management, conventional plant breeding and/or biotechnology (Broadley *et al.* 2010; Graham *et al.* 2001). Biofortification is based on the staple foods of a population and

the existing food distribution system, so, importantly, there is no need to change food habits. Biofortified crops may be grown locally and are easily available in the local market, in contrast to fortified food where a market system is required. Excess consumption of fortified foods may lead to toxicity in the humans. However, there is less risk of toxicity from biofortified foods. Once established the recurrent cost for biofortification is minimal. Sometimes, the added nutrition in the crops enhances germination, disease and insect resistance and crop yields (Graham *et al.* 1999).

Compared to supplementation and food fortification, biofortification is very cost effective. However, detailed cost-effectiveness estimates for biofortification are as yet unavailable (Horton 2006). Bouis (2002) made a comparative calculation of what $80 million could buy. It could provide vitamin A supplementation (capsules) to 6% of the population of South Asia for two years (one-fifteenth of the population); it could provide Fe fortification to 33% of the population of South Asia for two years; or it could develop six nutrient-dense crops for dissemination to the entire world's population for many years.

***Agronomic biofortification***

Agronomic biofortification includes the application of micronutrient fertilizer to soil, soaking seeds in micronutrient solution and the foliar application of micronutrient solution on standing crops (Broadley *et al.* 2010). It is a rapid and comparatively inexpensive means to deliver more micronutrients to the poor.

There are many factors that affect agronomic biofortification including the target nutrient, crop, variety, soil pH, texture, soil organic matter (Cary and Allaway 1969; Gissel-Nielsen *et al.* 1984; Johnsson 1991), chemical form of the target nutrient (Sima and Gissel-Nielsen 1985), time of nutrient application (Cary and Rutzke 1981), method of nutrient application (Curtin *et al.* 2006) and frequency of application (Smrkolj *et al.*

2005a). A suitable biofortification technique depends on environment and socio-economic conditions which are now discussed through examples of Zn, I and Fe biofortification.

For Zn biofortification, soil characteristics, the chemical form of fertilizer, time of application and method of fertilizer application are important factors. Zinc is more available for plants at low soil pH than at a high pH e.g. Zn fertilization was less effective in soybean (Payne *et al.* 1986) cultivated in high soil pH. Three chemical forms-Zn sulphate ($ZnSO_4$), Zn oxide (ZnO) and Zn-oxy-sulphate-are commonly used for agronomic biofortification (Lyons and Cakmak 2012). Due to high solubility and low cost, $ZnSO_4$ is more efficient than ZnO for biofortification in wheat (Cakmak 2008). Late fertilizer application is more effective than early application. In wheat, under field conditions, Zn foliar application at heading and early milk stages had significantly higher seed Zn concentration than at stem elongation and booting stages (Cakmak *et al.* 2010). Three methods are used for Zn biofortification-soil application, seed priming and foliar application - foliar application was found more effective than the other methods (Cakmak 2008). Two foliar applications were more effective than one application at achieving high grain Zn concentrations (Cakmak *et al.* 2010). The combination of soil and foliar applications were more effective for Zn biofortification in wheat compared to a single individual application (Yilmaz *et al.* 1997).

In contrast, soil and foliar fertilizer application was not efficient for iodine (I) biofortification in maize, wheat, soybean, potato (*Solanum tuberosum* L.) tubers and cassava storage roots (Lyons and Cakmak 2012). In Xinjiang province in north-west China, I supplementation and salt fortification was not successful due to lack of awareness about iodised-salt and preference for locally-produced rock salt to iodised salt (Cao *et al.* 1994). However, I application in the form of potassium iodate solution

(5%) drip in irrigation water for 2–4 weeks twice a year during mid or late-crop growth stages (mainly wheat) for four years increased the iodine concentration in soil solution, crops, sheep and chicken thyroid glands, and meat and the urine of children and women. As a consequence, infant mortality decreased by 50% and sheep production increased by 43%. The success of I biofortification was achieved at a cost of $0.04 per person per year (Cao *et al.* 1994; Jiang *et al.* 1997).

Agronomic biofortification for Fe is problematic. Both soil and foliar applications are less effective because when Fe fertilizers are applied to soil, plant available $Fe^{2+}$ is quickly changed to unavailable $Fe^{3+}$ (Frossard *et al.* 2000; Rengel *et al.* 1999; Zhang *et al.* 2008) and Fe is less mobile in phloem than sodium, potassium, phosphorus, chlorine, sulphur, Zn, copper, manganese (Bukovac and Wittwer 1957). Genetic biofortification may be a solution for Fe biofortification through conventional breeding and biotechnology.

***Plant breeding approach***

A breeding program to develop new varieties with high micronutrient concentrations requires useful genetic variation for micronutrient accumulation in the grain. Genetic variability is intensively exploited in the HarvestPlus Challenge Program (HarvestPlus 2013) and significant genetic variation has been found for both Fe and Zn concentrations in wheat (Welch and Graham 2002), rice (Gregorio *et al.* 2000), pearl millet (*Pennisetum glaucum* L.) (Govindaraj *et al.* 2011) and common bean (Beebe *et al.* 2000). Significant genetic variation was also found for Zn concentration in wild germplasm of emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) (Cakmak *et al.* 2004). As a result of micronutrient trait selection, a Fe-rich common bean variety have been developed for Congo and Rwanda through the HarvestPlus Challenge Program, which

has led to successful cultivation, especially of the common bean variety which is higher yielding than the traditional variety (HarvestPlus 2013).

Understanding the inheritance of a trait is important to optimize plant breeding. There is limited information available on the genetics of micronutrient uptake in plants and such mechanisms are not well understood. Iron uptake in soybean is controlled by a single dominant gene (Weiss 1943). The inheritance of Zn uptake in wheat is complex. Plant tolerance to low levels of soil Zn and high uptake of Zn concentration in seeds is controlled by separate, unrelated genetic systems (Cakmak *et al.* 1998; Lyons and Cakmak 2012). Since nutrient uptake inheritance is controlled by one or two genes (rather than polygenic), it is possible to transfer gene(s) to the cultivated variety by conventional plant breeding. Information on the inheritance of Se uptake is not available.

## 2.3 Selenium

Among micronutrients, Se is an essential element for human and animal health. It is a vital component of amino acids, selenoproteins, enzymes and antioxidants (Spallholz *et al.* 1990). Plants are the primary source of Se for human and animals. Selenium enters plant roots using the sulphate transporter due to the chemical similarity between selenate and sulphate (Abrams *et al.* 1990).

### 2.3.1 Selenium in human health

Selenium is fundamental to human health; it is required for proper functioning of the immune system (Arthur and Beckett 1994), and is an important nutrient for counteracting the development of viral infections and inhibiting HIV progression to AIDS. Selenium is also essential for sperm motility (Behne *et al.* 1997) and may reduce the risk of miscarriage. Two major human diseases have been associated with severe Se deficiency: Keshan disease (cardiomyopathy) and Kashin-Beck disease

(osteoarthropathy) (Reilly 1996). The organic form of Se comes from plant and animal sources, is digested and metabolized easily, and is more stable in the blood system than the inorganic form (as pill) (Johnson 2007); and is therefore more available in the time of stress (exercise, disease and injury). As a result organic Se is better for animal health than the inorganic form. The recommended daily allowance (RDA) of Se by the World Health Organization (WHO) is 55 µg/person/day (Monsen 2000). An elevated Se intake may be associated with reduced cancer risk. Importantly, Se is also toxic to humans at high doses and the range between deficiency levels <11 µg/day and toxic levels >900 µg/day is narrow (Yang and Xia 1995).

Globally over one billion people suffer from Se deficiency due to low bio-availability of soil Se (Lyons *et al.* 2003). Most soils in the world range from 100–2,000 µg/kg (Swaine 1955). Soil Se deficiency has been reported in New Zealand, Australia, UK, Thailand, Denmark, Finland, central Siberia, northeast to south central China, Turkey, parts of India, Nepal and Bangladesh (Fordyce 2005; Lyons *et al.* 2005; Spallholz *et al.* 2004; Spallholz *et al.* 2008b).

Selenium toxicity is less widespread than Se deficiency (Fordyce 2005). High soil Se levels are reported in some countries. Up to 10,000 µg/kg has been found in seleniferous soils in the Great Plains of the USA and Canada; Hubei Province, China; and parts of Ireland, Colombia and Venezuela (Combs 2001). The signs of Se toxicity are hair loss, brittle, thickened and stratified nails, garlic breath, and red and swollen skin (Whanger *et al.* 1996; Yang *et al.* 1983). The mean daily intake of 4,900 µg Se causes toxicity in the seleniferous region mentioned above.

### 2.3.2 Selenium in Bangladesh

Bangladeshi soils - developed from alluvial sediment deposition from the Himalayan mountains - are low in Se. Annual monsoon rainfall and sequential flooding might be

the causal factor for these low soil Se levels (Spallholz *et al.* 2008a). Soils from five locations in Bangladesh had less than 20 µg Se/kg (Spallholz *et al.* 2008b). The Se concentration in commonly consumed-foods from a local market at Jessore, Bangladesh were:105 µg/kg in rice, 488 µg/kg in wheat, 327 µg/kg in cabbage (*Brassica oleracea* var. *capitata* L.), 278 µg/kg in spinach (*Spinacia oleracea* L.), 181 µg/kg in potato (Spallholz *et al.* 2008a) and 1318 µg/kg in fish (Spallholz *et al.* 2008a). Mean dietary Se consumption in rural areas of Bangladesh is 26 µg per person per day (Spallholz *et al.* 2004), which is approximately half the WHO recommended level of 55 µg/person/day (Monsen 2000). This low dietary intake of Se may increase the risk of arsenicosis among people exposed to arsenic-contaminated water (Spallholz *et al.* 2004; Spallholz *et al.* 2008a).

***Relationship of selenium and arsenic***

The soil and ground water in Bangladesh has a high concentration of arsenic (As) (Chowdhury *et al.* 2000); the Bangladesh standard of As in drinking water is 50 µg/L (UNICEF Bangladesh 2011). More than 80 million people are at risk of drinking As-contaminated water in Bangladesh (Chowdhury *et al.* 2000). In 37 of 64 districts of Bangladesh, 7500 As-affected patients were identified (Huq *et al.* 2006a). Arsenic was also detected in rice, lentil (Huq *et al.* 2006b) and vegetables (Ali *et al.* 2003; Huq *et al.* 2006b; Smith *et al.* 2006). The capacity of As to enter via the food chain may increase human As exposure (Ali *et al.* 2003).

Selenium has an antagonistic effect on arsenic (As) (Levander 1977; Miyazaki *et al.* 2003), preventing its cyto-toxic effect (Biswas *et al.* 1999). A lethal dose of As can be counteracted by an equal dose of Se, a process known as mutual detoxification (Holmberg and Ferm 1969; Levander 1977). It has been hypothesized that dietary Se supplementation is important to prevent arsenicosis in Bangladesh (Spallholz *et al.*

2004; Thavarajah *et al.* 2007; Verret *et al.* 2005). There is some evidence to support this hypothesis: Se and As levels were negatively correlated in As-affected people in Bangladesh (Chen *et al.* 2007) and China (Huang *et al.* 2008; Wang *et al.* 2001; Xue *et al.* 2010). In addition, Se supplementation has mitigated the effects of As poisoning in clinical trials in Bangladesh (Verret *et al.* 2005) and China (Yang *et al.* 2002b). However, diet supplementation with Se as an approach is costly and not sustainable and not an economically-viable solution for poor people in developing countries. Selenium supplementation through crop biofortification may be an effective way to ameliorate As poisoning (Thavarajah *et al.* 2007).

### 2.3.3 Selenium in Australia

Selenium concentrations in Australian soils vary widely (Lyons *et al.* 2003). Areas of deficiency were reported in the Central and Southern Tablelands (Hart 1985), Northern Tablelands (Langlands *et al.* 1981) and some coastal areas of New South Wales; in south-eastern and coastal areas of Queensland (Judson and Reuter 1999; Knott and McCray 1959); in south-western areas of Western Australia (Gardiner and Gorman 1963); and in south-eastern areas of South Australia (Reuter 1975); and in high rainfall pockets of Victoria and Tasmania (Judson and Reuter 1999). In contrast, Se toxicity is reported in some areas in Queensland (Knott and McCray 1959).

### 2.3.4 Selenium in higher plants

Selenium is not an essential nutrient for plants (Terry *et al.* 2000). At low concentrations, Se has some beneficial effects on the growth of some species. For example, high germination rates were observed in lupin (*Lupinus angustifolius* L.) seed treated with Se (Frias *et al.* 2009). Selenium also has some beneficial effects against stress in some plant species. For example, Se application had increased aphid resistance in Indian mustard (*Brassica juncea* L.) (Hanson *et al.* 2004), drought tolerance in wheat

(Kuznetsov *et al.* 2003) and canola (*Brassica napus* L.) (Zahedi *et al.* 2009); salt tolerance in sorrel (*Rumex acetosella* L.) (Kong *et al.* 2005); cold tolerance in wheat (Chu *et al.* 2010); and yield increase in *Brassica rapa* L. (Lyons *et al.* 2009).

In contrast, higher amounts of Se are toxic to plants. Selenium toxicity was observed in plants when exposed to excess amounts of Se in soil or growth medium in rice (Liu and Gu 2008), potato (Poggi *et al.* 2000), lettuce (*Lactuca sativa* L.) (Ríos *et al.* 2008) carrot (*Daucus carota* L.) (Kápolna *et al.* 2009), canola, kenaf (*Hibiscus cannabinus* L.) and tall fescue (*Festuca arundinacea* Schreb.) (Banuelos *et al.* 1997). High soil Se delays and reduces the germination percentage, develops chlorosis on leaf margins and also reduces yield.

***Factors affecting selenium uptake by plants***

Many factors affect Se uptake by plants. Plant species itself is a factor that controls Se uptake from soil (Broadley *et al.* 2006; Rayman 2008). Based on Se uptake efficiency, plants can be classified into three groups namely accumulators, non-accumulators and secondary accumulators (Broadley *et al.* 2006). An accumulator takes up Se up to 4,160,000 µg/kg from seleniferous soil. Non-accumulators do not takes up Se much above 25,000 µg/kg from seleniferous soil. Secondary accumulators uptake up to 1,000,000 µg/kg where soil Se is low. (Brown *et al.* 1982; Moxon *et al.* 1939). As mentioned earlier, soil factors controlling Se availability are soil pH, organic matter, clay content, and moisture (Cary and Allaway 1969; Gissel-Nielsen *et al.* 1984; Johnsson 1991). Selenium is less available in acid and neutral soils (Terry *et al.* 2000) than in alkaline soils. At higher pH levels (near neutral), selenate ($SeO_4^{2-}$) concentration is more than selenite ($SeO_3^{2-}$) in soil (Johnsson 1991). Moreover, selenate is more soluble in water than selenite and therefore more available for root uptake and faster distribution throughout the plant (Hasanuzzaman *et al.* 2010; Terry *et al.* 2000). For

example, Se in wheat grain decreased by 63% when soil pH was reduced from 7 to 5 (Johnsson 1991). Selenium is more bioavailable in sandy soil than clay soils because of strong bonds between Se and clay particles (Hamdy and Gissel-Nielsen 1977). Wheat seed Se concentration decreased by 79% when soil clay content increased from 7% to 39% (Johnsson 1991). Selenium binds strongly with soil organic matter (OM) and is therefore less bioavailable to the plant under high OM soils than low OM soils (Eich-Greatorex *et al.* 2007; Johnsson 1991). Johnsson (1991) observed reduction in Se uptake in wheat seed by 88% when soil OM increased from 1.4% to 39%.

***Selenium biofortification in crops***

Agronomic biofortification of Se has been conducted in a number of food crops such as wheat, maize, rice, barley, soybean, pea, common bean, buckwheat, and pumpkin (Table 2.1). All reported experiments were conducted in the field except for one on common bean in the glasshouse (Smrkolj *et al.* 2007). In those experiments, either sodium selenite or sodium selenate was applied. In an experiment with barley, the foliar effect of sodium selenate and sodium selenite was compared, with selenate more effective than selenite (Sima and Gissel-Nielsen 1985). Application of different doses of Se (0 to 100 g/ha) resulted in seed Se concentrations ranging from 2 to 2524 µg/kg (Table 2.1). Doses between 10 and 50 g/ha were effective at increasing seed Se concentration. Selenium application rates below 10 g/ha produced low Se concentrations in seed, whereas those above 50 g/ha produced toxic levels. Selenium was applied at different stages of growth: by soaking seeds with Se solution before sowing, soil application, and foliar application at vegetative and flowering stage of crops. Selenium application at the flowering stage was more effective than at other stages.

Table 2.1. Summary of research conducted on Se application to different crops.

| Crop | Method of application | Crop growth stage during Se application | Se form | Se application rate (g/ha) | Seed Se concentration (µg/kg) | | Author(s) |
|---|---|---|---|---|---|---|---|
| | | | | | Control | Treated | |
| Wheat | Foliar | Main shoot and 9 or more tillers | $Na_2SeO_3$ | 0, 0.5, 1, 10, 20 | 39 | 47–192 | Ducsay *et al.* (2007) |
| | Foliar | Stem elongation: 1st node visible | $Na_2SeO_4$ | 0, 1, 5, 10, 15, 20, 50, 100 | 10 | 2524 FW | Broadley *et al.* (2010) |
| | Soil | Stem elongation: 1st node visible | $Na_2SeO_4$ fertilizer | 10 | 16 | 400 FW | Broadley *et al.* (2010) |
| | Seed | Sowing | $Na_2SeO_4$ | 0, 10, 20 | 23–51 | 33–86 | Curtin *et al.* (2006) |
| | Soil | Sowing | $Na_2SeO_4$ | 0, 10, 20 | 23–51 | 51–322 | Curtin *et al.* (2006) |
| | Soil | Stem elongation:1st node visible | $Na_2SeO_4$ | 0, 10, 20 | 23–51 | 178–467 | Curtin *et al.* (2006) |
| | Foliar | Flowering | $Na_2SeO_4$ | 0, 5, 10, 20 | 23–51 | 115–524 | Curtin *et al.* (2006) |
| | Soil | Stem elongation: 2nd node visible | $Na_2SeO_4$ fertilizer | 0, 5, 10, 15, 20 | 10–30 | 70–490 | Curtin *et al.* (2008) |
| Maize | Foliar | 4–6th leaf stage | $Na_2SeO_3$ | 0, 7.5, 15 | 10 | 50–120 | Cary and Rutzke (1981) |
| | Foliar | Tassels emerging | $Na_2SeO_3$ | 0, 7.5, 15 | 10 | 140–240 | Cary and Rutzke (1981) |
| Rice | Foliar | Heading | $Na_2SeO_3$ | 0, 18 | 2 | 411 | Hu *et al.* (2002) |
| | Foliar | Heading | $Na_2SeO_3$ enriched bio-fertilizer | 0, 14, 18 | 2–3 | 178–442 | Hu *et al.* (2002) |
| | Foliar | Heading | $Na_2SeO_3$ enriched bio-fertilizer | 0, 7.5, 15 | 35 | 48–107 | Fang *et al.* (2008) |
| Barley | Foliar | Stem elongation: 3rd node visible | $Na_2SeO_3$ | 0, 5, 10, 20, 40, 80 | 12–34 | 24–316 | Gupta *et al.* (1988) |
| | Foliar | Stem elongation: 1st node visible | Selenite | 0, 5, 25, 50 | 10 | 362 | Sima and Gissel-Nielsen (1985) |
| | Foliar | Stem elongation: 1st node visible | Selenate | 0, 5, 25, 50 | 10 | 702 | |

| Crop | Method of application | Crop growth stage during Se application | Se form | Se application rate (mg/l) | Seed Se concentration (µg/kg) | | Author(s) |
|---|---|---|---|---|---|---|---|
| | | | | | Control | Treated | |
| Soybean | Foliar | 78 DAS | $Na_2SeO_4$ | 0, 50 | 71 | 100 | Djanaguiraman *et al.* (2005) |
| Pea | Foliar | Flowering | $Na_2SeO_4$ | 0, 15, 30 | 21 | 383–743 | Smrkolj *et al.* (2005a) |
| Common Bean | Foliar | Flowering | $Na_2SeO_4$ | 0, 20 | 30–81 | 1892–2379 | Smrkolj *et al.* (2007) |
| | Seed | Sowing | $Na_2SeO_4$ | 0, 10 | 30–81 | 544–634 | Smrkolj *et al.* (2007) |
| Buckwheat | Foliar | Flowering | Selenate | 0, 1 | 43 | 394 | Stibilj *et al.* (2004) |
| | Foliar | Flowering | $Na_2SeO_4$ | 0, 15 | 55 | 3219 | Smrkolj *et al.* (2006) |
| Pumpkin | Foliar | Flowering | Selenate | 0, 1 | 105 | 381 | Stibilj *et al.* (2004) |
| | Foliar | Flowering | $Na_2SeO_4$ | 1.5 | 19 | 1100 | Smrkolj *et al.* (2005b) |

FW–Fresh weight, DAS– Days after sowing

Agronomic biofortification is also practiced in Finland by application of Se supplemented at 10 mg/kg fertilizers since 1984 (Eurola *et al.* 1990). After 25 years of Se enriched fertilizer application, Se concentration in spring cereals (wheat, rye and barley) increased by 15-fold, in beef by six, in pork by two, in milk by three-fold compared with the levels before Se fertilization. As a consequence, dietary Se intake increased from 30 to 70 µg/person/day and human plasma Se level increased by 60%. During the periods Se fertilization was monitored annually to maintain optimum Se levels in foods, dietary intake and the human body (Alfthan *et al.* 2010).

