American-Eurasian J. Agric. & Environ. Sci., 2 (1): 80-84, 2007
ISSN 1818-6769

Selenium Uptake by *Allium cepa* Grown in Se-spiked Soils

[1]*Shikha Yadav,* [1]*Saurabh Gupta,* [1]*Ranjana Prakash,*
[2]*Julian Spallholz and* [1]*Nagaraja Tejo Prakash*

[1]Department of Biotechnology and Environmental Sciences,
Thapar University, Thapar Technology Campus, Patiala-147 004, India
[2]Department of Food and Nutrition, College of Human Sciences,
Texas Technical University, Lubbock 79409, USA

Abstract: Selenium (Se) is efficiently transferred through the soil-plant-animal-human system. Geographic differences of Se in soils accounts for most variations in the Se content in foods. Strategies to enhance Se intake include increasing the consumption of high Se foods and food fortification. Se is efficiently taken up by the alliumsand these species produce organo-Se compounds that are active in the chemoprevention and anti-oxidant activity. Here we explore the possibility of growing *Allium cepa* in selenium spiked soils with an objective of introducing this species in seleniferous soils of Punjab. *A. cepa* plantlets showing near uniform height and weight were selected from an agricultural nursery for study. Six plantlets were grown in trays with soils spiked with Se (as selenate) soils of 25 μg gm^{-1} and 50 μg gm^{-1}, with one tray of plantlets maintained as control. Three plantlets were harvested after 30 and 70 days. Total Se was measured in plants and soils using GF-AAS. After 30 days of exposure, the weight of the plantlets exposed to 25 μg gm^{-1} averaged 558 mg with whole plant Se of 278.2 μg gm^{-1}. In plantlets exposed to 50 μg gm^{-1}, The average weight was 547 mg with a whole plant Se of 342.8 μg gm^{-1} with reduced bulb size. Further exposure to 25 μg gm^{-1} for 70 days inhibited growth of the plantlets and increased whole plant Se concentration to 885.4 μg gm^{-1}. Plants exposed to 50 μg gm^{-1} had Se increased to 1280.8 μg gm^{-1}. Conclusions: *Allium cepa* can accumulate Se in proportion to available selenate concentrations in soil. Onions which are commercially viable species can be introduced in the cropping profiles of seleniferous soils of the region for selenium mobilization as well as fortification.

Key words: Nutrient · biofortification · crops · onion

INTRODUCTION

Phytoremediation has received increasing recognition as a low-cost, environment friendly approach for managing the toxic effects of selenium (Se). Plantlets have the ability to absorb and sequester Se and to convert inorganic selenium to volatile forms of organic compounds that are released harmlessly into the atmosphere [1]. Once absorbed by plant roots, Se is translocated to the shoot where it may be harvested and removed from the site [2, 3]. The selenium supply in almost all European countries is below the recommended daily intake. In these countries, selenium fortification of foods and the use of selenium supplements are quite popular to compensate for low Se intake from diets. Wheat (*Triticum aestivum*) is known to be a good source for bioavailable seleniumand many studies have been performed to enrich selenium in wheat by selenium fertilization of the soil [4]. In addition, plantlets such as broccoli (*Brassica oleracea* var. *botrytis L.*), Indian mustard (*Brassica juncea* L.), sugarbeet (*Beta vulgaris* L.), rice (*Oryza sativa* L.), sunflower (*Helianthus annus*), white lupine (*Lupinus albus*) and garlic *(Allium sativum)* are also known to accumulate or fortify selenium in tissues in various forms [5-8]. These studies are of importance as some authors have considered the combination of this enriched material with non-enriched food as a source of selenium supplementation [9, 10].

For most of the world's cultures, alliaceous species are most sought after vegetables for salads and garnishes.

Corresponding Author: Dr. Nagaraja Tejo Prakash, Department of Biotechnology and Environmental Sciences, Thapar University, Thapar Technology Campus, Patiala-147 004, India

It is also one of the major processed vegetable crops being exported from Indian sub-continent to Europe and Americas. Among these species, onion consumption exceeds garlic consumption. Onion also produces greater edible bulb biomass than garlic, making it an additional and perhaps important target for Se-enriched vegetables for human consumption. The chemistry and biochemistry of selenium in these sulfur rich species is more easily expressed in terms of the better-known sulfur chemistry because of the great similarities in chemical properties that selenium and sulfur share by virtue of being adjacent group VIA elements [11-14].

