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Research Article

ANTIOXIDANT PROFILING OF SELENIUM FORTIFIED TOMATO (*Solanum lycopersicum*)

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ABSTRACT

In recent years the consumption of processed vegetables has been escalating. Similarly, food quality and its effects on human health have become crucial issues. Since selenium (Se) is an important microelement due to its ability to defend human against free radicals. We scrutinized the effects of Se on tomato and assessed the feasibility producing Se-enriched vegetable with enhanced antioxidant quality. The addition of 8 and 10 $mg.L^{-1}$ Se resulted in increased selenium concentration in the fruits, with positive effect on the plant yield. Selenium fortification was analyzed based on its application method to the plants. Entirely selenium accumulation enhanced all the antioxidant activities considered and decreased the production of phenylalanine ammonia lyase (PAL) activity. This Se fortified tomato could provide the rational Se intake for human nutrition in accordance with the recommended dietary allowance (RDA). The addition of Se by foliar application can be a useful system for providing enriched tomatoes with improved fruit quality. It can be used to modulate the availability of Se and to grow vegetables with the optimal Se content for human health. Hope an uncomplicated way to include selenium into human diet through tomatoes will ensure good health.

Keywords: Tomato, Selenium, Antioxidant activities.

INTRODUCTION

As a trace mineral, selenium (Se) is an essential nutrient of fundamental importance to human biology. Both Se deficiency and toxicity occurs worldwide, depending on Se availability in the environment. Dietary Se deficiency in humans is caused due to its low bioavailability in most crop soils. Since it is generally believed that organic Se compounds are better and safer as a dietary supplement. A variety of Se-enriched biological products including garlic, yeast and lactic acid bacteria etc. have been commercialized. The importance of Se to mammalian nutrition is paramount, since Se deficiency may promote cancer[1]. The possible action by Se on the human immune system includes resistance against viral infections, such as HIV[2, 3].

Plants vary considerably in their physiological response to selenium. Some plants are Se tolerant and accumulate very high concentrations of Se (Se accumulators), but most plants are Se non-accumulators and are Se sensitive[4]. It was thought that selenium might be essential for growth of the accumulator species but there is no definitive supporting evidence. The biological utilization ratio (absorption ability) of organic selenium in plants is higher than inorganic selenium. Selenium was shown to affect several physiological and biochemical processes, in the plant species[5, 6]. There is no doubt that plants are a good source of biologically active natural products. In the investigation of bioactive natural compounds, it is essential to have access to simple biological tests to locate required activities[7]. In this sense, works in rice, wheat and radish, demonstrating that Se fertilization increases the content of this trace element in plants and furthermore showing how this translates as Se ingestion in humans[8-10].

Cultivated tomato is considered one of the most important vegetable crops in the world. Tomato consumption is known to have health benefits because of their high content of phytonutrients, which includes numerous minerals and vitamin-C. These nutrients can vary largely depending on the growing conditions. Many analyzed the effect of Se on the nutritional quality of the plant products used as human food, particularly on the antioxidant capacity, when there is evidence that excessive Se application can have phytotoxic effects on plants[11]. This crop can be preferred in Se biofortification programs, as an efficient way of increasing intake of this element by the hefty population.

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. The antioxidant activities of some fruits and vegetables were highly correlated with their total phenolic contents. It is mainly due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[7]. Se induces the antioxidant capacity by its relationship with glutatione peroxidase (GSH-Px), which intensifies at higher foliar Se concentrations[12]. Se has also been defined to induce antioxidant capacity in plants, by increase in tocopherol and phenolic compounds[13, 11,14].

Generally the plants respond to oxidative stress through increase in the enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants may include superoxide dismutase (SOD), catalase (CAT), ascorbic acid peroxidase (APX) and guaiacol peroxidase (GPX) [15]. The non-enzymatic antioxidants may include glutathione, ascorbic acid and carotenoids[16]. Exogenous selenium (low concentration) can reduce the intensity of peroxide processes of membrane lipids and affect the activity of redox enzymes and thereby change the oxidation-reduction status of the cell[17]. The antioxidative effect of Se was related to an improved GSH-Px and

SOD activity and a decreased lipid peroxidation in Se treated plants, like lettuce and soybean[18, 19].

The study of the antioxidant capacity in the edible portion of plants used for human consumption is of great importance, given that in the last decade it was demonstrated that the ingestion of antioxidants of plant origin can neutralize the appearance of certain reactive-oxygen species (ROS), thereby diminishing the appearance of some diseases such as cancer and neurodegenerative diseases[20]. Hence the present study focuses on the effect of selenium on its antioxidant activities in the tomato plant. Enhancement in antioxidant activity will end up in a selenium enriched tomatoes with dual benefit. Hope an uncomplicated way to include selenium into human diet through tomatoes will ensure good health.

