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, Rotru 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 Shangxi 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 Kashinbeck disease, male fertility, pre-cleampsia, 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
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 levels 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 result 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 pinnata*, 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 Se⁶⁺ (as selenate, SeO₄²⁻) and Se⁴⁺ (selenite, SeO₃⁻) 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, Se⁶⁺ requires energy, while absorption of Se⁴⁺ is an initiative process [31]. The capacity of Se absorbing is largely different in plants, which are often divided into Se hyper-accumulated plants (≥ 1000 μg/g), Se-enriched plants (50-100 μg/g) and non-Se accumulated plants (≤ 50 μg/g) [32,33].

Most plants do not have the ability of Se enrichment, and their Se concentrations are low (≤ 100 μg/g DW). While some plants can enrich Se as high as over 1000 μg/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.), *Thlaspi arvense* 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 | *Astragalus Bisulcatus* [55,70] | Southwestern United States; Western of Hubei Province, China | 6000 (leaf) |
| | *Thlaspi arvense* L. [36] | China | 1427 (leaf) |
| | *Stanleya pinnata* [62] | Western United States | 1130 (bud) |
| Se-enriched plants | *Festucaarundinacea* [71] | USA, the Middle East, Europe etc. | 883 (bud) |
| | *Brassica rapa* [72] | Western Europe | 366-550 (bud) |
| | *Brassica juncea* [73] | China, USA, India etc. | 366-550 (bud) |
| | *Oryziasativa* [71] | Tropic (Asia) | 366-550 (bud) |
| | *Lesquerella fendleri* [74] | Western Europe | 503 (leaf) |
| | *Brassica canadensis* [71] | Europe, Mediterranean etc. | 288-470 (bud) |
| | *Atriplexnut tallii* [75] | Western United States | 300 |
| | *Beta vulgaris* [71] | Europe | 405 (bud) |
| | *Cardamine L.* [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 (MeSeCys) and methyl SeMet (MeSeMet). MeSeCys or MeSeMet 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 Methytransferase (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.
accumulation of sulfur was increased by about 30%.
proteins were also significantly reduced at the same time. The plant higher than the controls, the amount of Se accumulation increased [59]. The transgenic Arabidopsis almost 300 times higher than its activity toward cystine [45].

Cystathionine-γ-synthase (CGS) 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-β-lyase. Methionine synthase and S-adenosylmethionine synthetase form Methionine [60].

In 2005, Van et al. transformed AtCpNiF 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%.

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In 2003, Van Huyse 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 of the wild type in the condition of Se or selenate stress.

A TP 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].

A TP 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 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 usage 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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**References**

1. Rottruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, et al. (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179: 588-590.
2. Ellis DR, Salt DE (2003) Plants, selenium and human health. Curr Opin Plant Biol 6: 273-279.
3. Skorupa JP (1998) Selenium poisoning of fish and wildlife in nature: Lessons from twelve real-world experiences. Environmental chemistry of selenium, Marce Dekker, New York.
4. Salt DE, Blaylock M, Kumar NP, Dushenko V, Ensley BD, et al. (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology (N Y) 13: 468-474.
5. Ebbs SD, Kochian LV (1997) Toxicity of zinc to Brassica species: Implication for phytoremediation. J Environ Qual 26:776-781.
6. Hamilton SJ (2004) Review of selenium toxicity in the aquatic food chain. Sci Total Environ 326: 1-31.
7. Schweizer U, Schomburg L, Savaskan NE (2004) The neurobiology of selenium: lessons from transgenic mice. J Nutr 134: 707-710.
8. Riza M, Mehmood KT (2011) Selenium in human health and disease: a review. JPMI 26: 120-133.
9. Obićadaloj I (2012) Biological effects of selenium compounds with a particular attention to the ontogenetic development. Physiol Res 61 Suppl 1: S19-34.
10. Sonianair (2010) Selenium Deficiency Symptoms. Selenium in medicine and treatment. J Elem s 145-163.
11. Fraczek A, Pasternak K (2013) Selenium in medicine and treatment. J Elem s 145-163.
12. Ellis DR, Salt DE (2003) Plants, selenium and human health. Curr Opin Plant Biol 6: 273-279.
13. Lakin UW (1972) Geochemistry of selenium in relations to agriculture selenium consist of soils. Agriculture Handbook 200: 3-34.
14. Barbezat GO, Casey OC, Reabeck PG, Robinson MF, Thomson CD (1984) Selenium in: Current Topics in nutrition. Alan R. Liss Inc, New York, USA.
15. Frankie KW (1934) A new toxicant occurring naturally in certain samples of plant food stuffs. Result Obtained in preliminary feeding trials. J Nutr 6:597-608.
16. Hamilton SJ (2004) Review of selenium toxicity in the aquatic food chain. Sci Total Environ 326: 1-31.
17. Mézès M, Balogh K (2009) Proxidant mechanisms of selenium toxicity-a review. Acta Biologica Szegediensis 15-18.
18. Bodnar M, Konieczka P, Namiesnik J (2012) The properties, functions, and use of selenium compounds in living organisms. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 30: 229-252.
19. Beath OA (1937) The occurrence of selenium and seleniferous vegetation in Wyoming?Seleniumiferous vegetation.Wyoming Agric Exp Sta Bull 21: 29-64.
20. Beath OA, Gillett CS, Eppson HF (1939) The use of indicator plants on locating seleniferous area sin western United States. General American Journal of Botany 26: 257-269.
21. Beath OA, Gillett CS, Eppson HF (1939) The use of indicator plants on locating seleniferous areas in western United States. General American Journal of Botany 26: 296-315.
22. Beath OA, Gillett CS, Eppson HF (1940) The use of indicator plants on locating seleniferous areas in western United States. General American Journal of Botany 27: 564-573.
23. Beath OA, Draize JH, Eppson HF, Gilbert CS, McCreary OC (1934) Certain poisonous plants of Wyoming activated by selenium and their association with respect to soil types. Journal of the American Pharmaceutical Society 23: 94.
24. Cannon HL (1960) Botanical Prospecting for Ore Deposits. Science 132: 591-598.
25. Reeves RD, Baker AJM (2000) Metal-accumulating plants. Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, USA.
26. Byers HG (1935) Selenium occurrence in certain soils in the United States, with a discussion of related topics. US Dept Agric Tech Bull 482:1-47.
27. Virupaksha TK, Shiff A (1965) Biochemical differences between selenium accumulator and non-accumulator Astragalus species. Biochim Biophys Acta 107: 69-80.
28. Davis AM (1972) Selenium accumulation in Astragalus species. Agron J 6: 751-754.
29. Davis AM (1986) Selenium accumulation in Astragalus and Lupinus species. Agron J 78:727-729.
30. Galeas ML, Zhang LH, Freeman JL, Wegner M, Pillon-Smits EA (2007)
Seasonal fluctuations of selenium and sulfur accumulation in selenium hyperaccumulators and related nonaccumulators. New Phytol 173: 517-525.

31. Smith FW, Hawkesford MJ, Ealing PM, Clarkson DT, Vanden Berg PJ, et al. (1997) Regulation of expression of a cDNA from barley roots encoding a high affinity sulphate transporter. Plant J 12: 875-884.

32. Brown TA, Shrift A (1982) Selenium: toxicity and tolerance in higher plants. Biol Res 57:59-84.

33. Plon-Smiths EA, LeDuc DL (2009) Phyto Remediation of selenium using transgenic crops. Curr Opin Biotechnol 20: 207-212.

34. Zhao Chunmei, Cao Qimin, Tang Qunfeng, Li Xiaobo (2010) Research Advances on Selenium Accumulation in Plants. Chinese journal of tropical agriculture 30:82-86.

35. Pickering U, Prince RC, Salt DE, George GN (2000) Quantitative, chemically specific imaging of selenium transformation in plants. Proc Natl Acad Sci U S A 97: 10717-10722.

36. Xiang Tian yong (2006) Studies on Biological Characteristics of Cardamine Ensinensis and Selenium-containing Compounds in its Leaves. Changsha: Agricultural University Of Hunan.

37. Shao Shuxun, Zheng Baoshan, Su Hongcan, Luo Chong, Li Xiaoyan (2007) A new species of selenium hyperaccumulator identified in yutangba se deposit area. Acta Mineralogica Sinica 27: 566-570.

38. Freeman JL, Tamaoki M, Stushnoff C, Quinn CF, Cappa JJ, et al. (2010) Molecular mechanisms of selenium tolerance and hyperaccumulation in Stanleya pinnata. Plant Physiol 153: 1630-1632.

39. LeDuc DL, AbdelSamie M, Montes-Bayon M, Wu CP, et al. (2004) Over expression of selenocysteine methyltransferase in Arabidopsis and Indian mustard: increases selenium tolerance and accumulation. Plant Physiol 135:377-383.

