

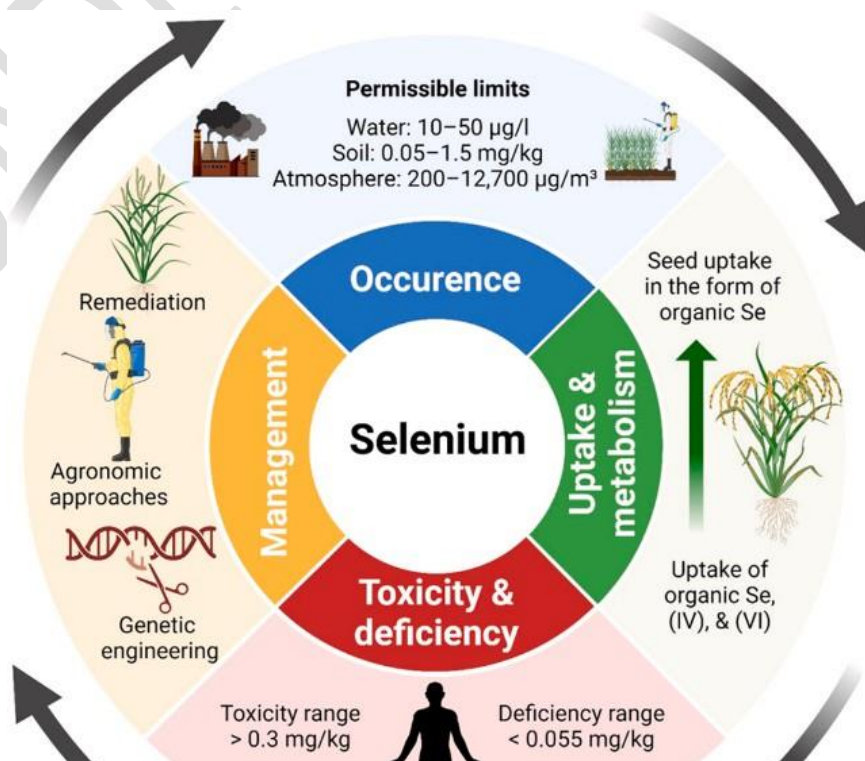
## Review Article

# “POSSIBILITY OF BIOFORTIFICATION BY SELENIUM FOR ABIOTIC STRESS MANAGEMENT USING EFFECTIVE MICROORGANISMS”

### ABSTRACT

Selenium biofortification aims to increase selenium accumulation or bioavailability in edible crops, tackling the global issue of hidden hunger for essential micronutrients. This review examines the vital role of selenium in human health, its interactions within soil-crop systems, and its potential to alleviate abiotic stresses in plants. By utilizing selenorhizobacteria as biotechnological tools, selenium biofortification presents an innovative method for enhancing crop nutrition and quality. The review emphasizes key mechanisms involved in selenium uptake, its function in activating plant antioxidant systems, and its dual role as both a beneficial nutrient and a prooxidant. Furthermore, it explores microbial contributions to selenium mobility and bioavailability, offering practical strategies to bolster crop resilience and productivity. This comprehensive analysis highlights the importance of selenium biofortification in fostering sustainable agriculture and improving global health outcomes.

### GRAPHICAL ABSTRACT



**Keywords:** Biofortification, Selenium, Abiotic stress, Crop improvement

## 1. Introduction

Biofortification is a process aimed to improve the nutritional value of crops by increasing the concentration of essential micronutrients in edible portions without sacrificing agronomic characteristics such as yield, or resistance to pests and drought. Biofortification through nanotechnology, where nano materials are applied to plants alone or as a component of conventional fertilizers (e.g. Zn, Fe or graphene nanoparticles), and green technologies, which involve the use of microorganisms to improve the nutrient status of the soil and the accessibility of nutrients to plants (Dhaliwal et al., 2022). The appropriate and healthy existence of humans, animals, archaea, and certain other microbes depends on the mineral micronutrient selenium. (El-Ramady et al., 2014).

The distribution of selenium varies around the world since it is found in the lithosphere layer of the earth's crust, which includes water, soil, and open areas (Hasanuzzaman et al., 2020). The primary source of selenium's role in functioning is its presence in selenium-containing amino acids such as selenomethionine (SeMet) and selenocysteine (SeCys). The total selenium concentration of food sources, including chemical forms (speciation), is crucial because it influences the nutritional value and bioavailability of selenium (Kikkert et al., 2013). Benefits derived from biofortifying crops with micronutrients, such as antioxidant qualities that can promote plant growth

and protect plants from many forms of abiotic stress. Although selenium is a necessary component of human and animal cells, its significance for plants is still being studied (Jozwiak et al., 2019).

Microorganisms are a novel biotechnological substitute for selenium biofortification since they are crucial to the transformations and availability of selenium. In addition to increasing plant productivity, fertilizing crops with selenium may also improve their nutritional value. The application of conventional agronomic selenium biofortification shows great promise in combating hidden hunger. (Haug et al., 2007).

The amount of selenium applied has a significant impact on its toxicity or benefits (Gupta et al., 2020). Se can activate plants' antioxidant systems at low concentrations, but at large concentrations, it functions as a prooxidant (Nawaz et al., 2015). Se has a number of beneficial impacts on plants, including boosting plant growth, reducing UV-induced oxidative damage, enhancing chlorophyll recovery from light stress, boosting senescing plants' ability to combat oxidative damage, and controlling drought-stricken plants' water status (Yao et al., 2009). Furthermore, Se has the ability to enhance plant growth and development as well as boost the plants' ability to withstand environmental stressors and produce antioxidants (Iqbal et al., 2015), hence contributing to increased grain yields (Hasanuzzaman et al., 2014). Research has shown that selenium (Se) can enhance plant development by fortifying stress tolerance mechanisms like antioxidant and secondary metabolite metabolism. (Kamran et al., 2020).

## **2. Importance of biofortification by selenium for abiotic stress management using effective microorganisms :**

### **2.1 Abiotic Stress in Agriculture**

Abiotic stress, including drought, heat, salinity, and heavy metal contamination, is a major constraint on agricultural productivity. These stressors significantly affect plant growth, yield, and quality. The global agriculture sector is facing an increasing number of challenges due to climate

change, which exacerbates the frequency and severity of abiotic stress conditions (Wang et al., 2020). The manuscript offers insights into how selenium, an essential micronutrient, can enhance plant tolerance to abiotic stress by acting as an antioxidant and promoting stress-related protein synthesis in plants (Tian et al., 2021). The potential of selenium to alleviate oxidative stress in plants makes it a promising candidate for improving crop resilience (Li et al., 2022).

## **2.2 Selenium in Biofortification**

Selenium is an essential micronutrient for both plants and humans, with significant health benefits, including antioxidant properties that help mitigate oxidative damage. Biofortification, which refers to the process of increasing the nutrient content of crops, is an important strategy for addressing micronutrient deficiencies in human diets. Selenium deficiency is a public health concern in many regions of the world, leading to conditions such as Keshan disease and weakened immune systems (Oldfield, 2022). Biofortifying crops with selenium can provide a sustainable way to improve both agricultural productivity and human nutrition. This manuscript's exploration of selenium biofortification is therefore crucial in the context of both agricultural science and public health.

