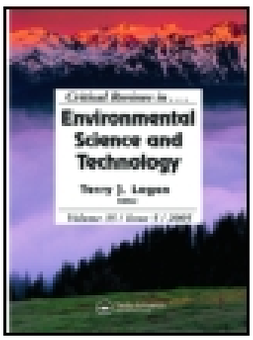

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Understanding selenium metabolism in plants and its role as a beneficial element

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ABSTRACT

Selenium (Se) is an essential element for many animals including humans, prokaryotes and a few green algae. For plants, Se essentiality is yet to be demonstrated. Nevertheless, it is well recognized that Se is a beneficial element for plants. For all organisms, while, a narrow range of Se is beneficial, at elevated levels it becomes toxic. This is due to accumulation of various toxic inorganic and organic Se forms during Se metabolism as well as nonspecific replacement by Se of chemically similar sulfur (S) analogs. Interestingly, Se can act both as an antioxidant and a pro-oxidant. Hence, Se chemistry and metabolism play crucial roles in determining its effects at specific concentration in an organism. A lot of knowledge has been gained regarding Se metabolites, however, the functions of many of these metabolites are yet to be resolved. Other Se containing metabolites and proteins might yet be awaiting their identification. Future research in this direction would pave the way towards identification of Se as an essential element for plants too. This review discusses the various aspects of Se uptake and metabolism with a major focus on functions of Se-containing metabolites in plants.

KEYWORDS

Beneficial element; humans; plants; selenium; sulfate transporters

1. Introduction

Selenium (Se) is an interesting element whose positive and negative impacts on the food chain and the environment have been debated through the years. Selenium was originally recognized as a toxic element to animals and humans (Spallholz, 1994). However, later on it was brought under the category of essential elements for microbes and many animals including humans, when glutathione peroxidase (GPx) and other selenoproteins were

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Table 1. Most common valence states of selenium.

S No.	Selenium Species	Valence state	Symbolic presentation
1	Selenide	−2	Se^{-2}
2	Elemental Selenium	0	Se^{0}
3	Thioselenate	+2	$SSeO_3^{-2}$
4	Selenite	+4	SeO_3^{-2}
5	Selenate	+6	SeO_4^{-2}

found to contain Se (Rotruck et al., 1973; Tinggi, 2003). Remarkably, there is a very fine line between the essentiality and toxicity of Se for most species (as little as one order of magnitude) and for this reason Se has been regarded as '*the essential poison*' (Reilly, 2006). As a consequence of this narrow window of adequacy, both deficiency and toxicity of Se are common problems worldwide (Terry, Zayed, De Souza, & Tarun, 2000). To prevent Se deficiency, Se is added in many commercially available food supplements for humans, and Se is commonly used as a nutritional feed additive for livestock too.

Selenium is a metalloid that lies between sulfur and tellurium in VIA group and between arsenic and bromine in 4th period of periodic table. Selenium shows chemical similarity with S in respect to bond energies, ionization potentials, atomic size, electronegativities and electron affinities. However, Se exists as reduced quadrivalent form while S in oxidized quadrivalent form; this is the major difference between Se and S (Tinggi, 2003). At physiological pH, selenium compounds (selenol) dissociate readily, which is important for its role in catalytic reactions (Tinggi, 2003). Selenium can exist in various oxidation states that allow it to form selenoamino acids and organoselenium compounds (Tinggi, 2003). The most common valence states of Se are −2, 0, +2, +4 and +6 (Table 1). The predominant available forms of Se to plants are inorganic, i.e. selenite (SeIV) and selenate (SeVI) (Schiavon & Pilon-Smits, 2017; White et al., 2004).

The presence of Se in soils worldwide is highly variable. While, Se levels in most soils are between 0.01 and 2 mg/kg (Dinh et al., 2018), seleniferous soils can contain up to 100 mg/kg Se (Pilbeam, Greathead, & Drihem, 2015) and some Se-rich areas contain up to 1200 mg/kg Se (Fordyce, 2013). The bioavailability of SeIV and SeVI in soil depends on environmental conditions (Zhao, Ren, Xue, & Lin, 2005). In oxic soil, the commonly present form of Se is SeVI while in anoxic soil SeIV is more abundant. Both forms are readily taken up and assimilated by plants. Plants show differential Se accumulation potential and have been categorized into three classes: hyper-accumulators, secondary-accumulators, and non-accumulators. Hyper-accumulators can tolerate Se more than 1000 mg/kg dry weight (DW), secondary-accumulators show no signs of toxicity between 100 and 1000 mg/kg DW while non-accumulators can tolerate Se only at <100 mg/kg DW (Beath, Gilbert, & Eppson, 1939). Hyper-accumulators have efficient

potential for converting Se to organic species while non-accumulators contain mostly inorganic Se species. Hyper-accumulators also typically translocate more Se to their shoot, especially their reproductive organs, and preferentially accumulate Se over S (Schiavon & Pilon-Smits, 2017).

In this review, we discuss the roles of Se in humans and plants, and Se uptake and transport mechanisms and effects in plants. The focus is on Se metabolism and known/possible functions of different Se containing metabolites in plants with cues from roles of Se-containing metabolites in animals and humans.

2. The effects of selenium on humans

In humans, dietary Se is converted to selenocysteine (SeCys), which is regarded as the 21st proteinogenic amino acid, an essential component of 25 different selenoproteins (Pappas, Zoidis, Surai, & Zervas, 2008). The translational incorporation of SeCys into proteins utilizes a specific t-RNA for SeCys that recognizes an opal UGA (stop) codon functioning as a SeCys codon in the presence of a SeCys insertion sequence in the adjacent mRNA (Lobanov, Hatfield, & Gladyshev, 2009). Selenocysteine functions in the catalytic center of several selenoproteins i.e. glutathione peroxidase, thioredoxin reductase, iodothyronine-deiodinases and selenophosphate synthetase. Apart from this specific SeCys insertion mechanism into selenoproteins, SeCys can also be inserted nonspecifically into other proteins, in place of cysteine. The incorporation of SeCys at the active site of enzymes (e.g. in case of methionine-R-sulfoxide reductase) in place of cysteine can alter their catalytic activity and electron donor specificity (Stadtman, 2005). This is thought to contribute to Se toxicity in humans.

