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BOLU ABANT İZZET BAYSAL UNIVERSITY
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EFFECTS OF SELENIUM ON SULFATE TRANSPORTER GENES IN RICE AND THE FUNCTIONAL CHARACTERIZATION OF THE *OSSULTR1;1* GENE IN SELENIUM HOMEOSTASIS IN ARABIDOPSIS

DOCTOR OF PHILOSOPHY

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APPROVAL OF THE THESIS

EFFECTS OF SELENIUM ON SULFATE TRANSPORTER GENES IN RICE AND THE FUNCTIONAL CHARACTERIZATION OF THE OSSULTR1;1 GENE IN SELENIUM HOMEOSTASIS IN ARABIDOPSIS submitted by **Noreen ASLAM** and defended before the Examining Committe Members listed below in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Department of Biology, Institute of Graduate Studies of Bolu Abant Izzet Baysal University in 16.07.2024** by

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ABSTRACT

EFFECTS OF SELENIUM ON SULFATE TRANSPORTER GENES IN RICE AND THE FUNCTIONAL CHARACTERIZATION OF THE *OsSULTR1;1* GENE IN SELENIUM HOMEOSTASIS IN ARABIDOPSIS
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Sulfate transporters in rice are reported to be involved in abiotic and heavy metal stresses. However, the regulation of these transporters and their functional characterization in relation to selenate treatment remain elusive. The focus of this research was to analyze the expression profiles of rice sulfate transporters at vegetative and reproductive stages. The transporters exhibited diverse expression patterns in response to Se treatment and displayed tissue specificity, suggesting their varied functional roles. *OsSULTR1;1* was selected as a candidate gene due to its expression under low sulfur (S) conditions (15 μM) and its high root tissue specificity, both of which were further enhanced by Se treatment. Subcellular localization revealed that the OsSULTR1;1 protein localizes to the plasma membrane, implicating its role in root uptake of Se. To further characterize this gene, it was genetically transformed into *Arabidopsis thaliana*. Overexpression (OX) of the gene in Arabidopsis resulted in enhanced growth, development, and uptake of mineral nutrients compared to wild-type (WT) plants, suggesting a role in efficient nutrient acquisition. OX plants subjected to time- and concentration-dependent Se treatment accumulated significant levels of Se in their tissues. Se was found to be toxic for both OX and WT plants at higher concentrations (10, 25, and 50 μM), suggesting its beneficial effects at low concentrations. The significantly enhanced Se levels in seed and shoot tissues of OX plants compared to WT plants indicate the functional role of *OsSULTR1;1* in Se uptake from the rhizosphere. A rhizosphere acidification assay revealed that OX Arabidopsis plants exude more efficiently, causing a significant change in rhizosphere pH compared to WT plants. This suggests a secondary active transport mechanism. OX plants exhibited increased tolerance to abiotic stresses such as heat, cadmium, lead, and aluminum. Taken together, these findings suggest that *OsSULTR1;1* is a potential candidate gene for Se accumulation for biofortification purposes. It may also play a role in plant tolerance to climate change and environmental stresses, potentially contributing to sustainable agricultural productivity under global warming conditions.

KEYWORDS: Basmati rice, Sulfate transporters, Selenium application, OsSULTR1;1, Transgenic OX *Arabidopsis thaliana*

ÖZET

SELENYUMUN ÇELTİK BİTKİSİNDE SÜLFAT TAŞIYICI GENLER ÜZERİNE ETKİLERİ VE ARABİDOPSİS'TE SELENYUM HOMEOSTAZINDA *OsSULTR1;1* GENİNİN FONKSİYONEL KARAKTERİZASYONU
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BİYOLOJİ BÖLÜMÜ ANABİLİM DALI

