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BIOFORTIFICATION OF HORTICULTURAL CROPS WITH SELENIUM

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ABSTRACT

Selenium (Se) is a naturally occurring element, structurally resembling with Sulphur, lying in group 6 of the periodic table. It is an essential micronutrient, required for proper functioning of many biological processes like hormone formation, immune system, hair growth, muscular movements and reproduction and helps in defense against many diseases like viral infections, Keshan's diseases and arthritis, as it is important component of selenoproteins and enzymes like glutathione peroxidase. Low selenium level in human body can lead to various diseases and disorders. To fulfill selenium dietary intake, selenium enriched diet is necessary. Plants are the main source of human diet. Plants are fortified with selenium by adding different forms of selenium through different methods. They uptake or absorb selenium through Sulphur assimilation pathway and store it into the tissues in either organic or inorganic form. Till now much research has been carried out in production of se-fortified crops. This review paper is aimed at reviewing recent studies which have been done on biofortification of horticultural plants with selenium, like fruits and vegetables, which contribute a major portion of human diet.

Keywords: Horticultural crops, Biofortification, Selenium

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1. INTRODUCTION

1.1. Environmental Selenium

Selenium is a non-metal element belonging to group 6 of the periodic table having 34 atomic number and 78.96 atomic mass (Fordyce 2005). It occurs in varying forms as elemental selenium (Se), selenide (Se^{-2}), selenite (SeO_3^{-2}), selenate (SeO_4^{-2}) (Chauhan et al. 2019). Naturally, selenium occurs in sedimentary rocks (White et al. 2004). Worldwide, average Se concentration in soils is 0.4mg.kg^{-1} while in seleniferous soils it can goes up to till 5000mg.kg^{-1} (Hartikainen 2005). Amount of Se vary in soils and depends upon various factors like type of soil, organic matter, and rainfall (Sors et al. 2005). Natural and anthropogenic activities are responsible for addition of selenium in environment (Winkel et al. 2012; Tan et al. 2016; Okonji et al. 2021). Natural activities like volcanic eruptions, forest fires and soil erosion and anthropogenic activities like burning of fossil fuels, plastics, rubbers, and paper etc. increase the concentration of selenium in the atmosphere (Mehdi et al. 2013). In air it is mostly present in volatile form as DMSe, DMDSe and methaneselenol. Se concentration in air is generally low, ranges from 1 to 10ng.m^{-2} (Mehdi et al. 2013). Selenium concentrations in plants vary depending upon specie, accumulation capacity and selenium levels in soils (Dumont et al. 2006; Pilon-Smits 2019; Ramkissoon et al. 2019; Naseem et al. 2021).

1.2. Importance of Selenium in Plants and Humans

Selenium is recognized as an essential micronutrient (El-Ramady et al. 2020; Li et al. 2021) after its discovery as an important component of enzyme glutathione peroxidase (Rotruck et al. 1973). It is crucial for proper growth and development and proper functioning of many biological functions like formation of thyroid hormones, muscle functioning, DNA synthesis, senescence, and reproduction (Hawrylak-Nowak 2013). Selenium is also involved in defense system and helps in disease prevention like cardiological disorders, viral infections, and arthritis (Shamberger 1981). Selenium is actively involved in immunity (Avery and Hoffmann 2018; Xia et al. 2021), thus preventing so many diseases. Its deficiency in human body can lead to various diseases like cystic fibrosis, Alzheimer's, heart diseases, disorders related to oxidative stress and hypothyroidism (Rayman 2012; Coppinger and Diamond 2001; Loscalzo 2014). Its optimal intake is necessary to overcome its deficiency, WHO has recommended 50-55µg/day selenium in dietary intake (World Health Organization 2011). Se enriched crops are considered ideal to increase the dietary intake of selenium (Dumont et al. 2006).

A plant growing in seleniferous soil absorbs selenium and assimilate, which varies on the capacity of plant, species, Se form and concentration in the soil (Banuelos and Meek 1990). Depending upon capacity of plant to absorb selenium, plants are classified as non-accumulators in which selenium accumulation levels are usually below $100mg.kg^{-1}$, accumulators which can store up to $100\text{-}1000mg.kg^{-1}$ and hyper-accumulators in which selenium accumulation levels exceeds than $1000mg.kg^{-1}$ (Ellis and Salt 2003). At appropriate levels selenium shows various beneficial effects in plants and affects seed germination and plant growth (Xue et al. 2001). Selenium act as antioxidant and helps in defense against biotic and abiotic stresses (Schiavon and Pilon-Smits 2017). It inhibits lipid peroxidation and increases glutathione peroxidase activity which results in delay in senescence and minimal post-harvest loses (Cartes et al. 2005). The antioxidant activity of selenium is related to enzyme glutathione peroxidase which shows that selenium is integral part of glutathione peroxidase (Xue et al. 2001).

