



## Advances in the Study of Genetic Enrichment of Selenium in Plants

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### Abstract

Selenium (Se) is a micronutrient essential for human and animal health. Due to the unbalanced distribution of Se resources in the world, Se deficiency is regarded as a major human health problem. Insufficient ingestion of Se in human caused a series of disease including Keshan disease and Kashin-Beck's disease. While some parts of the world was Se poisoning due to the Se ores on geological stratification or Se pollution. The using Se-enriched and/or hyper-accumulated green plants to treat these problems has acquired more and more attention worldwide from last decade. This paper describes current advances in the efforts of searching Se enriched plant genetic resources, clarifying the Se metabolism and accumulation mechanism in plants, and cloning key genes responsible for Se accumulation or hyper-accumulation and their transformation in plants.

**Keywords:** Plants; Selenium; Se enrichment genes; Cloning; Transgenic

### Introduction

Selenium (Se) was firstly found by Swedish scientist Jakob Berzelius in 1917. In the 1930s, it is generally accepted that Se is toxic or carcinogenic to human, while in 1957, Se was proved to be an important nutritional element of animal nutrition. In 1973, Se not only had been identified as the essential micronutrient of human life by the World Health Organization (WHO), but also called as "the kindling of life". In the same year, Rotruk et al. [1] illustrated that Se is an important part in the structure of antioxidant enzyme glutathione peroxidase (gsh-px) in animals. A large number of studies have shown that Se deficient was related to a number of diseases such as cancer, liver disease, diabetes, hypertension, cataract, age-related disease, anemia, reproductive system disease, etc [2].

Se distribution is scattered and scarce in nature, and more than forty countries and regions lack of Se sources in the world, including Zealand, Finland, Japan, etc. In China, about 700 million people live in 22 provinces that belong to the low Se areas. Thus it is significantly important to develop Se-enriched agricultural products for guaranteeing our human health.

Nevertheless, excess Se in the soil can cause abnormal endemic diseases for the human and animals. The most infamous cases of Se pollution was happened in Kesterson Reservoir in California of USA in the 1980s, which was highly Se contaminated, and resulted in a severe disaster to the inhabited wild life [3]. The major pollutant comes from agricultural sewage, in which excess Se can lead to the malformation or death of fish and birds. Most parts of China are short of Se, while some parts are rich with Se, such as Enshi county which lies in the southwestern of Anhui province, and Ziyang county which lies in Shanxi province. Enshi and Ziyang are the typical representation of Se-rich region in China.

To date, many ways for clearing and improving the environment of Se pollution were developed [4], among which the using se-enriched plants approach is highly fancied because of low cost, convenience, and environmental protection. This green technology is currently a branch of world trend of phytoremediation, which used the nature discovered Se accumulating or hyper-accumulating plants. However, currently the majority of known hyperaccumulators used to remedy the contaminated environment are usually slowly growing, small biomass, rosette leaves, and difficult for machine operation [5].

The rapid advances of modern molecular biology techniques make it become possible for obtaining the key genes for Se accumulation or hyper-accumulation in plants, and delivering them to target plants or crops to improve their capacity of Se enrichment. Here we described the current advances made in the studies of Se accumulating/hyper-accumulating plant resources, the mechanism of Se metabolism in plants, and the key genes responsible for Se enrichment in plants and their transgenic effects, in order to promote the application of this green technology for cleaning Se contaminated soil and water, and also for Se fortification to improve or guarantee our human health.

### Selenium Deficiency and Toxicity

Selenium (Se) is a metalloid that occurs naturally. It has gained attention for its role as both an essential element and a toxicant to humans and animals. The list of clinical disorders (aging, hypothyroidism, Keshan and Kaschinbeck disease, male fertility, pre-eclampsia, rheumatoid arthritis, cardiovascular disorders, loss of immune functions, etc.) expected to be influenced by Se deficiency is rapidly growing with time [6-12]. All living organism possess antioxidant defense system to combat oxidative stress. By means of its oxidative and reduction properties, Se is involved in the maintenance of the cell redox homeostasis. The effects of Se deficiency can include reduced T-cell counts, impaired lymphocyte proliferation and responsiveness. The deficiency symptoms vary from species to species, as is to be expected. In many warm-blooded animals, Se deficiency may be evidences by such symptoms as i) muscular dystrophy and pain, ii) inflammation of the muscles, iii) fragile red blood cells, iv) necrotic liver degeneration, v) hair or skin abnormal coloration and vi) exudative diathesis etc. [8,13]. There is one disease that is consistent

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in all livestock species, and that is Nutritional Muscular Dystrophy (NMD) or White Muscle Disease (WMD) which is caused by the deficiency of Se and/ or vitamin-E and S-containing amino acids [14]. However, the scientific information about selenium and neurological disorders still much that is not known.