Selenium plays an important role in the scavenging and regulation of free radicals (Kaur, Sharma, Kaur, & Nayyar, 2014). Selenoprotein P plays a major role in antioxidant defense system against harmful reactive oxygen species (ROS) and reactive nitrogen species (Steinbrenner, Alili, Bilgic, Sies, & Brenneisen, 2006; Steinbrenner, Steinbrenner, et al., 2006). Selenoprotein P is identified as a major selenoprotein that regulates plasma cholesterol level by protecting low density lipoproteins from oxidation by ROS (Traulsen, Steinbrenner, Buchczyk, Klotz, & Sies, 2004). Glutathione peroxidase also contributes to overall antioxidant capacity. The presence of SeCys in the active sites of antioxidant enzymes gives higher catalytic efficiency due to greater nucleophilic power of SeCys in comparison to cysteine (Eshdat, Holland, Faltin, & Ben-Hayyim, 1997; Campbell, 2001). Selenium is an excellent hydrogen ion donor at normal blood pH levels and is therefore much effective in the control of ROS as compared to sulfur (Longtin, 2004). The mutations causing defects in SeCys biosynthesis or

correct incorporation into Se-requiring proteins has been found to lead to severe neurological disorders or milder systemic disorders, respectively (Schmidt & Simonović, 2012). Thus, adequate Se intake is important for optimal antioxidant function. The beneficial effects of Se have been demonstrated in prevention against cancer and other diseases (Sanmartín, Plano, Font, & Palop, 2011). External Se supply has also been found to be beneficial in several animal models in terms of toxicity reduction of a drug/chemical or cure of a disease (Collery, 2018). However, Se administration is a critical process, as therapeutic effects of Se can be lost if the dose becomes high, this is further influenced by on other factors like Se species and bioavailability/bioaccessibility.

An insufficient intake of Se is linked to several diseases in humans like Keshan Disease and white muscle disease (Fordyce, 2013; Shi et al., 2017). Selenium deficiency in humans also makes them prone to several health risks, such as myxedematous endemic cretinism, growth retardation, impaired bone metabolism, abnormalities in thyroid function, reduced fertility and immune function, which can lead to cancers (Lobanov et al., 2009; Feng, Wei, & Tu, 2013; Gupta & Gupta, 2016). The recommended dose of Se is 50–55 µg/d by WHO and 50–70 µg/d by USDA (WHO, 2009; USDA, 2012). In humans, Se deficiency occurs when Se intake is below 40 µg/d (Winkel et al., 2011). Recently, Jones et al. (2017) reported that about 0.5 to 1 billion people are suffering from Se deficiency worldwide. On the other hand, higher dose exposure of Se can lead to adverse health problems, such as skin lesions, hair and nail loss, nervous disorders including paralytic symptoms and death (Fordyce, 2013). Inorganic Se is about 40 times more toxic than organic Se (Vinceti et al., 2013). High levels of Se (>400 µg/d) are toxic to humans (Winkel et al., 2011). The European Food Safety Authority (Scientific Committee on Food, 2006) guidelines have set the tolerable upper intake limit of Se to300 µg/d for adults, 60 µg/d for children aged 1 to 3 years and 250 µg/d for children aged 15 to 17 years.

In many regions of the world, extremely high concentration of Se in soil is the reason of chronic selenosis in humans. Symptoms of selenosis include nail brittleness, skin rash, gastrointestinal disorders, musculoskeletal disorders (stiff gait and lameness) hair loss, garlic like breath odor and irregular working of the nervous system (Reilly, 2006). Studies have also shown a positive correlation between high Se concentration and tumurogenesis via enhanced expression of thioredoxin reductase 1 in humans (Sun et al., 2014). Higher content of Se is also responsible for various chronic degenerative diseases such as cardiovascular disease and amyotrophic lateral sclerosis (Vinceti, Maraldi, Bergomi, & Malagoli, 2009). Disruption of endocrine function, impairement of thyroid hormones and insulin-like growth factor metabolism are additional toxic symptoms of Se (Ullah et al., 2018).

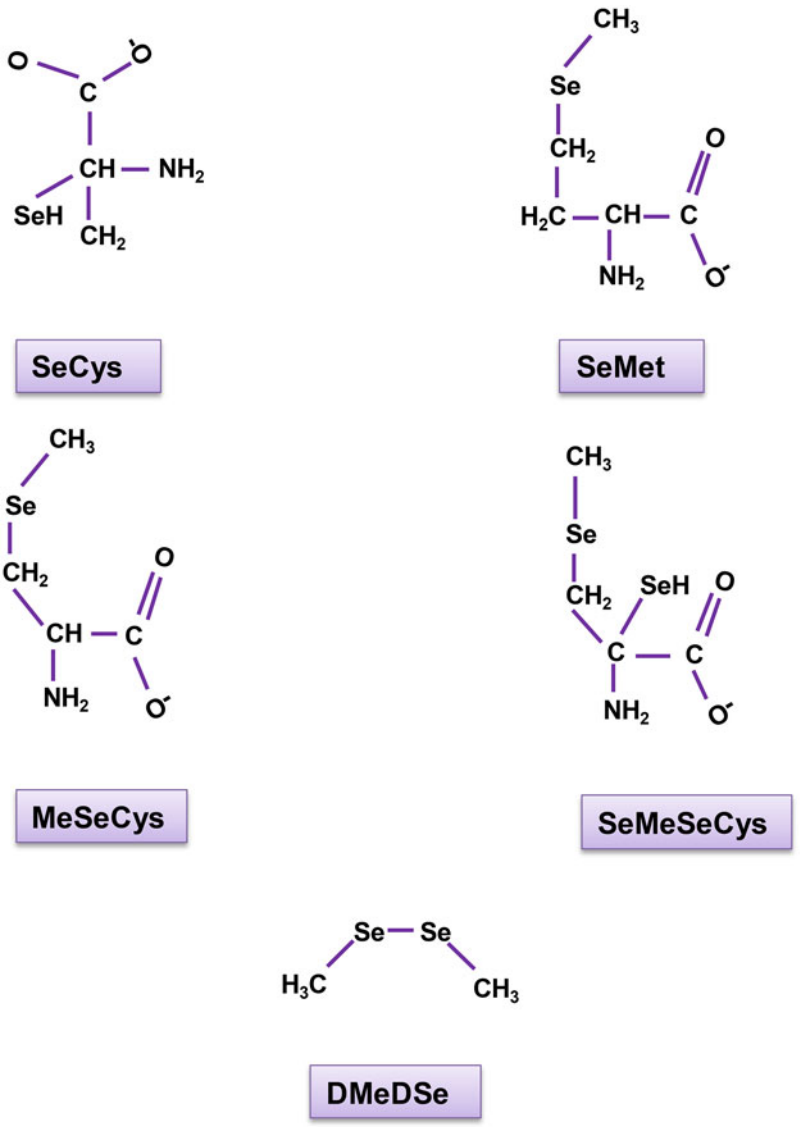

Figure 1. Structure of seleno-organic compounds. SeCys, Seleno Cysteine; SeMet, Seleno Methionine; MeSeCys, Methyl Seleno Cysteine; SeMeSeCys, Seleno Methyl Seleno Cysteine; DMeDSe, Di Methyl Di Selenide.