1.3. Selenium Supplementation in Plants

In order to fortify the plants with selenium, different forms of selenium are applied to the growing plants through different application methods. To produce selenium enriched foods, two types of selenium supplements are used i.e., inorganic selenium form (selenate or selenite salts) and in organic forms like selenomethionine, selenocysteine, selenium enriched yeast or addition of selinized plant into the soil to increase the selenium content of soil in which targeted crops are grown.

Commonly, selenium is applied in inorganic forms i.e., sodium selenate and sodium selenite. Both forms of selenium are toxic at higher levels but act as antioxidant at low levels. Sodium selenate is favored over selenite as it is readily absorbed by the plants and get accumulated. In 2010, Ramos et al. compared the effect of both forms by applying them on lettuce through hydroponic nutrient solution and results showed that selenate is better for fortification and increased the yield and biomass of lettuce. Different application methods are being practiced which mainly includes:

***1.3.1. Soil Addition*:** Addition of different forms of selenium supplements is most common application method to increase the soil selenium content for production of selenium fortified crops. Mostly, soil selenium content is increased by addition of selenate or selenite (Hart et al. 2011) or integration of selenium hyperaccumulator plant like *Stanleya pinnata* into the soil (Freeman and Banuelos 2011). Selenium hyperaccumulators used for phytoremediation can be used for biofortification of other plants by incorporating them into the soils. The decomposition of Se enriched plant increases the soil selenium content which is then absorbed by the growing plant and gets fortified (Bañuelos et al. 2010; Ramkissoon et al. 2019; D'Amato et al. 2020).

***1.3.2. Foliar Application*:** Foliar application of selenium is practiced on many horticultural crops but the effectiveness of this method depends upon many factors like leaf and fruit surface, presence of wax or hairs on leaves or fruits, epicarp and chemical composition of wax (Pezzarossa et al. 2014). Se accumulation in sprayed plants is found to be higher as compared to grown in hydroponics (Germ et al. 2007). It is also better than soil application because plants get fortified with minimal amount of salt and is more effective (MacLeod et al. 1998).

***1.3.3. Hydroponics*:** In this method, selenium salts are applied to the plant through nutrient solution flowing through the hydroponic system. This method is effective in controlling the selenium doses with minimal loss of salts as compared to soil and foliar application (Malorgio et al. 2009). Many horticultural crops like lettuce (Xue et al. 2001), spinach (Ferrarese et al. 2012), basil (Puccinelli et al. 2017; Hawrylak-Nowak et al. 2018), and tomatoes (Schiavon et al. 2013; Puccinelli et al. 2019) have been effectively selenium fortified through hydroponics.

***1.3.4. Seed Soaking*:** In this method seeds are soaked in different selenium concentration solutions because of which seeds absorb selenium and get fortified. This method gives better results with grain crops like wheat as compared to fruit crops. This method also induces tolerance against several stresses in the plants. In 2013, Nawaz et al. found that wheat grains soaked with selenium are drought tolerant as compared to non-treated seeds. It also induces metal (cadmium) stress tolerance in radish plants (Miao et al. 2008). Later on, lettuce seeds were investigated, and results shown that selenium-soaked lettuce seeds are tolerant towards salinity and gives good growth and yield (Shalaby and Ramadan 2017). However, selenium content in biofortified plants through seed soaking is lower than other methods.

1.4. Selenium Uptake and Accumulation by Plants

The uptake and accumulation of selenium in plants depends upon many factors like form of selenium (Organic or inorganic), plant species, growth stage of plant, soil conditions (pH, salinity) and plant translocation mechanism (Renkema et al. 2012). In soil, selenium is present in inorganic forms either as selenate or selenite. Selenate is mostly found in alkaline soils whereas selenite exist in acidic pH. Absorption rate and accumulation is different for both forms (Li et al. 2008). Both forms are absorbed by the transporters present in the root cells of the plants.