It can be concluded that the application of selenate at the flowering stage may be effective for Se biofortification. To date, no plant breeding approach in regard to Se biofortification has been attempted. However, after Se foliar application in a glasshouse experiment with four genotypes of common bean, no significant differences between genotypes were found for seed Se concentration (Smrkolj et al. 2007).

## 2.4. Lentils: A vehicle of Se biofortification

Lentil is an important cool season food legume and a rich source of protein (20–30%), prebiotic carbohydrates, essential fatty acids and a range of micronutrients (Bhatty 1988; Johnson *et al.* 2013; Thavarajah *et al.* 2011b). In addition, lentil is a rich source of Se, ranging from 22 to 672 µg/kg (Thavarajah *et al.* 2007; Thavarajah *et al.* 2011a), which is readily incorporated into proteins. Cooking lentil in boiling water does not change total Se concentration (Thavarajah *et al.* 2008). All these factors suggest that lentil is a whole food solution for micro-nutrition malnutrition (Thavarajah *et al.* 2011b).

Bangladesh is a producer, importer and consumer of lentil, whereas Australia is a major lentil producer and exporter. Lentil is one of the oldest and in terms of consumption, most popular grain legumes in Bangladesh. It was cultivated in Bangladesh from around

700 BC (Rashid et al. 2012). Today lentil is mostly cultivated in Faridpur, Jessore, Khustia, Natore, Pabna and Rajbari districts. In 2011 Bangladesh produced 80,442 t of lentil from 82,969 ha of land (FAO 2014). This production was insufficient to meet national consumption. In 2011, Bangladesh imported more than 74,696 t of lentil from Australia, Canada, India, Nepal, Turkey and the USA (FAO 2014). Australia grows lentil in wet-winter as a rotational crop with cereals and as an export commodity. During the 2007–2011 period, Australia produced on an average171,579 t of lentils (3% of world production) annually from 142,352 ha of land (3.7% of world lentil area) (FAO 2014). Australia was the third largest lentil-exporting country in 2011 (FAO 2014). On average during the 2007–2011 period, Australia exported 129,222 t of lentil annually which comprised 75% of local production (FAO 2014). Most Australian lentil exports go to South Asia and the Middle East. Clearly both Bangladesh and Australia are important for lentil production, consumption and trade and hence suitable venues for a lentil Se biofortification study.

## 2.5 Conclusions

The inadequate supply of a micronutrient in a diet that hampers normal body function is called micronutrient malnutrition ("hidden hunger"). Half of the world population suffers from micronutrient malnutrition. Supplementation and food fortification with micronutrients can reduce micronutrient malnutrition, but are relatively ineffective in rural areas due to poverty. Biofortification is a food-based approach that can supply micronutrients to poor people in rural areas at low cost through an agronomic and/or plant breeding approach. Agronomic biofortification is rapid but not suitable for all elements and under various socio-economic conditions. Agronomic biofortification of Zn through Zn-enriched fertilizer application has been effective in Turkey. For Fe deficiency, agronomic biofortification is less effective due to soil limiting factors but

genetic biofortification has been successful. Iron-enriched common bean varieties have been developed for Congo and Rwanda by the HarvestPlus Challenge Program.

Recently biofortification has concentrated on Fe, Zn, and vitamin-A. Other important micro-elements also need attention such as Se. Agronomic biofortification of Se has been practiced successfully in Finland through Se-enriched fertilizer application to soil. Foliar Se biofortification has also been successfully experimented in wheat, maize, rice, barley, soybean, field pea, common bean, buckwheat and pumpkin. The use of selenate is more effective than selenite both as soil and foliar applications, but foliar Se application is more efficient than soil application and late application during reproductive growth is better than early application. There are no data available in regard to the frequency of Se application. However, based on Zn application studies, it may be anticipated that two Se applications will be more effective than a single application.

Lentil is an important grain legume crop in terms of consumption and production in South Asia and the Middle East, where many countries are Se deficient. Lentil is rich in protein, essential fatty acids and micronutrients, and hence a good vehicle for Se biofortification. As, lentil biofortification with Se has never been examined, there is scope to test its efficacy in Se biofortification. Bangladesh, a producer, importer and consumer of lentil, and Australia, a producer and exporter of lentil, may be good options for experimentation. A survey on existing lentil cropping systems, a genotype × environment interaction study, a Se foliar application experiment and a lentil germplasm screening will generate new information on the Se status of soils and cultivated lentil varieties of Bangladesh and Australia. A genotype × environment interaction study will also provide a better understanding of how genotypes and/or environmental interactions affect the Se concentration of lentil seeds. The information

generated from such studies will also help to plan to produce Se-rich lentil in Australia, Bangladesh and elsewhere.

# Chapter 3

## Selenium biofortification in lentil (*Lens culinaris* Medikus subsp. *culinaris*): Farmers' field survey and genotype × environment effect

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(The farmers' field survey was designed by M.M. Rahman in consultation with W. Erskine and K.H.M. Siddique. The on-station yield trial was designed by Pulses Research Centre, Ishurdi Pabna, Bangladesh. M.S. Zaman contributed during sample collection from farmers' field. The seed and soil samples were collected and handled by M.M. Rahman. Selenium and arsenic was measured by M.M. Rahman under the supervision of P. Thavarajah and D. Thavarajah. The manuscript was written by M.M. Rahman and the co-authors were involved in the discussion of results, structure of the manuscript and editorial comments to finalise it.)

## Abstract

Selenium (Se) is an essential micronutrient for humans and animals, however more than one billion people around the world are Se deficient. Selenium helps to prevent several diseases in humans including arsenicosis, a major health problem in South Asia. Lentil is a popular staple food in South Asia; it can uptake Se from soil and is thus a potential source of Se for humans. A farmers' field survey and an on-station yield trial of seven advanced breeding lines at four locations were conducted in Bangladesh during 2010–11 to determine Se concentration in lentil. Total Se concentration was measured in soil and lentil seeds collected from both farmers' fields and yield trials. Mean of soil and lentil seed Se concentration in farmers' fields was 163 µg/kg and 312 µg/kg, respectively, with the highest being 173 µg/kg and 370 µg/kg in Rajshahi division, a major lentil growing area in Bangladesh. Consumption of 50 g of lentil provides 28% of the recommended daily allowance of Se (55 µg per person per day). There were significant genotype and location differences observed for seed Se, Se yield, and seed yield. However, genotype × location interaction was not significant for seed Se concentration and Se yield, but was significant for seed yield. Soil Se concentration in lentil growing regions of Bangladesh was moderate and overall it produced Se–rich lentils. Therefore, Se biofortification in lentil using agronomic and/or genetic approaches are possible to increase Se intake for Se deficient populations.

*Keywords*: Lentils, biofortification, selenium, arsenicosis, recommended daily allowance

## 3.1 Introduction

Selenium (Se) is an essential element for human and animal health. It is a vital component of amino acids, selenoproteins, enzymes, and antioxidants (Spallholz *et al.* 1990). It is also important in the prevention of several cancers (Yu *et al.* 1997), viral infections (Baum *et al.* 1997; Yu *et al.* 1997), oxidative stress, inflammation (Lyons *et al.* 2003) and suppression of HIV progression to AIDS (Dworkin 1994). Two major human diseases have been associated with severe Se deficiency: Keshan disease (cardiomyopathy) and Kashin-Beck disease (osteoarthropathy) (Reilly 1996). In addition, Se deficiency is also associated with immunodeficiency, thyroid problems, heart disease (Arthur and Beckett 1994) and male infertility (Behne *et al.* 1997).

Globally over one billion people suffer from Se deficiency due to low bio-availability of soil Se (Lyons *et al.* 2003). Most soils of the world have low levels of Se ranging from 100–2000 μg/kg (Swaine 1955). Soil Se deficiency has been reported in New Zealand, Australia, UK, Thailand, Denmark, Finland, central Siberia, northeast to south central China, Turkey, parts of India, Nepal and Bangladesh (Fordyce 2005; Lyons *et al.* 2005; Spallholz *et al.* 2004; Spallholz *et al.* 2008b). High soil Se levels are also reported in some countries. Up to 10,000 μg/kg has been found in seleniferous soils in the Great Plains of the USA and Canada; Enshi County, Hubei Province, China; and parts of Ireland, Colombia and Venezuela (Combs 2001).

Bangladeshi soils developed from alluvial sediment deposition from the Himalayan mountains are low in Se. Annual monsoon rainfall and sequential flooding may contribute to low soil Se levels in Bangladesh (Spallholz *et al.* 2008a). Soils from five locations in Bangladesh had less than 20 μg Se/kg (Spallholz *et al.* 2008b). The Se concentration in commonly consumed foods from a local market at Jessore, Bangladesh are as follows:105 μg/kg in rice (*Oryza sativa* L.), 488 μg/kg in wheat (*Triticum*

*aestivum* L.), 327 µg/kg in cabbage (*Brassica oleracea* var. *capitata* L.), 278 µg/kg in spinach (*Spinacia oleracea* L.), 181 µg/kg in potato (*Solanum tuberosum* L.) and 1318 µg/kg in fish (Spallholz *et al.* 2008a). Mean dietary Se consumption in rural areas of Bangladesh is 26 µg per person per day (Spallholz *et al.* 2004), approximately half of the WHO recommended level of 55 µg per person per day (Monsen 2000). This low dietary intake of Se may increase the risk of arsenicosis among people exposed to arsenic-contaminated water (Spallholz *et al.* 2004; Spallholz *et al.* 2008a).

The soil and ground water in Bangladesh has a high concentration of arsenic (As) (Chowdhury *et al.* 2000); the Bangladesh standard of As in drinking water is 50 µg/L (UNICEF Bangladesh 2011). More than 80 million people are at risk of drinking As-contaminated water in Bangladesh (Chowdhury *et al.* 2000). In 37 of 64 districts of Bangladesh, 7500 As-affected patients were identified (Huq *et al.* 2006a). Arsenic was also detected in rice, lentil (Huq *et al.* 2006b) and vegetables (Ali *et al.* 2003; Huq *et al.* 2006b; Smith *et al.* 2006). The capacity of As to enter via the food chain may increase human As exposure (Ali *et al.* 2003).

Selenium has an antagonistic effect on As (Levander 1977; Miyazaki *et al.* 2003), preventing the cyto-toxic effect of As (Biswas *et al.* 1999). It has also been known that a lethal dose of As can be counteracted by an equal dose of Se, a process known as mutual detoxification (Holmberg and Ferm 1969; Levander 1977). It has been hypothesized that dietary Se supplementation is important to prevent arsenicosis in Bangladesh (Spallholz *et al.* 2004; Thavarajah *et al.* 2007; Verret *et al.* 2005). There is some evidence to support this hypothesis: Se and As levels were negatively correlated in As-affected people in Bangladesh (Chen *et al.* 2007) and China (Wang *et al.* 2001; Xue *et al.* 2010). In addition, Se supplementation has mitigated the effects of As poisoning in clinical trials in Bangladesh (Verret *et al.* 2005) and China (Yang *et al.*

2002b). However, Se supplementation is costly and not a sustainable and economically-viable solution for poor people in developing countries. Selenium supplementation through lentils may be an effective way to ameliorate As poisoning (Thavarajah *et al.* 2007).

Biofortification is a process of enriching micronutrients in food crops using agronomic management, conventional plant breeding and modern biotechnology (Broadley *et al.* 2010; Graham *et al.* 2001). Agronomic biofortification includes application of Se fertilizer to soil, soaking seeds in Se solution and foliar application of Se (Broadley *et al.* 2010). It is a rapid method to produce Se-enriched food crops but not a complete and sustainable solution. A number of factors affecting agronomic biofortification include soil type and both economic and environmental factors. Selenium applications through fertilizers are not always a successful and sustainable approach because they increase the cost of production, especially in developing countries (Graham and Rengel 1993). In Se fertilization care needs to be taken because of the narrow window between toxic and beneficial Se levels (Terry *et al.* 2000). In contrast, genetic biofortification using conventional breeding for improved Se uptake by plants may be an effective and sustainable strategy in the long term (Nestel *et al.* 2006).

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is an important cool season food legume and a rich source of protein (20-30%), prebiotic carbohydrates, essential fatty acids and a range of micronutrients (Bhatty 1988; Johnson *et al.* 2013; Thavarajah *et al.* 2011b). In addition lentil is a rich source of organic Se, selenomethionine (Thavarajah *et al.* 2007; Thavarajah *et al.* 2008), which is readily incorporated into proteins. Cooking lentil in boiling water does not change total Se concentration (Thavarajah *et al.* 2008). Lentil is widely cultivated in Australia, Bangladesh, western Canada, China, India, Nepal, Syria, Turkey and the USA (Erskine 2009). The total Se concentration of

lentils grown in eight major lentil-producing countries was reported by Abhay and Krishnaswamy (1997) and Thavarajah et al. (2008; 2011a) as follows: Canada (672 μg/kg), India (208 μg/kg), Nepal (180 μg/kg), southern Australia (148 μg/kg), Turkey (47 μg/kg), Morocco (28 μg/kg), northwestern USA (26 μg/kg), and Syria (22 μg/kg). However, no detailed information is available on Se concentration in lentils grown in Bangladesh. In terms of production and consumption, lentil is the most important grain legume crop grown in Bangladesh. One cup or 50-100 g daily serving of high-Se lentils can supply recommended daily allowance (RDA) of Se (55 μg per person per day) . Therefore, biofortification approaches to Bangladesh may be beneficial as lentils can be bred for high Se.

The objectives of this study were to: (1) determine the variation of total Se concentration in lentil seeds grown in Bangladeshi farmers' fields; (2) assess the genotype × environment interaction of Se concentration in lentil seeds and (3) determine the variation of Se concentration in lentil growing soils in Bangladesh.

## 3.2 Materials and methods

### 3.2.1 Materials

Standards, chemicals, and high purity solvents used in digestion, extraction, and Se measurement were purchased from Alfa Aesar-A Johnson Matthey Company (Ward Hill, MA, USA), VWR International LLC (Batavia, IL, USA) and Sigma-Aldrich Chemical Company Inc. (Allentown, PA, USA). Water was distilled and deionized ($ddH_2O$) using a Milli-Q Water System (Milford, MA, USA) to a resistance of 18.5 mΩ or more.

### 3.2.2 Study 1: Farmers' field survey

#### *Location*

A survey was conducted to measure Se concentration of lentil cultivars and lentil-growing soils of Bangladesh during February and March 2011. Lentil seeds and soil samples were collected from 29 districts in six divisions (Fig. 3.1) of Bangladesh at the physiological maturity. The sample collection sites lie between latitudes 22º45′ to 26º00′ N and longitudes 88º13′ to 90º49′ E covering the major lentil growing regions of Bangladesh (Sarker *et al.* 2004).

#### *Field selection and data collection*

Farmers' field sampling was stratified based on the major lentil grown area of a division (unpublished DAE data, 2010). Field selection was random within a division. Most samples were collected from Khulna (26) division followed by Dhaka (23) and Rajshahi (19) where there is a large area under production. Approximately 15 km was maintained between fields for uniform sampling. Before sampling, the following information was collected from each farmer: name and address, preceding crop, date of sowing, name of the cultivar, whether the crop was irrigated and fertilizer use. Location of the field was recorded by a global positioning system (GPS). Lentil seed yield was calculated from a quadrat yield and converted to kg/ha. Se yield - the product of seed Se concentration and seed yield, reflects efficient plant uptake of Se from a unit of land - was expressed as mg/ha.

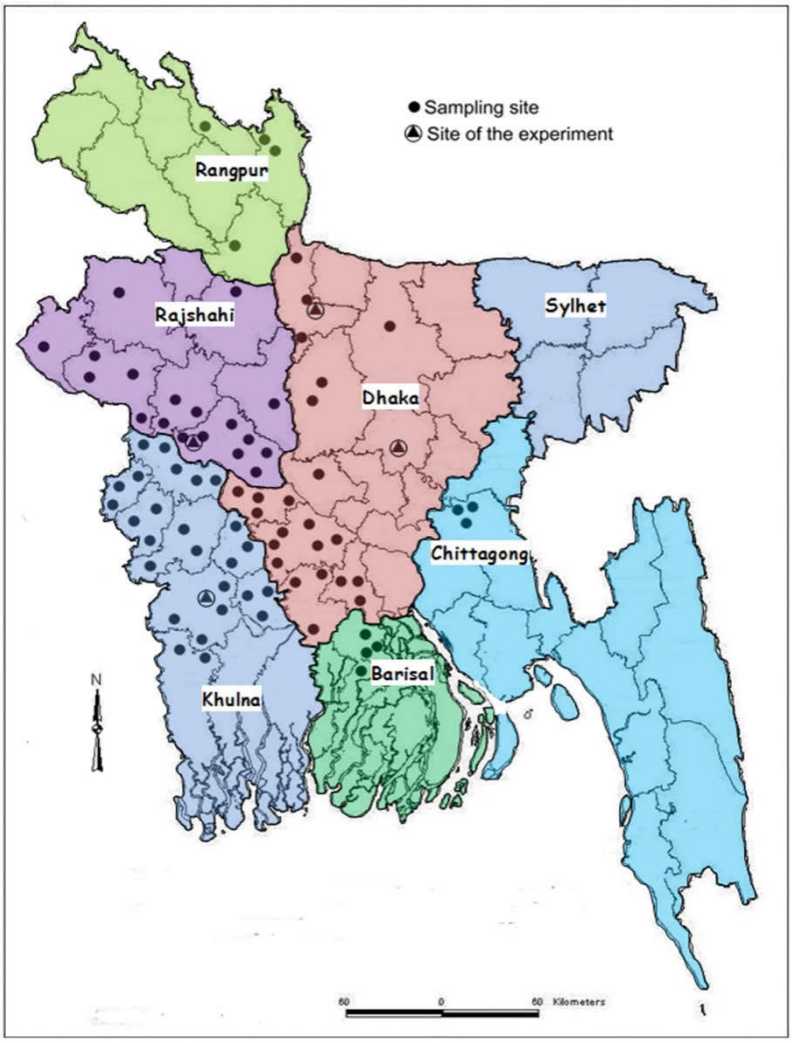


**Fig. 3.1.** Location of farmers' fields (●) and experimental sites (▲) in the six divisions of Bangladesh.

***Plant sample collection and preparation***

To determine the Se concentration in lentil seeds, whole lentil plants were collected randomly from 79 farmers' fields. Three samples were collected along a diagonal of each field. At first the sampling points were identified and marked with a quadrat (50 × 50 $cm^2$). Then, lentil plants from each quadrat area were counted, hand harvested and stored separately in cotton bags at room temperature. The lentil plants were air dried at ≤ 40°C inside the cotton bags, threshed, hand cleaned and seeds were separated. Immature seeds and foreign materials were removed to make a homogenous seed

sample. Cleaned seeds were oven dried at 40°C to reduce the moisture content to 14% and then weighed. Dried seeds were ground to powder using a mortar and pestle and stored at –20°C. Then, ground lentil seed samples were shipped from Bangladesh to Pulse Quality and Laboratory, North Dakota State University (Fargo, ND, USA) using an international courier where the samples were stored at –20°C until analysis.