Recent studies have shown that biofortifying cereals, vegetables, and other staple crops with selenium improves their nutritional value and addresses selenium deficiencies in human populations (McGrath et al., 2020). This manuscript contributes to this growing body of knowledge, highlighting the potential of selenium to improve both the nutritional quality of crops and their ability to cope with environmental stress.

## **2.3 Role of Effective Microorganisms (EM) in Stress Management**

Effective microorganisms (EM) refer to beneficial microbial consortia that can promote plant growth, enhance nutrient uptake, and improve stress tolerance. These microorganisms can aid in the bioremediation of contaminated soils, improve soil health, and increase the bioavailability of essential nutrients, including selenium (Zhang et al., 2021). The manuscript highlights the potential of combining selenium biofortification with EM, proposing that this synergy could enhance selenium uptake by plants and improve their resistance to abiotic stress.

Research has shown that EM can help plants withstand salinity stress (Sharma et al., 2022), drought (Shah et al., 2021), and heavy metal contamination (Chakraborty et al., 2020).

Furthermore, EM can promote the transformation of selenium into bioavailable forms, facilitating its uptake by plants (Rizvi et al., 2020). The manuscript's focus on this combined approach is significant for advancing sustainable agricultural practices.

## **2.4 Sustainability and Environmental Impact**

Selenium biofortification combined with EM offers a low-cost, environmentally friendly alternative to conventional agricultural practices that often rely on chemical fertilizers and pesticides. These traditional methods can lead to soil degradation, water pollution, and other ecological challenges (Singh et al., 2020). The use of biofortification and EM could mitigate these environmental issues while promoting sustainable agriculture. Recent studies have underscored the importance of integrating biofortification strategies with microbial approaches to reduce the environmental impact of farming while increasing crop yields and quality (Vargas et al., 2023).

By reducing dependency on chemical inputs and improving soil health through microbial activity, this approach aligns with the principles of sustainable and regenerative agriculture, which are vital for ensuring long-term food security.

## **2.5 Interdisciplinary Approach and Future Research**

The manuscript presents an interdisciplinary approach that integrates plant biology, microbiology, environmental science, and agricultural biotechnology. This cross-disciplinary perspective is important for addressing the complex challenges faced by modern agriculture. The findings could inspire future research on the genetic, molecular, and ecological aspects of selenium uptake and the role of EM in biofortification.

**2.5.1 Microbial Interactions and Selenium Uptake:** Future studies could focus on identifying specific microbial species that enhance selenium uptake in plants and investigating their mechanisms of action (Zhang et al., 2022).

**2.5.2 Genetic Engineering for Enhanced Biofortification:** Advances in plant genomics and genetic engineering could further improve the efficiency of selenium biofortification and stress tolerance (Sharma et al., 2021).

## 2.6 Global Health Implications

In addition to improving crop yield, selenium biofortification addresses global health concerns by combating selenium deficiency. Selenium plays a crucial role in immune function, thyroid hormone metabolism, and antioxidant defense. The manuscript's focus on biofortification as a solution to selenium deficiency is highly relevant for addressing public health issues, particularly in regions where soil selenium levels are low.

**2.6.1 Health Benefits of Selenium Biofortification:** Studies have demonstrated that selenium biofortified crops can significantly improve human selenium intake and reduce the incidence of selenium-related health problems (Sorensen et al., 2021). This manuscript's contribution to the biofortification field has the potential to influence policies aimed at improving global nutrition. This review will therefore attempt to spread more light to find out the ways for biofortification of crops to increase their tolerance towards drought and can produce reliable yield.

## 3. Significance of selenium in human health

Selenium is a trace element that is part of selenocysteine, an enzyme active site component, and is therefore required in trace amounts by both humans and animals. Se takes responsibility for a variety of metabolic activities in both animal and human systems. Se activates immune cells such natural killer (NK), cytotoxic T, and helper T in the immunological system (Razaghi et al., 2021).

Selenium has shown various benefits, including enhanced growth performance, immune functions, and nutritional quality of meats, with reduced oxidative stress and inflammation, and finally enhanced thyroid health and fertility in humans (Mojadadi et al., 2023). A vital trace element, selenium is fundamentally important for human health. For both humans and animals, selenium is an important mineral with antioxidant qualities (Jezek et al., 2012). Selenium deficiencies in the human body can result in or cause illnesses like Keshan and Kashin-Beck disorders (Fairweathe et al., 2011). Additionally, a shortage in selenium is linked to immune

system enhancement, muscular necrosis, hypothyroidism, cardio-cerebrovascular illness, male infertility, and an increased prevalence of several cancers (Fordyce et al., 2013). Se deficiency is thought to be associated with illnesses including acquired immune deficiency syndrome (AIDS) and coronavirus disease 2019 (COVID-19) (Zhang et al., 2020). A vital component of selenoproteins, selenium is involved in numerous biological processes with antioxidant qualities, including defense against free radical damage, thyroid hormone production, DNA synthesis, fertility and reproduction, HIV treatment, and defense against toxic heavy metals (Fairweather-Tait et al., 2011).

This metalloid's predicted significance stems from its association with selenoenzymes, including glutathione peroxidase, thioredoxin reductases, and proteins whose roles are unclear but which contribute to preserving the redox potential of cells (Ramos et al., 2010), as well as additional bodily structure and metabolic processes. A diet deficient in selenium can have a detrimental effect on human health by increasing the risk of heart disease, hypothyroidism, lower male fertility, impaired immune systems, and increased susceptibility to infections and malignancies (Hatfield et al., 2014). Numerous studies have demonstrated the protective effects of selenium compounds, such as SeMet, in the human diet against malignancies of the breast, prostate, lung, bladder, and liver (Fairweather-Tait et al., 2011). Thus, one practical way to lessen the issue of selenium insufficiency in humans and animals is to increase the content of selenium in food crops.

An approximate adequate daily intake of selenium for humans is 50–60  $\mu\text{g}$ , however hazardous levels of selenium ingestion range from 350–700  $\mu\text{g}$  (Badmaev et al., 2018).

It is possible for many microbes to transform inorganic selenite into organic forms, which are thought to be more effective and safe dietary sources of selenium. Additionally, selenium can bind to different polysaccharides and proteins to form complexes. The prevention of cancer and

cardiovascular disease is one of the main health benefits of selenium supplementation or ingestion in humans, even at low dosages. Significant increases in the amount of starch, reducing sugars, sulfur-containing amino acids, and other components are involved in selenium augmentation in cereals.

The percentage of people suffering from non-communicable, dietary-dependent illnesses such as obesity, hypertension, hyperinsulinemia, insulin resistance, and dyslipidemia is continuously rising (World health statistics, 2020). However, consuming too much selenium can also lead to the development of hypotension, tachycardia, tremor, muscle contractions, hair loss, and lesions on the skin and nails. Therefore, it's important to maintain a balanced daily intake of Se (Hossain et al., 2021). The amount of Se that is advised to be consumed each day varies based on factors like age, gender, stage of pregnancy, length of lactation, region, and food. In addition, the WHO's recommended daily intake of selenium (Se) is trending upward (Table 1). The body mostly absorbs selenium through diet. Products derived from plants and animals contain the trace element.