Selenium is a trace element that is essential for living organisms. It has three level of biological activity i) trace concentration are required for normal growth and development; ii) moderate concentrations can be stored and homeostatic functions maintained, iii) elevated concentrations can results in toxic effects. Even though, Se toxicity is a controversial topic, with numerous researches done on the subject. However, the high and low levels of this trace element in the living organism can be harmful, as it can lead to side effects of selenium, most prominent being toxicity. Acute human intoxication is rare and it is almost invariably fatal, manifested by stupor, hypotension and respiratory depression. A couple of studies have examined the chronic selenium poisoning, reported in some areas in china, produces selenosis in human and induces changes following symptoms: hair and nail loss, skin lesions, fatigue, liver and kidney damage, nausea, vomiting and abnormal blood clotting. However, the amount of Se in the soil is not equal in geographic area (China, USA, Canada, New Zealand) with high content of Se [8,12]. In most cases, the animals in the grazing on plants that have accumulated selenium show acute or chronic selenium poisoning. Chronic selenium toxicity (alkaline disease) is characterized by muscle degeneration, rough coat, labored breathing and cardiovascular failure. Acute selenium toxicity (blind staggers) manifests as weight loss, anorexia, excessive salivation, jaundice or necrosis of the heart and liver. On the other hands, the toxic amounts of Se can also cause birth defects in offspring from dams fed such levels [11,15-18]. There is no known treatment to reverse the effects of the poisoning, and oftentimes the animal dies before a diagnosis can be made.

### Plant Genetic Source for Se Accumulating/Hyperaccumulating

In the 1930s, Beath [19] found that the plant *Astragalus* can hyper-accumulate more than 1000 mg/kg (dry weight, DW) selenium during the investigation on the reason of livestock poisoning in the great plains of the west of America and the rocky mountain. Further investigation of more than a dozen plants of *Astragalus* and some other plants such as *Stanleya apinnata*, they found that these plants can accumulate a high content of selenium ranging from 30 to 3000 mg/kg [19-22]. By the end of the 20th century, dozens of se-enriched species have been successively found from families including *Brassicaceae*,

*Chenopodiaceae*, *Asteraceae*, *Lecythidaceae*, *Rubiaceae*, *Fabaceae*, and *Scrophulariaceae* [23-25]. Some of the plants, such as *Stanleya* (*Brassicaceae*) and *Astragalus* (*Fabaceae*) can hyper-accumulate Se to concentrations of 1,000 to 15,000 mg/kg (DW) in their shoots (0.1%-1.5%) while growing on soils containing only 2 to 10 mg Se (DW) [26-30]. Table 1 listed some of the popular Se-rich species found in nature and their maximum Se content in plant tissues. These provide substantial opportunity for further clarifying the Se tolerance and accumulation or hyper-accumulation mechanism in plants.

Plants take up Se mainly in the form of selenate, selenite or organic Se. The roots and leaves of plants have the ability of Se absorbing. The main forms of Se absorbing are  $Se^{6+}$  (as selenate,  $SeO_4^{2-}$ ) and  $Se^{4+}$  (selenite,  $SeO_3^{2-}$ ) valence states, however, due to selenite is much more easy to be absorbed onto clays and metal oxides than selenate under the condition of high soil pH and salinity, leaving selenate as the major form available for plant uptake. To be absorbed,  $Se^{6+}$  requires energy, while absorption of  $Se^{4+}$  is an initiative process [31]. The capacity of Se absorbing is largely different in plants, which are often divided into Se hyper-accumulated plants ( $\geq 1000 \mu g/g$ ), Se-enriched plants (50-100  $\mu g/g$ ) and non-Se accumulated plants ( $\leq 50 \mu g/g$ ) [32,33].