***Lentil seed selenium concentration***

Total Se concentration in lentil seeds was determined using the modified $HNO_3$-$H_2O_2$ method described by Thavarajah *et al.* (2008). Approximately 500 mg of finely-ground lentil seeds were weighed into digestion tubes. Digestion was conducted at 90°C using the following acids for specific durations: a) 6 mL of concentrated (70%) nitric acid ($HNO_3$) for 1 hour, b) 3 mL of hydrogen peroxide ($H_2O_2$) for 15 minutes, and c) 3 mL of 6 M hydrochloric acid (HCl) for 5 minutes. Upon complete digestion, the tubes were removed from the digestion block and cooled to room temperature. The total volume was adjusted to 10 mL, and then the solution was filtered (Whatman No. 1 filter papers) using a vacuum system (Gardener Denver Thomas Inc., Welch Vacuum Technologies, LA, USA). Measurement of total Se concentration was validated using the National Institute of Standards and Technology standard reference (1567a wheat flour, Se=1.1±0.1 mg/kg) and a laboratory reference sample (CDC Redberry, Se=700 µg/kg). The total Se concentration was determined using ICP-EMS (Inductively Coupled Plasma Emission Spectrometry) (ICP-EMS; ICP-6500 Duo, Thermo Fisher Scientific, PA, USA) with a detection limit of 75 µg/L. The Se concentration was quantified using a calibration curve that ranged between 1–50 mg/l.

***Soil sample collection and preparation***

Similar to the seed samples, three soil samples were collected along a diagonal of each field. Soil samples were collected from the centre of each quadrat by auger at a depth of 0–30 cm (Thavarajah *et al.* 2008) and stored separately in a polythene bag. The soil samples were air dried (≤40°C). Clods were broken, roots, leaves and other inert materials removed and then passed through a 2 mm sieve, and stored at –20°C until analysis. One set of samples was sent to Cornell Nutrient Analysis Laboratory, Cornell University (Ithaca, USA) by an international courier for soil Se measurement. Another set of samples was sent to the soil science laboratory, Bangladesh Agricultural Research Institute (BARI), Gazipur for soil texture, pH, and organic matter (OM) measurement.

***Soil physical and chemical characteristics measurement***

Sand, silt and clay contents were determined by hydrometer method. Soil pH was determined by glass electrode pH meter (Jackson 1962). Organic carbon was determined titrimetrically using the method of Walkley and Black (1934) by oxidation of organic carbon with potassium dichromate. The OM content of each sample was calculated by multiplying the organic carbon content by the Van Bemmelen factor, 1.73 (Nelson and Sommers 1982).

**Table 3.1.** Mean minimum and maximum temperature, total rainfall during crop growth period (November, 2010-February, 2011), soil physical and chemical characteristics of genotype × environment interaction experimental sites in Bangladesh (standard deviation in parentheses).

| Locations | Min temp (ºC) | Max temp (ºC) | Total rainfall (mm) | Soil OM (%) | Soil pH | Clay (%) | Soil Se (µg/kg) | Soil As (µg/kg) |
|---|---|---|---|---|---|---|---|---|
| Jamalpur | - | - | 28 | 0.7 (0.1) | 7.0 (0.1) | 29 (10) | 172 (23) | 3879 (302) |
| Joydebpur | 14 | 27 | 127 | 1.0 (0.2) | 7.0 (0.2) | 40 (11) | 171 (10) | 3499 (802) |
| Ishurdi | 16 | 23 | 83 | 1.2 (0.4) | 8.0 (0.1) | 32 (9) | 189 (10) | 5230 (239) |
| Jessore | 11 | 27 | 67 | 0.7 (0.1) | 8.5 (0.1) | 25 (12) | 115 (4) | 7357 (275) |

- Data are not available

***Soil Se measurement***

About 500 mg of soil sample was weighed into a 100 mL Teflon container. High purity sub boiled nitric acid ($HNO_3$) of 1.0 mL was added followed by 4.0 mL of high purity sub boiled perchloric acid ($HClO_4$) followed by 1.0 mL of reagent grade hydrofluoric acid (HF). Samples were heated to 180ºC using a programmable automated digestion system called a Q Block for 10 minutes (Questron Technologies Corp, Mississauga, Canada). Samples were dissolved then diluted to 50.0 mL with 5% nitric acid ($HNO_3$). The solutions were analyzed for Se using an Agilent 7500 series ICP-MS. The Plasma was operated at 1400 watts and 5.0 mL of hydrogen gas was used in the Octopole Reaction System (ORS) to remove Ar-Ar ion interferences. Selenium concentration was measured at mass 78.

### 3.2.3 Study 2: Genotype × environment effect

***Experimental details***

Yield trials were conducted at four sites Jamalpur, Joydebpur, Ishurdi, and Jessore during the 2010–2011 winter season (Fig. 3.1). Mean minimum, maximum temperature and total rainfall during growing period (November- February) and soil properties of the four sites are presented in Table 3.1. Each experiment had seven lentil genotypes including five advanced breeding lines–BLX-02009-04–1, BLX-02009-04-5, BLX-02009-06-2, BLX-02009–19-2, LR-9-25–and two cultivars–BARI masur-5 and BARI masur-6. The breeding lines were developed by Pulses Research Centre, BARI, Ishurdi, Pabna, Bangladesh. The lines were homozygous, small seeded (< 2.5 g/100 seed) and had red cotyledon. The experiment was sown at Jessore on 4$^{th}$, Joydebpur on 8$^{th}$, Jamalpur and Ishurdi on 10$^{th}$ November 2010. The experimental design was a randomized complete block design (RCBD) with three replications. The land was

ploughed, harrowed, levelled and fertilizer applied at 20 kg N/ha (urea: 46% N), 40 kg P/ha (triple super phosphate: 20% P) and 20 kg K/ha (muriate of potash: 50% K). Sowing was at a depth of approximately 3 cm in plots 3.2 m wide × 4 m long with an inter-row distance of 40 cm. Post-sowing irrigation was applied to ensure seed germination. Weeding and other cultural practices were done as required.

***Plant sample collection and measurement***

The plants were hand harvested from each plot separately and stored in cotton bags at room temperature. Seed samples were prepared as described in section *2.2.3*. Total Se concentration was measured as described in section *2.2.4*. Seed yield was calculated from the whole plot and converted to kg/ha. Se yield was calculated as described previously and expressed as mg/ha.

***Soil sample collection and measurement***

One composite soil sample was collected from each of the three replicate blocks at each site at a depth of 0–30 cm. Then, twelve composite soil samples were collected from four experimental sites and stored separately at –20ºC. Soil samples were prepared as described in section *2.2.5.* and total Se concentration was measured as described in section *2.2.7*.

### 3.2.4 Statistical analysis

***Study 1: Farmers' field survey***

Means of locations, soil properties and agronomic practices of the survey data were analysed using one–way analysis of variance (ANOVA) in GenStat 14th edition (© 2000–2011 VSN International Ltd, Hemel Hempstead, UK). Least significant differences (LSD) were calculated by Fisher's test at $P < 0.05$ in 'agricolae' package of

R (Felipe de Mendiburu 2012). Correlation coefficients for the farmers' field survey were also estimated in the same package of R.

***Study 2: Genotype × environment effect***

Means of location, genotype, and location × genotype interaction were estimated using the linear mixed model of restriction maximum likelihood (REML) procedure in GenStat 14 edition. Genotypes, locations, and genotype × location were fixed factors in the model. Least significant differences (LSD) were calculated by Fisher's test at $P < 0.05$ in 'agricolae' package of R (Felipe de Mendiburu 2012).

## 3.3 Results

### 3.3.1 Study 1: Farmers' field survey

Analysis of variance (ANOVA) showed significant variation in different divisions, farmers' fields, soil properties, and agronomic practice for soil OM, soil pH, soil Se, soil As, seed Se, Se yield, and seed yield (Table 3.2).

***Location effect on seed Se concentration***

The seed Se concentration of 79 farmers' fields ranged from 74 to 965 µg/kg and the mean was 312 µg/kg (Table 3.3). Seed Se concentration in different divisions differed significantly. Rajshahi, Barisal and Khulna divisions had relatively high seed Se concentration of 370 µg/kg, 351 µg/kg, and 329 µg/kg, respectively. Overall, consumption of 50 g of lentil grown in Rajshahi, Barisal and Khulna divisions will provide approximately 30 % of Se RDA (Table 3.3).

**Table 3. 2.** Significance levels from one-way analyses of variance (ANOVA) by different variables of soil OM, soil pH, soil Se, soil As, seed Se, Se yield and seed yield in the farmers' field survey (n=225).

| Source of variation | df | P values | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Soil OM | Soil pH | Soil Se | Soil As | Seed Se | Se yield | Seed yield |
| Location | | | | | | | | |
| Division | 5 | *** | *** | *** | *** | ** | *** | *** |
| Farmers' field | 78 | *** | *** | *** | *** | *** | ***Ψ | *** Ψ |
| Latitude | 6 | *** | ** | *** | *** | *** | *** | *** |
| Longitude | 5 | ** | *** | NS | *** | NS | NS | ** |
| Soil properties | | | | | | | | |
| OM | 4 | - | ** | *** | *** | NS | NS | *** |
| pH | 6 | *** | - | * | *** | NS | ** | *** |
| Sand | 7 | * | NS | * | *** | NS | NS | ** |
| Silt | 7 | * | NS | * | *** | NS | NS | ** |
| Clay | 7 | * | NS | * | *** | NS | NS | ** |
| Agronomic practice | | | | | | | | |
| Sowing week | 6 | ** | *** | * | *** | *** | *** | ** |
| Cultivar | 6 | * | *** | *** | * | * | NS | *** |
| Preceding crop | 9 | *** | NS | NS | *** | ** | * | NS |

df, degrees of freedom

Ψ df =76

- P values not available

* Significantly different at P <0.05

** Significantly different at P <0.01

*** Significantly different at P <0.001

NS, not significant

***Agronomic practice effects on seed Se concentration***

Sowing time

Sowing in late October, first week of November and mid-November onwards are considered early, optimum and late sowing, respectively (Afzal *et al.* 2003). There was significant variation in seed Se, Se yield, and seed yield for different sowing dates (Table 3.2). Early sowing produced high seed Se concentration and Se yield compared to optimum and late sowing. However, optimum sowing produced lower seed Se, Se yield, and seed yield than early and late sowing (data not shown).

Preceding crop effect

Ten different crops were reported by farmers to have preceded lentil, namely black gram (*Vigna mungo* (L.) Hepper), eggplant *(Solanum melongena* L.*)*, ginger *(Zingiber officinale* Roscoe*)*, jute (*Corchorus olitorius* L.), maize (*Zea mays* L.), okra (*Abelmoschus esculentus* (L.) Moench), rice (*Oryza sativa* L.), sesbania (*Sesbania aculeata* (Willd.) Pers.), sugarcane (*Saccharum officinarum* L.), and tobacco (*Nicotiana tabacum* L.). Rice was the most frequent preceding crop followed by jute. There was significant variation for seed Se and Se yield in lentil associated with the different preceding crops (Table 3.2). A comparison of rice with other preceding crops showed that lentil grown after rice had low seed Se concentration and Se yield (data not shown). Lentil cultivated after okra had a high concentration of seed Se concentration with medium seed yield. Lentil cultivated after tobacco had a low concentration of seed Se concentration and Se yield, but a high seed yield.

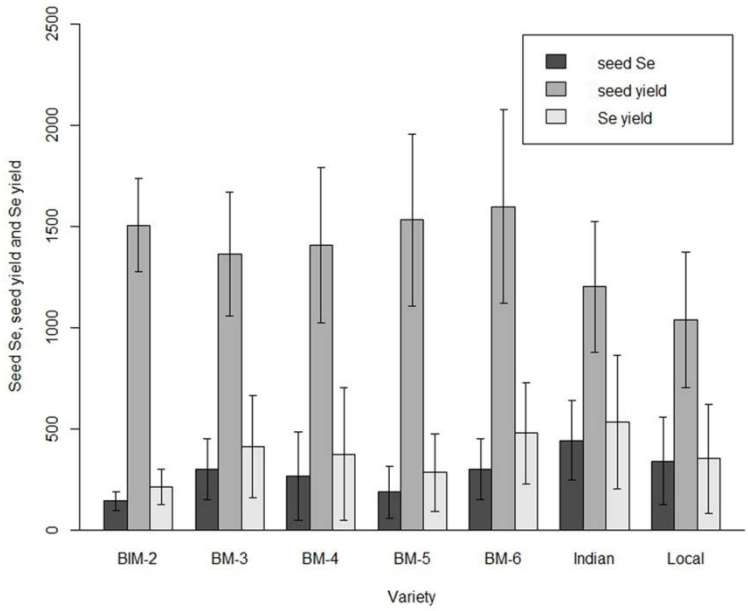


**Fig. 3.2.** Comparison of seven lentil cultivars for seed Se concentration (µg/kg), seed yield (kg/ha) and Se yield (mg/ha) with standard deviation in the farmers' field study. BIM- BINAmasur, BM- BARImasur.

Lentil cultivar

Farmers reported growing seven lentil cultivars–BARImasur-3, BARImasur-4, BARImasur-5, BARImasur-6, BINAmasur-2, Indian, and local. All these cultivars were small seeded with red cotyledon because of consumers' preference. Approximately 60% of farmers were using the local cultivar. There was significant variation among cultivars for seed Se concentration and seed yield but not for Se yield (Table 3.2). Indian and local cultivars had the highest seed Se concentration but lower seed yield (Fig. 3.2.). In contrast, improved cultivars had higher seed yields but lower seed Se.

**Table 3.3.** Mean seed Se (µg/kg) concentration, Se yield (mg/ha), seed yield (kg/ha) and recommended daily allowance (RDA) (%) of Se for lentil grown in farmers' fields in six divisions in Bangladesh (standard deviation in parentheses). The table includes the number of districts and the number of fields from each division used in this study.

| Division | No. of districts | No. of fields | No. of samples | Seed Se | Se yield | Seed yield | % RDA |
|---|---|---|---|---|---|---|---|
| Barisal | 2 | 4 | 11 | 351 (135) | 306 (76) | 955 (316) | 32 |
| Chittagong | 1 | 3 | 7 | 199 (86) | 195 (83) | 979 (85) | 18 |
| Dhaka | 8 | 23 | 68 | 261 (156) | 319 (193) | 1219 (322) | 23 |
| Khulna | 8 | 26 | 72 | 329 (221) | 430 (312) | 1346 (365) | 30 |
| Rajshahi | 7 | 19 | 57 | 370 (225) | 521 (316) | 1406 (456) | 33 |
| Rangpur | 3 | 4 | 10 | 201 (142) | 115 (77) | 599 (216) | 18 |
| Total/Mean | 29 | 79 | 225 | 312 (201) | 395 (282) | 1263 (409) | 28 |

% RDA was calculated based on 50 g lentil consumption (Monsen 2000)

Soil Se

The soil Se concentration of Bangladesh across 79 farmers' fields ranged from 70 to 296 µg/kg, and the mean was 163 µg/kg (Table 3.4). Soil Se concentration was significantly ($P$<0.001) higher in Rajshahi, Dhaka, Khulna and Chittagong divisions.

*Soil As*

The soil As concentration of Bangladesh across 79 farmers' fields ranged from 1408 to 16415 µg/kg and the mean was 7166 µg/kg (Table 3.4). Soil As was significantly ($P$<0.001) higher in Dhaka and Khulna divisions.

***Location effect on Se yield and seed yield***

The Se yield of 79 farmers' fields ranged from 45 to 1048 mg/ha and the mean was 395 mg/ha (Table 3.3). Se yield in different divisions differ significantly. Rajshahi, Khulna

and Dhaka divisions had higher Se yield of 521 mg/ha, 430 mg/ha, and 319 mg/ha, respectively.

**Table 3.4.** Mean of soil OM (%), soil pH, clay content (%), soil As (µg/kg) and soil Se (µg/kg) in farmers' fields from six divisions in Bangladesh (standard deviation in parentheses). Number of districts, fields and samples were as in Table 3.

| Division | OM (%) | Soil pH | Clay (%) | Soil As | Soil Se |
|---|---|---|---|---|---|
| Barisal | 0.6 (0.2) | 7.9 (0.4) | 33 (6) | 5619 (1286) | 132 (25) |
| Chittagong | 0.7 (0.2) | 6.6 (0. 9) | 48 (18) | 4556 (1319) | 160 (30) |
| Dhaka | 0.8 (0.4) | 7.9 (0.5) | 46 (20) | 8357 (3230) | 167 (44) |
| Khulna | 0.8 (0.3) | 8.2 (0.4) | 35 (18) | 7673 (2399) | 163 (42) |
| Rajshahi | 0.8 (0.2) | 8.0 (0.6) | 41 (14) | 6551 (1844) | 173 (35) |
| Rangpur | 0.5 (0.2) | 7.2 (0.8) | 47 (20) | 2954 (1149) | 113 (21) |
| Mean | 0.8 (0.3) | 8.0 (0.6) | 41 (18) | 7166 (2750) | 163 (41) |

The seed yield of farmers' fields ranged from 420 to 1892 kg/ha and the mean was 1263 kg/ha (Table 3.3). Seed yield in different divisions differed significantly. Like Se yield, Rajshahi, Khulna and Dhaka divisions had high seed yields of 1406 kg/ha, 1346 kg/ha, and 1219 kg/ha, respectively.

***Correlation***

Soil OM was a key factor, which correlated positively and significantly with soil As, soil Se, and seed yield (Table 3.5). Moreover, soil Se was another important factor that correlated positively and significantly with seed Se, Se yield, and seed yield. Amongst all traits, soil Se affected seed Se concentration the most, accounting for 12% of variation. When added to sowing time, the % variation in seed Se concentration accounted for increased to 19%.

### 3.3.2 Study 2: Genotype × environment interaction

The combined analysis of variance over locations indicated that the effects of location and genotype were significant for seed Se, Se yield, and seed yield. However, the location × genotype interaction was not significant for seed Se concentration and Se yield, but was significant for seed yield. Genotypes BARImasur-6 (337 µg/kg) and LR-9-25 (325 µg/kg) produced the highest seed Se concentration (Table 3.6). Genotype LR-9-25 (1300 kg/ha) produced significantly higher seed yield than BLX-02009-06-2 (784 kg/ha), while the rest of the genotypes did not differ. The highest seed yields were found in Ishurdi (1717 kg/ha) followed by Jamalpur (1181 kg/ha) (Table 3.6). Both seed Se concentration and Se yield were highest in Jessore followed by Ishurdi.

## 3.4 Discussion

Soil Se concentration in lentil growing regions of Bangladesh was moderate and overall it produced Se–rich lentils. One cup or 50 g serving will provide 28% of the recommended daily allowance. The farmers' field survey showed large locational variation for seed and soil Se concentrations. In a multi-location trial of advanced breeding lines, genotypes and locations differed significantly in Se uptake but their interaction was not significant. The cultivar BARImasur-6 had high seed Se concentration (337 µg/kg), Se yield (457 mg/ha) and seed yield (1217 kg/ha) in both survey and the multi-location genotypic evaluation trial. Replacing local cultivars with BARImasur-6 will increase the seed Se concentration. Therefore, naturally Se-rich local lentil will be an effective tool against arsenic toxicity and there is opportunity for Se biofortification in Bangladesh.