**Table1.: Recommended daily intake levels of Se**

country	Men mg/day	Women mg/day	Pregnancy mg/day	Lactation period mg/day	Maximum allowable level mg/day	Toxic dose mg/day	Reference
WHO	42 (1996); 34 (2004); 55 (2019)	39 (1996); 26 (2004); 55 (2019)	57-59	64-71	400	900	Kieliszek et al., 2019
Russia	70	55	55	55	-	-	World Health Organization; 2017
United Kingdom	75	60	-	-	400	-	Kieliszek et al., 2019
United States	55	55	60	70	200	400	The National Academy Press, 2000.
Europe	60	53	-	-	400	-	Commission Directive 2008
India	55	55	60	70	200	-	Alexander et al., 2020

#### 4. Status of selenium in soil and crop

The physical, chemical, and biological characteristics of the soil have a significant impact on the effectiveness of selenium-treated crops in terms of yield (Zhao et al., 2005). The majority of soils have extremely low bioavailability of selenium content, ranging from 0.01 to 2 mg/kg on average (0.4 mg/kg); nevertheless, in select seleniferous locations, greater amounts of up to 1200 mg/kg have been reported (Fordyce et al., 2005). Selenium concentrations in vegetation on most soils are less than 1 mg/kg. Most plant species on seleniferous soils have a selenium content of 1–10 mg/kg, while plants that are hyper accumulators of selenium, such as *Astragalus* and *Stanleya* genera, can collect 1000–15000 mg/kg at low soil concentrations.

##### 4.1 Distribution of Se worldwide



**Figure 1. Map showing the prominent regions having selenium in the world. (Selenium rich areas are marked red while selenium deficient are marked blue).**

In India, the soil of Gujarat has a very low selenium status and is regarded as deficient in the element; the total amount of selenium in the soil ranges from 0.142 to 0.678 mg/kg, with an average of 0.375 mg/kg (Patel et al., 1970). In agroecosystems, selenium can be present in both inorganic and organic forms. Elemental selenium, selenide, selenite, and selenate are the four oxidation states of inorganic selenium. Uneven distribution of selenium in the soil might result in selenium insufficiency. Low quantities of plant-available forms of selenium in soil can reduce the amount of selenium consumed through food due to crops' slow uptake of the mineral (Winkel et al., 2015). Only around 5% of the selenium that is added to the soil is used by plants. Plant species differ widely in their capacity for selenium buildup in their tissues (White, 2016). In general,  $\text{SeO}_4^{2-}$  and  $\text{SeO}_3^{2-}$  have a strong affinity for plants. Conversely, some organic forms of selenium, such as selenocysteine (Se-Cys) and selenomethionine (Se-Met), are employed as active ingredients because they have a higher phytoavailability.

The amount of selenium in the soil and the concentration of selenium in grown food plants in a given area determine the selenium status and intake in a given human population. Therefore, controlling the amount of Se in plants is a major way to manage human intake of Se and its status, which is also influenced by soil Se level, bioaccumulation of Se, and the effectiveness of soil microbes (Stoffaneller and Morse, 2015; Winkel et al., 2015). The average selenium content of soils worldwide ranges from 0.1 to 0.7 mg kg<sup>-1</sup>. Clay soils typically have an average Se content of 0.8 to 2 mg kg<sup>-1</sup>, whereas tropical soils have an average Se level of 2-4.5 mg kg<sup>-1</sup>. As a result, clay soils contain more Se than soils with coarse minerals. (Hartikainen, 2005). Rainfall, organic matter, and soil texture all affect the amount of selenium present in the soil (El-Ramady et al., 2016).

Se content is extremely low in igneous rocks and explosive soils. These kinds of soils are found in hilly nations like Sweden, Finland, and Scotland. Se is abundant in sedimentary rocks. Se is typically prevalent in rocks found in the world's arid regions. These rocks are linked to the detrimental effects of selenium on animals. (McNeal and Balistrieri, 1989; Gupta and Gupta, 2000). By using microorganisms that can metabolize inorganic selenium and be used as seed inoculants or as biotechnological tools for crop nutrition and quality, it may be possible to reduce the complexity of selenium behavior in plants and soils, particularly in soils that are specifically deficient in the mineral. A novel biotechnological approach to address the toxicity and selenium deficit in some agroecosystems could involve combining biofortification and bioremediation (Acuna et al., 2013).

The distribution, mobility, and bioavailability of selenium in soils are significantly impacted by the presence of microbes in the surrounding environment. The bioprocesses involved in the metabolism of bacteria also affect the relative amounts of selenium oxidation states and selenium compounds in the atmosphere. For the purpose of bioremediation of contaminated soils, sediments, industrial effluents, and agricultural drainage waters, bacteria have the capacity to convert inorganic selenium into elemental forms (Dungan et al., 2003). Nearly 80% of the world's total Se reserves are distributed across Australia, Peru, China, the United States, Chile, Canada, New Guinea, Zambia, the Philippines, and Zaire (Liu et al., 2011). There are places in about forty countries where human selenium intake is 10%  $\mu\text{g day}^{-1}$  or even significantly lower, while soil selenium concentrations are limited. Conversely, Switzerland, New Zealand, Australia, Finland, and South Korea are among the nations with Se-abundant to Se-limited areas. (Wu et al., 2015).

Total Se concentration ranged from 0.023 to 4.91  $\text{mg kg}^{-1}$  in 0–15 cm surface soil and 0.64–515.0  $\text{mg kg}^{-1}$  in vegetation samples in the northwest region of Indian soil (Dhillon et al., 2014). Plants, soils, rocks, and groundwater are all parts of the agroecosystem that contain

selenium. The range of selenium amounts in animal diets for both adequacy and toxicity is 0.05–0.10 mg kg<sup>-1</sup> and 4.0–5.0 mg kg<sup>-1</sup>, respectively (Zanetti et al., 2015).

## **5. Need of Selenium for abiotic stress management in rainfed rice cultivation**

Rice (*Oryza sativa* L.) is one of the major food crop globally ranked third after wheat and maize in terms of production and is the staple food crop for nearly two-third of the world's population (Abbade et al., 2021). About 52% of net sown area in India falls under rainfed agriculture, contributing 46% of food grain production and supporting livelihood for 40% of the population in the country (NRAA- National rainfed area authority, 2020). Because the livelihood of a majority of the world's population depends on rice, it is vital to investigate how abiotic stress influences the variability of rice yields (Song et al., 2022). Due to unpredictable, insufficient, and inconsistent rainfall during the growing season. Selenium application with effective microorganisms are needed to reduce rice crop yield losses in rainfed lowland areas and increase overall rice production. It is estimated that approximately 90% of global arable land is prone to one or more environmental stresses, resulting in 70% yield losses in major crops (Waqas et al., 2019). Severe droughts witnessed in India during 2002, 2003, 2009 and 2010 caused significant reduction in rice yields particularly in eastern Indian states of Jharkhand, Bihar, Uttar Pradesh, Chhattisgarh, and Odisha.

## **6. Role of soil microbes in mobilization of selenium**

Enhancing selenium biofortification through crop-microorganism interactions is the focus of this emerging field of study. Plant growth promoting bacteria (PGPB) are a diverse group of soil bacteria that, when coexisting with a host plant, stimulate the host's growth. Nowadays, using bacteria that promote plant growth can serve as an alternative to conventional techniques for improving plants' uptake of micronutrients. (Mora et al., 2015).