3. The effects of selenium on plants

Crops are the major source of dietary Se intake for many of the human populations worldwide and thus, Se enriched crops might be envisioned as a tool to counteract Se deficiency (Schiavon & Pilon-Smits, 2017). Plants readily take up and metabolize Se. Different plant species have been found to accumulate a number of Se containing metabolites (Figure 1): selenomethionine (SeMet), SeCys, Seleno-methylselenocysteine (SeMeSeCys), methylselenocysteine (MeSeCys), selenotaurine, selenobetaine, selenoecholine,

dimethylselenine, dimethyldiselenide (DMDSe), and trimethylselenium (Kabata-Pendias, 2011). However, the present knowledge states that Se is not an essential part of proteins in plants (Pilon-Smits, Quinn, Tapken, Malagoli, & Schiavon, 2009) and hence, plants Se metabolism in plants occurs nonspecifically via sulfur metabolic pathways (Schiavon & Pilon-Smits, 2017). It cannot be excluded that in future studies some plant species might be found to require Se, or to contain essential selenoproteins. The essentiality of Se for plants could be conclusively proven in experiments where plants are shown not to survive under totally Se-free conditions (Stadtman, 1996), or if proteins/enzymes containing SeCys as an integral amino acid (incorporated via translation process) can be discovered (De Filippis, 2010). There are several green (eukaryotic) algae, including *Chlamydomonas reinhardtii* that possess a GPx homolog with SeCys and also have UGA opal codon decoding SeCys tRNA (Fu et al., 2002). Algae contain several selenoproteins as evidenced by identification of 12 selenoproteins in *C. reinhardtii* (Grossman et al., 2007). Other algae (about 33 algae) are also known to require Se for selenoproteins, such as *Cyanidioschyzon* (Cyanidiaceae), *Ostreococcus* (Prasinophyceae) and *Emiliania huxleyi* (Haptophytes) (Schiavon & Pilon-Smits, 2017). The alga *Ulva australis* also has specific SeVI transport system (Schiavon et al., 2012). However, algae have been classified in different ways either exclusively or partly in Protista and Plantae Kingdoms (Whittaker & Margulis, 1978). Hence, essentiality of Se in algae cannot be considered as a hint of Se being essential in plants too. It appears that higher plants might have lost essential Se metabolism or the identification of selenoproteins might still be awaiting in plants (Lobanov et al., 2009).

It is reported that Se at low concentrations can act as a priming agent augmenting the stress tolerance of plants (Pilon-Smits et al., 2009). A low dose of Se can stimulate plant growth, improve photosynthesis and help in homeostasis of essential nutrient elements (Chauhan et al., 2017). Selenium serves as an anti-senescent and helps in maintenance of cellular structure and function, thus, contributes towards improved plant growth and development (Kaur et al., 2014; Balk & Pilon, 2011). Selenium has also been found to inhibit the uptake and accumulation of toxic metals in plants (Chauhan et al., 2017). The levels of ROS like superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$), and lipid peroxide radicals ($LOO^{\bullet}$, $ROO^{\bullet}$) that are produced in plants at low concentrations in unstressed conditions, increase rapidly in response to various abiotic stresses (Feng et al., 2013). Selenium works as a stress modulator and inhibits the accumulation of ROS during stress by acting as a ROS quencher and as an antioxidant (Feng et al., 2013). Selenium can regulate the production and accumulation of ROS in

stressed plants by these pathways: (1) stimulation of the spontaneous dismutation of $O_2\bullet-$ into H_2O_2, (2) regulation of enzymatic and non-enzymatic antioxidant systems and (3) direct quenching of ROS through Se species. Another possibility lies in the positive effects of Se on the integrity of photosynthetic complexes and photosynthesis machinery that in turn leads to a decrease in ROS production (Feng et al., 2013; Chauhan et al., 2017).

However, Se functions as an antioxidant only at low concentration, whereas at higher concentrations, it acts as a pro-oxidant, inducing an increase in ROS and lipid peroxidation (Hartikainen, Xue, & Piironen, 2000; Terry et al., 2000). This narrow range of essentiality and toxicity differs among plant species and also depends on the species of Se (Shahid et al., 2018). Toxicity of Se in plants results in stunted growth, chlorosis, withering and drying of leaves, and decreased protein synthesis, as well as premature death of plants (Breznik, Germ, Gaberščik, & Kreft, 2004, Kaur et al., 2014). The biochemical basis of Se toxicity lies in the fact that Se and S are chemical analogs. Hence, SeVI and SeIV assimilated into SeCys and SeMet. The replacement of cysteine and Met in proteins by SeCys and SeMet can result in loss of functionality of proteins that is in turn attributed to misfolded or malformed proteins (Van Hoewyk, 2013). In addition, Se interaction with proteins can lead to formation of selenotrisulphides, selenylsulphide bonds and diselenides (Ganther, 1999). Indeed, proteomics analyses have found Se-induced increase in levels of heat shock proteins and proteasomal subunits involved in protein refolding and degradation of misfolded proteins respectively (Sabbagh & Van Hoewyk, 2012). Selenium can also affect protein functions through replacement of S in Fe-S clusters to form Fe-Se clusters (Balk & Pilon, 2011). A third process by which Se induces toxicity in plants is increased oxidative stress due to pro-oxidant properties of Se via decreased level of reduced glutathione (GSH) concentrations and by direct reaction with reduced GSH to form superoxide (Van Hoewyk, 2013). Inorganic Se species react with GSH to form seleno-trisulphides that in turn propagate a chain reaction with other thiols leading to the generation of ROS. The conversion of diselenides into selenols by thiols is another route for ROS generation (Mézes & Balogh, 2009). Further, when excess Se is present, accumulation of SeCys inhibits the methylation of Se and increases the level of hydrogen-selenide, which is a toxic Se species (Mézes & Balogh, 2009). To prevent or alleviate Se toxicity, plants can metabolize Se into volatile dimethylselenide or DMDSe that can be released into the atmosphere (Terry et al., 2000). Some species also can convert SeCys to MeSeCys as a Se detoxification process, because MeSeCys is not incorporated into proteins (Terry et al., 2000).