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Pirinçteki sülfat taşıyıcılarının abiyotik ve ağır metal streslerinde rol oynadığı bilinmektedir. Ancak, bu taşıyıcıların düzenlenmesi ve fonksiyonel karakterizasyonları selenat muamelesi ile ilişkisi belirsizdir. Bu çalışma ile pirinçte sülfat taşıyıcılarının vejetatif ve üreme aşamalarındaki ekspresyon desenleri araştırılmıştır. Taşıyıcılar Se muamelesi ile çeşitli ekspresyon desenleri sergilemiştir ve dokuya özgü özellikler göstermiştir, bu da onların çeşitli fonksiyonel rollerini işaret etmektedir. Düşük kükürt (S) koşullarında (15 µM) ifadesi ve kök dokuya yüksek özgüllüğü nedeniyle *OsSULTR1;1* aday gen olarak seçilmiştir ve her iki özellik de Se muamelesiyle daha da artmıştır. Subselüler lokalizasyon, OsSULTR1;1 proteininin plazma zarına lokalize olduğunu ve Se'nin kök tarafından alınmasında rol oynadığını ortaya koymuştur. Daha ayrıntılı olarak karakterizasyon için ilgili gen *Arabidopsis thaliana*'ya aktarılmıştır. Arabidopsis'te genin aşırı ekspresyonu (OX), vahşi tip (WT) bitkilere kıyasla büyüme, gelişme ve mineral besin maddelerinin alımında artışa neden olarak etkin besin alımında rol oynadığını göstermiştir. Zaman ve konsantrasyona bağlı Se muamelesine maruz bırakılan OX bitkileri, dokularında önemli seviyelerde Se biriktirmiştir. Se'nin OX ve WT için yüksek konsantrasyonlarda (10, 25 ve 50 µM) toksik olduğu, düşük konsantrasyonlarda yararlı olduğu gösterilmiştir. OX bitkilerin tohum ve sürgün dokularında WT bitkilere kıyasla önemli ölçüde artan Se seviyeleri, *OsSULTR1;1*'in rizosferden Se alımındaki fonksiyonel rolünü göstermektedir. Rizosfer asitleştirme deneyi, OX Arabidopsis rizosfer pH'sında WT bitkilere kıyasla önemli bir değişiklik yaratarak daha verimli bir şekilde dışarı attığını ortaya koymuştur. Bu, ikincil aktif taşıma mekanizmasını işaret etmektedir. OX bitkiler ısı, kadmiyum, kurşun ve alüminyum gibi abiyotik streslere karşı artan tolerans sergilemiştir. Bulgular bir araya getirildiğinde, OsSULTR1;1'in biyofortifikasyon amaçlı Se birikimi için potansiyel bir aday gen olduğunu öne sürülmekye olup genin iklim değişikliği ve çevresel streslere karşı bitki toleransında rol oynayabileceği, küresel ısınma koşulları altında sürdürülebilir tarıma katkıda bulunabileceği öne sürülmektedir.

ANAHTAR KELİMELER: Basmati pirinci, Sülfat taşıyıcıları, Selenyum uygulaması, OsSULTR1;1, Transgenik OX *Arabidopsis thaliana*

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LIST OF ABBREVIATIONS AND SYMBOLS

µ : Micro
µg :Microgram
µL : Microliter
µM : Micromolar
ºC : Celsius degree
ADP : Adenosine diphosphate
Al : Aluminium
ANOVA : Analysis of variance
As : Arsenic
ATP : Adenosine triphosphate
B : Boron
BLAST : Basic local alignment search tool
bp : Base pair
Ca : Calcium
Cd : Cadmium
cDNA : Complementary DNA
Cu : Cupper
dH2O : Distilled water
DMF : Dimethylformamide
DNA : Deoxiribonucleic acid
dNTPs : Deoxyribonucleotide triphosphate
Fe : Iron
GFP : Green fluorescent protein
g : Gram (s)
K : Potassium
L : Liter
LB : Luria Bertani medium
mM : Millimolar
Mg :Magnesium
Mo : Molybdenum
Mn : Manganese
Ni : Nicklel

OX **:** Overexpression

PCR **:** Polymerase chain reaction

RNA **:** Ribonucleic acid

RNAase **:** Ribonuclease

ROS **:** Reactive oxygen species

S **:** Sulfur

Se **:** Selenium

SE **:** Standard error

SeCys **:** Selenocysteine

SeMet **:** Selenomethionine

Tris **:** 2-amino-2-(hydroxymethyl)-1,3-propanediol

UV **:** Ultraviolet

WT **:** Wild type

w/v **:** Weight to volume ratio

Zn **:** Zinc

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1. INTRODUCTION

1.1 Se Element Importance for Human

Scientists discovered selenium (Se), a crucial element for living things, in 1817 (1, 2). Initially overlooked, it gained attention after revealing its toxic properties (3). Later research found selenium prevents liver damage in rats, leading to the discovery of its essential functions in animals (1). Selenium acts as an antioxidant, protecting cells from harm by being a part of an enzyme called glutathione peroxidase (2, 4). When selenium levels are low, the body becomes more susceptible to various diseases, including cancers, infections, and heart problems (4-6). In severe deficiencies, specific diseases like Keshan and Kashin-Beck can develop (7, 8). Interestingly, recent studies suggest a link between low selenium levels and COVID-19. This finding indicates that selenium deficiency might be a marker for worse outcomes in COVID-19 patients (9).