In studies using Se-enriched *Allium* species, demonstrated that organoselenium compounds are more active than S analogs in chemoprevention, suggesting that Se-enriched vegetables may be a better delivery source for organoselenium analogs than the commonly used selenite or selenomethionine [12, 15]. By controlling the intensity and frequency of the crop fertilization with water-soluble selenite salts it is possible to cultivate Se-enriched species with 100-1355 μg g^{-1} of Se [16].

For the past few years the attention of several researchers has been engaged in the study of the role of Se in seleniferous soils and crops of Nawanshahr-Hoshiarpur Region of Punjab, Indiaand its impact on human and livestock health [17-19]. In this area, the average selenium intake of both men and women was more than nine times that in the non-seleniferous areas [20] The malformations appearing in livestock were also attributed to feeding of fodder grown in seleniferous soils [17, 21].

The present study was undertaken to determine the absorption and accumulation patterns of Se in *Allium cepa* (onion) within the harvest period of 30 and 70 days. The study is focused towards a broader objective of introducing this species in Se-impacted areas as selenium fortified vegetables and other crops can result in commercially valuable Se-fortified crop products which can be blended with un-enriched crop produce from non-seleniferous regions.

MATERIALS AND METHODS

Ten day old *Allium cepa* plantlets (variety Nasik Red) were obtained from seed store Patiala. The test soil was taken from agricultural land from non-seleniferous agricultural soils. Trays with capacity of holding 11 kg soil per tray were used for the study for sowing of plantlets. The soils were sieved to remove coarse material and other soils debris before the layout of the plantlets.

Plantlets (6 plantlets per tray) were grown in trays with soil pre-spiked with 25 μg gm^{-1} and 50 μg gm^{-1} of Se as selenate with three trays set for each level of Se. Plantlets were grown in one tray kept as control without Se. The experimental and control plantlets were irrigated at regular intervals with single distilled water. Estimation of selenium in plantlets and soil was carried out before sowing of plantlets and after 30 and 70 days. Plantlets were harvested carefully and soil samples were taken from their respective rhizosphere. The samples were oven-dried (60°C) for 2 days to remove moisture. Dried soil samples were sieved before digestion and dry weight of the whole plantlets were recorded.

1 gm soil sample and whole plant were digested separately in perchloric acid:nitric acid mixture [22]. Digested samples were left for cooling followed by dilution and treatment with 5 ml of 0.2% nitric acid, followed by filtration using 0.45 μm filter. Final dilutions were made by using 0.2% nitric acid. Filtrate was analyzed for selenium content by graphite furnace atomic absorption spectrophotometer (GFAAS-Perkin-Elmer Analyst 600) with detection limit 4 μg l^{-1}. Analytical programming was carried out using WinLab 3.0 software. GF-AAS temperature profiles were-drying at 110°C amd 130°C, pyrolysis at 1300°C, atomization/read step at 1900°C and cleanout at 2400°C. Matrix modifier used was mixture of 5 μg palladium nitrate and 3 μg magnesium nitrate (Sigma-Aldrich). Standard solutions were made from Perkin-Elmer stock AAS standards. Statistical calculations were carried out using GraphPad Prism 4.0.

RESULTS AND DISCUSSION

Observations were taken for the weight of plantlets in triplicates of the control set as well as plantlets harvested after the duration of 30 and 70 days (Fig. 1). Their respective Se concentrations (per gram dry weight) along with the Se levels in rhizosphere soils were estimated using GF-AAS (Fig. 2). Background concentration of selenium in the soil estimated before the start of the experiment was found to be below detection limits.

As indicated in the results, growth was observed of plantlets upto 30 days in 25 μg gm^{-1} which did not further increase by 70 days. However, growth at 50 μg gm^{-1} showed retardation. In case of control set, plants had considerable bulb formation with normal leaves and root density. Reduction in bulb size, length of leaves and root density was noted in plantlets grown in soil spiked with 50 μg gm^{-1} Se. Similar visible reduction in foliar mass