4. Selenium uptake, transport and detoxification in plants

In plants, Se uptake depends on the species of Se and its concentration (Figure 2), as well as on the activity of membrane transporters (Zhao et al., 2005; Zhang et al., 2014). Selenate finds its way into plant cells through sulfate transporters because of structural analogy of SeVI and sulfate (White, 2016). In fact, it is hypothesized that all type of sulfate transporters are likely involved in the uptake and transport of SeVI in plants (Yoshimoto, Inoue, Saito, Yamaya, & Takahashi, 2003). Constitutively expressed (low-affinity) sulfate transporters select SeVI over sulfate even when available sulfate is high in the external environment, while the high-affinity sulfate transporters that are induced at low external sulfate, select sulfate over SeVI in *Arabidopsis thaliana* (White et al., 2004). In *A. thaliana*, high-affinity sulfate transporters; AtSULTR1;1 and AtSULTR1;2, are involved in SeVI uptake (White, 2016).

After uptake, SeVI can be transported from the root to the shoot with the help of sulfate transporter AtSULTR2;1, AtSULTR2;2 and AtSULTR3;5 via xylem (White, 2016). The influx of SeVI into plastids might be mediated by AtSULTR3;1, while AtSULTR4;1 and AtSULTR4;2 are proposed to act as efflux transporters of SeVI allowing its release from the vacuoles (Boldrin et al., 2016; White, 2016). In contrast to SeVI, the uptake of SeIV is mediated by phosphate transporters. Zhang et al. (2014) reported the role of *Oryza sativa* phosphate transporter (OsPT2) in SeIV uptake and Se accumulation in rice grains. They found that over-expression and knockout lines of *OsPT2* accordingly affected SeIV uptake in rice plants. A recent report by Song et al. (2017) suggested that the over-expression of *OsPT8* in transgenic tobacco lines improved phosphate and Se acquisition in shoots. Additionally, a silicon influx transporter in rice (Figure 2), nodulin 26-like intrinsic membrane protein (OsNIP2;1), can mediate SeIV uptake under acidic conditions (Zhao, Mitani, Yamaji, Shen, & Ma, 2010). Translocation of SeVI occurs more readily from root to shoot in comparison to other Se forms (Kikkert & Berkelaar, 2013). Translocation of Se within the plant not only depends on the different Se forms but also on plant species. Carey et al. (2012) reported that SeMet and SeMeSeCys were more efficiently transported to the grain of rice plants in comparison to SeIV and SeVI, whereas in wheat and canola the following order of Se translocation has been reported: SeVI > SeMet > SeIV/SeCys (Kikkert & Berkelaar, 2013).

It is reported that Se induces the activity of phytochelatin synthase (PCS) in the plant cells (Kumar et al., 2014), which catalyzes the synthesis of phytochelatins (PCs) from GSH. The synthesized PCs are complexed with selenide and the complex transported into the vacuole (Aborode et al., 2016). It has been reported that co-exposure of Se with other metals can enhance PC concentrations in plants (Kumar et al., 2014). However, unlike other