MATERIALS AND METHODS

Field Experiment

It was carried out in the greenhouse condition with 12h light and 12h dark period, at the temperature of 28-35°C at day and 20-28°C at night. Tomato seeds were purchased from the Super Agri Seeds Private Limited, India. The soil characteristics includes; pH of 7.7, nitrogen, potassium and phosphorous content as 84500, 500 and 20kg/h respectively. Calcium carbonate content was in minimal evidence and EC value of 1.0 d/sm. The initial selenium content in the soil was 0.04µg/g.

Fortification method includes; seed soaking, soil application and foliar spray. Each treatment includes five pots in triplicates. The 10 days old seedlings (two leaf stage) were transplanted to the plastic pots (7.5 × 15 cm) containing soil and sand mixture with fertilizers (cow dung, P and N in 3: 1: 1). After four weeks of seedlings growth, sodium selenate (Na_2SeO_4) was supplemented at four concentrations (0, 2, 4, 8 and 10mg/L). Seed soaking involves 2 hours treatment, before sowing to the nursing bed for germination. In soil application and foliar spray; sodium selenate was supplied, weekly once from 50 to 90 days growth stage directly to the soil and sprayed on the leaves respectively. The plants were maintained away from the rain water till their fruit yield for analysis, along with the control plants.

Total selenium content

The plant materials were rinsed with distilled water, dried at 70ºC and 0.5 g was acid digested with perchloric acid and HCl reduction. Selenium content in leaves, root and fruits (at harvest, post-harvest and after blanching) were determined. The digests were analyzed by hydride generation atomic absorption spectrophotometry (HG-AAS).

Antioxidant activities

All the antioxidant activity analysis was carried out with the Se fortified and control plants (both in leaves and fruit samples). Antioxidant profiling was analyzed by its total antioxidant activity, PAL, CAT, POX and DPPH radical scavenging activities. Enzyme extraction was carried out at 4ºC, with potassium phosphate buffer (pH 7.8) containing 1mM EDTA and the supernatant was collected and stored in -20ºC for further use.

Total antioxidant activity was measured by the Phosphomolybdenum method[21]. PAL activity was measured on the rate of cinnamic acid production[22]. Catalase (EC.1.11.1.6; CAT) activity was analyzed by Aebi's method[23]. Peroxidase (EC 1.11.1.7; POX) activity was measured by the oxidation of guaiacol to tetra-guaiacol[24]. Scavenging effects on free DPPH radicals of methanol extracts was determined by Shimada's method[25]. The absorbance of a mixture of 1 ml of the extract and 1 ml of the DPPH solution was measured at 517 nm. The radical scavenging activity was calculated from the equation:

$$\% \text{ of radical scavenging activity} = (\text{A control} - \text{A sample})/\text{A control} \times 100.$$

The total ascorbate content was estimated by the 2, 4-dinitrophenylhidrazine method in the fruit samples[26].

Characterization of Se fortified tomatoes

Chemical group variation on selenocompounds accumulation was analyzed by FT-IR analysis by conventional KBr pellet method. Samples were based on maximum selenium accumulation by foliar application. The spectrum was analyzed by Origin software (version 8.0). The site of accumulation and the size of the particle were observed in the Se fortified leaf and fully ripened fruit samples by SEM analysis with the fine powdered samples.