It is widely acknowledged that a large range of bacterial species exist in the rhizosphere and that these species perform vital roles in agriculture, including nutrition, plant growth, and

disease prevention (Hawkesford et al., 2007). Similar to this, microbes are crucial to the biogeochemical cycle of selenium in the natural world (Ike et al., 2000). Selenium is modified during bacterial metabolism by a variety of processes (**oxidation, reduction, or methylation**), as well as by selenium respiration in bacteria that are tolerant of selenium and linked to the processes of assimilation and metabolization of this metalloid within the cells. This has demonstrated a great deal of promise for usage in selenium-contaminated sites bioremediation and phytoremediation (Ghosh et al., 2008; Tong et al., 2014). The amount of accessible EXC-Se and SOL-Se in soil and plants was considerably enhanced by the selenium-oxidizing bacteria *Dyella* sp. LX-1, *Rhodanobacterium* sp. LX-100, and *Agrobacterium* sp. T3F4. Concentrations of Se (Guo et al., 2024). Excessive levels of heavy metals, such Cd, in seleniferous soils need to be addressed, despite the fact that microbial selenium fortification in crops is an environmentally beneficial biotechnology (Jiao et al., 2022)

In both aquatic and terrestrial ecosystems, specific bacterial species are crucial for the transformation of selenium. Se serves as the terminal acceptor for the anaerobes' respiration. *Thauera selenatis*, which was found in a bioreactor containing Se-oxyanion and agricultural wastewater in California, and *Sulfurospirillum barnesii*, which was found in drainage containing Se, are the first microbes to be isolated to use  $\text{SeO}_4^{2-}$  for such a process (Macy et al. 1993; Oremland et al. 1994). While  $\text{SeO}_4^{2-}$  is produced in small quantities, autotrophic bacteria like *Bacillus megaterium* typically oxidize  $\text{Se}^0$  into  $\text{SeO}_3^{2-}$ . Previous investigations into soils and slurries have demonstrated that the oxidation process of  $\text{Se}^0$  results in the formation of  $\text{SeO}_4^{2-}$  and  $\text{SeO}_3^{2-}$ , respectively (Dowdle and Oremland 1998). A number of other microbial species, such as *Shewanella*, *Anaeromyxobacter dehalogenans*, *Desulfitobacterium* sp. *D. chlororespirans*, *Geobacter sulfurreducens*, and *Enterobacter cloacae* etc. were furthermore examined for their capacity to reduce, specifically for conversions from  $\text{SeO}_3^{2-}$  &  $\text{SeO}_4^{2-}$  into  $\text{Se}^0$ , respectively (Schilling et al. 2020).

## 6.1 Se-metabolising bacteria

### Selenate-metabolising bacteria

Bacteria that consume selenium undergo reduction of  $\text{SeO}_4^{2-}$  in two primary stages: first, selenium reductases convert  $\text{SeO}_4^{2-}$  into  $\text{SeO}_3^{2-}$ , and then they further reduce selenite into  $\text{Se}^0$ .



Microbes such as *B. selenatarsenatis* and *E. cloacae* are capable of undergoing these kinds of reactions, which take place in the presence of anaerobic and aerobic circumstances. That are often engaged in the reduction of  $\text{SeO}_4^{2-}$  (Nancharaiah and Lens 2015). SerABC selenate reductase is a trimeric molybdenum enzyme that aids in the reduction of  $\text{SeO}_4^{2-}$  into  $\text{SeO}_3^{2-}$  in the periplasm.

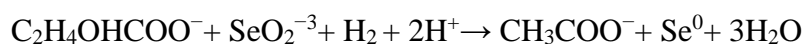
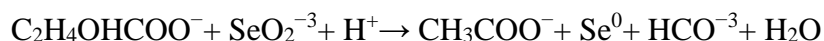
However, the purest form of selenate reductase enzyme was identified, screened, characterized, and purified by Schroder et al. (1997). The unique way that selenium-metabolizing bacteria operate allows them to break down selenium inside their cells.

## 6.2 Selenite-metabolising bacteria

Many different microbial strains reduce  $\text{SeO}_3^{2-}$ , contributing up approximately 43% of the soil microbial population that takes part in the conversion of  $\text{SeO}_3^{2-}$  and  $\text{SeO}_4^{2-}$  into  $\text{Se}^0$ .

Additionally, anaerobic respiration or detoxification both promote this process (Sura-de Jong 2015). Different bacterial strains that reduce  $\text{SeO}_4^{2-}$  also exhibit the capacity to dissimilatory reduce  $\text{SeO}_3^{2-}$ , which mostly promotes the synthesis of lactate and acetate, respectively (Oremland et al. 1994).

## 6.3 The following reactions involve the conversion of $\text{SeO}_3^{2-}$ into $\text{Se}^0$ :



This is successfully aid in the detoxification mechanism by mediating the process of  $\text{SeO}_3^{2-}$  reduction in bacteria, which typically occurs in the cytoplasm or periplasm and is then translocated

to the cell exterior as  $\text{Se}^0$  (Kessi and Hanselmann 2004). Additionally, the reduction of  $\text{SeO}_3^{2-}$  using organic carbon (lactate, propionate, butyrate, and acetate) with the help of *Cronobacter sp.* has been demonstrated. In this case, organic carbon acts as an electron donor in the microaerobic environment where microorganisms require oxygen to completely decrease  $\text{SeO}_3^{2-}$  (Estrada et al. 2020). Therefore, all of these bacterial strains can be investigated for their ability to convert inorganic forms of selenium and for use as a source of selenium supplement in food. Potential biotechnological uses for PGPR include serving as a carrier for agricultural biofortification. The selenium linked with the bacterial inoculum may be incorporated and translocated into leaves and other plant parts by plants treated with bacteria that were tolerant of selenium.

This bacterial inoculum enhanced with selenium can be employed as a biotechnological instrument for plant selenium biofortification. Wheat plant tissue had higher selenium concentrations after being inoculated with selenium-enriched rhizobacteria, or seleniorhizobacteria, which can metabolize selenium (Acuna et al., 2013). The selenium content of grain was enhanced in plants co-inoculated with a combination of *selenibacteria* strains and *G. claroideum*. A potential substitute for increasing the selenium content of cereals cultivated on soils poor in selenium is the use of microorganisms that are tolerant of selenium.

Many research investigations have demonstrated a variety of aerobic bacteria that are resistant of selenium, including *Pseudomonas aeruginosa*, *Bacillus sp.*, *Stenotrophomonas sp.*, *Acinetobacter sp.*, and *Klebsiella sp.* (Acuna et al., 2013) etc. possess the capacity to accumulate selenium, and these bacteria may be employed as inoculants to enhance cereal wheat with selenium (Table 2). *Selenobacteria* could transform the species and valencies of Se in the soil, facilitating Se uptake by plants. Whole-genome analysis unveiled the genetic elements of the two *selenobacteria* with contrasting Se utilization profiles and identified candidate genes related to Se utilization. This work significantly advances our understanding of the potential molecular mechanisms underlying Se biofortification by *selenobacteria* (Liao et al., 2024)

**Table 2.: Plant growth promoting bacteria used for selenium biofortification in crops.**

No	Microorganisms	Plant	Method of fertilization	Source of Se	Results	References
1.	<i>Enterobacter</i> sp. B16, <i>Stenotrophomonas</i> sp. B19	Triticum aestivum L. cv. Fritz	Inoculation Root	Selenobacteria	Enhanced selenium content in Shoot	Acuna et al. (2013)
2.	<i>Bacillus</i> sp. R8, <i>Enterobacter</i> sp. B16, <i>Pseudomonas</i> sp. R12, <i>Stenotrophomonas</i> sp. B19	Triticum aestivum L. cv. Puelche	Inoculation Root	Selenobacteria	Enhanced selenium content in Grain	Duran et al. (2013)
3.	<i>Bacillus</i> sp. YAM2	Triticum aestivum L.	Seed palletization	Selenobacteria	Enhanced selenium content in wheat kernels & stems	Yasin et al. (2015)
4.	<i>Acinetobacter</i> sp. E6. 1, <i>Bacillus</i> sp. R8, <i>Klebsiella</i> sp. E2	Triticum aestivum L. cv.	Seed palletization	Endophytic Selenobacteria	Enhanced selenium content in Shoot	Duran et al. (2015)