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Table 1: Summary of Se- enriched horticultural crops with form, application method, dose applied, increased Se-content and effect on antioxidants and yield

Plant Species	Selenium Form	Application Method	Dose	Se Content in Edible Portion	Yield and Biomass	Antioxidants	References
Fragaria x *ananassa* Duch.	Selenium nanoparticles (Se-NPs)	Foliar application	10 and 20 $mg.L^{-1}$	-	Increased	Superoxide dismutase (SOD) and Peroxidase (POD) activity increased	Zahedi et al. (2019)
Valerianella locusta L.	Sodium selenate (Na_2SeO_4)	Foliar and soil application	$50mgdm^{-3}$	0.1-61 $mg.kg^{-1}$ DW	30-48% increased	Glutathione (GSH), Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Ascorbic acid, Glutathione peroxidase (GPOX), Ascorbate peroxidase (APOX), Catalase (CAT) activity increased	Hawrylak-Nowak et al. (2018)
Lactuca sativa L. var, capitata	Sodium selenate	Hydroponic Nutrient film technique	0 – 40µM	20–140 $mg.kg^{-1}$ DW	5.7% increased	Increased total phenolic contents (TPC) and carotenoids	Pannico et al. (2019)
Malus domestica	Sodium selenate and Sodium selenite (Na_2SeO_3)	Foliar application	$0.15kg.ha^{-1}$	4.5-5.6 µg/100g FW	-	Antioxidant activity and total phenolic content increased	Groth et al. (2020)
Cichorium endivia L. var. *crispum* Hegi	Sodium selenate	Foliar and soil application	1, 2, 4 and $8\mu mol.L^{-1}$	0.71-17.61 $mg.kg^{-1}$	27.8-42.6% increased	Ascorbic acid, Total phenolic content (TPC) increased	Sabatino et al. (2019)
Brassica juncea L.	Sodium selenate	Foliar application	$50mg.L^{-1}$	8577-8922 $\mu g.kg^{-1}$ DW	Increased	Ascorbic acid, Flavonoids increased	Golubkina et al. (2018)
Lactuca sativa L.	Sodium biselenite ($HNaO_3Se$)	Foliar and soil application	50, 75 and 100ppm	200-1700 ppb	43.1% increased	Catalase, Ascorbate peroxidase, increased	Shalaby et al. (2017)
Allium cepa L. *Aggregatum*	Sodium selenate and Selenocysteine (SeCys)	Soil application	0.26mM solution	530% increased	51.6% increased	Ascorbic acid, flavonoids, phenolic content and antioxidant activity increased	Golubkina et al. (2019)
Daucus carota L. cv *Brasília*	Sodium selenate and Sodium selenite	Foliar and soil application	$1.0mgdm^{-3}$ through soil and 50 $\mu m.L^{-1}$ through foliar	$14mgkg^{-1}$	14-16% increased	7-33% carotenoid contents increased	Oliveira et al. (2018)
Lactuca sativa L. cv. *Vera*	Sodium selenate and Sodium selenite	Nutrient solution	0, 2, 4, 8, 16, 32, and $64\mu molL^{-1}$	10-22 $mg.kg^{-1}$	3.69–5.67% increased	Superoxide dismutase and catalase activity increased	Ramos et al. (2010)
Allium sativum L.	Sodium selenate and Sodium selenite	Soil application	0, 5, 10 and 15kg/ha Se	$23\mu gg^{-1}$ DW	-	-	Pérez et al. (2019)
Brassica oleracea L. var. *Marathon* and *Daucus carota* L.	Addition of hyperaccumulator *Stanleya pinnata*	Soil application	25, 50, 100, 200g	0.5 to 3.5µg Se g^{-1} DW	Increased	-	Bañuelos et al. (2015)

Selenite compound is absorbed by phosphate transport mechanism whereas selenate is transported from the soil to the plant by sulfate transporters of the root cells (Li et al. 2008).