1.2 Se Importance for Plant

1.2.1 Plant Uptake of Se

Selenium occurs naturally as Se^{2-}, Se^{0}, Se_2O3^{2-}, SeO_3^{2-}, and SeO_4^{2-} (10). Se is uptake by plant roots in different forms such as selenocysteine (SeCys), Selenomethionine (SeMet), selenite (Se(IV)) and selenate (Se(VI)) (11). With the exogenous level of Se, the level of inorganic Se increases than the organic Se content in leaves and roots.

Plants absorb selenium via root tissues primarily in two forms: selenate and selenite (12, 13). Interestingly, the same transporters (sulfate transporters) that uptake sulfate, a mineral like selenate, are also responsible for selenate uptake in plants. This phenomenon was discovered by studying a mutant of Arabidopsis plant that was resistant to selenate. It was found that these resistant plants had mutations in a gene that encodes a transporter with a high affinity for sulfate, called AtSultr1;2 (14, 15). This suggests that this transporter also plays a role in selenate uptake.

1.2.2 Beneficial Effects of Se on Rice

Rice, a food source for over half the world, is grown in many countries, with most production coming from Asia (16). Unfortunately, rice plants can absorb harmful elements like arsenic (As), cadmium (Cd) and lead (Pb) from the soil. These elements accumulate in significant amounts in edible parts and can cause health problems. However, it was found that fine aromatic rice (Basmati rice)

accumulates much lower levels of As compared to coarse rice cultivars. A study showed that almost 70% reduction in As uptake in consuming 500 grams of aromatic rice compared to non-aromatic rice. Additionally, aromatic rice accumulates significantly high level of Se than in coarse varieties (17). Therefore, consuming aromatic rice as a staple food would not only reduce intake of toxic heavy metals but also improve human health by intake of Se. Se priming was performed for improving emergence and seedling growth of two basmati rice cultivars (Super and Shaheen Basmati). Treating rice seeds with selenium (Se priming) led to faster sprouting and stronger seedlings in both types of Basmati rice tested, compared to untreated seeds. This suggests Se priming benefits rice growth regardless of the specific variety. Investigators observed several changes in Se-primed seeds that might explain these improvements. The electrical conductivity of the seed leachates was lower, indicating less damage to cell membranes. Additionally, Se priming reduced lipid peroxidation and increased the activity of α-amylase enzyme that breaks down starches for energy. Se-primed seeds also contained higher levels of soluble sugars, readily available for seedling growth. Finally, Se priming boosted the activity of several antioxidant enzymes (SOD, POX, CAT, and GPX) that protect cells from stress. These findings suggest that Se priming improves rice emergence and seedling vigor by enhancing starch metabolism, stabilizing cell membranes, and increasing antioxidant activity (18). Previous studies showed that foliar spray with a selenium-rich fertilizer significantly increased the amount of selenium in the rice grains. This fertilizer, applied at a rate of 20 grams of selenium per hectare, came in two forms: sodium selenite and sodium selenate. Interestingly, selenate proved more effective, boosting selenium content in rice by 35.9% compared to selenite. This research suggests a promising method for creating selenium-rich rice. By consuming 400 grams of rice grown with this method and containing a controlled amount of selenium (between 0.3 and 0.5 micrograms per gram), people could increase their daily selenium intake by 100 to 200 micrograms (19).

1.2.3 Negative Effects of Se on Plant Growth

Although Se can benefit rice growth, however, high concentration proved to be phytotoxic. Studies showed that excessive Se exposure stunted rice plant growth and reduced overall plant mass. This was linked to a decrease in chlorophyll,