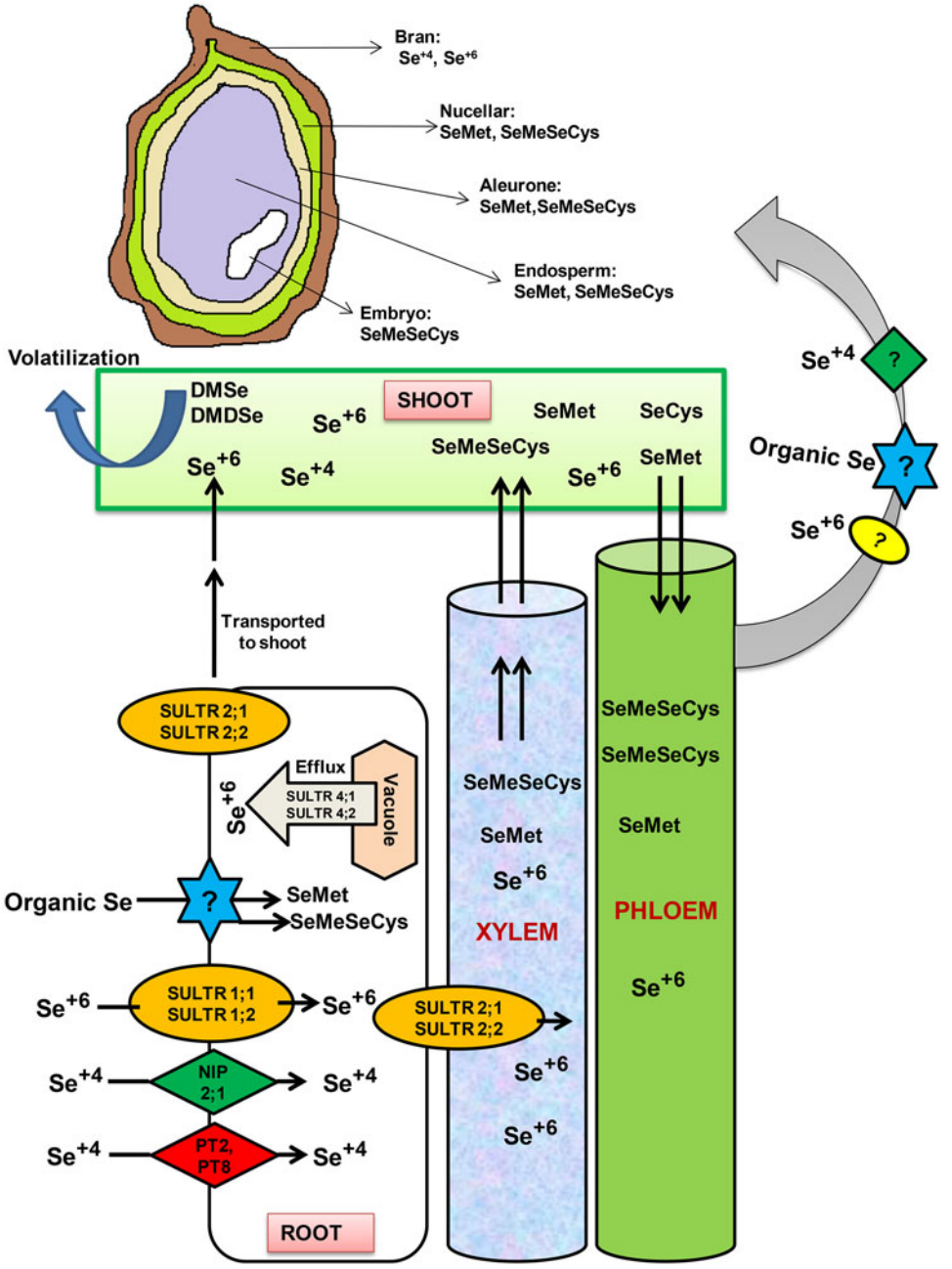

Figure 2. Flow diagram representing the transporters involved in the uptake and transport of different selenium species through xylem and phloem, up to the grains. Sulfate transporters (SULTR1;1, SULTR1;2) and aquaporins (NIP2;1) are involved in uptake of selenate (Se^{+6}) and selenite (Se^{+4}), respectively in rice plants. Phosphate transporters: PT2 and PT8 are also involved in uptake of Se^{+4}. SULTR2;1 and SULTR2;2 help in direct transportation of Se^{+6} into shoot cells and also contribute in xylem loading of Se^{+6} in rice plants. Organic Se species like SeMet: Seleno Methionine, SeCys: Seleno Cysteine and SeMetSeCys: Seleno Methyl Seleno Cysteine are also taken up by rice roots and transported upto rice grains but the transporters are still unknown. DMSe: Di Methyl Selenide and DMDSe: Di Methyl Di Selenide are the volatile forms of selenium. Selenium is transported into seeds through phloem. Different Se species are reported to be present in different layers of the rice seed.

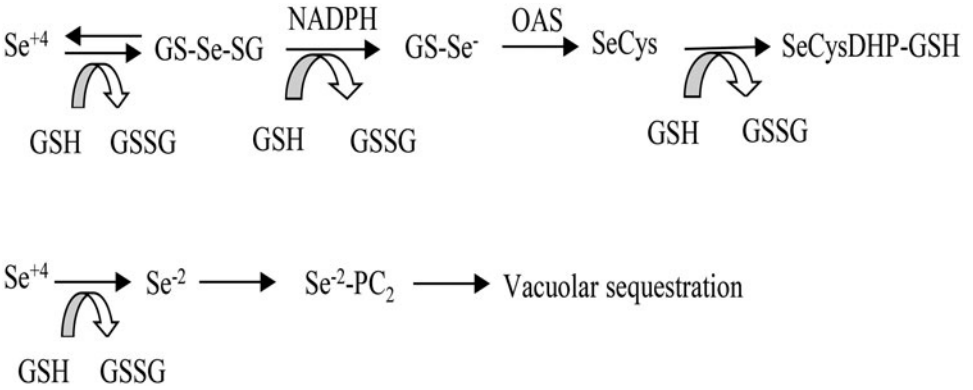

Figure 3. Transformation of Selenium into selenopeptides using GSH (Reduced Glutathione). Se^{+4}, Selenite; GS-Se-SG, Selenodiglutathione; GSSG, Oxidized Glutathione; NADPH, Nicotinamide Adenine Dinucleotide Phosphate Hydrogen; $GS\text{-}Se^{-}$, Glutathione Selenide Anion; OAS, O-Acetyl Serine; SeCys, Selenocysteine; SeCysDHP-GSH, Seleno Cysteinyl-2,3-dihydroxylpropionyl Glutathione; Se^{-2}, Selenide; Se^{-2}-PC_2, Selenide-Phytochelatin Complex.

metals like arsenic, lead and cadmium, there is very little information about Se-induced PC generation inside plants and PC-dependent Se detoxification. It is hypothesized that Se 'in the form of selenite' induces synthesis of GSH and extensively uses it for its biotransformation into selenopeptides at the expense of PC biosynthesis (Aborode et al., 2016). One Se-containing peptide has been identified as Se^{II}cysteinyl-2,3-dihydroxyproprionyl glutathione in the vine, *Thunbergia alata* (Aborode et al., 2015) (Figure 3).

5. Selenium metabolism

The metabolic fate of Se also determines its distribution, spatial and developmental, in plants. Pickering, Prince, Salt, and George (2000) reported the differential distribution of Se compounds in plants through spatially resolved X-ray absorption spectroscopy showing that SeVI concentrated in older leaves while organic Se compounds (C-Se-C compounds, mainly MeSeCys) were found more in the young leaves of the Se hyper-accumulator *Astragalus bisulcatus.* In the following sections, Se metabolism is discussed in a step by step manner.

5.1. Conversion of selenate into selenite and selenide

Selenium metabolism starts with the assimilation of SeVI in leaf chloroplasts by the sulfate assimilation pathway (Terry et al., 2000). In Se assimilation pathway, Se can be converted into organic, nontoxic and volatile forms of Se (Figure 4) by SeVI reduction to SeIV, which is the rate limiting and crucial step in this pathway (Pilon-Smits & Quinn, 2010). Selenate is largely transported to the shoots where it is assimilated predominantly in