**Table 3.5.** Correlation coefficients (r) of soil parameters, seed Se concentration and seed yield in farmers' fields.

| | Soil pH | Sand | Silt | Clay | Soil As | Soil Se | Seed Se | Se yield | Seed yield |
|---|---|---|---|---|---|---|---|---|---|
| Soil OM | 0.28* | –0.24* | –0.20 | 0.27* | 0.48** | 0.43** | –0.11 | 0.09 | 0.49** |
| Soil pH | | –0.08 | 0.03 | 0.04 | 0.35** | 0.13 | 0.13 | 0.25* | 0.28* |
| Sand | | | 0.40** | –0.87** | –0.33** | –0.19 | –0.06 | 0.00 | 0.04 |
| Silt | | | | –0.80** | –0.25* | –0.24* | –0.07 | –0.03 | 0.07 |
| Clay | | | | | 0.35** | 0.26* | 0.08 | 0.02 | –0.06 |
| Soil As | | | | | | 0.47** | 0.17 | 0.23* | 0.32** |
| Soil Se | | | | | | | 0.36** | 0.46** | 0.41** |
| Seed Se | | | | | | | | 0.85** | –0.10 |
| Se yield | | | | | | | | | 0.31** |

** Significantly different at P <0.01

* Significantly different at P <0.05

Seed Se concentration and Se yield varied geographically by division within the country. Seed Se concentration was relatively high in Rajshahi (370 µg/kg), Barisal (351 µg/kg) and Khulna (329 µg/kg) divisions, but was lower in Chittagong (199 µg/kg), and Rangpur (201 µg/kg) (Table 3.3). This variation of seed Se concentration and Se yield was associated with relatively higher soil pH and low clay content in the respective division (Table 3.4). Unlike pH, soil Se concentration is more plant available in soil with low clay content (Gissel-Nielsen 1971). Furthermore, Se is less plant available in clayey soil because of strong bonds between Se and clay particles (Hamdy and Gissel-Nielsen 1977). In the genotype × environment trial, higher seed Se concentration and Se yield at Jessore might be also associated with high pH, low OM, and low clay content in the soil (Table 3.1).

In the farmers' field study, soil Se was positively and significantly correlated with seed yield and Se yield. Selenium is not an essential nutrient to plants (Terry *et al.* 2000). However it has some beneficial effects on plant growth i.e. increased aphid resistance in Indian mustard (Hanson *et al.* 2004), drought tolerance in wheat and canola (Kuznetsov *et al.* 2003; Zahedi *et al.* 2009) and yield increase in *Brassica rapa* L. (Lyons *et al.* 2009).

In the farmers' survey, lentil genotypes differed in their seed Se concentration. The multi-location trial offered a more reliable comparison among genotypes and significant genotypic variation was observed for seed Se concentration and Se yield across the four locations. The lack of significance in the location × genotype interaction for seed Se concentration and Se yield showed that differences between genotypes were consistent over locations suggesting the possibility of improving traits with high selection efficiency in the early stages of a breeding program.

**Table 3.6.** Mean of seed Se (µg/kg) concentration, Se yield (mg/ha) and seed yield (kg/ha) of seven lentil genotypes grown in four locations in Bangladesh (standard deviation in parenthesis). (n=21 for each location).

| Location | Parameters | Genotype | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | BLX-04-1 | BLX-04-5 | BLX-06-2 | BLX-19-2 | LR-9-25 | BAM-5 | BAM-6 | Location mean |
| Jamalpur | Seed yield [a] | 1255 | 954 | 868 | 1166 | 1534 | 1045 | 1444 | 1181 |
| | Seed Se | 171 (63) | 181 (45) | 208 (67) | 187 (6) | 244 (78) | 246 (92) | 401 | 234 (133) |
| | Se yield [a] | 215 | 172 | 181.3 | 218 | 375 | 257 | 580 | 286 |
| Joydebpur | Seed yield | 473 (136) | 258 (63) | 241 (50) | 320 (52) | 253 (33) | 243 (86) | 212 (94) | 286 (108) |
| | Seed Se | 138 (51) | 104 (46) | 103 (4) | 138 (20) | 130 (3) | 121 (31) | 112 (10) | 121 (29) |
| | Se yield | 70 (40) | 27 (17) | 25 (5) | 43 (1.39) | 33 (4) | 31 (15) | 24 (12) | 36 (22) |
| Ishurdi | Seed yield | 1359 (79) | 1483 (224) | 1123 (128) | 1848 (172) | 2295 | 1855 | 2054 | 1717 (431) |
| | Seed Se | 214 [b] | 187 (30) | – | 106 (34) | 335 (184) | 224 (54) | 330 | 235 (123) |
| | Se yield | 310 [b] | 276 (56) | – | 193 (51) | 789 (495) | 412 (76) | 647 | 438 (300) |
| Jessore | Seed yield | 1459 (92) | 1125 (314) | 905 (358) | 1094 (347) | 1120 | 1146 | 1159 | 1144 (278) |
| | Seed Se | 349 (245) | 301 (147) | 311 (158) | 271 (132) | 590 (152) | 502 (304) | 502 | 404 (222) |
| | Se yield | 503 (334) | 310 (101) | 312 (273) | 284 (161) | 683 (314) | 597 (390) | 576 | 467 (305) |
| Genotype mean | Seed yield | 1136 (414) | 955 (494) | 784 (380) | 1106 (589) | 1300 | 1072 | 1217 | 1082 (587) |
| | Seed Se | 219 (153) | 193 (102) | 208 (125) | 176 (88) | 325 (207) | 273 (201) | 337 | 248 (176) |
| | Se yield | 275 (243) | 197 (127) | 239 (188) | 185 (117) | 471 (400) | 325 (279) | 457 | 307 (288) |

BLX = BLX-02009; BAM= BARImasur

[a] Only mean data were available

[b] Data for only one replication were available

– Data unavailable

There were seven identified cultivars in the farmers' field survey and two cultivars and five advanced breeding lines were in the multi-location trial. BARImasur-6 was a common cultivar with high seed Se, Se yield and seed yield in both investigations and may be a good choice for a lentil Se improvement program. Around 60% of farmers were using a local cultivar. The replacement of local cultivars with the cultivar BARImasur-6 may help not only to increase lentil Se content but also seed yield in Bangladesh.

There may be hope for soil Se application in Bangladesh. Overall, soil Se (163 μg/kg) was below the worldwide mean Se level of 400 μg/kg (Fordyce 2005). Low levels of an essential plant nutrient in soil are typically rectified by fertilizer application. Khulna, Dhaka, and Rajshahi divisions are the major lentil growing areas in Bangladesh with relatively high seed Se concentrations. These areas would be worth assessing for agronomic biofortification. Soil and foliar applications of Se might be an effective way to improve Se concentration in lentil. For example, annual application of Se fertilizer as little as 10 g/ha increased food Se concentration significantly in Finland. As a consequence, human plasma Se also increased significantly (Varo *et al.* 1988). Bangladesh might consider following the same strategy in the major lentil growing areas. Safe maximum limit of Se in food grain is 1000 μg/kg (Tan 1989). So there is scope to increase seed Se concentration up to threefold. A detailed study is needed to evaluate and calibrate fertilizer application rate for Bangladesh conditions. Manipulation of agronomic practices may also help to increase Se uptake. The farmers' survey identified different agronomic practices for lentil cultivation e.g., different cultivars, fertilizer treatments, sowing times, irrigation regimes and preceding crops. These practices correlated with Se uptake in different ways, for example early sowing led to increased Se uptake probably from the extended period available for greater root

and shoot biomass and increased Se uptake from the soil. Further research is needed to clarify crop management practices that increase Se uptake.

Soil As in Bangladesh (7166 µg/kg) was higher than the worldwide mean (6000 µg/kg) (Baker and Chesnin 1975). In rice production, As has been detected in surface soil (0–15 cm) from 0 to 83,000 µg/kg (Alam and Sattar 2000; Meharg and Rahman 2003). Our soil As data agree with previous surveys in Bangladesh soils. In Bangladesh, As has also been detected in rice grain (358 µg/kg), vegetables (333 µg/kg) (Ali *et al.* 2003; Smith *et al.* 2006) and lentil (1000 µg/kg) (Huq *et al.* 2006b). However, in our study seed As concentration was very low, below the detectable limit of 5.3 µg/kg. This was probably because lentils, unlike rice, are grown under rainfed conditions in Bangladesh and not irrigated with As-rich water and/or plants can control As movement into seeds.

The soil As levels found in this survey were not high enough to reduce lentil seed yield. In the farmers' field survey, soil As was positively and significantly correlated with Se yield and seed yield, in contrast to other studies. Arsenic is highly toxic to plants (National Research Council 1977) and toxicity varies among plant species (Smith *et al.* 1998). Arsenic showed negative effect on germination, chlorophyll content, growth and yield of rice (Rahman *et al.* 2007), wheat (Geng *et al.* 2006), mungbean (*Vigna radiata* (L.) R. Wilczek) (Singh *et al.* 2007), and tomato (*Solanum lycopersicum* L.) (Barrachina *et al.* 1995).

Selenium consumption in Bangladesh is very low, at only 26 µg/day which is correlated with As poisoning (Spallholz *et al.* 2004). In Bangladesh the mean consumption of lentil is only 4 g/day (calculated from FAO 2013) , which supplies approximately two percent of the Se RDA. Lentil is also a good source of protein, iron, zinc, phosphorus, potassium and vitamins (Bhatty 1988). Increasing the consumption of lentil in Bangladesh to 50 g would supply 28% of Se RDA and other important elements. Thus

Se-rich and As-free lentil may be an effective tool to combat As poisoning in Bangladesh.

## 3.5 Conclusion

In Bangladesh lentil could be a potential source of dietary Se for arsenic affected people. Despite being grown on moderate soil Se, the lentils surveyed in this study contained relatively high seed Se, suggesting that the species is efficient at taking up and accumulating Se. The genotype × environment interaction in lentil for seed Se concentration and Se yield was not significant. Therefore, there is scope for agronomic biofortification and efficient plant selection/breeding for Se.

# Chapter 4

## Enhancing selenium concentration in lentil (*Lens culinaris* subsp. *culinaris*) through foliar application

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## Abstract

Selenium (Se) is an essential micronutrient for human and animal health. Globally, more than one billion people are Se deficient due to low dietary Se. Low dietary intake of Se can be improved by Se supplementation, food fortification and biofortification of crops. Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is a popular cool-season food legume in many parts of the world; it is naturally rich with Se and therefore has potential for Se biofortification. A Se foliar application experiment at two locations and a multi-location experiment of 12 genotypes at seven locations were conducted from April to December 2011 in South Australia and Victoria, Australia. Screening of 12 diverse germplasm accessions, including five common genotypes with the Se foliar application and the multi-location experiment, for response to Se foliar application was conducted from July to December 2012 in a glasshouse at The University of Western Australia. Foliar application of a total of 40 g/ha of Se as potassium selenate ($K_2SeO_4$) - 10 g/ha during full bloom and 30 g/ha during the flat pod stage - increased seed Se concentration from an average of 201 to 2772 µg/kg over two locations, but had no effect on seed size or seed yield. Consumption of 20 g of biofortified lentil can supply 100% of the recommended daily allowance of Se. After Se foliar application, cultivars PBA Herald XT (3327 µg/kg), PBA bolt (3212 µg/kg) and PBA Ace (2957 µg/kg) had high seed Se concentrations. These cultivars may be used in lentil biofortification. In the genotypic evaluation trial, significant genotype and location variation was observed for seed Se concentration, but the interaction was non-significant. In the germplasm screening in the glasshouse, significant genotypic variation was also observed for seed Se concentration. In conclusion, foliar application of Se as $K_2SeO_4$ is an efficient agronomic approach to improve seed Se concentration for lentil consumers and there is also scope for genetic biofortification in lentil.

**Keywords:** Australia, lentil, genotype × environment interaction, selenium, biofortification, foliar application

## 4.1 Introduction

Selenium (Se) is an essential element for humans, animals and micro-organisms. It was classified among toxic elements until 1957 when the nutritional benefits of Se were first published (Schwarz and Foltz 1957). Since then, Se has been identified as a key component of amino acids, selenoproteins, enzymes, antioxidants and hormones (Rayman 2002; Spallholz *et al.* 1990). It is also important in the prevention of several cancers (Clark *et al.* 1996; Yu *et al.* 1997), viral infections (Beck *et al.* 1995), oxidative stress, inflammation (Lyons *et al.* 2003), suppression of HIV progression to AIDS (Dworkin 1994), Keshan disease (cardiomyopathy), Kashin-Beck disease (osteoarthropathy) (Reilly 1996), immunodeficiency, thyroid problems, heart disease (Arthur and Beckett 1994) and male infertility (Behne *et al.* 1997).

Worldwide over one billion people suffer from Se deficiency due to low dietary intake of Se (Lyons *et al.* 2003). Plants absorb Se from the soil as selenite or selenate that are incorporated into organic or inorganic Se forms, which are then eaten by animals. Most soil Se levels range from 100 to 2000 µg/kg (Swaine 1955). Soil Se deficiency has been reported in New Zealand, Australia, UK, Thailand, Denmark, Finland, central Siberia, northeast to south central China, Turkey, parts of India, Nepal and Bangladesh (Fordyce 2005; Lyons *et al.* 2005; Spallholz *et al.* 2004; Spallholz *et al.* 2008b) which is reflected in low Se levels in the diet locally. These low levels can be rectified by dietary diversification, Se supplementation (seleno yeast or selenomethionine), food fortification during processing and the new approach of biofortification (Rayman 2002, 2004).

Biofortification is the production of a nutrition-dense crop by means of agronomic management, plant breeding and modern biotechnology (Chen *et al.* 2009; Nestel *et al.* 2006). Agronomic management includes the application of Se fertilizer to soil, soaking seeds in Se solution and foliar application (Broadley *et al.* 2010). Soil and foliar application are commonly practiced in Finland, New Zealand, China and Australia. Soil Se application is less efficient than foliar application to plants (Tveitnes *et al.* 1995) due to higher fertilizer doses, leaching losses (Gissel-Nielsen *et al.* 1984) and Se fixing in the soil (Gissel-Nielsen 1977) by organic matter and clay particles (Reilly 1996).

The two forms of Se, selenate and selenite, can be used for foliar application (Sima and Gissel-Nielsen 1985). The uptake of selenate and its distribution in plants is faster than that of selenite (Arvy 1993), hence selenate is more efficient at increasing plant Se concentration (Sima and Gissel-Nielsen 1985).

Foliar application increased Se concentration significantly in seeds of wheat (*Triticum aestivum* L.) (Broadley *et al.* 2010; Ducsay *et al.* 2007), barley (*Hordeum vulgare* L.) (Sima and Gissel-Nielsen 1985), maize (*Zea mays* L.) (Cary and Rutzke 1981), soybean (*Glycine max* [L.] Merr.) (Djanaguiraman *et al.* 2005; Yang *et al.* 2002a), pea (*Pisum sativum* L.) (Smrkolj *et al.* 2005a) and common bean (*Phaseolus vulgaris* L.) (Smrkolj *et al.* 2007). Cary and Rutzke (1981) demonstrated that Se foliar application at 15 g/ha as sodium selenite ($Na_2SeO_3$) at the reproductive stage (240 µg/kg) in maize was more efficient than at the vegetative stage (120 µg/kg), yet no Se application effect was found on yield. Sima and Gissel-Nielsen (1985) found that foliar application of Se as selenate at 5–50 g/ha increased barley seed Se concentration from deficient to adequate levels for human consumption.

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is an edible pulse and a rich source of protein (20–30%), prebiotic carbohydrates, essential fatty acids and a range of

micronutrients including Se (Bhatty 1988; Johnson *et al.* 2013; Thavarajah *et al.* 2011b). Selenium in lentil ranged from 22–672 µg/kg and is easily incorporated with proteins (Thavarajah *et al.* 2007; Thavarajah *et al.* 2011a). Cooking lentil seeds in boiling water does not change the total Se content (Thavarajah *et al.* 2008). All these factors make lentil an appropriate crop for Se biofortification.

Australia grows lentil as an export commodity in rotation with cereals. During the 2007–2011 period Australia produced on an average 171,579 t of lentil (3% of world production) annually from 142,352 ha of land (3.7% of world lentil area) (FAO 2014). Australia was the 3rd largest lentil exporting country in 2011 (FAO 2014). On average during 2007–2011 period, Australia exported 129,222 t of lentil annually which comprised 75% of local production (FAO 2014) to Bangladesh, Egypt, India, Pakistan, Saudi Arabia, Sri Lanka, Turkey and the United Arab Emirates (Pulse Australia 2011). Dietary Se is low in many of these lentil-consuming countries. Seed Se concentrations of non Se fertilized Australian lentil are low (Thavarajah *et al.* 2011a). Selenium-rich biofortified Australian lentil could benefit these consumers, but biofortification has not been attempted. Therefore, the objectives of this study were to evaluate: (1) the effect of foliar Se application at two locations on lentil seed yield, seed size and seed Se concentration, (2) genotypic variability in Se uptake and seed Se concentration among lentil genotypes at seven locations in South Australia (SA) and Victoria, the main lentil growing States in Australia and (3) the variation in Se response in a diverse sample of germplasm (12 lentil accessions) in the glasshouse of The University of Western Australia.

## 4.2 Materials and methods

Three experiments were conducted in winter/spring of 2011 and 2012. The first and second experiments had 12 common lentil genotypes (Table 4.1, Sl. No. 1–12) including one green *macrosperma* lentil, Boomer and 11 genotypes of red *microsperma* lentils. The third experiment also had 12 genotypes of which five genotypes were in common with the Se foliar application and the genotype × environment interaction experiment (Table 4.1, Sl. No. 1–4, 9, 13–19). The genotypes included five green *macrosperma* lines and seven lines of red *microsperma* lentils from six countries. Country of origin, identity, cotyledon colour and seed size of these genotypes are presented in Table 4.1.

***Experiment 1: Foliar Se application***

Experiment 1 was a two-factor – Se application and genotypes – experiment in a split-plot design with three replications at Horsham, Victoria and Melton, SA (Table 4.2). Two levels of Se application (+/– Se) were the main plots (application details in Table 4.3) and 12 genotypes were the sub-plots. Plot size was 8.75 $m^2$ in Victoria and 9.45 $m^2$ in SA. The experiment was sown on 3 June 2011 at Horsham and on 9 June 2011 at Melton. Sowing was at 125 seeds/$m^2$ with an inter-row distance of 30 cm in Victoria and 22.5 cm in SA. A total of Se of 40 g/ha was applied over two applications with firstly 10 g Se/ha applied at full bloom (flowers open on nodes 10–13 of the basal primary branch) and secondly with 30 g Se/ha applied at flat pod stage (pods on nodes 10–13 of the basal primary branch have reached full length and are largely flat; seeds fill less than half of the pod area but can be felt as a bump between the fingers) (Erskine *et al.* 1990). At maturity in November 2011 each plot was machine-harvested. The weight of 100 randomly-selected seeds from each plot was measured after air drying. Seed yield was measured on an individual plot basis and converted to kg/ha. Se yield

was calculated as the product of seed yield and seed Se concentration and expressed in mg/ha.

**Table 4.1.** Country of origin, type of germplasm accession, cotyledon colour and seed size of lentil genotypes used in the foliar Se application (Sl. No.1-12), genotype × environment interaction (Sl. No.1-12) and germplasm screening experiment (Sl. No.1-4, 9, 13-19).

| Sl. No. | Genotype name | Country of origin | Accession type | Cotyledon colour | 100 seed weight (g) |
|---|---|---|---|---|---|
| 1. | Nugget | Australia | Cultivar | Red | 4.22 |
| 2. | Boomer | Australia | Cultivar | Yellow | 6.52 |
| 3. | Nipper | Australia | Cultivar | Red | 3.46 |
| 4. | Northfield * | Jordan | Landrace | Red | 3.41 |
| 5. | PBA Blitz | Australia | Cultivar | Red | 4.98 |
| 6. | PBA Bounty | Australia | Cultivar | Red | 4.00 |
| 7. | PBA Flash | Australia | Cultivar | Red | 4.24 |
| 8. | PBA Herald | Australia | Cultivar | Red | 3.28 |
| 9. | PBA Jumbo | Australia | Cultivar | Red | 5.10 |
| 10. | PBA Bolt | Australia | Cultivar | Red | 4.41 |
| 11. | PBA Ace | Australia | Cultivar | Red | 4.33 |
| 12. | CIPAL 902 | Australia | Breeding line | Red | 4.83 |
| 13. | ATC 71131 | Australia | Landrace | Yellow | 5.14 |
| 14. | ATC 70860 | Bangladesh | Landrace | Red | 2.05 |
| 15. | ATC 70487 | Chile | Landrace | Yellow | 6.41 |
| 16. | ATC 71309 | Chile | Landrace | Yellow | 6.91 |
| 17. | ATC 70401 | Syria | Landrace | Red | 3.82 |
| 18. | ATC 70402 | Syria | Landrace | Red | 3.70 |
| 19. | ATC 70507 | Turkey | Landrace | Yellow | 6.19 |

* Released as a cultivar in Australia

**Table 4.2.** Latitude (°S), longitude (°E), total seasonal rainfall (mm), average minimum and maximum temperatures (°C) at seven locations over the growing period (April–November, 2011).

| Location | Latitude | Longitude | Total rainfall | Min. temp | Max. temp |
|---|---|---|---|---|---|
| Beulah, Vic | 35.94 | 142.42 | 204 | 6 | 19 |
| Hopetoun, Vic | 35.73 | 142.36 | 193 | 6 | 21 |
| Horsham, Vic | 36.71 | 142.20 | 274 | 6 | 19 |
| Minyip, Vic | 36.46 | 142.59 | 242 | 7 | 20 |
| Mallala, SA | 34.44 | 138.51 | 269 | 8 | 21 |
| Melton, SA | 34.08 | 137.98 | 235 | 9 | 20 |
| Willamulka, SA | 33.93 | 137.86 | 260 | 8 | 21 |

SA, South Australia; Vic, Victoria

**Table 4.3.** Type of sprayer, date and rate of application used in foliar Se application experiment at Melton and Horsham.

| Spray application | Melton | | Horsham | |
|---|---|---|---|---|
| | 1st | 2nd | 1st | 2nd |
| Knapsack Sprayer | Power-operated | | Hand held | |
| Date | 20.09.2011 | 07.10.2011 | 5.10.2011 | 20.10.2011 |
| Water (l/ha) | 200 | 200 | 370 | 370 |
| Se as $K_2SeO_4$ (g/ha) * | 10 | 30 | 10 | 30 |

*Spray volume same as water volume

***Experiment 2: Genotypic evaluation***

Experiment 2 compared the same 12 genotypes in a randomized complete block design (RCBD) with three replications at seven locations (Table 4.2). Plot size, sowing rate and spacing were as Experiment 1. Plots were sown between Week 3 of May and Week 2 of June 2011. The location trials were machine-harvested between Week 1 of November and Week 1 of December 2011. Hundred seed weight, seed yield and Se yield were measured as described previously.