Most plants do not have the ability of Se enrichment, and their Se concentrations are low ( $\leq 100 \mu g/g$  DW). While some plants can enrich Se as high as over 1000  $\mu g/g$  DW, even if they were grown in Se-poor soil. These plants are called Se-super-accumulating plants [32,33].

Cruciferae rapeseed has the strongest Se accumulation capacity, followed by Leguminosae, and cereal is the lowest. Wheat has the maximum accumulation of Se in cereals. The Se content in dry weight (g/g) is sorted as tubers and bulbs crops>field crops>leafy crops>crop seeds and aquatic>vegetables and fruit crops>fruit crops. The field crops ranked for their accumulation of Se ability as cruciferae>rye grass>beans>cereal [34]. In traditional Chinese medicine, the *Astragalus membranaceus* enriched more Se than other plants.

To date, the representative of Se hyper-accumulating plants discovered as Table 1 listed are *A. bisulcatus* (Mainly grows in the southwestern United States) [35], *Cardamine sp* [36] (*Cardamine L.*), *Thlaspiarvensis L.* [37] and *Stanleya pinnata* (*Cruciferae*) [38], etc. However, the overwhelming majority is *Astragalus*, and the Se-rich genes have been cloned mostly from this sort of plants.

### Mechanism of Se Metabolism in Plants

In the 1960s, the study of Se metabolism was compared with sulfur because of the properties of Se and sulfur were similar. Se glutathione peroxidase was found for the first time in 1970s, which laid a foundation

Types of Se-rich plants	Species and references	Origin	The maximum Se content ( mg/ kg)
Se hyperaccumulated plants	<i>Astragalus Bisulcatus</i> [35,70]	Southwestern United States; Western of Hubei Province, China	6000 (leaf)
	<i>Thlaspi arvense L.</i> [36]	China	1427 (leaf)
	<i>Stanleya apinnata</i> [62]	Western United States	1130 (bud)
Se-enriched plants	<i>Festucaarun dinacea</i> [71]	USA, the Middle East, Europe etc.	883 (bud)
	<i>Brassicao leracea</i> [72]	Western Europe	366~550 (bud)
	<i>Brassica juncea</i> [73]	China, USA, India etc.	366~550 (bud)
	<i>Oryzasativa</i> [71]	Tropic( Asia)	366~550 (bud)
	<i>Lesquerella fendleri</i> [74]	Western Europe	503 (leaf)
	<i>Brassi canapus</i> [71]	Europe, Mediterranean etc.	288~470 (bud)
	<i>Atriplexnut tallii</i> [75]	Western United States	300
	<i>Betavulgaris</i> [71]	Europe	405 (bud)
	<i>Cardamine L.</i> [35]	Temperate zone	200

Table 1: Some popular Se-rich plants and the maximum Se contents.

for the biochemistry of Se. In 1980s, some research results showed that only one-third of Se have Se glutathione peroxidase activity in the body.

In recent years, with the continuing efforts made by scientists, the key pathways of plant Se metabolism become more and more clear, we summarized them in Figure 1 [33,39-41]. Owing to its similarity, Se can make use of S transporters and metabolic pathways. Selenate could be absorbed into the plant chloroplast, then it was activated by ATP Sulfurylase and reduced to selenite by further reduction, and assimilated into the selenoamino acids, selenocysteine (SeCys), and selenomethionine (SeMet), which produce SeMet and Met in final. Non-specific incorporation of these selenoamino acids into proteins in place of Met and Cys is toxic [42]. Methylation of SeCys (in cytosol) and SeMet (in chloroplast) leads to accumulation of the non-protein amino acids methyl-SeCys (MetSeCys) and methyl SeMet (MetSeMet). MetSeCys or MetSeMet can be further metabolized to volatile dimethylselenide (DMSe, in non-hyperaccumulators) or dimethyldiselenide (DMDSe, in hyperaccumulators) [43,44]. SeCys may be broken down into alanine and elemental Se [45,46], and some new Se containing proteins were also found [47].

A variety of Se proteins are found in the plant. Selenomethionine are the major form of Se in Se non-accumulation plants [48], which presents at least 50% in wheat, 70% in alfalfa. Se is also an intermediate for sulfur-containing amino acid metabolism when detoxification products exist, such as SeCyst and MeSeCys [49].