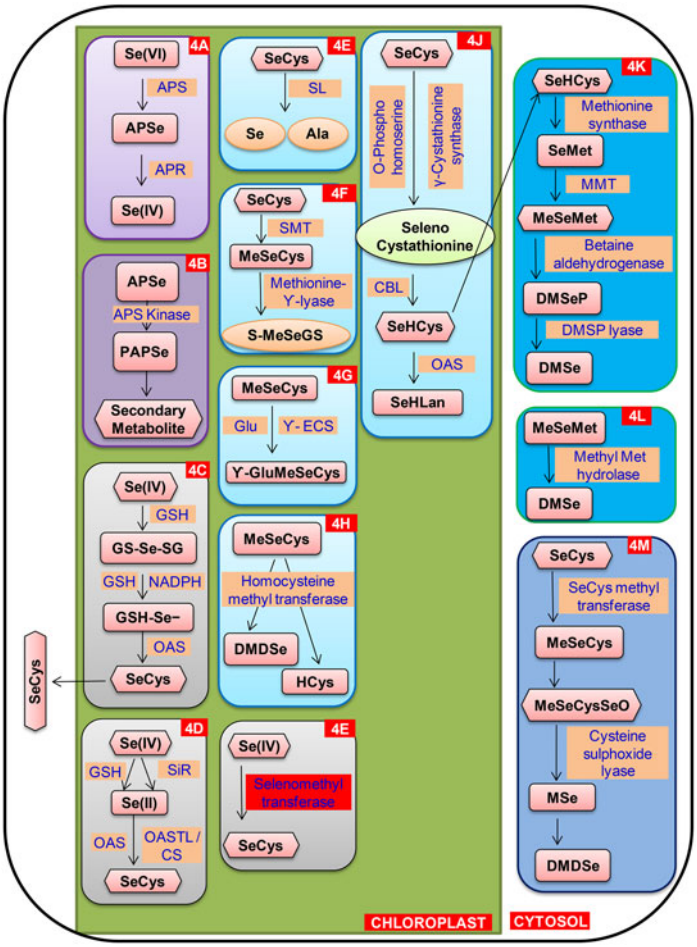

Figure 4. Schematic representation of metabolic fate of selenium in plants. Selenate is transported into the shoot cell and assimilated into the selenium metabolites by the action of a series of metabolic enzymes. APS, ATP Sulfurylase; APSe, Adenosine Phospho Selenate; APR, Adenosine Phosphosulfate Reductase; APSe, Adenosine Phospho Selenate; APSK, APS Kinase; PAPSe, Phosphoadenosine Phosphoselenate; GSH, Reduced glutathione; NADPH, Nicotinamide Adenine Dinucleotide Phosphate Hydrogen; GS-Se-SG, Diglutathione Selenium; OAS, O-Acetyl Serine; SeCys, Selenocysteine; SiR, Sulfite Reductase; OASTL/CS, OAS Thiol lyase/Cysteine Synthase; SL, Selenocysteine Lyase; Ala, Alanine; Se, Elemental Selenium; SMT, Selenocysteine Methyl Transferase; MeSeCys, Methy Seleno Cysteine; S-MeSeGS, S-Methyl-Selenoglutathione; Glu, Glutamate; ϒECS, γGlutamyl Cysteine Synthetase; GluMeSeCys, GlutamylMethylSelenoCysteine; DMDSe, DiMethylDiSelenium; HCys, HomoCysteine; CBL, Cystathionine β lyase; SeHCys, SelenoHomoCysteine; SeHLan, SelenoHomoLanthionine; SeMet, SelenoMethionine; MMT, Methionine Methyl Transferase; MeSeMet, MethylSelenoMethionine; DMSeP, DiMethylSelenoPropionate; DMSe, Di Methyl Selenium; MeSeCysSeO, MethylSelenoCysteine Selenooxide; MSe, Methaneseleniol; DMDSe, Di Methyl Di Selenide.

leaf chloroplasts, while SeIV is rapidly assimilated in roots and translocated to a much lower degree. Consecutive actions of two enzymes i.e. ATP sulfurylase (APS) and adenosine phosphosulfatereductase (APR) are involved

in the conversion of SeVI to SeIV. First, APS couples SeVI to ATP and forms adenosine phosphoselenate (APSe) (Sors, Ellis, & Salt, 2005), which is subsequently reduced to SeIV by the action of APR (Pilon-Smits & Quinn, 2010) (Figure 4A). In a parallel direction, APSe can be directed to the synthesis of secondary metabolites by forming phosphoadenosine phosphoselenate (PAPSe), an intermediate compound (Figure 4B), in the presence of APS kinase (Pilon-Smits, 2015). Isozymes for APS and APR are present in both chloroplast and cytosol, but most of the SeVI reduction occurs in chloroplasts.

5.2. Selenite to selenocysteine

Selenocysteine can be synthesized from SeIV by both enzymatic and non-enzymatic reduction steps. In non-enzymatic reduction, SeIV is first reduced into selenodiglutathione (GS-Se-SG) in the presence of GSH. Another reduction step leads to formation of selenopersulfide/glutathionyl-selenol (GS-SeH) with the use of NADPH as a reducing agent. This reduction is followed by conversion of GS-SeH to SeCys (Figure 4C) by its coupling with O- acetylserine (OAS). This SeCys can be further transported into the cytosol (Terry et al., 2000). In a process involving both enzymatic and non-enzymatic steps, SeIV is first reduced to selenide (Se^{-2}) either by sulfite reductase (Pilon-Smits & Womg, 2012) or by non-enzymatic reduction in the presence of GSH (Terry et al., 2000; Figure 4D). The enzymatic synthesis of SeCys from Se^{-2} is catalyzed by cysteine synthase (CS), a complex of serine acetyl transferase (SAT) and O-acetylserinethiollyase (OAS-TL) subunits (Pilon-Smits & Womg, 2012; Figure 4D). This enzyme catalyzes the reaction of Se^{-2} with OAS to form SeCys (Figure 4E). Alternatively, SeCys can form directly from SeIV by the action of seleno methyltransferase (SMT) (Pilon-Smits & Quinn, 2010).

5.3. Metabolism of selenocysteine

Selenocysteine can be further converted to several different selenocompounds. Selenocysteine can be converted to elemental selenium (Se^0) and alanine by the action of SeCyslyase (SL) (Figure 4F). This process contributes to Se tolerance, as Se^0 does not interfere as much with cellular processes compared to SeCys (Pilon-Smits & Quinn, 2010). Another fate of SeCys is its methylation by SeCys methyltransferase (SeCysMT) to produce MeSeCys (Shahid et al., 2018). This MeSeCys have several metabolic fates. One is conversion of MeSeCys into S-methylselenoglutathione (S-MeSeGS) in the presence of methionine-ϒ-lyase (Figure 4G). Another route is the formation of ϒ-glutamylmethyl-SeCys (ϒ-GluMeSeCys) by conjugation

with glutamate (Figure 4H) in the presence of ϒ-glutamyl cysteine synthetase (Freeman et al., 2007). ϒ-glutamylmethylselenocysteine is a major storage form of Se in the Se hyper-accumulator *Astragalus* species (Gupta & Gupta, 2016). Methylselenocysteine can also become converted into volatile DMDSe, or into homocysteine by the action of homocysteine methyl transferase (Figure 4I).