As selenium structure resembles with that of Sulphur, it is easily absorbed by the sulfate transporters of the root cells. After entering the plants, selenate or selenite is further assimilated by the Sulphur assimilation pathway and converted into organic selenium compounds (White et al. 2004). First, by the action of enzyme ATP sulfurylase (APS) and APS reductase (APR), selenate is converted into selenite. APS hydrolyzes the ATP and convert it into adenosine phosphoselenate which is reduced by APR to selenite. Selenite is further reduced to selenide by enzyme sulfite reductase or glutathione (Wallenberg et al. 2010). In the presence of enzyme cysteine synthase, selenide is converted into selenocysteine (SeCys). SeCys is then either converted to elemental Se with the help of enzyme SeCys lyase or into selenomethionine (SeMet) by the action of series of enzymes. Two or more than two SeMet

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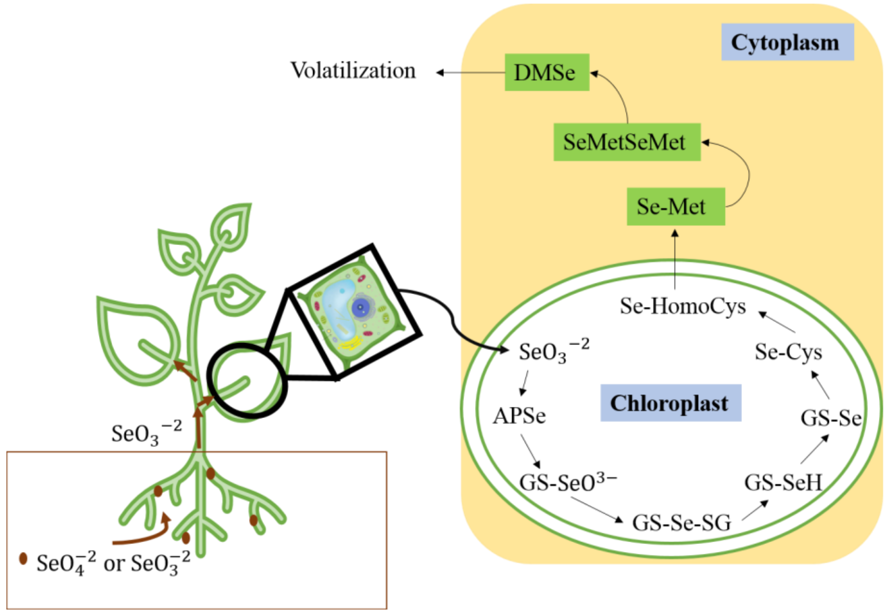

Fig. 1: Selenium uptake and assimilation pathway in plants.

compounds join to form selenoproteins or can be converted into volatile dimethylselenide, which escapes out in the atmosphere from the stomata of the plants (Pilon-Smits and Quinn 2010).

Capacity of each plant to assimilate selenium in them varies according to species. Depending upon the capacity of plant to accumulate selenium, plants are classified as accumulators, non-accumulators and hyper accumulators (Bodnar et al. 2012). Hyperaccumulators are those plants which can store more than 1000mgSe.kg^{-1} D.W and easily grows in seleniferous soils. *Stanleya,* and *Asparagalus* species are prominent examples of hyperaccumulators. Leafy vegetables are considered as accumulator plants as they can accumulate up to 100-1000 mg Se/kg dry weight. These mainly include *Lactuca sativa, Brassica juncea, Helianthus* and *Brassica napus* etc. Non accumulators are those plants which are sensitive to the selenium. Presence of high levels of selenium causes toxicity and results in retarded growth. However, they can store less than 100mgSe/kg dry weight e.g., grasses and crops (Bodnar et al. 2012).

1.5. Selenium Enrichment Effect on Horticultural Plants

Horticultural crops like vegetables and fruits contribute a major portion of human diet. To increase selenium content in human diet, many horticultural crops have been selenium fortified. Many studies have been carried out to produce se enriched horticultural crops by using different form of selenium (selenate, selenite, organic forms) through different application methods (foliar, soil, hydroponic nutrient solution). Some of the recent studies are summarized in the Table 1.

Conclusion: In conclusion, selenium has been recognized as an essential micronutrient element, required for proper functioning and health maintenance of human body. Deficiency of selenium can lead to various diseases and disorders. To overcome its deficiency, crops are fortified with selenium. Horticultural crops contribute a major portion of human diet. Se-fortified horticultural crops are efficient source of Se intake. Studies have shown that when it is applied to the plants, it also imposes positive beneficial effects on growth, nutritional profile, antioxidant composition, and induces tolerance against many stresses. However, further studies are required to explore the mechanism of action of selenium in the physiology of plants.

Author's Contributions
All authors have contributed equally and critically revised the manuscript and finalized the manuscript.

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