## 7. Selenium biofortification mediated by micro-organisms for effective and sustainable crop production

Microbes help with Se-biofortification, which raises crop nutrient levels and uses them as fertilizer for both biotechnological and conventional breeding techniques. In areas where soil nutrients are scarce, this procedure improves the nutritional content of crops (Hossain et al. 2021). Additionally, in Se-deficient soils, Se-biofortification is carried out by choosing a plant species that can better absorb micronutrients through their edible organs, enhancing the diets of humans and animals. The most effective method for ensuring that plants absorb selenium is through biofortification, which allows diverse agricultural soils deficient in selenium to recoup it through animal excrement as well (Ye et al. 2020). Certain microorganisms help plants absorb nutrients more efficiently, and in addition to increase plant growth and productivity, these microbes also assist plants resist stress. Microflora, including endophytic fungi, mycorrhizae, and rhizobacteria

that promote plant growth, are being employed in biofortification processes. (Hossain et al. 2021). Mycorrhizae act as intermediaries. Plant uptake of selenium (Se) improved the uptake and accumulation of selenium and selenite, especially in *Glomus versiform* and *Funneliformis sp.* (Patel et al., 2018), (Table 3). In this three-year field experiment, soybean was found to be a good candidate for biofortification, able to accumulate up to 16.22 mg/kg of Se in the seeds with no obvious negative impact on yield (or quality). This enrichment could lead to a reduction of Se deficiency in the diet of livestock and populations. Soybean is able to convert almost 95% of the mineral forms of selenium into organic forms, even at a rate of 100 g/ha Se. (Mrstina et al. 2024).

**Table 3.: Microbes mediating Se-biofortification for sustainable agricultural practices**

S.No.	Micro-organism	Plant species	References
1.	<i>Acinetobacter sp.</i>	<i>Triticum aestivum</i>	Duran et al. (2013)
2.	<i>Alcaligenes faecalis</i> , <i>Paraburkholderia gapolita</i>	<i>Ricinus communis</i> , <i>Glycine max</i>	Trivedi et al. (2020)
3.	<i>Anabaena sp.</i>	<i>Triticum aestivum</i>	Abadin et al. (2017)
4.	<i>Bacillus amyloliquefaciens</i>	<i>Arabidopsis thaliana</i>	Wang et al. (2017)
5.	<i>Bacillus axarquiens</i>	<i>Triticum aestivum</i>	Duran et al. (2013)
6.	<i>Bacillus cereus</i>	<i>Triticum aestivum</i>	Yasin et al. (2015)
7.	<i>Glomus fasciculatum</i>	<i>Allium sativum</i>	Patharajan and Raaman (2012)
8.	<i>Glomus intraradices</i>	<i>Allium sativum</i>	Larsen et al. (2006)
9.	<i>Rhizophagus intraradices</i>	<i>Lactuca sativa</i> , <i>Asparagus officinalis</i> , <i>tuca sativa</i> , <i>Allium cepa</i>	Sanmartin et al. (2018)
10.	<i>Glomus versiform</i>	<i>Triticum aestivum</i>	Luo et al. (2019)
11.	<i>Glomus mosseae</i>	<i>Lolium perenne</i> , <i>Allium sativum</i> , <i>M. sativa</i> , <i>Glycine max</i> , <i>Zea mays</i>	Patharajan and Raaman (2012); Yu et

			al.(2011)
12.	<i>Trichoderma harzianum</i>	<i>Allium cepa</i>	Sanmartin et al. (2018)
13.	<i>Alternaria seleniiphila, Alternaria astragali, Aspergillus leporis, Fusarium acuminatum</i>	<i>Stanleya pinnata, Astragalus bisulcatus, Stanleya pinnata, Astragalus racemosus</i>	Lindblom et al. (2013)
14.	<i>Bacillus</i> sp. R12, <i>Enterobacter</i> B16, <i>Pseudomonas</i> sp. R8, <i>Stenotrophomonas</i> sp. B19	<i>Triticum aestivum</i>	Duran et al. (2013)
15.	<i>Bacillus subtilis</i>	<i>Allium cepa</i>	Golubkina et al. (2019)
16.	<i>Bacillus licheniformis, Bacillus pichinotyi</i>	<i>Triticum aestivum</i>	Yasin et al. (2015)
17.	<i>Bacillus mycoides</i>	<i>Brassica juncea</i>	Lampis et al. (2009)
18.	<i>Bacillus</i> sp. E6.1, <i>Enterobacter ludwigii, Klebsiella oxytoca</i>	<i>Triticum aestivum</i>	Duran et al. (2014)
19.	<i>Calothrix</i> sp.] <i>Providencia</i> sp]	<i>Triticum aestivum</i>	Rana et al. (2012); Yasin et al. (2017)
20.	<i>Rhizobium</i> sp.	<i>Astragalus bisulcatus, A. drummondii</i>	Alford et al. (2014)
21.	<i>Stenotrophomonas maltophilia</i>	<i>Ricinus communis, Glycine max, Brassica juncea</i>	Businelli et al., (2015)

## 8. On Selenium Biofortification and Nanotechnology :

### 8.1 Nano Materials for the Application of Selenium:

Selenium is an important micronutrient for animals and humans. Though it is not an essential nutrient element, a low dose of selenium in soil protects plants from various abiotic stresses like drought, cold, and toxic metals (Feng et al., 2013). As it has chemical similarity with sulphur, the uptake to the plant cell is carried by sulphur transporters through the root plasma membrane through the sulphur assimilatory pathway (Gupta et al., 2017). Many researchers have published a comparison of efficacy in the diffusion of nano selenium over inorganic selenium usage

(Galic et al., 2021; Schiavon et al., 2020). On the other side, Domokos-Szabolcsy et al., (2012) observed no growth stimulation with nano selenium in tobacco (*Nicotinia tabacum* L.), but the application of inorganic selenium at less than 50 mg l<sup>-1</sup> inhibited plant growth. Conversely, the application of nano selenium at 50–100 mg kg<sup>-1</sup> stimulated the growth of root system and organogenesis in elephant grass (*Arundo donax* L.) by nearly 40%, while the use of inorganic selenium did not show any effect on the root system, but in turn, it inhibited the growth at 50–100 mg kg<sup>-1</sup> (Domokos-Szabolcsy et al., 2014).