Statistical analysis

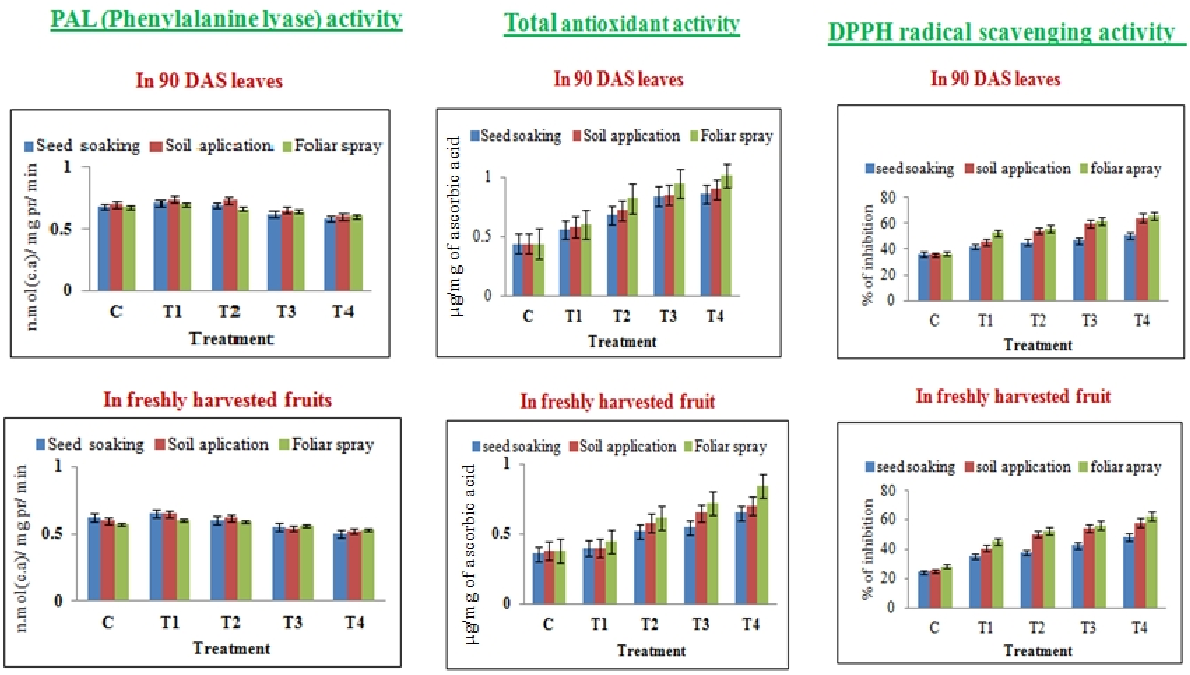
The biochemical changes of selenium fortified tomatoes were analyzed in three different treatment conditions. The changes of three replicates were analyzed statistically based on completely randomized block design. Data were subjected to analysis of variance (one way ANOVA) and compared by Dunnett's test ($P< 0.05$) in Graphpad prism version 5.0.

RESULTS AND DISCUSSION

The functional foods are of increasing interest in the prevention and treatment of various diseases. There is growing evidence indicating the additive and synergetic effects of antioxidative compounds from fruits and vegetables on human health, as they can diminish the risk of much pathology related to oxidative stress[27]. Interest in Se metabolism derived primarily due to its nutrition and multiple health benefits. Our results are consistent with others referring to different plants in which selenate provoked a greater Se accumulation in plants[8, 10, 12 and 13]. The reduction in shoot biomass with increase in selenium concentrations is in agreement with reports in the mustard and lettuce plants respectively[28, 29]. Selenium accelerated all stages of reproductive growth considerably in the concentrations studied. The length of inflorescence, fruit yield per plant and the total yield increased with increasing concentration. Foliar spray method showed the significant yield. The taste index >0.7 indicates the good quality fruits when compared to the control. The reports were agreed by previous studies with Se fortification in tomatoes[30, 31].

The plant cells own extremely effective antioxidative defense system, which gets rid of the harmful effect of oxidative stress. Among the phytochemicals; phenolic compounds, an extensive group with wide range of chemical structures are the most important one. A noteworthy aspect to be considered here is that phenols can act as chelators of metals as well as inhibitors of enzymes such as oxidase xanthine and thereby prevent enzymatic and non-enzymatic formation of ROS. Furthermore, some are attributed with a possible anti-carcinogenic role[32]. In addition to total phenolic and flavonoids, phenylpropanyl glycosides (PPGs), defined as intermediary compounds in phenolic metabolism, have an antioxidant function in plant cells[33].

The total antioxidant activity increased gradually with increasing concentration, irrespective of the methods and significant in foliar spray method (Figure1). PAL activity significantly reduced with increasing sodium selenate concentration than the control. Both CAT and POX activity increased by 45.45% and 52.85% respectively, with T4 concentration and high in foliar spray method (Figure 2). Selenate induced significant activities of catalase and ascorbate oxidase enzymes.