Another process by which SeCys can be metabolized includes its coupling with O-phosphohomoserine leading to the production of seleniumhomocysteine (SeHCys) by forming an intermediate selenocystathionine. The two reactions are catalyzed by the γ-cystathionine synthase and cystathionine-β-lyase, respectively (Pilon-Smits & Quinn, 2010). Seleniumhomocysteine conjugates with OAS to form selenohomolanthionine (SeHLan) (Pilon-Smits & Quinn, 2010) (Figure 4J). SeHCys can subsequently be transported to cytosol where it undergoes a sequential conversion to form volatile dimethylselenide. In this sequential conversion process, SeHCys converts into SeMet and methyl-SeMet (MeSeMet) by the action of methioninesynthase and methioninemethyltransferase (MMT), respectively (Figure 4K). The compound MeSeMet is also known to have two different metabolic routes. In one route, by decarboxylation and transamination reactions, MeSeMet converted into dimethylselenopropionate (DMSeP) and finally into DMSe (Figure 4K) by the action of betainealdehydrogenase and di-methylselenopropionate lyase (DMSePlyase), respectively (Pilon-Smits & Quinn, 2010). In another route, methylmethionine hydrolase directly converts MeSeMet into DMSe (Bodnar, Konieczka, & Namiesnik, 2012; Figure 4L). The biosynthesis of SeMet is limited by the conversion of the precursor SeCys into non-protein amino acids like MeSeCys, γ-GMeSeCys and selenocystathionine (Shahid et al., 2018). Selenium metabolism through the different routes mentioned above (Figure 4) allows plants to either minimize 'or completely avoid' the nonspecific incorporation of SeCys and SeMet into proteins and consequently ameliorate Se toxicity. In the cytosol, SMT also catalyzes the synthesis of MeSeCys from SeCys, which is subsequently oxidized into methyl-SeCys-selenooxide (MeSeCysSeO). Cysteine sulfoxidelyase cleaves MeSeCysSeO into methaneseleniol (Figure 4M) that is finally converted into volatile DMDSe (Bodnar et al., 2012).

6. Function of selenium metabolites

6.1. Role of selenium metabolites in plants

Selenium has been extensively applied as a base fertilizer or through foliar spray to enhance plant productivity, minimize the injuries generated due to environmental stresses and to increase the Se content in the edible parts of crops (Pezzarossa, Remorini, Gentile, & Massai, 2012). It is apparent that there is a fine line between required and toxic levels of Se, and the

practices of Se biofortification can be more safely and accurately applied if the functions and fates of the various seleno-metabolites are more thoroughly understood. In addition to already known seleno-metabolites, several others might yet be identified. As discussed, plants have several different selenocompounds whose nutritional values and toxicity levels are not completely elucidated. The regulation of ROS levels by Se, as described earlier, can be a key mechanism for counteracting environmental stress in plants. Selenoaminoacids i.e. SeMet and SeCys act as direct antioxidants or help in the synthesis of Se-dependent antioxidants (e.g. glutathione peroxidases, thioredoxinreductases, methionine sulfoxidereductases) and in repair of proteins. The nucleophilic properties of the ionized selenol (RSe^{-}) and the ease of oxidation of SeMet and SeCys raise the direct antioxidant potential of these amino acids. The catalytic activity of Se-dependent antioxidant enzymes against biological oxidants has been found to be greater than sulfur-containing enzymes due to the presence of SeCys in the catalytic sites of seleno enzymes (Rahmanto & Davies, 2012). Hartikainen et al. (2000) reported the increased activity of GPx under different treatments of Se in ryegrass seedlings. The activation of the GPx is also observed in many Se-treated plants subjected to diverse stresses e.g. cadmium (Filek et al., 2008), salt (Hasanuzzaman & Fujita, 2011), drought (Hasanuzzaman, Hossain, & Fujita, 2011) and arsenic (Chauhan et al., 2017). The increased activity of GPx on Se supplementation suggests a crucial role of this enzyme in counteracting oxidative stress in plants. In addition, other antioxidant enzymes i.e. superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR) have been found to be enhanced after Se addition in many stressed plants (Feng et al., 2013; Kumar et al., 2014). Other antioxidants activated in stressed plant to diminish oxidative stress upon Se addition are some low molecular substances such as ascorbate, tocopherol and polyphenols (Hasanuzzaman & Fujita, 2011).

Another Se species, SeHLan, has also been isolated from a range of organisms including *Brassica juncea* (Indian mustard), *Raphanus sativus* L. (Japanese radish cultivar), (*Triticum aestivum*) wheat and a Se enriched yeast (Duncan et al., 2017). While knowledge on its biological role is limited to rats, SeHLan is considered as a precursor of SeCys (Anan, Mikami, Tsuji, & Ogra, 2011). SeHLan has also been proposed to have lower toxicity to mammals than SeMet, and thus to be a desirable species for Se biofortification purpose (Anan et al., 2011).

6.2. Role of selenium metabolites in animals and humans

Methyl-SeCys, a methylated product of SeCys, has been reported to have a vital role in prevention of mammary cancer in a rat model system (Ellis