### The Choice of Receptor Plants

*Arabidopsis thaliana* and tobacco are usually selected for the receptor plant in the study of gene function. It is noteworthy that *alfalfa* is chosen as receptor plant materials in many research papers

for phytoremediation or as fodder. The high Se containing *Medicago sativa* (Se content 8~10 times more than ordinary hay) was fed to cows, which obviously increased the cow body length, chest circumference and body quality. In the same time, the day milk yield of *Medicago sativa* fed cows increased by 8.6%, which was 12.17% lower than the material milk, and the cows' milk fat content was increased by 22.15% [50]. *Medicago sativa* was also proved to be an ideal plant to be used in the bioremediation of Se contaminated soil, due to its wide adaptability, fast growing and large dry biomass [51,52]. Indian mustard is another model plant used for phytoremediation technology, because of its high enrichment ability for dozens of heavy metals and fast growth rate. Many years of research results showed that Indian mustard has evolved special molecular, physiological mechanisms and structure features to adapt to the heavy metal enriched environment and accumulated poisonous heavy metal ions in the body. Table 2 listed the key genes for Se accumulation cloned from plants and their transformation and the practical application, which will be discussed in detail in the next section.

### Main Cloned Genes for Se Hyper-Accumulating and their Transformation in Plants

#### Selenocysteine Methyltransferase (SMT)

SMT has played an important role in metabolic processes. It can efficiently transform a majority of SeCys to non-poisonous of MetSeCys through methylation and reduce the intracellular concentration of SeCys and SeMet [53]. This process greatly reduces the chances of misincorporation of SeCys and SeMet into protein which significantly increased Se tolerance in plants [54].

In 2004, Ellis et al. [55] successfully inserted *SMT1* into *Arabidopsis*

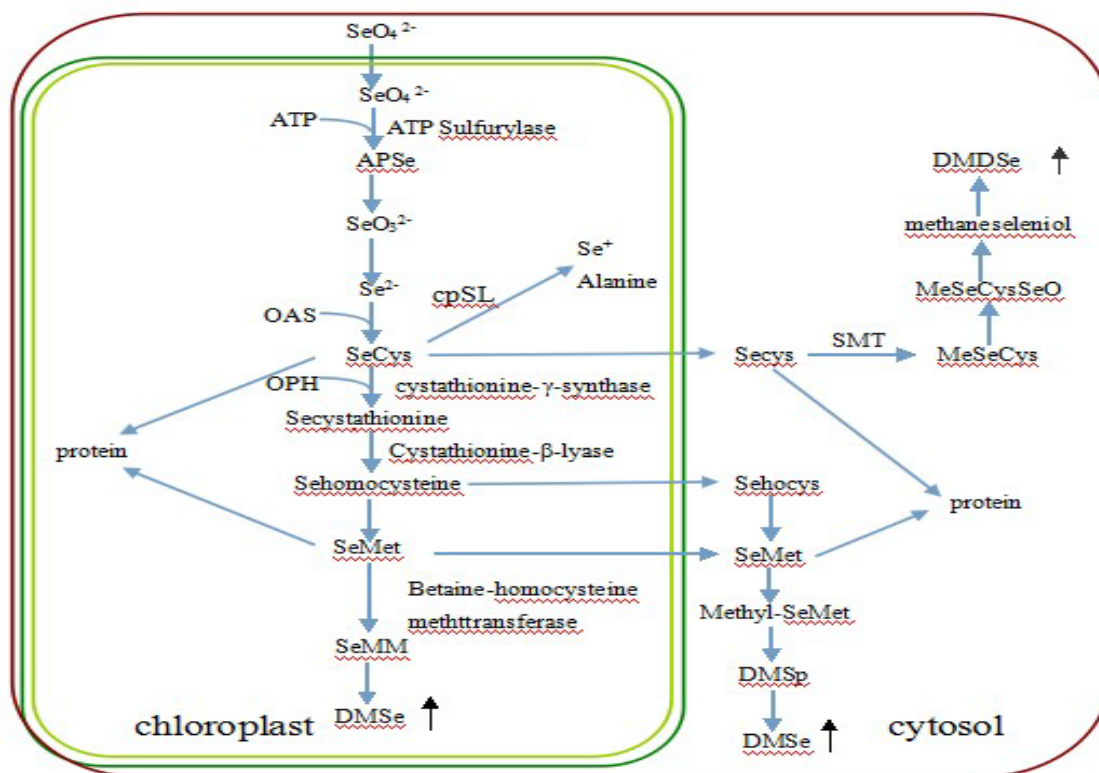


Figure 1: Selenium metabolic fate in plants.