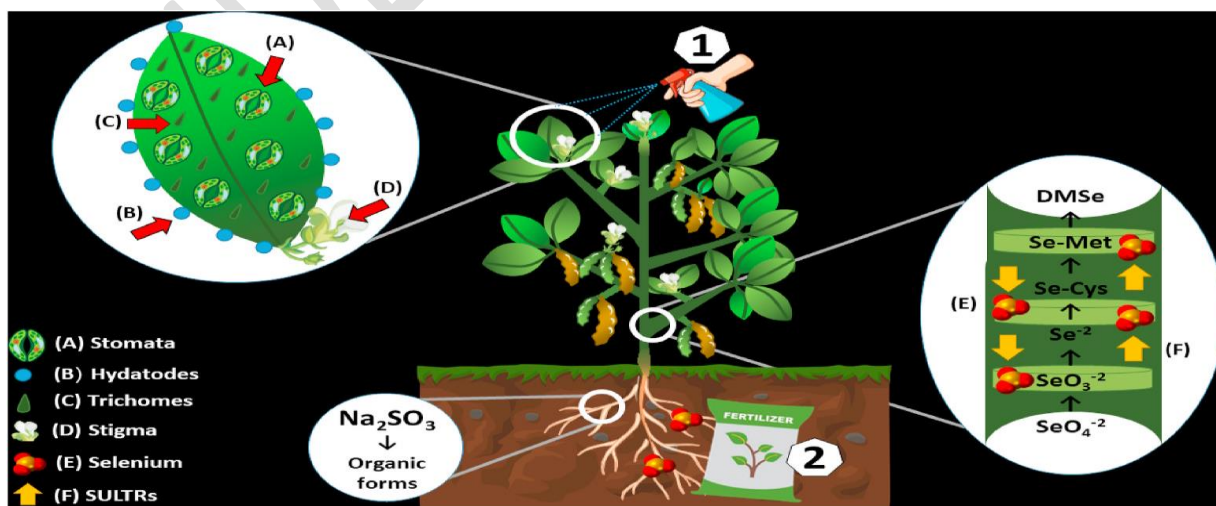
To overcome the present issue; biofortification, a method to enhance nutritional status of food crops, can address the issue of hidden hunger. Nanotechnology may contribute to improving the quality of food through biofortification and may prove to be an effective and sustainable remedy to this issue by foliar application of essential nutrients (Zn, Cu, Fe and Se) nanoparticles and their nano-based fertilizers in the soil to improve nutrient deficiency. (Ul din et al., 2023).

## **9. Abiotic stress management by selenium**

In crops, selenium's function in reducing environmental stress has been widely documented. At modest concentrations, selenium can promote plant development and help plants adapt to a variety of environmental stressors, including abiotic stressors like drought, cold, and heavy metal stress (Oancea et al., 2015; Mora et al., 2015; Handa et al., 2016). Drought stress is one of the most significant environmental stressors, which has negative effects on plant growth and yield. Se plays an active role in the regulation of Plant Antioxidants, Chlorophyll Retention, and Osmotic Adjustment under Drought Conditions (Dar et al. 2021). Plants may grow and develop to their full potential if selenium levels in the soil are raised during stressful times (Sieprawska et al., 2015). Increased water retention in plant tissue may result from selenium stimulation's rise in the contents of both organic and non-organic osmo-protectants (Hajiboland et al., 2015). It was widely accepted that adding selenium lessens these pressures' detrimental effects on plants' and fruits' ability to produce biomass (Xue et al., 2001).

The activation of antioxidative enzymes by selenium in appropriate amounts helps modulate oxidative stress (Hartikainen et al., 2000). Three mechanisms exist by which selenium can control

the amounts of reactive oxidative species (ROS) in stressed plants: (1) by stimulating the  $O_2^{\cdot-}$  spontaneous dismutation into  $H_2O_2$ ; (2) by a direct interaction between molecules containing selenium and ROS; (3) by regulating the activity of antioxidant enzymes. One important way that selenium may help plants avoid stress is by controlling the amount of ROS they produce. Plant cells produce very little ROS under normal circumstances. Stress conditions like as drought, excessive water, intense light, cold, salt, and heavy metals, on the other hand, can cause a buildup or increase in ROS levels in plants. An increase in ROS generation can be harmful to plants. The primary types of ROS are singlet oxygen ( $O_2$ ), hydroxylic free radical ( $OH$ ), hydrogen peroxide ( $H_2O_2$ ), superoxide anion ( $O_2^{\cdot-}$ ), and methyl radical ( $CH_3$ ). When plants are exposed to various environmental stressors, a small amount of selenium added to the growth substrates can lower the excess ROS formation (Cartes et al., 2010), (Fig 2). Foliar Se application provides the deposit of droplets containing elements on the leaves, The main pathway for nutrients to enter the leaves is through a passive process due to difference in concentration, which occurs on the external surface, where there is a higher concentration of solute, towards the internal, with a lower concentration of solutes, through the aqueous pores present in the cuticles, proceeding to the mesophylic cells through specific transporters (Yang et al., 2019).



## Fig 2. - Selenium uptake and assimilation mechanisms by plants

Table 4.: summarizing selenium's effects on different abiotic stresses :

Abiotic Stress	Effect of Selenium	References
<b>Drought Stress</b>	Enhances water use efficiency, reduces oxidative damage, improves survival under drought conditions.	Liu et al. (2014), Wu et al. (2017)
<b>Salinity Stress</b>	Reduces salt toxicity by improving antioxidant activity, ion balance, and osmotic regulation.	Zhang et al. (2018), Zhang et al. (2020)
<b>Temperature Stress</b>	Mitigates heat and cold stress by boosting antioxidant defense systems and stabilizing cellular structures.	Hasan et al. (2019), Yang et al. (2020)
<b>Heavy Metal Stress</b>	Reduces heavy metal toxicity (e.g., Cd, Pb) by enhancing antioxidant mechanisms and improving metal tolerance.	Zhang et al. (2019), Azhari et al. (2021)
<b>UV Radiation Stress</b>	Protects plants from UV-induced damage by promoting antioxidant systems and reducing oxidative stress.	Djanaguiraman et al. (2013), Panda et al. (2017)

The field experimental results revealed that induced drought stress at panicle initiation stage had drastically reduced the growth characters, tiller production, and grain and straw yields. Under both normal and drought induced conditions, foliar application of Se (either 10 ppm or 20 ppm) resulted in a significant increase in growth parameters (plant height, LAI, drymatter production) and tiller development, which subsequently enhanced rice grain and straw yields. Hence, the field study confirmed that selenium foliar spraying to be an efficient strategy for improving rice yield under drought conditions (Monisha et al., 2021).

Selenium can be added to stressed plants to increase their antioxidant levels, which will control the amount of reactive oxygen species. In order to counteract the increased ROS levels,

plants typically activate two different forms of antioxidants. Low molecular weight compounds like glutathione and ascorbic acid are one type of antioxidant; enzymes such as superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, glutathione reductase, and glutathione peroxidase are another type of antioxidant (Hartikainen et al., 2000). These antioxidants have the ability to interact with ROS directly or indirectly through the activity of enzymes. Selenium has the ability to directly or indirectly regulate the synthesis and scavenging of reactive oxygen species (ROS) by modulating antioxidant levels. It is generally known that glutathione and selenium can combine directly to create selenocysteine, selenio-methionine, and eventually proteins that contain selenium (Terry et al., 2000).

The mechanism behind this beneficial effect of selenium on antioxidant capacity could be either indirect—caused by selenium-induced activation of general stress resistance mechanisms—or direct—caused by the antioxidant activity of selenio-compounds. Additionally, adequate selenium levels can shield plants from the harm that heavy metals like As, Hg, Pb, Cd, Zn, Cu, and Cr can inflict (Malik et al., 2012). Selenium may limit the uptake and translocation of heavy metals from plant roots, which could be a relevant heavy metal detoxification process.