*Soil sample collection, preparation and Se measurement for both experiments*

Three soil samples were collected from each location at 0–30 cm depth. Samples were stored separately in polyethylene bags and air dried (≤ 40°C). Clods were broken; roots, leaves and others inert material were removed from the samples, passed through a 2 mm sieve and stored at –20°C until analysis. The samples were shipped to Cornell Nutrient Analysis Laboratory, Cornell University, Ithaca, USA for soil Se measurement. Total soil Se was measured by the perchloric acid digestion method (Rahman *et al.* 2013).

*Tissue and seed sample collection, preparation and Se measurement*

Three lentil branches were collected at two crop growth stages from each plot in the foliar Se application trial. The first sample was collected at full bloom stage, before Se application. The second sample was collected two weeks after the first application, at flat pod stage prior to the second foliar application. Lentil branches were air dried at 40°C for 24 h. Immature pods were removed from branches, then the branches along with leaves were ground into powder. The ground samples were shipped to the Pulse Quality Laboratory, North Dakota State University (NDSU), Fargo, USA where the samples were stored at –20°C until analysis. Total Se concentration in lentil branches was determined using the modified $HNO_3$–$H_2O_2$ method described by Thavarajah *et al.* (2008). Measurement of total Se concentration was validated using the National Institute of Standards and Technology standard reference (1567a wheat flour, Se=1.1±0.1 mg/kg) and a laboratory reference sample (CDC Redberry, Se=700 µg/kg). Total Se concentration was determined using ICP-ES (Inductively Coupled Plasma Emission Spectrometry) (ICP-ES; ICP-6500 Duo, Thermo Fisher Scientific, PA, USA) with a detection limit of 75 µg/l and quantified using a calibration curve that ranged from 1–5 mg/l.

Seed samples were collected from both Experiments after harvesting and threshing individual plots. Seeds were air dried (≤ 40°C), cleaned and ground into a fine powder (<0.5 mm sieve). The samples were shipped to the Pulse Quality Laboratory, NDSU and stored at –20°C until analysis. Seed Se concentration was determined using the modified $HNO_3$–$H_2O_2$ method described by Thavarajah *et al.* (2008) and Rahman *et al.* (2013).

***Experiment 3: Germplasm screening***

The experiment was conducted in the glasshouse of the Plant Growth Facility, The University of Western Australia during July-December, 2012. Initially 150 germplasm accessions from ten countries of origin were sown in pots on June 13, 2012. However, the experiment was infested with foot rot in the pots with only 143 out of 900 pots containing healthy plants. The experiment was abandoned. However, 12 diverse germplasm accessions were re-sown as a separate experiment on July 11, 2012. The experiment was a two-factor randomized complete block design (RCBD) with three replications. The two factors were Se application (two levels, +/– Se) as one factor and lentil genotypes (12) as another factor. Seeds were sown in 72 pots. The individual pot volume of each pot was three litres, with a hole in the bottom for drainage. Pots were filled first with 2 cm gravel, then with river sand. During sowing, sand was inoculated with rhizobia at 7.28 kg/ha (Becker Underwood Company). Ten seeds were sown per pot and later thinned down to six plants per plot. Pots were watered regularly with de-ionized (DI) water as required. Each pot was fertilized weekly, starting one month after sowing until physiological maturity of each genotype. Water or Se solution was applied once at 50% flowering stage (first opening of flower at 50% plant of a pot) with a hand-pump sprayer to each pot with either 5.5 mL of water or Se solution containing 17.5 mg/l of Se as $K_2SeO_4$ (equivalent to 40 g Se/ha). Pots were harvested individually at

physiological maturity. The plants were air-dried at 40°C for 24 h. Pods were picked and counted. The numbers of seeds per pods were counted from 10 randomly selected pods. The total number of seeds and 100 seeds were counted by a seed counter (Contador E, Baumann Saatzuchtbedarf, Germany) and weights recorded. Numbers of seeds per plant were calculated by dividing total number of seeds by number of plants per pot. Dried seeds were ground into a fine powder (<0.5 mm sieve). The samples were shipped to the Pulse Quality Laboratory, NDSU and stored at –20°C until analysis. Seed Se concentration was determined using the method described earlier in this chapter.

**Statistical analysis**

Two-factor split-plot analysis of variance was performed in GenStat 14.1 (VSN International Ltd) for each foliar Se application trial and then a combined analysis was performed. For the genotypic evaluation trials, a general linear mixed model analysis of variance (ANOVA) was performed using restriction maximum likelihood (REML) procedure in ASReml–R version 3. For the germplasm screening, two-factor analysis of variance was performed in 'agricolae' package of R (Felipe de Mendiburu 2012). For the three experiments, means were separated by Fisher's least significant difference test (LSD) at $P<0.05$. Genotypic and phenotypic correlation coefficients were estimated by the formulae of Johnson *et al.* (1955), Miller *et al.* (1958) and Singh and Chaudhary (1979).

## 4.3 Results

### 4.3.1 Experiment 1: Foliar Se application

There were significant differences between the foliar application of water and Se for both tissue ($P<0.001$) and seed Se concentration ($P<0.001$) and Se yield ($P<0.001$), but not for seed size ($P<0.275$) and seed yield ($P<0.403$) (Table 4.4). The average tissue Se

concentration increased from 29 µg/kg with water application to 478 µg/kg with Se application at Melton, and similarly from 85 to 659 µg/kg at Horsham (Fig. 4.1). On average, after Se application tissue Se concentration increased from 57 to 568 µg/kg, seed Se concentration from 201 to 2772 µg/kg (Table 4.5) and Se yield from 464 to 6917 mg/ha.

With the water treatment, seed Se concentration was significantly ($P$<0.001) higher in Horsham (230 µg/kg) than Melton (172 µg/kg); but this became statistically equivalent after Se application (Fig. 4.1).

Significant ($P$<0.001) variation was also observed for Se yield at the two locations with Melton significantly higher than Horsham (Fig. 4.1). In general, foliar Se application increased Se yield by 40-fold compared with the water application at both locations.

### 4.3.2 Experiment 2: Genotypic evaluation

The ANOVA identified significant genotypic and location variation for seed Se concentration, seed size, and seed yield (Table 4.4). There was also significant location variation for Se yield. However, the genotype × location interaction was non-significant for all these characters except 100 seed weight.

Seed Se concentration ranged from 164 to 236 µg/kg among the 12 genotypes with an average of 199 µg/kg. The cultivars Northfield (236 µg/kg), Boomer (225 µg/kg), PBA Herald XT (216 µg/kg) and PBA Jumbo (211 µg/kg) had significantly higher seed Se concentrations than other cultivars (Table 4.6). Seed Se concentration across the seven locations ranged from 106 to 444 µg/kg, with the highest at Horsham (Fig. 4.2).

**Table 4.4.** Analyses of variance showing degrees of freedom (df) and F-test (P) values for different variables of the foliar Se application, genotypic evaluation and germplasm screening experiments.

| Sources of variation | df | P value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Maturity days | No. of pods per plant | No. of seeds per pod | Tissue Se | Seed Se | Se yield | 100 seed wt | Biological yield | Seed yield |
| Exp. 1: Foliar Se application [*] | | | | | | | | | | |
| Spray | 1 | | | | <0.001 | <0.001 | <0.001 | 0.275 | | 0.403 |
| Genotype | 11 | | | | 0.061 | <0.001 | 0.852 | <0.001 | | 0.001 |
| Location | 1 | | | | <0.001 | 0.171 | <0.001 | <0.001 | | <0.001 |
| Spray × genotype | 11 | | | | 0.434 | <0.001 | 0.836 | 0.364 | | 0.587 |
| Spray × location | 1 | | | | <0.001 | 0.005 | <0.001 | 0.520 | | 0.458 |
| Genotype × location | 11 | | | | 0.589 | 0.560 | 0.469 | <0.001 | | <0.001 |
| Spray × genotype × location | 11 | | | | 0.922 | 0.608 | 0.643 | 0.747 | | 0.812 |
| Exp. 2: Genotypic evaluation [†] | | | | | | | | | | |
| Genotype | 11 | | | | | <0.001 | <0.001 | <0.001 | | <0.001 |
| Location | 6 | | | | | 0.021 | 0.167 | <0.001 | | <0.001 |
| Genotype × location | 66 | | | | | 0.997 | 0.963 | <0.001 | | 0.081 |
| Exp. 3: Germplasm screening [††] | | | | | | | | | | |
| Spray | 1 | 0.568 | 0.108 | 0.486 | | <0.001 | | 0.640 | 0.687 | 0.816 |
| Genotype | 11 | <0.001 | <0.001 | <0.001 | | <0.001 | | <0.001 | <0.001 | <0.001 |
| Spray × genotype | 11 | 0.261 | 0.670 | 0.839 | | <0.001 | | 0.418 | 0.936 | 0.910 |
| Spray × country | 5 | | | | | 0.024 | | | | |
| Spray × cotyledon colour | 1 | | | | | 0.655 | | | | |
| Spray × seed size | 1 | | | | | 0.655 | | | | |
| Spray × accession type | 1 | | | | | 0.746 | | | | |

Number of samples, n = [*] 144, [†] 252 and [††] 72; df, degrees of freedom

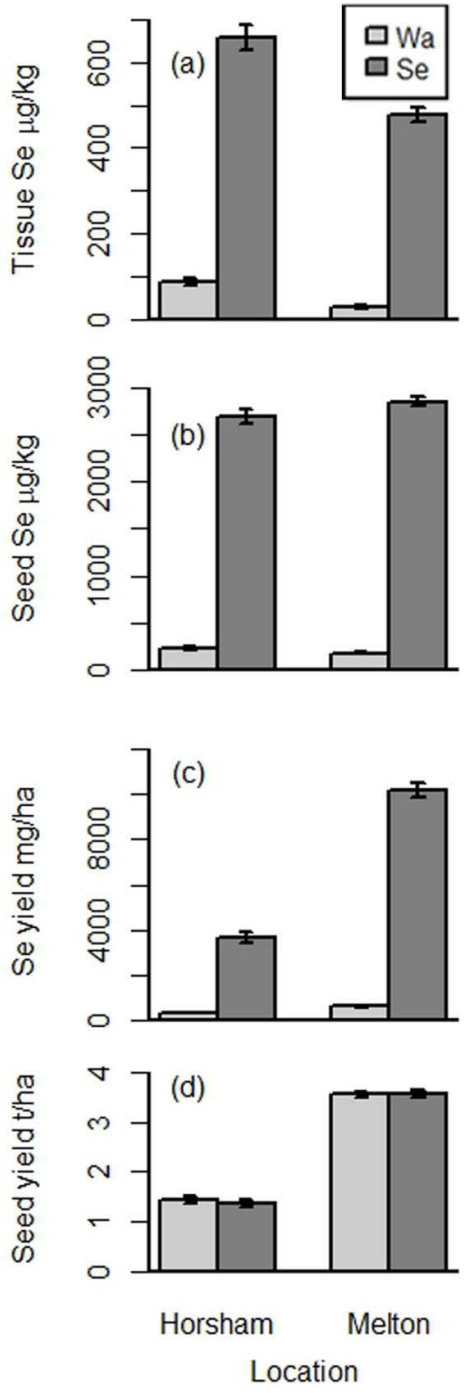


**Fig. 4.1.** Effect of water vs. Se foliar application on mean (n=36) and standard error (SE-bar) of (a) tissue Se concentration (µg/kg), (b) seed Se concentration (µg/kg), (c) Se yield (mg/ha) and (d) seed yield (t/ha) in Horsham and Melton in the foliar Se application experiment.

Seed yield varied significantly for genotypes and locations. The cultivars PBA Ace (2.88 t/ha), PBA Bolt (2.67 t/ha), PBA Bounty (2.64 t/ha) and CIPAL 902 (2.60 t/ha) had significantly higher seed yield than other cultivars (Table 4.6). Across the seven locations, seed yield ranged from 1.45 to 3.32 t/ha with Melton, Minyip and Mallala producing significantly higher seed yield than the other locations (Fig. 4.2).

Soil Se concentration across the seven locations varied from 151 to 737 µg/kg with an average of 281 µg/kg. Significantly higher soil Se concentrations were observed at Mallala and Willamulka (Fig. 4.2).

Significant cultivar effects were observed for seed size ranging from 3.28 to 5.10 g per 100 seed among the red lentil genotypes, while the large-seeded green cultivar Boomer had a seed size of 6.52 g/100 seed. A significant location effect was also observed for seed size with the largest seeds produced at Mallala.

**Table 4.5.** Effect of water and Se foliar application on seed Se concentration (µg/kg) of 12 lentil cultivars grown in the foliar Se application experiment.

| Cultivars | Seed Se concentration | |
|---|---|---|
| | – Se application | + Se application |
| Nugget | 211 | 2460 |
| Boomer | 241 | 2646 |
| Nipper | 164 | 2700 |
| Northfield | 206 | 2766 |
| PBA Blitz | 172 | 2783 |
| PBA Bounty | 207 | 2662 |
| PBA Flash | 170 | 2684 |
| PBA Herald XT | 239 | 3327 |
| PBA Jumbo | 204 | 2528 |
| PBA Bolt | 201 | 3212 |
| PBA Ace | 239 | 2957 |
| CIPAL 902 | 159 | 2533 |
| Mean | 201 | 2772 |
| Spray × genotype S.E.D.* | 141 | |

* S.E.D, Standard error of difference

**Table 4.6.** Performance of 12 lentil cultivars for seed Se concentration (μg/kg) and seed yield (t/ha) across seven locations in the genotypic evaluation trial.

| Cultivars | Seed Se concentration | Seed yield |
|---|---|---|
| Nugget | 164 | 2.30 |
| Boomer | 225 | 2.15 |
| Nipper | 201 | 2.17 |
| Northfield | 236 | 2.19 |
| PBA Blitz | 205 | 2.39 |
| PBA Bounty | 184 | 2.64 |
| PBA Flash | 168 | 2.35 |
| PBA Herald XT | 216 | 2.23 |
| PBA Jumbo | 211 | 2.44 |
| PBA Bolt | 179 | 2.67 |
| PBA Ace | 193 | 2.88 |
| CIPAL 902 | 197 | 2.60 |
| Mean | 199 | 2.43 |
| S.E.D.* | 21 | 0.11 |

* S.E.D., standard error of difference

The genotypic correlation coefficient for seed Se concentration and seed yield ($r_g$= –0.60, $P$<0.001) was negative, strong and highly significant, and the phenotypic correlation coefficient ($r_p$= –0.21, $P$<0.01) was negative, weaker but significant. Seed yield (r=0.44, $P$<0.05) was positively and significantly associated with soil Se concentration. The genotypic correlation coefficient between seed Se concentration and seed weight ($r_g$=0.184, $P$<0.01) was positive, weak, but significant; while the equivalent phenotypic correlation was non-significant.

### 4.3.3 Experiment 3: Germplasm screening

The striking genetic diversity within this small sample of 12 genotypes is illustrated for seed size by a range among genotypes from 2.05 to 6.91 g/100 seed (Table 4.1) and for time to maturity by the spread of two months from 110 to 172 days.

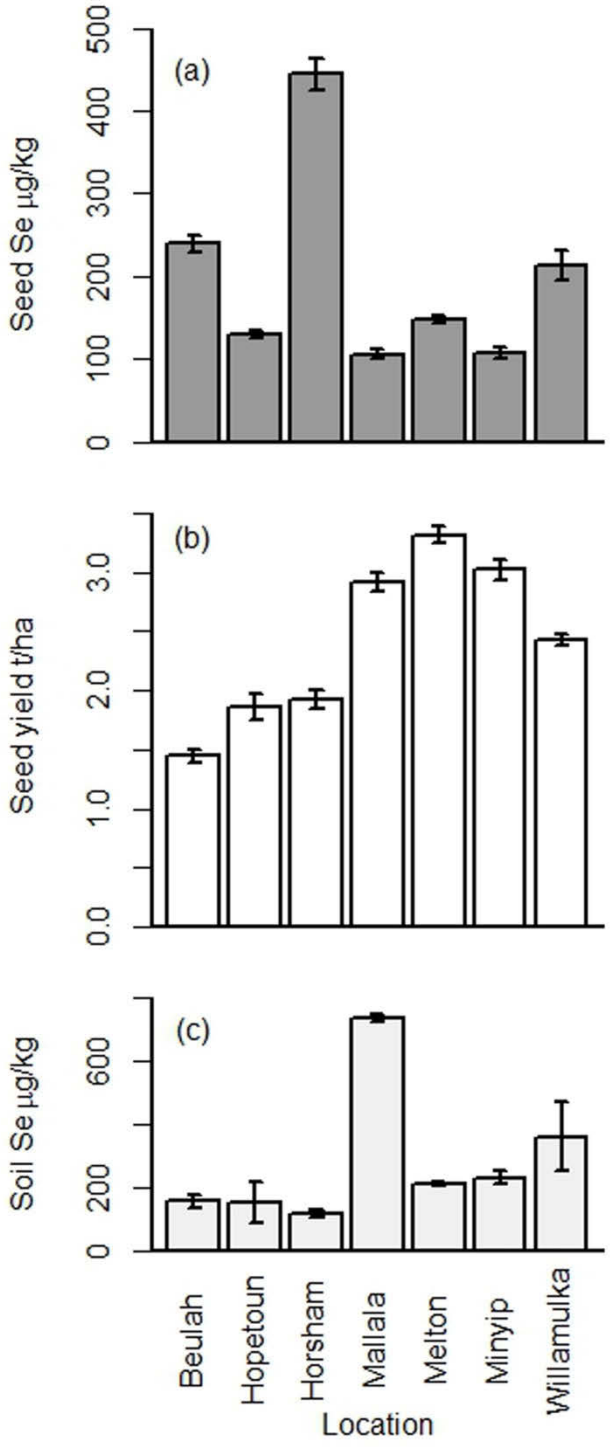


**Fig. 4.2.** Mean and standard error (SE-bar) at seven locations for (a) seed Se concentration* (µg/kg), (b) seed yield* (t/ha), (c) soil Se concentration† (µg/kg) across 12 cultivars in the genotypic evaluation trial.

*n=36; †n=3

Significant spray effect, genotypic variation and differences between countries of origin of the genotypes were found for seed Se concentration (Table 4.4). However, there was no effect of Se application on seed size, seed yield and other yield components. There was also no effect of Se application between the *micro* and *macrosperma* lentil for seed Se concentration. On average seed Se concentration increased from 142 µg/kg with water application to 436 µg/kg with Se application. Genotypes Northfield, Nugget, ATC 71309, Boomer, ATC 70860 and Nipper had significantly higher seed Se concentration than the other genotypes with Se application. Northfield had the lowest seed Se concentration with water treatment, but ranked the highest with Se foliar spray treatment (Fig. 4.3).