Gene transferred	Origin	Target transgenic plant species	References
<i>AtCpNifS</i>	<i>A. thaliana</i>	<i>A. thaliana</i>	[58,76]
<i>APS</i>	<i>A. thaliana</i> and <i>Camellia sinensis</i> Qimen-chuyezhong	<i>B. juncea</i> and <i>Camellia sinensis</i>	[39,64,65,66]
<i>CGS</i>	<i>A. thaliana</i>	<i>B. juncea</i>	[60]
<i>ESC</i>	<i>A. thaliana</i>	<i>B. juncea</i>	[39,64]
<i>GS</i>	<i>A. thaliana</i>	<i>B. juncea</i>	[39,53]
<i>SMT</i>	<i>B. juncea</i> and <i>A. bisulcatus</i>	<i>B. juncea</i> <i>A. thaliana</i> Tobacco <i>Medicago sativa</i> L. and tomato	[54,55,56,57,77]

**Table 2:** Major Se-enrichment genes that have been reported.

*thaliana* genome by pollen dropper for the first time and found that Se content in the transgenic plants was nearly 8 times higher than the controls. The *SMT1* plants can synthesize MeSeCys while wild *Arabidopsis thaliana* plants cannot [55].

LeDuc et al. [54] cloned *SMT* from Indian mustard and *Astragalus bisulcatus* in 2006 and transformed it into *Brassica juncea*. The transgenic plants successfully improved the tolerance and accumulation of selenite. But similar results did not produce for supplying selenate. After transforming the *APS* and *SMT* genes at the same time, the transgenic plants greatly improved the Se accumulation ability when supplying selenate.

Yao et al. [56] inserted *SMT* into the tobacco genome in 2009, and the over-expression of *SMT* significantly improved the transgenic tobacco's tolerance of selenate. In 2011, Brummel et al. [57] developed *SMT* transgenic tomatoes. Selenate or selenite was added into the medium during the period of fruit development, as a result, the content of MeSeCys increased by 16% of the total Se content in the transgenic tomato fruits.

In 2012, Lijing et al. [58] successfully inserted *SMT* into the genome of *Medicago sativa* by Agrobacterium-mediated transformation method. However, they have not yet got the performance data of the transgenic *Medicago sativa*.

### *AtCpNifS*

*AtCpNifS* was found as a kind of chloroplast protein in *Arabidopsis thaliana*, and it was the first characterized *NifS*-like protein from higher plants. *AtCpNifS* can convert SeCys into Se and alanine by Selenocysteine lyase. Also, it contains activity of cysteine desulfurization enzyme and selenocysteine lyase, especially for its activity toward SeCys, which was almost 300 times higher than its activity toward cystine [45].

In 2005, Van et al. transformed *AtCpNifS* into *Arabidopsis thaliana* [59]. The transgenic *Arabidopsis* had significantly improved ability of Se-resistant and Se accumulation, in which the root growth is 1.9 times higher than the controls, the amount of Se accumulation increased to 2~3 times higher than the controls, and the Se incorporated proteins were also significantly reduced at the same time. The plant accumulation of sulfur was increased by about 30%.

### Cystathionine- $\gamma$ -synthase (CGS)

Cystathionine- $\gamma$ -synthase is the key enzyme for methionine synthesis. It competes with threonine synthase for the same substrate O-phospho-L-homoserin, and catalyzes it to form cystathionine. Cystathionine was catalyzed by cystathionine- $\beta$ -lyase. Methionine synthase and S-adenosylmethionine synthetase form Methionine [60].

In 2003, Van Huysen et al. [61] transformed *CGS* into *Brassica juncea*. They found that the ability of selenite tolerance and enrichment in transgenic plants have been obviously improved in comparison with

that of wild plants. The Se volatilization rate of *CGS* transgenic *Brassica juncea* was 2-3 times higher than that the wild type in the condition of Se or selenate stress.

### ATP Sulfurylase (APS)

ATP Sulfurylase (APS) is the first enzyme catalyzing sulfate activation in the sulfur assimilation pathway in plants, which catalyzes sulfate and forms phosphorus acid adenosine, the further reduction reaction can form sulfide such as cysteine, etc. [62] Se metabolizes in higher plants through the sulfur metabolism pathways and synthesizes organic Se in the final [63,64].