40. Plon-Smiths EA, Hwang S, Mel Lytle C, Zhu Y, Tai JC, et al. (1999) Overexpression of AT sulphurase in Indian mustard leads to increased selenate uptake, reduction, and tolerance Plant Physiol 119: 123-132.

41. de Souza MP, Pickering U, Wally M, Terry N (2002) Selenium assimilation and volatilization from selenocyanate-treated Indian mustard and mugskrass. Plant Physiol 128: 625-633.

42. Brown TA, Shrift A (1981) Exclusion of selenium from proteins of selenium-tolerant astragalus species. Plant Physiol 67: 1051-1053.

43. Anderson JW (1993) Selenium interactions in sulfur metabolism. In Sulfur nutrition and assimilation in higher plants-Regulatory, agricultural and environmental aspects. SPB Academic Publishing 49-60.

44. Lewis B, Johnson C, Delwiche C (1966) Release of volatile selenium compounds by plants: collection procedures and preliminary observations. J Agric Food Chem 14:638-644.

45. Plon-Smiths EA, LeDuc DL (2009) Phyto Remediation of selenium using transgenic crops. Curr Opin Biotechnol 20: 207-212.

46. Plon-Smiths EA, Garfullina GF, Abdel-Ghany S, Kato S, Mihara H, et al. (2002) Characterization of a NIS-like chloroplast protein from Arabidopsis. Implications for its role in sulfur and selenium metabolism. Plant Physiol 130: 1309-1318.

47. Novoselov SV, Rao M, Onoshko NV, Zhi H, Kryukov GV, et al. (2002) Selenoproteins and selenocysteine insertion system in the model plant cell system, Chlamydomonas reinhardtii. EMBO J 21: 3681-3693.

48. Toyoda H, Himeno S, Imura N (1990) Regulation of glutathione peroxidase mRNA level by dietary selenium manipulation. Biochim Biophys Acta 1049: 213-215.

49. Hawkes WC, Wilhelmsen EC, Tappel AL (1985) Abundance and tissue distribution of selenocysteine-containing proteins in the rat. J Inorg Biochem 23: 77-92.

50. Guo Xiao, Je Xiaolei, Li Ming, Liu Shiliang, Hua Dangling (2008) Analysis on the Effect of Alfalfa Hay with Different High Concentration Trace Mineral on Holstein. China Cattle Science 5: 54-57.

51. Cao Hong, Zhang Huiling, Ma Yongxiang, Chen Hong, Fang Gang, et al. (2009) A regional test of alfalfa varieties in the Long dong area. Acta Prataculturae Sinica 8:184-191.

52. Xia SuYin, Yan XueBing, Wang ChengZhang, Li HaiYan (2010) The application of alfalfa bioactive phytochemicals to animal husbandry. Pratacultural Science 27:133-140.

53. Li Yingsheng, Li Yanan, Chen Daqing (2003) Biological Functions of Selenium and the Mechanism of Selenium Enrichment in Plant. Journal of Hubei Agricultural College 23: 476-480.

54. LeDuc DL, Abdel Samie M, Montes-Bayon M, Wu CP, Reisinger SJ, et al. (2006) Over expressing both AT sulphurase and selenocysteine methyltransferase enhances selenium phyto remediation traits in Indian mustard. Environmental Pollution 144: 70-76.

55. Ellis DR, Tins TG, Brunk DG, Albrecht C, Orser C, et al. (2004) Production of Se-methylselenocysteine in transgenic plants expressing selenocysteine methyltransferase. BMC Plant Biol 4: 1.

56. Yao Xin, Chen Daqing, Xiao Chun, Li Yanan (2009) Overexpression of Selenocysteine Methyltransferase Gene on Physiological Effect of Selenium Stress in Tobacco. Hubei Agricultural Sciences 48: 1551-1553.

57. Brumwell DA, Watson LM, Pathirana R, Joyce NI, West PJ, et al. (2011) Biofortification of tomato (Solanum lycopersicum) fruit with the anticancer compound methylselenocysteine using a selenium methyltransferase from a selenium hyperaccumulator. Journal of Agricultural and Food Chemistry 59: 10987-10994.

58. Li Jing, Ren Weibo, Guo Huiguo, Wang Maoyan, Liu Yaxue (2012) Transformation of alfalfa with smt1 gene mediated by agrobacterium. Pratacultural Science 1224-1228.