## **10. Selenium for crop yield improvement**

Many studies have been conducted to ascertain the advantageous function of Se in enhancing yield (Broadley et al., 2010; Ekanayake et al., 2015; Nawaz et al., 2016). Studies on the effects of selenium (Se), including fertilizer, foliar spraying, and nanoparticles, on crop yield enhancement. Under drought stress, exogenous Se-foliar spray (40 mg L<sup>-1</sup>) boosted the crude protein by 47%, fiber synthesis by 10%, and Se contents by 36% without changing the crude ash contents (Nawaz et al., 2016). Ekanayake et al. (2015) examined the effects of field-applied selenium (30 g ha<sup>-1</sup> of SeO<sub>3</sub><sup>2-</sup> and SeO<sub>4</sub><sup>2-</sup>) on the production of lentil grains at the 50% flowering and seedling phases. The production of lentil seeds increased by 10 and 4%, respectively, upon the

use of these fertilizers. Sweet corn's response to exogenous Se spray has been reported by Huang et al. (2019). A spray applied at different growth stages with different doses of Se fertilizer (0.80–1.00 g Se L<sup>-1</sup>) increased the sugar content and had a beneficial influence on seed production.

Recent reports describe the application of Se nanoparticles to increase crop output. Hernandez-Hernandez et al. (2019) shown that utilizing 10 mg L<sup>-1</sup> of Se nanoparticles increased tomato yield by 21%. Additionally, vitamin C, GSH, flavonoid levels, firmness, total soluble solids (TSS), and fruit acidity were all improved by the selenium treatment. In addition, Zahedi et al. (2019b) showed how Se (Na<sub>2</sub>SeO<sub>4</sub>) foliar spray and Se nanoparticles affected various pomegranate growth indices. Applications made in tandem significantly enhanced yield, peel diameter, and fruit count.

### **11. Improvement of crop quality by selenium biofortification**

Enhancing the essential nutritional content in edible portions of plants, animals, or microorganisms using agronomic or biotechnological processes is known as biofortification. To mitigate micronutrient deficiencies, agronomic biofortification is a simple, effective, and environmentally friendly method (Yuan et al., 2012). The best way to enrich crops with this element is one of the most important aspects of Se biofortification. Selenium can be sprayed on the foliage, added to mineral fertilizers, or mixed into the nutrient medium in hydroponic farming. (Hawrylak-Nowak, 2013; Banuelos et al., 2017; Golubkina et al., 2018). The efficiency, simplicity, and lack of multidirectional chemical changes of selenium in the soil, along with its limited translocation to aboveground organs, appear to be the benefits of applying selenium topically over soil application. There seems to be less of a risk of environmental pollution because the foliar spray requires a minimal consumption of Se salts (Puccinelli et al., 2017; Hawrylak-Nowak et al., 2018c).

The foliar application of Se has an advantage over the other methods, according to a recent study by Moteszarehadeh et al. (2020) on alfalfa grown in calcareous and non-calcareous

soils supplied with Se (Se soil application, selenobacteria inoculation, Se foliar application, combined soil and foliar Se application). Additionally, they proposed that a natural way of enhancing the quality of alfalfa feed could be by the inoculation of plants with selenobacteria. In a closed soilless system, Pannico et al. (2019) found that Se treatments (8–40  $\mu\text{M}$  as  $\text{SO}_4^{2-}$ ) decreased the production of green butterhead lettuce (FW), while yield reduction in the red cultivar was noted at  $\geq 32$   $\mu\text{M}$  Se. Significantly, both cultivars showed a rise in the foliar Se content; however, the red butterhead lettuce absorbed around 57% more Se than the green one. Further, the red cultivar's carotenoid content increased with 32  $\mu\text{M}$  Se, while the phenolic acid and anthocyanin contents increased with 16  $\mu\text{M}$  Se.

Selecting the appropriate type of selenium (Se) is essential for successful biofortification. The majority of research has shown that  $\text{SeO}_4^{2-}$  is a more effective form than  $\text{SeO}_3^{2-}$  for foliar and soil treatment. (Lyons, 2018). Wang et al. (2020) reported that the uptake of inorganic selenium from foliar spray was found to be more efficient than that of its organic forms. Additionally, the phloem was found to transport tiny amounts (less than 10%) of selenium deposited in the shoots to other organs. On the other hand, organic Se was absorbed by the roots at a significantly higher rate than inorganic Se. The most significant selenium uptake and translocation by the xylem and phloem was brought about by the application of selenium methyl cysteine. In four rice cultivars, Lidon et al. (2019) observed that foliar  $\text{SeO}_3^{2-}$  fertilization increased grain Se content by 427–884 times, whereas  $\text{SeO}_4^{2-}$  treatment increased grain Se concentrations by 128–347 times.

In addition to raising the concentration of Se in the rice grains, the foliar application of Se also enhanced the concentration of other bioactive compounds. Lidon et al. (2018) supplied various rice genotypes with 30–300  $\text{g Se ha}^{-1}$  as  $\text{SeO}_3^{2-}$  or  $\text{SeO}_4^{2-}$  in a previous agronomic biofortification study. Both Se forms induced an increase in total lipids (particularly oleic, linoleic, and palmitic acids), sugars, and proteins; however, the macronutrient content of the rice flour varied within the rice genotypes. Se malnutrition may be effectively decreased in Se-deficient

areas by raising the Se content of cereals (rice and wheat) using a process known as Se biofortification. The application of 10 g Se ha<sup>-1</sup> boosted the Se content of wheat grain in the UK by up to 10 times, according to research by Broadley et al. (2010). Another potential method of Se-biofortification is the use of arbuscular mycorrhizal fungi (AMF) (Golubkina et al., 2020).

**Table 5: Scalability of Selenium Biofortification for Smallholder Farmers:**

Factor	Impact on Scalability	References
<b>Soil Selenium Availability</b>	Selenium biofortification depends on the selenium content in soil. In regions with selenium-deficient soils, selenium fertilization is necessary for biofortification.	White & Broadley (2009), Bañuelos et al. (2015)
<b>Cost of Fertilizers and Inputs</b>	The cost of selenium fertilizers can be a significant barrier, especially for smallholder farmers. Cost-effective solutions or subsidies may be required.	Ali et al. (2016), Gupta et al. (2017)
<b>Crop Selection and Adaptation</b>	Certain crops (e.g., wheat, rice) accumulate selenium better than others. Biofortification efforts must focus on crops that are adapted to the local environment.	Tiwari et al. (2017), Ghasemi et al. (2020)
<b>Local Knowledge and Training</b>	Education and training programs for smallholder farmers are essential for effective selenium biofortification, improving both yield and health	Ríos et al. (2018), Tiwari et al. (2019)

	outcomes.	
<b>Market Demand and Education</b>	The success of biofortified crops also depends on consumer awareness and demand for selenium-enriched foods, which can drive the adoption of biofortification practices.	Meacham et al. (2017), Ortiz et al. (2020)
<b>Environmental and Policy Support</b>	Government policies, subsidies, and agricultural extension services play a crucial role in supporting selenium biofortification at a larger scale.	Bañuelos & Lin (2018), Ríos et al. (2020)

## 12. Potential Risks of Selenium Overuse and Regulatory Challenges

Selenium is an essential micronutrient for both plants and humans, but like many nutrients, its overuse can lead to toxicity and environmental issues. Overuse of selenium, especially in agricultural settings, may pose several risks to ecosystems, human health, and agricultural practices. Additionally, regulatory frameworks for selenium use in agriculture, especially for biofortification and fertilization, can be complex and require careful management.