Note: Error bars represents Standard deviation among the triplicates, Values are mean± SE, n=3

Figure 1: Antioxidant activity in selenium fortified tomato plant

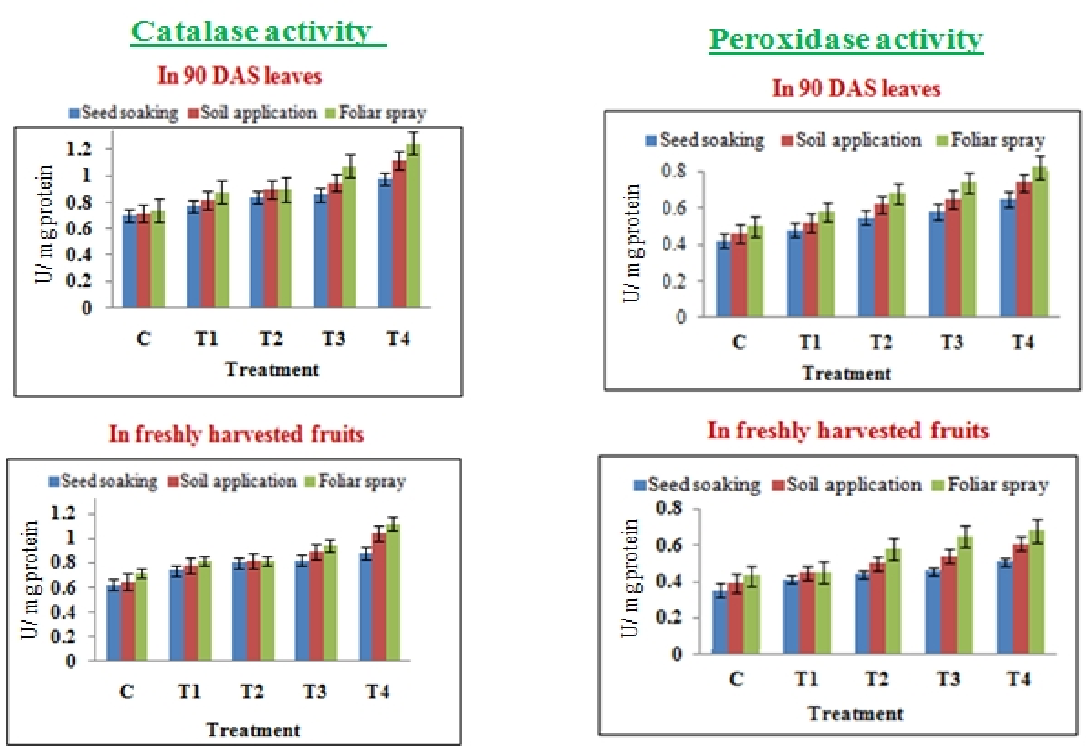


Figure 2: Catalase and Peroxidase activities in selenium fortified tomato plant

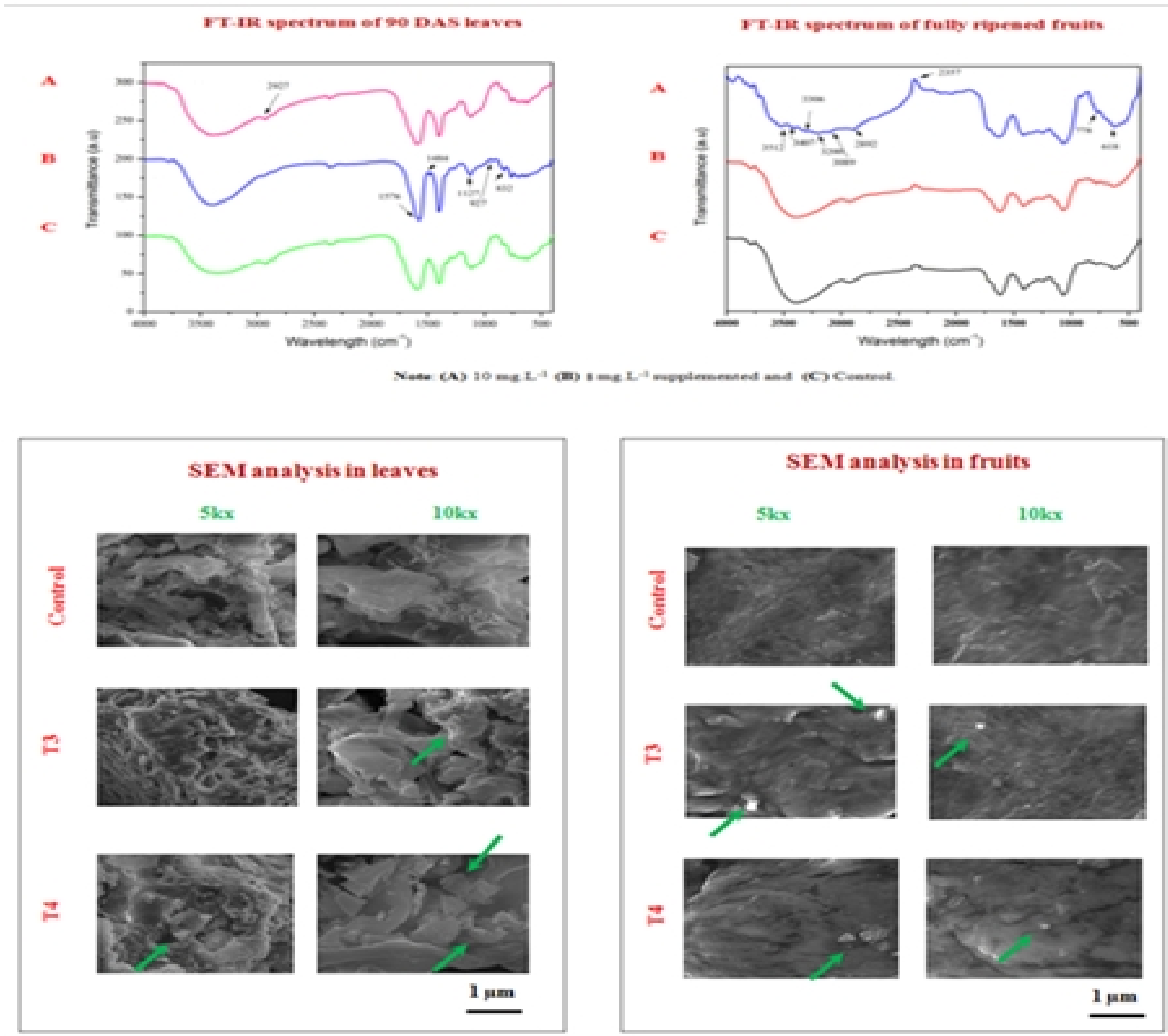


Figure 3: FT-IR spectrum and SEM image of selenium fortified tomato plant

It can be proposed that the selenate-induced superoxide radicals in rocket tops are converted to H_2O_2 by SOD. In DPPH radical scavenging activity analysis, extraction by methanol has highest phenolic content, due to the ability to inhibit the reaction of polyphenol oxidase that causes oxidation of the phenolics[34]. Similar results were reported in fenugreek, ryegrass and lettuce[35, 11, 29].

Higher antioxidant activities explain the lower levels of ROS accumulation in presence of Se. The reason for enhancement in antioxidant activities may be due to glutathione peroxidase (GSH-Px), the first selenoenzymes that reduces H_2O_2 to water[36]. Our results coincide with the literature, given that the application of selenate in different plants and under different conditions bolsters the antioxidant capacity[13, 18 and 37]. The selenium modifies the plant redox balance probably due to increasing activities of antioxidant enzymes as glutathione peroxidase and superoxide dismutase[29]. The gradual increase in total ascorbate content is in agreement with the antioxidant activities of the Se fortified fruits. Ascorbate is the major antioxidant in every plant cell compartment that gradually increases the antioxidant activities[38].