59. Van Hoevck D, Garfullina GF, Ackley AR, Abdel-Ghany SE, Marcus MA, et al. (2005) Overexpression of ATCSNIFS enhances selenium tolerance and accumulation in Arabidopsis. Plant Physiol 139: 1518-1528.

60. Wang Yiping, Yu Yang, Sun Shi, Xu Yanli, Hou Wensheng (2011) Preparation of Polyclonal Antibody against D-ACTGS and Detection of D-ACTGS in Transgenic Soybean Plants. Soybean science 30: 537-540.

61. Van Huysen T, Abdel-Ghany S, Hale KL, LeDuc DL, Terry N, et al. (2003) Overexpression of cysteinhione-gamma-synthase enhances selenium volatilization in Brassica juncea. Plantas 218: 71-78.

62. Pickering U, Wright C, Bubner B, Ellis D, Persans MW, et al. (2003) Chemical form and distribution of selenium and sulfur in the selenium hyperaccumulator Astragalus bisulcatus. Plant Physiol 131: 1460-1467.

63. Parker DR, Feit LJ, Varvel WT, Thomas on ND, Zhang YQ (2003) Selenium phyto remediation potential of Stanleya pinnata. Plant and Soil 249: 157-165.

64. Lyi SM, Heller Li, Rutzie M, Welch RM, Kochian LV, et al. (2005) Molecular and biochemical characterization of the Selenocysteine Se-Methyltransferase gene and Se-Methylselenocysteine synthesis in broccoli. Plant Physiology 138: 409-420.

65. Zhu YL, Plon-Smiths EA, Tarun AS, Weber SU, Jouanin L, et al. (1999) Cadmium tolerance and accumulation in Indian mustard is enhanced by overexpressing gamma-glutamylcysteine synthetase. Plant Physiol 121: 1169-1178.

66. Tao Shaoqiang, Li Juan, Gu Xungang, Wang Yanan, Xiang Q, et al. (2012) Quantitative Analysis of ATP Sulfurylase and Selenocysteine Methyltransferase Gene Expression in Different Organs of Tea Plant(Camellia sinensis). American Journal of Plant Sciences 1: 51-59.

67. Wang Yanan, Zhu Lin, Ye Aihu, Tao Shaoqiang, Qin Bing (2013) The Gene Cloning of ATP Sulfurylase of Tea Plant, the Key Enzyme Related to Selenium Metabolism and the Structure Analysis of Its Promoter. Chinese Journal of Tropical Crops 34: 654-661.

68. Mai Weijun, Zeng Jingqiu, Zhang Mingyong, Cai Zhaoyan (2006) Stress Inducible Expression Analysis of Glutathione Synthetase Gene in Rice (Oryzazativa). Journal of Tropical and Subtropical Botany 4: 451-459.

69. Bafuelos G, Terry N, Leduc DL, Plon-Smiths EA, Mackey B (2005) Field trial of transgenic Indian mustard plants shows enhanced phyto remediation of selenium contaminated sediment. Environ Sci Technol 39: 1771-1777.

70. Zu Song Cheng (1995) The Yutangba selenium mining area in south-western Hu Bei and origin of selenium pollution. Geological Review 41: 121-1261.

71. Wu L, Huazong Z, Burau RG (1988) Selenium accumulation and Se salt co-tolerance in five grass species. Crop Sci 28:517-522.

72. Banuelos GS, Ajwa HA, Mackey B, Wu L, Cook C, et al. (1987) Evaluation of plant species used for phyto remediation of high soil Se. J Environ Qual 26: 639-646.
73. Zayed A, Lytle C M, Terry N (1998) Accumulation and volatilization of different chemical species of selenium by plants. Planta 206: 284-292.

74. Grieve CM, Poss JA, Suarez DL, Dierig DA (2001) Lesquerella growth and selenium uptake affected by saline irrigation water composition. Industrial Crops and Products 13: 57-65.

75. Vickerman DB, Shannon MC, Baruelos GS, Grieve CM, Trumble JT (2002) Evaluation of Atriplex lines for selenium accumulation, salt tolerance and suitability for a key agricultural insect pest. Environ Pollut 120: 463-473.

76. Li Yanan, FENG Xia, DU Yuxiao, Chen DaQing (2010) Cloning and Prokaryotic Expression of AtCpNIfS from Arabidopsis thaliana. Hubei Agricultural Sciences 1:1-4.

77. Wang YD, Wang X, Wong YS (2012) Proteomics analysis reveals multiple regulatory mechanisms in response to selenium in rice. J Proteomics 75: 1849-1866.