### 12.1 Potential Risks of Selenium Overuse

#### 12.1.1 Toxicity to Plants:

- **Selenium toxicity in plants:** While selenium is necessary for plant growth at low levels, excessive selenium can be toxic, leading to reduced plant growth and yield. Plants that accumulate too much selenium may suffer from chlorosis, necrosis, and overall poor health. Selenium can also disrupt the uptake of other essential nutrients like sulfur and phosphorus. Toxicity symptoms include the inhibition of root and shoot growth, which ultimately leads to poor crop performance (Zhang et al., 2019; Ghasemi et al., 2020).

## 12.2 Environmental Pollution:

- **Soil and water contamination:** Excessive application of selenium fertilizers can lead to selenium accumulation in soils, potentially contaminating water sources. This can harm aquatic life, as selenium is highly toxic to fish and other organisms when concentrations exceed certain thresholds. Over-application can lead to selenium leaching into groundwater and nearby water bodies, affecting biodiversity and ecosystem health (Bañuelos et al., 2015).

## 12.3 Human Health Risks:

- **Selenium toxicity in humans:** Overconsumption of selenium through biofortified crops or dietary supplements can lead to selenosis, a condition caused by excessive selenium intake. Symptoms of selenosis include nausea, diarrhea, fatigue, and hair loss. In severe cases, selenium toxicity can lead to nerve damage or even death (Yang et al., 2005). While selenium deficiency is a concern in many regions, biofortification practices must be carefully managed to avoid the risk of overconsumption in populations that are already selenium-replete.

## 12.4 Disruption of Ecosystem Balance:

- **Bioaccumulation in food chains:** Selenium can bioaccumulate in food chains, particularly in aquatic ecosystems, where organisms like fish may accumulate selenium to toxic levels. This poses risks to wildlife and can affect species diversity in ecosystems that are already sensitive to heavy metal contamination (Ohlendorf, 2003).

## 12.5 Regulatory Challenges

### 12.5.1 Lack of Standardized Guidelines:

- **Absence of universal standards:** Selenium biofortification and its use in agriculture lack standardized guidelines across different regions. The recommended levels of selenium in soils and crops vary between countries, making it difficult to establish consistent regulatory frameworks for selenium use. The risk of over-

application arises when local agricultural practices do not align with scientifically established safe thresholds for selenium concentration in crops (Bañuelos & Lin, 2018).

### **12.5.2 Regulation of Fertilizer Use:**

- **Monitoring and regulation of selenium fertilizers:** While there are regulations on the application of fertilizers, specific standards for selenium use are less common. In many regions, selenium fertilizers are not regulated in the same way as other micronutrients or pesticides. This can lead to inconsistent use and possible over-fertilization, especially if farmers are unaware of the risks of selenium overuse. Regulatory authorities may face challenges in monitoring the correct application rates, especially in low-income regions where training and resources are limited (Bañuelos et al., 2015).

### **13. Challenges in Biofortification:**

- **Ensuring safe biofortification levels:** The regulatory challenge in biofortifying crops with selenium lies in ensuring that biofortified foods contain enough selenium to address nutritional deficiencies without reaching levels that could be harmful. Regulatory bodies need to define upper limits for selenium content in biofortified foods to avoid unintentional overconsumption in vulnerable populations (Petry et al., 2017). Balancing nutritional needs with safety is key to successful selenium biofortification programs.

#### **13.1 Lack of Awareness and Education:**

- **Education and awareness programs:** Farmers and consumers may not be fully aware of the risks of selenium overuse, both in the form of fertilizers and biofortified crops. Regulatory agencies must invest in awareness programs to educate farmers on safe selenium application methods and help them understand how to monitor and manage selenium levels in soils and crops. Similarly, consumers need to be informed about the benefits and potential risks of biofortified foods (Rios et al., 2018).

#### **14. Concluding remarks and future direction**

Application of selenium and microorganisms (selenorhizobacteria) pelleted seeds when applied in soil or the rhizosphere will stimulate natural processes to enhance nutrient uptake, productivity, abiotic stress tolerance and crop quality which can be considered as a safe, cost effective approach to achieve this target in selenium deficient areas. Utilizing selenium-tolerant bacteria (selenorhizobacteria) in biotechnological applications is a viable approach to selenium biofortification. Further investigation is necessary to identify the selenium forms found in grains as well as the genetic and biochemical pathways that underlie the biofortification of plants with selenium in order to develop innovative technologies for selenium biofortification initiatives. Therefore, management techniques should concentrate on building a link between agricultural enriched food products, selenium concentration, and bioavailability in soil in order to minimize selenium shortage and associated diet related disruptions by the plant growth boosting bacteria. The fundamental mechanisms of selenium uptake, distribution, and metabolism, as well as any favorable or detrimental effects on plant physiology and plant performance under abiotic stressors, have all been covered in this study.

Additionally, we proposed the biofortification topic. At low quantities, selenium has positive impacts on plant growth and development. Furthermore, it has been documented that both hyperaccumulator and non-hyperaccumulator plants benefit from growth effects associated with the ideal Se concentration. Whether Se is a necessary plant nutrient or not is still up for debate. For superior plant performance brought about by the administration of Se, the ideal dose of Se should be determined for each species of plant, as well as for each development stage, size, growth substrate, application method, form, and—above all—the concentration of Se in the tissue. Se can effectively increase tolerance to a variety of abiotic stressors, as demonstrated by a substantial body of experimental data, which calls for further, in-depth research. Furthermore, just a few crops

have been researched in this area, although Se has potential as a biofortification or phytofortification agent enhancing the nutritional quality of meals. The synthesis of non-specific S-proteins, pro-oxidative creation of ROS, and oxidative stress, which impede physiological functions, have been linked to selenium phytotoxicity. It boosts the creation of reactive oxygen species (ROS) because it is a pro-oxidant; nevertheless, it has been shown that a modest amount of selenium (Se) can upregulate the antioxidant defense system, which is an intriguing area of research. The results of the study showed that selenite resistance is regulated by ethylene and jasmonate acid signaling. Plant growth and development under both normal and stress conditions are thought to be influenced by the interplay between phytohormones and selenium (Se), which in turn regulates genes involved in Se production, uptake, and assimilation. (Tamaoki et al., 2008; Van Hoewyk et al., 2008; Freeman et al., 2010; Wang et al., 2018). The study should be expanded to take into account various growth environments, both in the lab and in the field, and to use a variety of grown plants as test plants. It is important to extensively utilize omics technologies, such as transcriptomics, proteomics, metabolomics, and genomics, in various plant species to determine the mechanism of selenium's presence as a beneficial or harmful component. Furthermore, the engineering of Se-mediated metabolic pathways can help identify the true mechanism behind Se-mediated stress tolerance and offer fresh perspectives on current understanding.

The future of selenium biofortification lies in the integration of cutting-edge technologies like **omics** (genomics, proteomics, metabolomics, etc.) and **CRISPR/Cas9 gene editing**. These technologies will not only enhance the efficiency and precision of biofortification but will also ensure that crops are tailored for both high selenium content and minimal toxicity risk. Additionally, continued research into the human health implications of biofortified foods and the environmental sustainability of selenium use will be essential for the successful scaling of selenium biofortification in agriculture.

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Option 1:

Author(s) : hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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