Lentil genotypes from Jordan (93 vs 529 µg/kg), Bangladesh (203 vs 475 µg/kg) and Australia (130 vs 441 µg/kg) had significantly higher seed Se concentration than lentil from Chile (195 vs 407 µg/kg), Turkey (108 vs 406 µg/kg) and Syria (134 vs 402 µg/kg) with Se foliar application treatment.

## 4.4 Discussion

Lentil exhibited a high capacity to store Se in seed with foliar Se application increasing from 201 to 2772 µg/kg in the field and 93 to 529 µg/kg in the glasshouse. Selenium application had no effect on seed size or seed yield. The cultivars PBA Herald XT (3327 µg/kg), PBA Bolt (3212) and PBA Ace (2957 µg/kg) had higher seed Se concentration with Se applications than other cultivars.

Based on information from wheat (Broadley *et al.* 2010; Ducsay *et al.* 2007), barley (Sima and Gissel-Nielsen 1985), maize (Cary and Rutzke 1981), soybean (Djanaguiraman *et al.* 2005; Yang *et al.* 2002a) and pea (Smrkolj *et al.* 2005a), we

applied a total of 40 g/ha Se as $K_2SeO_4$ in two applications at full bloom and flat pod stage and found this regime very effective for lentil Se biofortification.

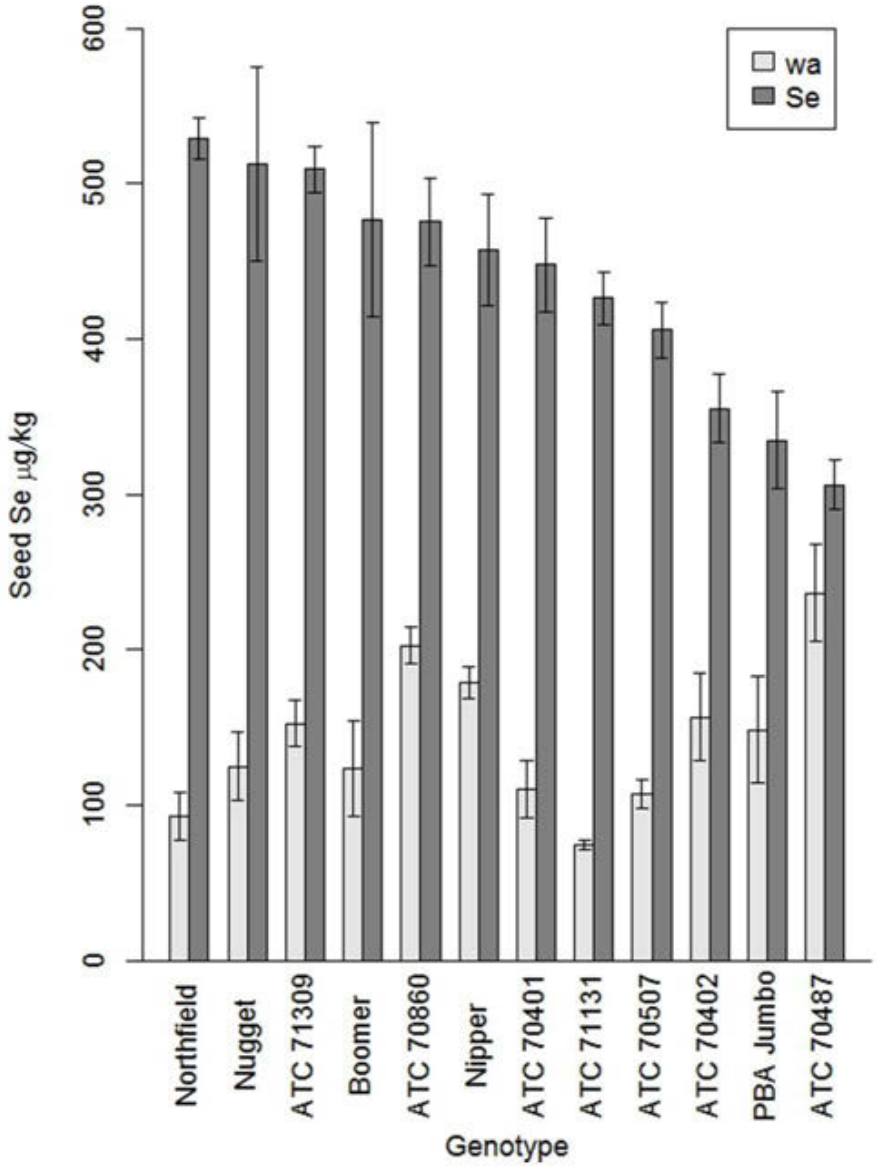


**Fig. 4.3.** Effect of water and Se foliar application on average seed Se concentration (µg/kg) and standard error (SE) bar of 12 diverse lentil genotypes.

Tissue Se concentration in lentil increased approximately ten-fold after Se application from 58 to 569 µg/kg. Similarly, tissue Se concentration increased in barley from 30 to 527 µg/kg (Sima and Gissel-Nielsen 1985) and in soybean from 100 to 300 µg/kg (Djanaguiraman *et al.* 2005).

Lentil seed Se concentration increased sharply from 201 to 2772 µg/kg with Se foliar application in the field. At various application rates, foliar Se application significantly increased seed Se concentration in barley from 10 to 702 µg/kg (Sima and Gissel-Nielsen 1985), wheat from 16 to 2524 µg/kg (Broadley *et al.* 2010), soybean from 28 to 1211 µg/kg (Yang *et al.* 2002a) pea from 21 to 743 (Smrkolj *et al.* 2005a) and common bean from 30 to 2379 µg/kg (Smrkolj *et al.* 2007).

Selenium concentration in the seed was greater in the field than the germplasm screening experiments in the glasshouse. This may be due to two applications of Se in the field and the lower temperature (13° C) compared to the glasshouse (21° C) over the growing period. In addition, field sites had high soil Se concentration compared to nil Se in the sand medium used in the glasshouse experiment.

In the current genotype × environment interaction experiment, without Se application, the seed Se concentration in Australian lentil is 199 µg/kg, which is lower than in Canadian (672 µg/kg) and Bangladeshi (312 µg/kg) lentil; but higher than lentil grown in India (208 µg/kg), Nepal (180 µg/kg), Turkey (47 µg/kg), Morocco (28 µg/kg), northwestern USA (26 µg/kg), and Syria (22 µg/kg) (Abhay and Krishnaswamy 1997; Rahman *et al.* 2013; Thavarajah *et al.* 2008; Thavarajah *et al.* 2011a). However, agronomic biofortification of Australian lentil by Se fertilization increased seed Se concentration beyond that reported for the no-Se applied lentil from Canada and Bangladesh.

Biofortified lentil represents a rich source of Se and is also safe for those with Se-rich diets. The World Health Organization (WHO) recommended daily allowance (RDA) of Se is 55 µg/person/day (Monsen 2000). Consumption of 20 g of this biofortified lentil will provide 100% of the RDA. Data on the consumption of individual food grains, including pulse crops, are not as readily available as that for area, production and trade

(Akibode and Maredia 2012). Based on current pulse consumption, the average maximum lentil consumption in developing countries does not exceed 22 g/person/day (Akibode and Maredia 2012). In developed countries such as Australia where Se is not deficient in the population, average daily consumption of lentil is 5.34 g/person/day (calculated from FAO 2014). Without changing food habits, consumption of biofortified lentil would supply adequate Se to consumers, similar to the supply of vitamin A and Fe to children in biofortified sweet potato and common bean, respectively in Uganda (Wagabaza 2013).

Selenium biofortification is more economic than Se supplementation, but from the farmers' point of view Se biofortification of lentil is not economic. Foliar Se application costs were estimated as US$ 483/ha (113 g potassium selenate $461, two applications $22). Average seed yield of biofortified lentil was 2.47 t/ha which can provide 100% RDA of Se to 123,500 persons/day or 338 persons/year this is equivalent to $1.42/person/year. Cost of equivalent Se supplementation with Se tablets commercially available in Australia is US$ 31/person/year (http://www.vitamingrocer.com.au) which is higher than Se biofortification. The cost of Se biofortification in other crops is unavailable. However, compared with wheat flour fortification with iron (Fe) and zinc (Zn), Se biofortification is costly. Fortification of wheat flour with Fe costs $0.12/person/year (Layrisse *et al.* 1996) and with Zn costs $0.06–0.24/person/year (Horton 2006). Farmers are unlikely to adopt Se foliar application unless there is a premium for high seed Se concentration. In our experiments high quality of Se was applied for research purpose and further research is required with commercial Se fertilizers to reduce the cost of Se application.

There was a significant cultivar effect observed for Se application. The cultivars PBA Herald XT, PBA Bolt and PBA Ace were efficient in Se uptake with significantly

higher seed Se concentration than other cultivars in Se application in the field. This result differs with other crops, where cultivar differences were found non-significant in common bean (Smrkolj *et al.* 2007), soybean (Yang *et al.* 2002a) and wheat (Broadley *et al.* 2010; Curtin *et al.* 2006) for seed Se concentration with foliar Se application. The lentil cultivars PBA Herald XT, PBA Bolt and PBA Ace may be used in a lentil biofortification program.

Significant location effects for lentil seed Se concentration were found in the genotypic evaluation trial with the highest seed Se concentration observed at Horsham. Melton and Horsham were previously reported as contrasting for seed Se concentration in Australia (Thavarajah *et al.* 2011a) and hence selected as sites for the Se application experiment. However, the difference between locations disappeared with Se application. Clearly, the massive response to Se application allows biofortification at contrasting sites.

Selenium foliar application did not affect lentil seed size or seed yield in both foliar Se application and germplasm screening in the glasshouse, confirming research on seed yield in wheat (Lyons *et al.* 2004), barley (Sima and Gissel-Nielsen 1985), rice (*Oryza sativa* L.) (Hu *et al.* 2002) and soybean (Yang *et al.* 2002a). However some beneficial effects have been reported on plant growth i.e. drought tolerance in wheat (Kuznetsov *et al.* 2003) and canola (*Brassica napus* L.) (Zahedi *et al.* 2009), a 40% yield increase in *Brassica rapa* L. (Lyons *et al.* 2009), and increased aphid resistance in Indian mustard (*Brassica juncea* L.) (Hanson *et al.* 2004).

Agronomic biofortification is a rapid way to increase Se concentration in crops but not a complete and sustainable solution (Nestel *et al.* 2006). In contrast, breeding to improve Se uptake by plants may be an effective and sustainable strategy for the long term (Nestel *et al.* 2006). Our main objective was to observe the effect of foliar Se

application on lentil. However, we also observed some genetic variation for seed Se concentration in the genotypic evaluation trial, which reflects similar findings in lentil (Rahman *et al.* 2013; Thavarajah *et al.* 2008), wheat, rice, brassica vegetables (Combs 2001) and soybean (Wei 1996). Though the number of lentil germplasm was small however, significant genotypic variation was found in the germplasm screening. Significant genotypic variation was also found for seed Fe and Zn concentration in lentil (Kumar *et al.* 2013) and cereals (Graham *et al.* 1999). The results of the genotype × environment interaction study are consistent with the results of our previous study on lentil in Bangladesh (Rahman *et al.* 2013) and Canada (Thavarajah *et al.* 2008). Both genotype and environment effects exist for seed Se concentration irrespective of seed size. However, the genotype × environment interaction was non-significant for seed Se concentration. Wherever genotypic variation exists, there is scope for breeding (Nestel *et al.* 2006). These findings strongly suggest the possibility of selecting for Se uptake in the early stages of a breeding program.

Soil Se concentration in this study ranged from 119–738 µg/kg across locations. Although the soil Se concentration in Australian lentil-growing soil was higher than in Canada (37–301 µg/kg) (Thavarajah *et al.* 2008) and Bangladesh (113–189 µg/kg) (Rahman *et al.* 2013), seed Se concentration in Australian lentil was lower than both these countries. Other factors that also indicate plant-available Se are soil texture, pH, organic matter, CEC, and redox potential (Cary and Allaway 1969; Gissel-Nielsen *et al.* 1984; Johnsson 1991). Clearly further investigation is required to identify the main driving factor(s) of seed Se concentration in lentil.

## 4.5 Conclusion

A total of 40 g/ha foliar application of Se in field experiment during the reproductive stage increased seed Se concentration from 201 to 2772 µg/kg which is effective for

lentil biofortification in Australia. Without changing food habits, biofortified lentil would provide adequate dietary Se which is low in many lentil-consuming countries. Therefore, biofortified lentil might be a good source of dietary Se to consumers.

# Chapter 5

# General Discussion

Micronutrient malnutrition is a major global health problem that affects more than half of the world population. Due to poverty and food habits, people in rural areas especially in the developing world have limited access to technologies to minimise micronutrient malnutrition. Biofortification is likely to address micronutrient malnutrition in an affordable and sustainable way. This thesis explored the scope of Se biofortification in lentil by a large field survey followed by a series of designed experiments in Bangladesh and Australia.

The hypothesis that soil Se concentration and lentil seed Se concentration are low in Bangladesh was tested in a farmers' field survey. It was determined that soil Se concentration in Bangladesh was moderate and lentil seed Se concentration was relatively high compared with lentil grown in Australia, India, Morocco, Nepal, Syria, Turkey and the USA. However, from Bangladesh perspective, seed Se concentration in Bangladeshi lentil is not sufficiently high to provide adequate dietary Se because of low consumption of lentil, only 4 g/person/day that supplies approximately two percent of Se RDA (Rahman *et al.* 2013). If seed Se concentration increased, for example by 100% with biofortification, existing lentil consumption will supply only four percent of the Se RDA. Thus lentil biofortification alone will be insufficient to increase Se concentration to consumers in Bangladesh. Biofortification of lentil along with rice and/or wheat may supply adequate Se to Bangladesh consumers. However, further research is needed in Bangladesh to test the feasibility of agronomic biofortification in lentil, rice and/or wheat on large scale basis. Both soil and foliar Se application could be tested in Bangladesh to determine the most effective biofortification method for local environments and socio-economic conditions.

The hypothesis that soil Se concentration and lentil seed Se concentration are high in Australia was tested in the genotypic evaluation experiment. It was identified that soil

Se concentration is higher compared to Canadian and Bangladeshi soils, but seed Se concentration is lower in Australian lentil than both Canadian and Bangladeshi lentil seeds. However, soil Se in Australia is less available to plants, so there is scope of agronomic biofortification through foliar application. The possibility for Se biofortification by foliar application was tested at two contrasting locations – Horsham, Victoria and Melton, South Australia – through the application of 40 g Se/ha as potassium selenate at the flowering and early podding stages of growth. Lentil exhibited a high capacity to store Se in seed with foliar Se application, increasing from 201 to 2772 µg/kg with no effect of Se application on seed size or seed yield. Consumption of 20 g of biofortified lentil can supply 100% of the recommended daily allowance of Se. There was no difference in response to foliar applications with Se application between locations, thus the experiment also showed that biofortification is possible at contrasting sites.

Foliar Se application in Australia is not economic from the farmers' point-of-view due to the cost of the application of the pure grade potassium selenate used in this study. Research is needed with commercial Se fertilizer to determine the appropriate Se application rate and plant growth stage for the application. Selenium foliar application can be incorporated with other agrochemicals such as fungicides or insecticides to reduce the application cost. In Australia, soil Se concentration was high yet seed Se concentration was low. Further research is needed to identify lentil genotypes efficient in soil Se uptake. The physiology of Se uptake in lentil has not been studied; it is important to understand the mechanisms of Se uptake, assimilation, translocation and storage in seed tissue to fine-tune biofortification protocols. Several micro-nutrients may be deficient concurrently. Research is also needed to develop a biofortification method for multiple elements e.g. Fe, Zn, Se and other elements as required.

The hypothesis that significant genotypic variation exists in lentil for seed Se concentration was tested in a farmers' field survey in Bangladesh, in designed genotypic evaluation experiments in both Bangladesh and Australia, and also in Se foliar application experiments in the field and glass-house in Australia. All experiments demonstrated that significant genetic variation exists for seed Se concentration in lentil. No significant genotype × environment interaction for seed Se concentration was observed in either Bangladesh or Australia. The significant genotypic variation and low environmental control of seed Se concentration will allow the development of cultivar(s) that perform well in diverse environments.

In this study, only 19 genotypes from six countries of origin were used which is a fraction of the world's lentil germplasm collection. The International Center for Agricultural Research in Dry Areas (ICARDA) has a lentil genetic resources collection that comprises 8860 accessions of cultivated lentil from more than 70 countries and 583 accessions of wild *Lens* taxa (Furman *et al.* 2009). There are also germplasm collections available at the Australian Temperate Field Crops Collection (ATFCC) in the Department of Primary Industries, Victoria, Australia with 5250 accessions, Pullman United States Department of Agriculture (USDA) Agricultural Research Service (ARS) with 2797 accessions, the N.I. Vavilov All-Russian Research Institute of Plant Industry (VIR) with 2396 accessions, and the National Bureau of Plant Genetic Resources, India with 2212 accessions (Dwivedi *et al.* 2010). More of these germplasm collections and wild relatives of lentil should be screened to identify extreme phenotypes and gene(s) for Se uptake. Selenium measurement in seeds requires time to seed development and the analytical ICP-MES measurement is costly. For these reasons molecular markers linked with Se uptake gene should also be developed. Marker-assisted selection can reduce the time and cost of Se research in lentil by accelerating germplasm screening and cultivar development.

The eventual success of biofortification will depend on Se absorption in the human body. Clinical studies are needed with biofortified lentil on animal models first and then on humans to determine if biofortified Se effectively eliminates Se deficiency before the adoption of a large-scale Se biofortification program.

The major finding from the thesis are: (1) lentil seed Se concentration is not closely dependent on soil Se concentration, (2) Se foliar application has no effect on seed size and seed yield, (3) significant genotypic variation exists for seed Se concentration, (4) no significant genotype × environment interaction exists for seed Se concentration and (5) Se biofortification of lentil is possible through agronomic and genetic approaches. The results from this research have potential application in the food and health areas. The information generated from this research will assist potential production of Se and other micronutrient-rich food grains in Australia, Bangladesh and elsewhere.

## Literature Cited

Abhay, K. and Krishnaswamy, K. (1997). Selenium content of common Indian cereals, pulses and spices. *Journal of Agricultural and Food Chemistry, 45*(7), 2565-2568. doi: 10.1021/jf960920u

Abrams, M.M., Shennan, C., Zasoski, R.J. and Burau, R.G. (1990). Selenomethionine uptake by wheat seedlings. *Agronomy Journal, 82*(6), 1127-1130.

Afzal, M.A., Bakr, M.A., Hamid, A., Haque, M.M. and Aktar, M.S. (2003). *Lentil in Bangladesh,* Gazipur: Lentil blackgram and mungbean development pilot project, Bangladesh Agricultural Research Institute.

Akibode, S. and Maredia, M. (2012). *Global and regional trends in production, trade and consumption of food legume crops*. Michigan, United States: Department of Agricultural, Food, and Resource Economics, Michigan State University. Available: http://EconPapers.repec.org/RePEc:ags:midasp:136293 [Accessed 12 July 2013].

Alam, M.B. and Sattar, M.A. (2000). Assessment of arsenic contamination in soils and waters in some areas of Bangladesh. *Water Science and Technology, 42*(7-8), 185-192.

Alfthan, G., Aspila, P., Ekholm, P., Eurola, M., Hartikainen, H., Hero, H., Hietaniemi, V., Root, T., Salminen, P. and Venéiléiinen, E. (2010). Nationwide supplementation of sodium selenate to commercial fertilizers: History and 25-year results from the Finnish selenium monitoring programme. *In:* Thompson, B. and Amoroso, L. (eds.) *Combating Micronutrient Deficiencies: Food-based Approaches.* CABI, Wallingford.

Ali, M.A., Badruzzaman, A.B.M., Jalil, M.A., Hossain, M.D., Ahmed, M.F., Masud, A.A., Kamruzzaman, M. and Rahman, M.A. (2003). *Arsenic in plant-soil*

*environment in Bangladesh*. Available: http://www.bvsde.ops-oms.org/bvsacd/arsenico/arsenic/ali2.pdf [Accessed 2 October 2010].