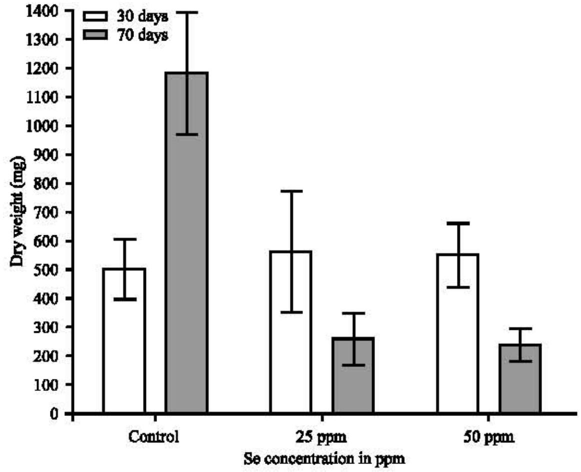

Fig. 1: Plant dry weight (mg) in control and selenium exposed plantlets

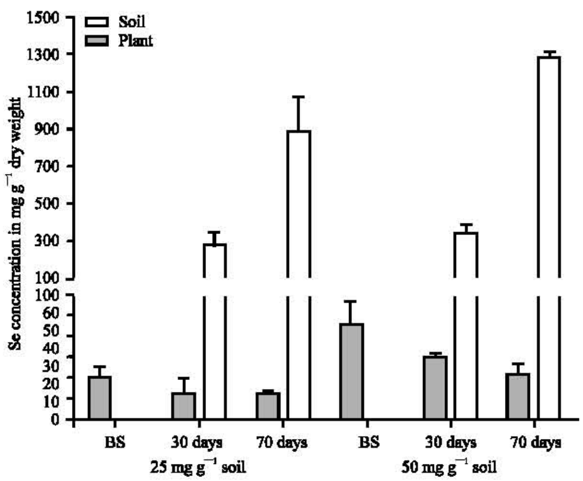

Fig. 2: Selenium levels in soil and plants before sowing (BS) and after 30 and 70 as of growth

was observed under high Se treatments (up to 20 ppm) in "Granex" onions by Kopsell and Randle [14] The researchers suggested that the reduction in biomass might be due to interruption of sulphur metabolism through replacement of S proteins by Se amino acids, producing retarded growth effects. Sulphur metabolism in onion supports plant growth and fertility levels [23, 24]. Decrease in plant growth and yield with increased Se application were also reported for alfalfa (*Medicago sativa* L. Var. African) and subterranean clover (*Trifolium subterraneum* L. var. Mt. Baker) [25].

The estimation of the accumulation of Se content in onion plantlets showed that onions take up and accumulate Se in proportion to available selenium (Na_2SeO_4). Uptake also increased with age of plant. Observations showed that average accumulation of Se in plantlets after 30 days in 25 μg gm^{-1} was 278.7 μg and in 50 μg gm^{-1} plantlets was 343 μg After 70 days, it was 885.5 μg and 1280.8 μg respectively. Two-way ANOVA analysis of the triplicate samples taken at 30 and 70 days indicated that there is interaction ($p>0.05$) between selenium concentration and duration of exposure on the Se levels in *Allium* biomass. Similar observations in increasing uptake of Se with increase in age of plant were recorded by Kopsell and Randle [14]. Whanger *et al.* [26] also reported that the Se uptake in ramps (*Allium tricoccum*) increased despite difference in the growth media, namely peatmoss (I), vermiculite (II) and hydroponics (III). In I and II, Se added to mixture was 30, 60, 90, 120 mg kg^{-1} and content of Se in ramp bulb was 120, 140, 177, 235 mg kg^{-1} respectively. In case III, concentration of Se added to nutrient solution was 10, 20, 30, 50, 70, 90 mg L^{-1} and content of Se in bulbs was observed to be 88, 142, 252, 325, 335, 432 mg kg^{-1}. The results of the present study also indicated that as growth in the biomass of *A. cepa* does not get affected upto 25 μg gm^{-1} within harvesting time of 30 days. Therefore these species can be grown in seleniferous soils or Se spiked soils and harvested at green onion stage, with medium bulb size for further blending and processing.

Crop plants such as alliaceous species with an augmented capacity to accumulate Se from soils can thus be used to aid sustainable agriculture and to improve human health through balanced mineral nutrition vis-à-vis to mobilize selenium from seleniferous soils, thus using the phytoremediation and biofortification concepts together. *A. cepa,* examined in the present study exhibits potential to mobilize selenium from soil as well as accumulate in the biomass. If the concepts of fortification by plants which have augmented capacity to accumulate Se hold a market value in terms of crops that can be used as Se supplements, these food products can be exported in the specific geographical regions which are naturally deficient for Se. This will serve two main purposes-(a) remediation of selenium-laden soil thereby putting an end to the trauma faced by human and livestock of the region affected by selenium impactand (b) raising financial standards of the farmers of affected regions if such land is taken up for research, consequently for projects dealing with production of export quality fortified foods used as supplements to compensate for low Se intake from diets.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support by CSIR, Govt. of India through sponsored project. The analytical facilities provided by CORE-Agro-Industrial Biotechnology, Patiala for the study are also duly acknowledged.

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