ATP Sulfurylase is a rate-limiting enzyme in Se metabolic pathways. The limited function of APS was restricted in some early studies, in which *APS* was overexpressed in *Brassica juncea*, resulted in the rapid transformation of selenate into selenite and forms organic Se. The ability of Se tolerance and accumulation in transgenic plants has been significantly improved by *APS* overexpression compared with untransformed controls [53].

In 1999 LeDuc et al. and Zhu et al. [39,65] transformed *APS* and *ESC* (Glutamylcysteine synthetase) into *Brassica juncea*, respectively. As a result, the accumulation of Se in genetically modified *Brassica juncea* was 4.3 and 2.8 times higher than that of wild type.

Tea plant (*Camellia sinensis*) has unique biological features for the study of Se metabolism. Using qRT-PCR technology, Tao et al. [66] checked the expression levels of *APS1*, *APS2* and *SMT* in the different tissues of tea plant, they found that the expression of these genes in the roots of selenium-enriched tea plants were 1.6, 4.8 and 3.3 times higher than that in ordinary tea trees respectively. Their results confirmed the correlation between selenium assimilation and expression levels of these genes. In 2013, Wang et al. [67] cloned *APS* from *Camellia sinensis* and analyzed its promoter structure characters.

### Glutathione Synthetases (GS)

Glutathione is an important antioxidant in plants and it is a tripeptide distributed in living biological cells widely. The activity of its hydrosulphonyl can react with free radicals to protect the body through converting to the oxidized glutathione. Glutathione has certain treatment effects on liver disease, cancer and even AIDS [68].

Lyi et al. transformed *GS* into *Brassica juncea* [39,64]. Their results showed that Se accumulation in GM *Brassica juncea* was 2.3 times higher than that in the wild type plants.

In 2005, Bañuelos et al. [69] tested selenium-enrichment ability of three transgenic Indian mustard lines under field conditions. The *APS*, *ECS*, and *GS* transgenic plants accumulated 4.3, 2.8, and 2.3 times higher Se in leaves than the wild type plants respectively. The *GS* plants grew better on contaminated soil than the wild type, they grew

to almost 80% of their own that on clean soil, while the wild type only grew to 51% of their own on clean soil.

## Conclusion and Prospect

Enormous progress has been made in recent decades, reflecting advances in molecular biology, our knowledge about the mechanism of Se metabolism and accumulation in plants has been largely improved.

Currently, in the aspect of Se-enriched gene cloning, a majority of these genes were cloned from Se-rich plants in legumes and crucifer, and at least 25 Se proteins were also found and separated in mammals, however, the exact function of many of the proteins has not yet been established. In the aspect of absorption mechanism of Se, plants absorb selenate by sulfur transporter, but the mechanism of selenite absorption in plants is not very clear. Because of a lack of evidence, plants were thought to absorb selenite by passive way for a long time. A very newly study found that selenite is absorbed by phosphate transporter in rice. In this study, researchers identified a phosphate transporter which has strong transport activity for selenite. This result not only greatly enriched and perfected the theory for plants absorbing selenite, but also provided a very effective way to produce Se-rich rice and even Se-enriched products. So far, dozens of Se-rich plants were found in nature, however the practical using potential of these plants is limited because of their slowly growing, small biomass and the limited living environment. Now according to the actual application the transgenic receptor group has been purposefully expanded, making the transgenic Se-rich plants a wider application.

Furthermore, Se-rich plants have very good application value in human health and environmental security. On the one hand, the plants can act as a "Se release system" returned to the soil in Se deficiency by the cultivation techniques or in the form of food additive, etc. to supply more Se to people and animals living in Se deficiency regions. On the other hand, these plants can be used for Se cleaning by absorbing Se from the Se polluted soil or water to make the environment more healthy. However, the exploitation of the genetic material of these specialized plants also offers us the opportunity to enhance both the nutritional properties of crop plants and engineer plants. Although progress is being made in understanding the genetic basis of Se-hyper-accumulation, a more complete understanding will be necessary before we can take full advantage of the genetic potential of these plants.

Therefore, with the more Se concentrating genes to be identified and separated, we will know more about the mechanism of Se-enriching in plants, thus will greatly promote the development and utilization of the Se-rich plant resources for the service of a better human health and clean environment.

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