Allen, L.H., De Benoist, B., Dary, O. and Hurrell, R. (2006). *Guidelines on food fortification with micronutrients,* Geneva: World Health Organization.

Arthur, J.R. and Beckett, G.J. (1994). New metabolic roles for selenium. *Proceedings of the Nutrition Society, 53*(3), 615-624. doi: 10.1079/PNS19940070

Arvy, M.P. (1993). Selenate and selenite uptake and translocation in bean plants (*Phaseolus vulgaris*). *Journal of Experimental Botany, 44*(6), 1083-1087.

Aung, M.S., Masuda, H., Kobayashi, T., Nakanishi, H., Yamakawa, T. and Nishizawa, N.K. (2013). Iron biofortification of Myanmar rice. *Frontiers in Plant Science, 4*, 1-14. doi: 10.3389/fpls.2013.00158

Baker, D.E. and Chesnin, L. (1975). Chemical monitoring of soils for environmental quality and animal and human health. *Advances in Agronomy, 27*, 305-374.

Banuelos, G.S., Ajwa, H.A., Mackey, B., Wu, L., Cook, C., Akohoue, S. and Zambruzuski, S. (1997). Evaluation of different plant species used for phytoremediation of high soil selenium. *Journal of Environmental Quality, 26*(3), 639-646.

Barrachina, A.C., Carbonell, F.B. and Beneyto, J.M. (1995). Arsenic uptake, distribution, and accumulation in tomato plants: effect of arsenite on plant growth and yield. *Journal of Plant Nutrition, 18*(6), 1237-1250.

Baum, M.K., Shor-Posner, G. and Lai, S. (1997). High risk of HIV related mortality is associated with selenium deficiency. *Journal of Acquired Immune Deficiency Syndromes, 15*(5), 370-374.

Beck, M.A., Shi, Q., Morris, V.C. and Levander, O.A. (1995). Rapid genomic evolution of a non-virulent coxsackievirus B3 in selenium-deficient mice results in selection of identical virulent isolates. *Nature Medicine, 1*(5), 433-436.

Beebe, S., Gonzalez, A.V. and Rengifo, J. (2000). Research on trace minerals in the common bean. *Food and Nutrition Bulletin, 21*(4), 387-391.

Behne, D., Kyriakopoulos, A., Kalcklösch, M., Weiss-Nowak, C., Pfeifer, H., Gessner, H. and Hammel, C. (1997). Two new selenoproteins found in the prostatic glandular epithelium and the spermatid nuclei. *Biomedical and Environmental Sciences, 10*(2-3), 340-345.

Bender, D.A. (2009). *Nutritional biochemistry of the vitamins, 2nd Edition.*New York, USA: Cambridge University Press.

Bhatty, R.S. (1988). Composition and quality of lentil (*Lens culinaris* Medik): A review. *Canadian Institute of Food Science and Technology, 21*(2), 144-160.

Biswas, S., Talukder, G. and Sharma, A. (1999). Prevention of cytotoxic effects of arsenic by short-term dietary supplementation with selenium in mice in vivo. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 441*(1), 155-160. doi: 10.1016/s1383-5718(99)00028-5

Bouis, H., Boy-Gallego, E. and Meenakshi, J.V. (2012). Micronutrient malnutrition: causes, prevalence, consequences, and interventions. *In:* Bruulsema, T. W., Heffer, P., Welch, R. M., Cakmak, I. and Moran, K. (eds.) *Fertilizing crops to improve human health: a scientific review.* International Plant Nutrition Institute, Norcross, GA, USA and International Fertilizer Industry Association, Paris, France.

Bouis, H.E. (2002). Global efforts to breed for more nutritious crops. *In:* Roos, N., Bouis, H. E., Hassan, N. and Kabir, K. A., eds. Alleviating malnutrition through agriculture in Bangladesh: biofortification and diversification as suatainable solutions, Dhaka and Gazipur, Bangladesh. 29-34.

Broadley, M.R., Alcock, J., Alford, J., Cartwright, P., Foot, I., Fairweather-Tait, S.J., Hart, D.J., Hurst, R., Knott, P., McGrath, S.P., Meacham, M.C., Norman, K.,

Mowat, H., Scott, P., Stroud, J.L., Tovey, M., Tucker, M., White, P.J., Young, S.D. and Zhao, F.-J. (2010). Selenium biofortification of high-yielding winter wheat (*Triticum aestivum* L.) by liquid or granular Se fertilisation. *Plant and Soil, 332*(1-2), 5-18. doi: 10.1007/s11104-009-0234-4

Broadley, M.R., White, P.J., Bryson, R.J., Meacham, M.C., Bowen, H.C., Johnson, S.E., Hawkesford, M.J., McGrath, S.P., Zhao, F.-J., Breward, N., Harriman, M. and Tucker, M. (2006). Biofortification of UK food crops with selenium. *Proceedings of the Nutrition Society, 65*(02), 169-181. doi: doi:10.1079/PNS2006490

Brown, T.A., Brown, A. and Shrift. (1982). Selenium: toxicity and tolerance in higher plants. *Biological Reviews, 57*(1), 59-84.

Bukovac, M.J. and Wittwer, S.H. (1957). Absorption and mobility of foliar applied nutrients. *Plant Physiology, 32*(5), 428-435. doi: 10.2307/4259213

Cakmak, I. (2008). Enrichment of cereal grains with zinc: agronomic or genetic biofortification? *Plant and Soil, 302*(1-2), 1-17.

Cakmak, I., Kalayci, M., Kaya, Y., Torun, A.A., Aydin, N., Wang, Y., Arisoy, Z., Erdem, H., Yazici, A. and Gokmen, O. (2010). Biofortification and localization of zinc in wheat grain. *Journal of Agricultural and Food Chemistry, 58*(16), 9092-9102.

Cakmak, I., Torun, A., Millet, E., Feldman, M., Fahima, T., Korol, A., Nevo, E., Braun, H.J. and Ozkan, H. (2004). *Triticum dicoccoides*: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. *Soil Science and Plant Nutrition, 50*(7), 1047-1054.

Cakmak, I., Torun, B., Erenoğlu, B., Öztürk, L., Marschner, H., Kalayci, M., Ekiz, H. and Yilmaz, A. (1998). Morphological and physiological differences in the response of cereals to zinc deficiency. *Euphytica, 100*(1-3), 349-357.

Cao, X.Y., Jiang, X.M., Dou, Z.H., Rakeman, M.A., Kareem, A., Zang, M.L., Ma, T., O'Donnell, K., DeLong, N. and DeLong, G.R. (1994). Iodination of irrigation water as a method of supplying iodine to a severely iodine-deficient population in Xinjiang, China. *The Lancet, 344*(8915), 107-110.

Cary, E.E. and Allaway, W. (1969). The stability of different forms of selenium applied to low-selenium soils. *Soil Science Society of America Journal, 33*(4), 571-574.

Cary, E.E. and Rutzke, M. (1981). Foliar application of selenium to field corn. *Agronomy Journal, 73*(6), 1083-1085.

Chen, Y., Hall, M., Graziano, J.H., Slavkovich, V., van Geen, A., Parvez, F. and Ahsan, H. (2007). A prospective study of blood selenium levels and the risk of arsenic-related premalignant skin lesions. *Cancer Epidemiology Biomarkers and Prevention, 16*(2), 207-213. doi: 10.1158/1055-9965.epi-06-0581

Chen, Y.W., Truong, H.Y. and Belzile, N. (2009). Abiotic formation of elemental selenium and role of iron oxide surfaces. *Chemosphere, 74*(8), 1079-1084. doi: 10.1016/j.chemosphere.2008.10.043

Chowdhury, U.K., Biswas, B.K., Chowdhury, T.R., Samanta, G., Mandal, B.K., Basu, G.C., Chanda, C.R., Lodh, D., Saha, K.C., Mukherjee, S.K., Roy, S., Kabir, S., Quamruzzaman, Q. and Chakraborti, D. (2000). Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environmental Health Perspective, 108*(5), 393-397. doi: sc271_5_1835 [pii]

Chu, J., Yao, X. and Zhang, Z. (2010). Responses of wheat seedlings to exogenous selenium supply under cold stress. *Biological Trace Element Research, 136*(3), 355-363. doi: 10.1007/s12011-009-8542-3

Clark, L.C., Combs, G.F., Turnbull, B.W., Slate, E.H., Chalker, D.K., Chow, J., Davis, L.S., Glover, R.A., Graham, G.F., Gross, E.G., Krongrad, A., Lesher, J.L., Park, H.K., Sanders, B.B., Smith, C.L. and Taylor, J.R. (1996). Effects of selenium

supplementation for cancer prevention in patients with carcinoma of the skin. *Journal of the American Medical Association, 276*(24), 1957-1963.

Combs, G.F., Jr and Welch, R.M. (1998). *Creating a healthful food system: linking agriculture to human needs,* Ithaca, NY: Cornell International Institute for Food, Agriculture, and Development.

Combs, G.F., Jr. (2001). Selenium in global food systems. *British Journal of Nutrition, 85*(5), 517-547. doi: S0007114501000782 [pii]

Combs, G.F., Welch, R.M., Duxbury, J.M., Uphoff, N.T. and Nesheim, M.C. (1996). Food-based approaches to preventing micronutrient malnutrition: an international research agenda. Ithaca, NY: Cornell International Institute for Food, Agriculture and Development, Cornell University.

Curtin, D., Hanson, R., Lindley, T.N. and Butler, R.C. (2006). Selenium concentration in wheat *(Triticum aestivum*) grain as influenced by method, rate, and timing of sodium selenate application. *New Zealand Journal of Crop and Horticultural Science, 34*(4), 329-339.

Curtin, D., Hanson, R. and Van Der Weerden, T.J. (2008). Effect of selenium fertiliser formulation and rate of application on selenium concentrations in irrigated and dryland wheat (*Triticum aestivum*). *New Zealand Journal of Crop and Horticultural Science, 36*(1), 1-7. doi: 10.1080/01140670809510216

Djanaguiraman, M., Devi, D.D., Shanker, A.K., Sheeba, J.A. and Bangarusamy, U. (2005). Selenium - an antioxidative protectant in soybean during senescence. *Plant and Soil, 272*(1/2), 77-86. doi: 10.1007/s11104-004-4039-1

Ducsay, L., Lozek, O., Varga, L. and Losak, T. (2007). Effect of winter wheat supplementation with selenium. *Ecological Chemistry and Engineering, 14*(3-4).

Dwivedi, S.L., Upadhyaya, H.D., Balaji, J., Buhariwalla, H.K., Blair, M.W., Ortiz, R., Crouch, J.H. and Serraj, R. (2010). Using genomics to exploit grain legume

biodiversity in crop improvement. *Plant breeding reviews.* John Wiley and Sons, Inc.

Dworkin, B.M. (1994). Selenium deficiency in HIV infection and the acquired immunodeficiency syndrome (AIDS). *Chemico-Biological Interactions, 91*(2-3), 181-186. doi: 10.1016/0009-2797(94)90038-8

Eich-Greatorex, S., Sogn, T.A., Øgaard, A.F. and Aasen, I. (2007). Plant availability of inorganic and organic selenium fertiliser as influenced by soil organic matter content and pH. *Nutrient Cycling in Agroecosystems, 79*(3), 221-231.

Erskine, W. (2009). Global production, supply and demand. *In:* Erskine, W., Muehlbaur, F. J., Sarker, A. and Sharma, B. (eds.) *The lentil botany, production and use.* Oxfordshire, UK: CAB International.

Erskine, W., Muehlbauer, F.J. and Short, R.W. (1990). Stages of development in lentil. *Experimental Agriculture, 26*(1), 297-302.

Eurola, M., Ekholm, P., Ylinen, M., Koivistoinen, P. and Varo, P. (1990). Effects of selenium fertilization on the selenium content of cereal grains, flour, and bread produced in Finland. *Cereal Chemistry, 67*(4), 334-337.

Fang, Y., Wang, L., Xin, Z., Zhao, L., An, X. and Hu, Q. (2008). Effect of foliar application of zinc, selenium, and iron fertilizers on nutrients concentration and yield of rice grain in China. *Journal of Agricultural and Food Chemistry, 56*(6), 2079-2084. doi: 10.1021/jf800150z

FAO. (2013). *FAOSTAT Statistical database of the United Nations Food and Agriculture Organization*. Available: http://faostat.fao.org/site/342/default.aspx [Accessed 17 February 2013].

FAO. (2014). *FAOSTAT Statistical database of the United Nations Food and Agriculture Organization*. Rome, Italy. Available:

http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor [Accessed 30 January 2014].

Felipe de Mendiburu. (2012). Agricolae: Statistical procedures for agricultural research (R package version 1.1-2). Retrieved from http://CRAN.R-project.org/package=agricolae

Fordyce, F.M. (2005). Selenium deficiency and toxicity in the environment. *In:* Selinus, O. (ed.) *Essentials of medical geology: impacts of the natural environment on public health.* London, UK: Elsevier.

Frias, J., Gulewicz, P., Martínez-Villaluenga, C., Pilarski, R., Blazquez, E., Jiménez, B.a., Gulewicz, K. and Vidal-Valverde, C.n. (2009). Influence of germination with different selenium solutions on nutritional value and cytotoxicity of lupin seeds. *Journal of Agricultural and Food Chemistry, 57*(4), 1319-1325. doi: 10.1021/jf8028368

Frossard, E., Bucher, M., Mächler, F., Mozafar, A. and Hurrell, R. (2000). Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *Journal of the Science of Food and Agriculture, 80*(7), 861-879.

Furman, B.J., Coyne, C., Redden, B., Sharma, S.K. and Vishnyakova, M. (2009). Genetic resources: collection, characterization, conservation and documentation. *In:* Erskine, W., Muehlbauer, F. J., Sarker, A. and Sharma, B. (eds.) *The lentil, botany, production and uses.* Oxfordshire, UK: CAB International.

Gardiner, M. and Gorman, R. (1963). Further observations on plant selenium levels in Western Australia. *Australian Journal of Experimental Agriculture, 3*(11), 284-289. doi: 10.1071/EA9630284

Geng, C.N., Zhu, Y.G., Tong, Y.P., Smith, S.E. and Smith, F.A. (2006). Arsenate (As) uptake by and distribution in two cultivars of winter wheat (*Triticum aestivum* L.). *Chemosphere, 62*(4), 608-615.

Gissel-Nielsen, G. (1971). Influence of pH and texture of the soil on plant uptake of added selenium. *Journal of Agricultural and Food Chemistry, 19*(6), 1165-1167. doi: 10.1021/jf60178a030

Gissel-Nielsen, G. (1977). *Control of selenium in plants,* Roskilde, Denmark: Riso National Laboratory.

Gissel-Nielsen, G., Gupta, U.C., Lamand, M. and Westermarck, T. (1984). Selenium in soils and plants and its importance in livestock and human nutrition. *Advances in Agronomy, 37*, 397-460. doi: 10.1016/S0065-2113(08)60459-9

Govindaraj, M., Selvi, B., Rajarathinam, S. and Sumathi, P. (2011). Genetic variability and heritability of grain yield components and grain mineral concentration in India's pearl millet (*Pennisetum glaucum* (L) R. Br.) accessions. *African Journal of Food, Agriculture, Nutrition and Development, 11*(3), 4758-4771.

Graham, R., Senadhira, D., Beebe, S., Iglesias, C. and Monasterio, I. (1999). Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field Crops Research, 60*(1-2), 57-80. doi: 10.1016/s0378-4290(98)00133-6

Graham, R.D. and Rengel, Z. (1993). Genotypic variation in zinc uptake and utilization by plants *In:* Robson, A. D. (ed.) *Zinc in soils and plants.* Netherlands: Springer.

Graham, R.D., Welch, R.M. and Bouis, H.E. (2001). Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: Principles, perspectives and knowledge gaps. *Advances in Agronomy, 70*, 77-142. doi: dx.doi.org/10.1016/S0065-2113(01)70004-1

Gregorio, G.B., Senadhira, D., Htut, H. and Graham, R.D. (2000). Breeding for trace mineral density in rice. *Food and Nutrition Bulletin, 21*(4), 382-386.

Gupta, U.C., Mcrae, K.B. and Winter, K.A. (1988). Selenium enrichment of crops through foliar applications. *Canadian Journal of Soil Science, 68*(3), 519-526. doi: 10.4141/cjss88-050

Hamdy, A.A. and Gissel-Nielsen, G. (1977). Fixation of selenium by clay minerals and iron oxides. *Zeitschrift für Pflanzenernährung und Bodenkunde, 140*(1), 63-70.

Hanson, B., Lindblom, S.D., Loeffler, M.L. and Pilon-Smits, E.A.H. (2004). Selenium protects plants from phloem-feeding aphids due to both deterrence and toxicity. *New Phytologist, 162*(3), 655-662. doi: 10.1111/j.1469-8137.2004.01067.x

Hart, K. (1985). Recent observations on selenium and sheep health. *Selenium and ruminant health.* New South Wales Department of Agriculture.

HarvestPlus. (2013). *Breeding crops for better nutrition*. Washington, USA: HarvestPlus Challange Program, Consultative Group on International Agricultural Research. Available: http://www.harvestplus.org/content/iron-beans [Accessed 20 November 2013].

Hasanuzzaman, M., Hossain, M.A. and Fujita, M. (2010). Selenium in higher plants: physiological role, antioxidant metabolism and abiotic stress tolerance. *Journal of Plant Sciences, 5*, 354-375. doi: 10.3923/jps.2010.354.375

Holmberg, R.E., Jr and Ferm, V.H. (1969). Interrelationships of selenium, cadmium, and arsenic in mammalian teratogenesis. *Archives of Environmental Health, 18*(6), 873-877.

Horton, S. (2006). The economics of food fortification. *Journal of Nutrition, 136*, 1068-1071.

Hu, Q., Chen, L., Xu, J., Zhang, Y. and Pan, G. (2002). Determination of selenium concentration in rice and the effect of foliar application of Se-enriched fertiliser or sodium selenite on the selenium content of rice. *Journal of the Science of Food and Agriculture, 82*(8), 869-872. doi: 10.1002/jsfa.1115

Huang, Z., Pei, Q., Sun, G., Zhang, S., Liang, J., Gao, Y. and Zhang, X. (2008). Low selenium status affects arsenic metabolites in an arsenic exposed population with skin lesions. *Clinica Chimica Acta, 387*(1–2), 139-144. doi: 10.1016/j.cca.2007.09.027

Huq, S.M.I., Correll, R. and Naidu, R. (2006a). Arsenic accumulation in food sources in Bangladesh. *In:* Naidu, R., Smith, E., Owens, G. and Bhattacharya, P. (eds.) *Managing arsenic in the environment: From soil to human health.* Melbourne: CSIRO.

Huq, S.M.I., Joardar, J.C., Parvin, S., Correll, R. and Naidu, R. (2006b). Arsenic contamination in food-chain: transfer of arsenic into food materials through groundwater irrigation. *Journal of Health, Population and Nutrition, 24*(3), 305.

Jackson, M.L. (1962). *Soil chemical analysis,* N.J., USA: Prentice Hall.

Jiang, X.M., Cao, X.Y., Jiang, J.Y., Tai, M.A., James, D.W., Rakeman, M.A., Dou, Z.H., Mamette, M., Amette, K. and Zhang, M.L. (1997). Dynamics of environmental supplementation of iodine: Four years' experience of iodination of irrigation water in Hotien, Xinjiang, China. *Archives of Environmental Health, 52*(6), 399-408.

Johnson, C.R., Thavarajah, D., Combs, G.F., Jr and Thavarajah, P. (2013). Lentil (*Lens culinaris* L.): A prebiotic-rich whole food legume. *Food Research International, 51*, 107-113.

Johnson, H.W., Robinson, H.F. and Comstock, R.E. (1955). Estimates of genetic and environmental variability in soybeans. *Agronomy Journal, 47*(7), 314-318.

Johnson, R.D. (2007). Selenium: The organic-inorganic debate explained. *Cascade Horseman.* California.