et al., 2004). A mono-methylated form of Se, methylseleninic acid (MSA) has also been shown to inhibit the growth of mouse mammary hyperplastic epithelial cells by arresting the G1 phase of the cell cycle. The effect was accompanied by a reduction in total cellular levels of cyclin D1 (Zhu, Jiang, Ganther, & Thompson, 2002). Organic forms of Se, like SeMet, methylselenol (MSe), γ-GluMeSeCys and MeSeCys, were reported to have anti-carcinogenic properties (Fernandes & Gandin, 2015). This has sparked interest in increasing and/or modifying the Se compounds in plants (Ellis et al., 2004). Several studies have reported that MeSeCys isolated from garlic and broccoli is an effective chemoprotectant in reducing the incidence of mammary and colon cancer in rats (Finley et al., 2001; Ip et al., 2000). Rayman (2008) also confirmed MeSeCys and γ-GluMeSeCys to be more effective tumor inhibitors in comparison to other Se species. Selenomethionine and MeSeCys that are produced by different plant species, grown in Se rich soil, or Se fertilized soil have shown several beneficial properties in humans e.g. prevention of different types of cancers, alleviation of thyroid disorders, improved immune system response and treatment of male infertility (Sepúlveda, Barrientos, Mahn, & Moenne, 2013; Roman, Jitaru, & Barbante, 2014). Selenium mediated induction of apoptosis of cancerous cells and inhibition of tumor angiogenesis prove that Se controls progression of early cancerous lesions (Ip, Dong, & Ganther, 2002; Lu & Jiang, 2001; Jiang, Wang, Ganther, & Lü, 2002). The proposed mechanism of Se mediated induction of apoptosis includes activation of BCL2 family proteins and caspases and arrest of cell cycle at G1 and S phase (Wallenberg, Misra, & Björnstedt, 2014). Treatment with SeIV and GS-Se-SG has been reported to induce single-strand breaks of DNA. However, SeMeSeCys, SeMet and MSA generates methylselenol has been reported to inhibit cancer either by activation of caspase-mediated apoptosis or DNA fragmentation (Jiang et al., 2002; Wallenberg et al., 2014). Jiang et al. (2002) reported that SeIV treatments induce S-phase arrest, caspase-independent DNA fragmentation and apoptosis. Selenite-mediated activation of apoptosis involves increase in the expression of phosphorylated serine/threonine kinase, JNK1 and p38 mitogen-activated protein kinase. In contrast, MSA caused G1-phase arrest, DNA fragmentation and a caspase-dependent poly (ADP-ribose) polymerase 1 cleavage, leading to apoptosis. On the other hand, SeVI has other important functionalities as to inhibit adipogenesis via transforming growth factor β1 signaling (Wallenberg et al., 2014). Similarly, other Se metabolites like GS-Se-SG, MSA, SeCys and SeMeSeCys also prevent cancer and induce apoptosis via glutathionylation of membrane protein, DNA fragmentation, cell cycle arrest at G1, inhibition of cell cycle regulators (cyclin A and D) and activation of caspases especially caspase 3 and 12 (Wallenberg et al., 2014; Figure 5).

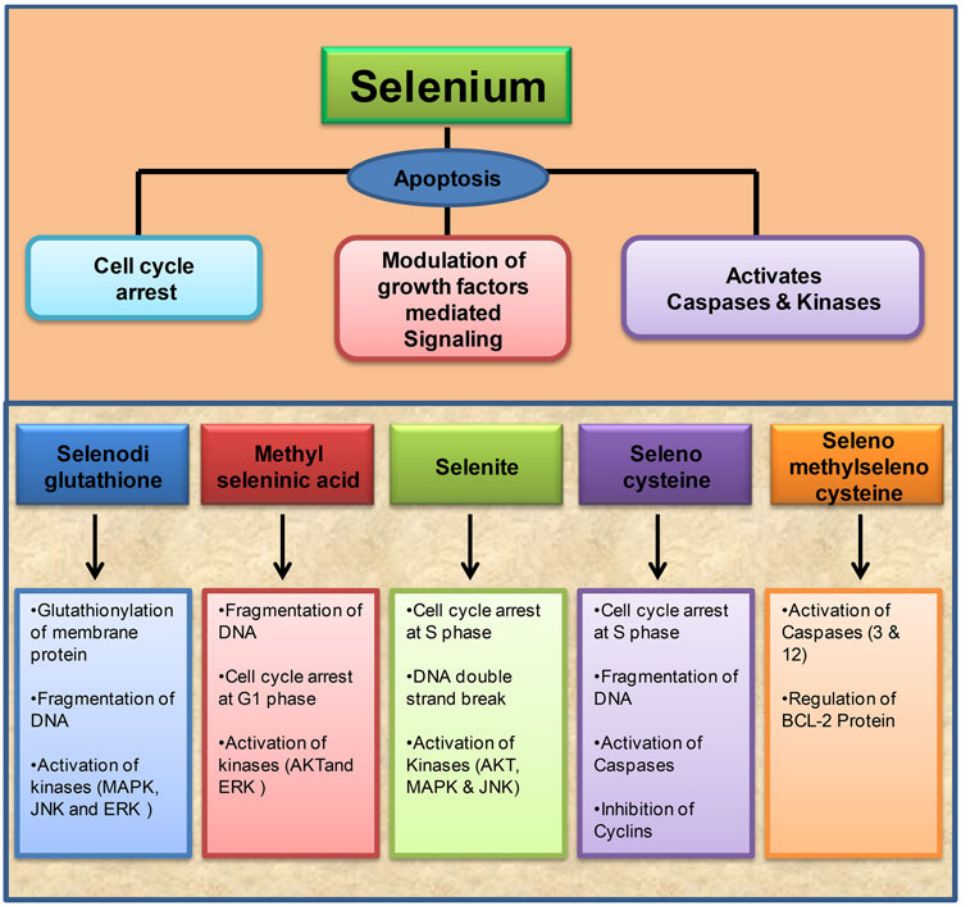

Figure 5. Regulation of signaling pathways mediated by selenium metabolites in animal cells for the treatment of various diseases. Selenium can regulate apoptosis through processes like cell cycle arrest, growth factor changes, and activation of caspases and kinases. These processes are, in turn, modulated by different Se metabolites, as presented in the lower panel.

7. Conclusions and future prospects

In recent years, the approach of Se biofortification has been practiced in many countries to obtain Se-enriched food for improving human nutrition and health (Bañuelos, Arroyo, Pickering, Yang, & Freeman, 2015). However, more detailed information is warranted on Se metabolism and formation and functions of selenocompounds in plants. As it is clearly apparent that the total Se content is no longer sufficient to fully evaluate the potential biological effects of an enriched source of Se, it is the Se-containing metabolites, which have unique functions and properties and contribute towards beneficial and toxic impacts in animals and humans. Therefore, future research should aim to understand how Se affects plants in the light of Se speciation and metabolites. The use of more sensitive

analytical techniques and accurate methodologies should fill this gap. The purpose should not be limited to quantify total Se concentrations but also to detect the various Se metabolites. Targeted experiments are required to unravel Se as an essential element for higher plants. This basic knowledge would also be helpful in the utilization of Se as a priming agent/biostimulant to augment crop growth and yield amidst various abiotic and biotic stresses.

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