FTIR results envisaged the possible selenocompounds synthesized in Se fortified plant materials. The peaks corresponding 800 to 1200 cm^{-1} and 2800 to 3400 cm^{-1} region, were reported due to the presence of selenocompounds, as supported by previous reports in radish and pumpkin [39, 40]. The SEM analysis pictured that the Se are accumulated around the leaf edges in the epidermal cells as inorganic form in the Se fortified leaf samples (Figure 3). It may be converted to protein and accumulated as selenocompounds in fruit samples.

Our main objective was to achieve an accumulation of the element in such a way that it satisfies the recommended daily amount (RDA) stipulated for adults (50–200mcg.day^{-1}) after the ingestion of these tomatoes, without producing phytotoxic effects or reducing agricultural production. In short, our findings define the selenium fortified tomatoes have enhanced antioxidant properties and selenocompounds accumulation. It is the good indicator for human usage. Further studies on the genes involved for maximum organo-seleno compounds accumulation will lead a new hope to prevent various ailments in humans and a nutritional food.

CONCLUSION

According to our results, Se application in the form of selenate would be more beneficial for a biofortification. Because it is less phytotoxic, prompts more biomass growth and causes greater Se accumulation. In terms of antioxidant capacity, Se in general augments antioxidant compounds (thereby increasing the nutritional value of the crop). In short, for maximum antioxidant capacity and Se concentration, the appropriate rate and form to be applied is $10mg.L^{-1}$ sodium selenate by foliar application. A significant relationship with the different antioxidant compounds, ascorbate being the compound that most reflects the effect of Se on the antioxidant capacity under our experimental conditions.

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