Johnsson, L. (1991). Selenium uptake by plants as a function of soil type, organic matter content and pH. *Plant and Soil, 133*(1), 57-64. doi: 10.1007/BF00011899

Judson, G.J. and Reuter, D.J. (1999). Selenium. *In:* Peverill, K. I., Sparrow, L. A. and Reuter, D. J. (eds.) *Soil analysis: an interpretation manual.* Melbourne: The Commonwealth Scientific and Industrial Research Organisation.

Kápolna, E., Hillestrøm, P.R., Laursen, K.H., Husted, S. and Larsen, E.H. (2009). Effect of foliar application of selenium on its uptake and speciation in carrot. *Food Chemistry, 115*, 1357-1363.

Knott, S.G. and McCray, C.W.R. (1959). Two naturally occurring outbreaks of selenosis in Queensland. *The Australian Veterinary Journal, 35*(4), 161-165.

Kong, L., Wang, M. and Bi, D. (2005). Selenium modulates the activities of antioxidant enzymes, osmotic homeostasis and promotes the growth of sorrel seedlings under salt stress. *Plant Growth Regulation, 45*(2), 155-163.

Kumar, H., Dikshit, H., Singh, A.M., Singh, D., Kumari, J., Singh, A. and Kumar, D. (2013). Characterization of elite lentil genotypes for seed iron and zinc concentration and genotype x environment interaction studies. *Indian Journal of Genetics and Plant Breeding, 73*(2), 169-176.

Kuznetsov, V.V., Kholodova, V.P., Kuznetsov, V.V. and Yagodin, B.A. (2003). Selenium regulates the water status of plants exposed to drought. *Doklady Biological Sciences, 390*(1), 266-268. doi: 10.1023/a:1024426104894

Langlands, J.P., Bowles, J.E., Smith, A.J. and Donald, G.E. (1981). Selenium concentration in the blood of ruminants grazing in Northern New South Wales. Part II Relationship with geological, pedological and other variables. *Australian Journal of Agricultural Research, 32*(3), 523-533. doi: 10.1071/AR9810523

Layrisse, M., Chaves, J.F., Bosch, V., Tropper, E., Bastardo, B. and González, E. (1996). Early response to the effect of iron fortification in the Venezuelan population. *The American Journal of Clinical Nutrition, 64*(6), 903-907.

Levander, O.A. (1977). Metabolic interrelationships between arsenic and selenium. *Environmental Health Perspectives, 19*, 159-164.

Liu, K. and Gu, Z. (2008). Selenium accumulation in different brown rice cultivars and its distribution in fractions. *Journal of Agricultural and Food Chemistry, 57*(2), 695-700. doi: 10.1021/jf802948k

Lyons, G. and Cakmak, I. (2012). Agronomic biofortification of food crops with micronutrients. *In:* Bruulsema, T. W., Heffer, P., Welch, R. M., Cakmak, I. and Moran, K. (eds.) *Fertilizing crops to improve human health: a scientific review.* Norcross, GA, USA: International Plant Nutrition Institute and International Fertilizer Industry Association.

Lyons, G., Monasterio, I.O., Stangoulis, J. and Graham, R. (2005). Selenium concentration in wheat grain: Is there sufficient genotypic variation to use in breeding? *Plant and Soil, 269*(1-2), 369-380. doi: 10.1007/s11104-004-0909-9

Lyons, G., Stangoulis, J. and Robin, G. (2003). High-selenium wheat: biofortification for better health. *Nutrition Research Reviews, 16*(1), 45-60.

Lyons, G.H., Genc, Y., Soole, K., Stangoulis, J.C.R., Liu, F. and Graham, R.D. (2009). Selenium increases seed production in Brassica. *Plant and Soil, 318*(1), 73-80.

Lyons, G.H., Lewis, J., Lorimer, M.F., Holloway, R.E., Brace, D.M., Stangoulis, J.C.R. and Graham, R.D. (2004). High-selenium wheat: agronomic biofortification strategies to improve human nutrition. *Food, Agriculture and Environment, 2*(1), 171-178.

Mayer, J.E., Pfeiffer, W.H. and Beyer, P. (2008). Biofortified crops to alleviate micronutrient malnutrition. *Current Opinion in Plant Biology, 11*(2), 166-170.

Meharg, A.A. and Rahman, M.M. (2003). Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption. *Environmental Science and Technology, 37*(2), 229-234.

Miller, P.A., Williams, J.C., Robinson, H.F. and Comstock, R.E. (1958). Estimates of genotypic and environmental variances and covariances in upland cotton and their implications in selection. *Agronomy Journal, 50*(3), 126-131.

Miyazaki, K., Ushijima, K., Kadono, T., Inaoka, T., Watanabe, C. and Ohtsuka, R. (2003). Negative correlation between urinary selenium and arsenic levels of the residents living in an arsenic-contaminated area in Bangladesh. *Journal of Health Science, 49*(3), 239-242.

Monsen, E.R. (2000). Dietary reference intakes for the antioxidant nutrients: vitamin C, vitamin E, selenium, and carotenoids. *Journal of the American Dietetic Association, 100*(6), 637-640.

Moxon, A.L., Olson, O.E. and Searight, W.V. (1939). *Selenium in rocks, soils, and plants*: Agricultural Experiment Station, South Dakota State College of Agriculture and Mechanic Arts.

National Research Council (1977). Biological effects of arsenic on plants and animals. *Arsenic: Medical and biological effects of environmental pollutants.* Washington, DC: National Academy Press.

Nelson, D.W. and Sommers, L.E. (1982). Total carbon, organic carbon, and organic matter. *In:* Page, A. L., Miller, R. H. and Keeney, D. R. (eds.) *Methods of soil analysis. Part 2-chemical and microbial properties.* 2nd ed. Madison, Wisconsin, USA: American Society of Agronomy.

Nestel, P., Bouis, H.E., Meenakshi, J.V. and Pfeiffer, W. (2006). Biofortification of staple food crops. *Journal of Nutrition, 136*(4), 1064-1067.

O'Dell, B.L. and Sunde, R.A. (1997). *Handbook of nutritionally essential mineral elements,* New York: Marcel Decker Inc.

Payne, G.G., Sumner, M.E. and Plank, C.O. (1986). Yield and composition of soybeans as influenced by soil pH, phosphorus, zinc, and copper. *Communications in Soil Science and Plant Analysis, 17*(3), 257-273. doi: 10.1080/00103628609367712

Poggi, V., Arcioni, A., Filippini, P. and Pifferi, P.G. (2000). Foliar application of selenite and selenate to potato (*Solanum tuberosum*): effect of a ligand agent on selenium content of tubers. *Journal of Agricultural and Food Chemistry, 48*(10), 4749-4751. doi: 10.1021/jf000368f

Pulse Australia. (2011). *Australian lentil exports*. Available: http://www.cicilsiptic.org/downloads/0_71377800_1357723359AustralianLentilExports.pdf [Accessed 1 July 2013].

Rahman, M.A., Hasegawa, H., Rahman, M.M., Islam, M.N., Miah, M.A.M. and Tasmen, A. (2007). Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. *Chemosphere, 67*(6), 1072-1079.

Rahman, M.M., Erskine, W., Zaman, M.S., Thavarajah, P., Thavarajah, D. and Siddique, K.H.M. (2013). Selenium biofortification in lentil (*Lens culinaris* Medikus subsp. *culinaris*): Farmers' field survey and genotype x environment effect. *Food Research International, 54*(2), 1596-1604. doi: dx.doi.org/10.1016/j.foodres.2013.09.008

Rashid, M.H.-o., Schäfer, H., Gonzalez, J. and Wink, M. (2012). Genetic diversity of rhizobia nodulating lentil (*Lens culinaris*) in Bangladesh. *Systematic and Applied Microbiology, 35*(2), 98-109. doi: 10.1016/j.syapm.2011.11.008

Rayman, M.P. (2002). The argument for increasing selenium intake. *Proceedings of the Nutrition Society, 61*(2), 203-215.

Rayman, M.P. (2004). The use of high-selenium yeast to raise selenium status: how does it measure up? *British Journal of Nutrition, 92*(4), 557-574.

Rayman, M.P. (2008). Food-chain selenium and human health: emphasis on intake. *British Journal of Nutrition, 100*(2), 254–268. doi: 10.1017/S0007114508939830

Reilly, C. (1996). Biological role of selenium. *Selenium in food and health.* London, UK: Blackie.

Rengel, Z., Batten, G.D. and Crowley, D.E. (1999). Agronomic approaches for improving the micronutrient density in edible portions of field crops. *Field Crops Research, 60*(1–2), 27-40. doi: dx.doi.org/10.1016/S0378-4290(98)00131-2

Reuter, D.J. (1975). Selenium in soils and plants. *Agricultural Record, 2*, 44-55.

Ríos, J.J., Rosales, M.A., Blasco, B., Cervilla, L.M., Romero, L. and Ruiz, J.M. (2008). Biofortification of Se and induction of the antioxidant capacity in lettuce plants. *Scientia Horticulturae, 116*(3), 248-255. doi: 10.1016/j.scienta.2008.01.008

Sarker, A., Erskine, W., Rahman, M.M., Bakr, M.A., Afzal, M.A. and Saxena, M.C. (2004). Lentil improvement in Bangladesh. *A success story of fruitful partnership between the Bangladesh Agricultural Research Institute and International Center for Agricultural Research in the Dry Areas.* Thailand: Asia-Pacific Association of Agricultural Research Institutions.

Schwarz, K. and Foltz, C.M. (1957). Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *Journal of the American Chemical Society, 79*(12), 3292-3293.

Sima, P. and Gissel-Nielsen, G. (1985). Spraying of crops with selenium. *Acta Agriculturae Scandinavica, 35*(2), 161-164.

Singh, H.P., Batish, D.R., Kohli, R.K. and Arora, K. (2007). Arsenic-induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative

stress resulting from enhanced lipid peroxidation. *Plant Growth Regulation, 53*(1), 65-73.

Singh, R.K. and Chaudhary, B.D. (1979). *Biometrical methods in quantitative genetic analysis,* New Delhi: Kalyani.

Smith, E., Naidu, R. and Alston, A.M. (1998). Arsenic in the soil environment: a review. *Advances in Agronomy, 64*, 149-195.

Smith, N.M., Lee, R., Heitkemper, D.T., Cafferky, K.D., Haque, A. and Henderson, A.K. (2006). Inorganic arsenic in cooked rice and vegetables from Bangladeshi households. *Science of the Total Environment, 370*(2-3), 294-301. doi: 10.1016/j.scitotenv.2006.06.010

Smrkolj, P., Kreft, I. and Stibilj, V. (2005a). Selenium uptake and species distribution in peas after foliar treatment with selenate. *In:* Eurola, M., ed. Twenty years of selenium fertilization, Helsinki, Finland. MTT Agrifood Research Finland.

Smrkolj, P., Osvald, M., Osvald, J. and Stibilj, V. (2007). Selenium uptake and species distribution in selenium-enriched bean (*Phaseolus vulgaris* L.) seeds obtained by two different cultivations. *European Food Research and Technology, 225*(2), 233-237.

Smrkolj, P., Stibilj, V., Kreft, I. and Germ, M. (2006). Selenium species in buckwheat cultivated with foliar addition of Se(VI) and various levels of UV-B radiation. *Food Chemistry, 96*(4), 675-681. doi: 10.1016/j.foodchem.2005.05.002

Smrkolj, P., Stibilj, V., Kreft, I. and Kapolna, E. (2005b). Selenium species determination in selenium-enriched pumpkin (*Cucurbita pepo* L.) seeds by HPLC-UV-HG-AFS. *Analytical Sciences, 21*, 1501-1504.

Spallholz, J.E., Boylan, L.M. and Larsen, H.S. (1990). Advances in understanding selenium's role in the immune system. *Annals of the New York Academy of Sciences, 587*, 123-139.

Spallholz, J.E., Boylan, L.M., Rahman, M.M., Katz, D., Robertson, J.D., Zakaria, A.B.M., Khan, A.H., Alauddin, M., Bhattacharjee, M. and Sultana, S. (2008a). Selenium content of rice, mixed plant foods and fish from Bangladesh. *Toxicological and Environmental Chemistry, 90*(2), 211-220. doi: 10.1080/02772240701419404

Spallholz, J.E., Boylan, L.M. and Rhaman, M.M. (2004). Environmental hypothesis: is poor dietary selenium intake an underlying factor for arsenicosis and cancer in Bangladesh and West Bengal, India? *Science of the Total Environment, 323*(1-3), 21-32. doi: 10.1016/j.scitotenv.2003.09.034

Spallholz, J.E., Boylan, L.M., Robertson, J.D., Smith, L., Rahman, M.M., Hook, J. and Rigdon, R. (2008b). Selenium and arsenic content of agricultural soils from Bangladesh and Nepal. *Toxicological and Environmental Chemistry, 90*(2), 203 - 210.

Stein, A.J., Meenakshi, J.V., Qaim, M., Nestel, P., Sachdev, H.P.S. and Bhutta, Z.A. (2005). *Health benefits of biofortification: an ex-ante analysis of iron-rich rice and wheat in India*. Paper presented at the *American Agricultural Economics Association Annual Meeting*, Providence, RI, USA.

Stibilj, V., Kreft, I., Smrkolj, P. and Osvald, J. (2004). Enhanced selenium content in buckwheat (*Fagopyrum esculentum* Moench) and pumpkin (*Cucurbita pepo* L.) seeds by foliar fertilisation. *European Food Research and Technology, 219*(2), 142-144.

Swaine, D.J. (1955). Selenium. *The trace element content of soils.* England: Commonwealth Agricultural Bureaux.

Tan, J. (1989). Selenium ecological landscape map of China. *The atlas of endemic diseases and their environments in the People's Republic of China.* Beijing: Science Press.

Terry, N., Zayed, A.M., de Souza, M.P. and Tarun, A.S. (2000). Selenium in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology, 51*(1), 401-432. doi: 10.1146/annurev.arplant.51.1.401

Thavarajah, D., Ruszkowski, J. and Vandenberg, A. (2008). High potential for selenium biofortification of lentils (*Lens culinaris* L.). *Journal of Agricultural and Food Chemistry, 56*(22), 10747-10753. doi: 10.1021/jf802307h

Thavarajah, D., Thavarajah, P., Sarker, A., Materne, M., Vandemark, G., Shrestha, R., Idrissi, O., Hacikamiloglu, O., Bucak, B. and Vandenberg, A. (2011a). A global survey of effects of genotype and environment on selenium concentration in lentils (*Lens culinaris* L.): Implications for nutritional fortification strategies. *Food Chemistry, 125*(1), 72-76. doi: 10.1016/j.foodchem.2010.08.038

Thavarajah, D., Thavarajah, P., Wejesuriya, A., Rutzke, M., Glahn, R., Combs, G.F., Jr. and Vandenberg, A. (2011b). The potential of lentil (*Lens culinaris* L.) as a whole food for increased selenium, iron, and zinc intake: preliminary results from a 3 year study. *Euphytica, 180*(1), 123-128. doi: 10.1007/s10681-011-0365-6

Thavarajah, D., Vandenberg, A., George, G.N. and Pickering, I.J. (2007). Chemical form of selenium in naturally selenium-rich lentils (*Lens culinaris* L.) from Saskatchewan. *Journal of Agricultural and Food Chemistry, 55*(18), 7337-7341. doi: 10.1021/jf070681i

The Daily Star. (2013). High zinc rice released. *The Daily Star*, 27 August, Available: http://archive.thedailystar.net/beta2/news/high-zinc-rice-released/.

Tveitnes, S., Singh, B.R. and Ruud, L. (1995). Selenium concentration in spring wheat as influenced by basal application and top dressing of selenium-enriched fertilizers. *Nutrient Cycling in Agroecosystems, 45*(2), 163-167. doi: 10.1007/bf00790666

UNICEF Bangladesh (2011). Bangladesh national drinking water quality survey of 2009. Dhaka.

Varo, P., Alfthan, G., Ekholm, P., Aro, A. and Koivistoinen, P. (1988). Selenium intake and serum selenium in Finland: effects of soil fertilization with selenium. *The American Journal of Clinical Nutrition, 48*(2), 324-329.

Verret, W.J., Chen, Y., Ahmed, A., Islam, T., Parvez, F., Kibriya, M.G., Graziano, J.H. and Ahsan, H. (2005). A randomized, double-blind placebo-controlled trial evaluating the effects of vitamin E and selenium on arsenic-induced skin lesions in Bangladesh. *Journal of Occupational and Environmental Medicine, 47*(10), 1026-1035. doi: 10.1097/01.jom.0000183095.45050.97

Wagabaza, O. (2013). Sweet potatoes, beans perform miracles for Buyende children. *New Vision*, 2 October, Available: http://www.newvision.co.ug/news/647891-sweet-potatoes-beans-perform-miracles-for-buyende-children.html.

Walkley, A. and Black, I.A. (1934). An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science, 63*(4), 251-263.

Wang, W., Yang, L., Hou, S. and Tan, J. (2001). Mitigation of endemic arsenocosis with selenium, an example from China. *Current Science, 81*(9), 1215-1218.

Wei, A. (1996). Soybean sprout. *Zhengzhou Liangshi Xueyuan Xuebao, 17*, 67-70.

Weiss, M.G. (1943). Inheritance and physiology of efficiency in iron utilization in soybeans. *Genetics, 28*(3), 253-268.

Welch, R.M., Combs, G.F. and Duxbury, J.M. (1997). Toward a “greener” revolution. *Issues in Science and Technology, 14*, 50-58.

Welch, R.M. and Graham, R.D. (1999). A new paradigm for world agriculture: meeting human needs: productive, sustainable, nutritious. *Field Crops Research, 60*(1), 1-10.

Welch, R.M. and Graham, R.D. (2002). Breeding crops for enhanced micronutrient content. *Plant and Soil, 245*(1), 205-214.

Whanger, P., Vendeland, S., Park, Y.-C. and Xia, Y. (1996). Metabolism of subtoxic levels of selenium in animals and humans. *Annals of Clinical and Laboratory Science, 26*(2), 99-113.

White, P.J. and Broadley, M.R. (2009). Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist, 182*(1), 49-84.

Xue, W., Wang, Z., Chen, Q., Chen, J., Yang, H. and Xue, S. (2010). High selenium status in individuals exposed to arsenic through coal-burning in Shaanxi (PR of China) modulates antioxidant enzymes, heme oxygenase-1 and DNA damage. *Clinica Chimica Acta, 411*(17), 1312-1318.

Yang, F., Chen, L., Hu, Q. and Pan, G. (2002a). Effect of the application of selenium on selenium content of soybean and its products. *Biological Trace Element Research, 93*(1-3), 249-256.

Yang, G., Wang, S., Zhou, R. and Sun, S. (1983). Endemic selenium intoxication of humans in China. *The American Journal of Clinical Nutrition, 37*(5), 872-881.

Yang, G.Q. and Xia, Y.M. (1995). Studies on human dietary requirements and safe range of dietary intakes of selenium in China and their application in the prevention of related endemic diseases. *Biomedical and Environmental Sciences, 8*(3), 187-201.

Yang, L., Wang, W., Hou, S., Peterson, P.J. and Williams, W.P. (2002b). Effects of selenium supplementation on arsenism: an intervention trial in Inner Mongolia. *Environmental Geochemistry and Health, 24*(4), 359-374.

Yilmaz, A., Ekiz, H., Torun, B., Gultekin, I., Karanlik, S., Bagci, S.A. and Cakmak, I. (1997). Effect of different zinc application methods on grain yield and zinc

concentration in wheat cultivars grown on zinc-deficient calcareous soils. *Journal of Plant Nutrition, 20*(4-5), 461-471. doi: 10.1080/01904169709365267

Yu, S.Y., Zhu, Y.J. and Li, W.G. (1997). Protective role of selenium against hepatitis B virus and primary liver cancer in Qidong. *Biological Trace Element Research, 56*(1), 117-124.

Zahedi, H., Noormohammadi, G., Rad, A.H.S., Habibi, D. and Boojar, M.M.A. (2009). The effects of zeolite and foliar applications of selenium on growth, yield and yield components of three canola cultivars under drought stress. *World Applied Sciences Journal, 7*(2), 255-262.

Zhang, F., Fan, M., Gao, X., Zou, C. and Zuo, Y. (2008). Soil and crop management for improving iron and zinc nutrition of crops. *In:* Banuelos, G. S. and Lin, Z.-Q. (eds.) *Development and uses of biofortified agricultural products.* Boca Raton, USA: CRC Press.