carotenoids, and soluble proteins which are essential for healthy plant development. It was also found that Se accumulated most heavily in the leaves, followed by the leaf sheaths and roots. Additionally, high Se levels appeared to worsen its toxic effects by disrupting the plants' oxidative metabolism. This was seen through increased levels of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radicals, and lipid peroxidation products which are the indicators of oxidative stress (20). When grown in soil high in selenium (seleniferous soil), two rice varieties, PR116 and Pusa Basmati 1121, experienced stunted growth during their early development. Additionally, flowering was delayed by 10 days in these plants compared to those grown in normal soil. Selenium content also increased significantly in both rice varieties. Leaves showed the highest increase, with levels going up 3 to 20 times higher in Pusa Basmati 1121 and 13 to 14 times higher in PR116. Grain selenium content also increased, but to a lesser extent, with Pusa Basmati 1121 showing an 18-fold increase and PR116 a 3-fold increase. Interestingly, high selenium levels also triggered the production of enzymes and biochemicals associated with reactive oxygen species (ROS). ROS can damage cells, so this suggests a cellular stress response to the excess selenium (21). It is also evident that Basmati rice cultivar has a higher level of Se accumulation capacity. These findings show that doses of Se based fertilizers should be optimized in advance for the rice cultivars and the Se content of the soil of the field, to avoid the loss in plant growth and development as well as yield loss.

1.2.4 Synergisitic or Antagonistic Effects of Other Nutrient Elements on Se Uptake

Se and auxin have synergistic effect in rice to reduce the accumulation of heavy toxic metal As which poses health risk in human. When plants are exposed to both Se and auxin, their tolerance to As improves. This improved tolerance likely results from a combination of beneficial effects happening at the cellular, the molecular, and through biochemical level (22). These results show that simultaneous application of auxin and Se reduce the uptake of toxic heavy metal at root interface. The best crops for people to eat are those that are high in essential nutrients, like selenium, but don't take up too many harmful elements like arsenic in the parts we consume. Some mineral elements like N, P, S impact the accumulation and bioavailability of Se in plant tissues. Reis, de Queiroz Barcelos (33)) shown that applying more nitrogen fertilizer (N-fertilizer) to crops like rice

and wheat can lead to higher levels of selenium (Se) in the edible parts. Similarly, Chen, Liang (34) demonstrated, when plants receive more nitrogen, it boosts their sulfur (S) metabolism. This interaction between N and Se can be explained as follows: (1) This is because nitrogen increases a key molecule called O-acetyl serine, which is crucial for making cysteine, an amino acid containing sulfur (35). Since selenium can use the same pathway as sulfur in plants, this increased activity also promotes Se uptake and its incorporation into selenoproteins, beneficial proteins containing selenium. (2) Nitrogen fertilizer helps plants grow more vigorously, leading to a stronger root system. This improved root system is better at absorbing various minerals from the soil, including essential nutrients like phosphorus (P), potassium (K), sulfur (S), and even Se. (34); (3) Nitrogen fertilizer applied to soil is mostly in the form of nitrate (NO_3-N). When applied in high amounts, this nitrate can actually bump selenium (Se) anions off the surfaces of soil particles. This releases more Se into the soil solution in the form of selenate (SeO_4^2). Since plants absorb nutrients dissolved in the soil solution, this increase in Se availability makes it easier for plants to take up selenium (36). Similarly, Klikocka, Szostak (37) found that spring wheat plants accumulated more selenium (Se) in their grains as the amount of nitrogen fertilizer (N-fertilizer) applied to the soil increased. This suggests that using nitrogen fertilizer can be a helpful strategy for plants to take up more selenium from the soil.

Zhang, Dong (23) reported that adding phosphorus (P) fertilizer to wheat grown in high-calcium soil (calcareous soil) impacted selenium (Se) uptake differently depending on the Se form: Selenite (Se(IV)): P fertilizer decreased Se uptake by wheat. Selenate (Se(VI)): P fertilizer increased Se uptake by wheat (24). This difference is because P makes selenate more available for plants, but also lowers soil pH, which activates iron ions converting soluble Se in the soil into iron oxide Se and organic Se (38). Being neighbors on the periodic table (both chalcogens), sulfur (S) and selenium (Se) share a lot of chemical similarities. This translates to plants using the same pathways to absorb and incorporate both elements. Interestingly, selenate (Se(VI)), with its structure resembling sulfate ($SO4^2$-), gets taken up by plants through the sulfate transporters in the root cell membranes (12). The type and amount of S fertilizer used on soil can significantly affect how much Se plants can absorb. This applies to a wide variety of plant species (25).

1.3 Sulfur and Sulfate Transporters for Selenate Uptake

Sulfur is an important mineral which is categorized as essential macro-element for the plant growth and development. Sulfur is taken up from rhizosphere by group of proteins making a gene family called sulphate transporters. Sulphate transporters are classified into various clades based on their affinity for sulphate as a substrate and also their compartmentalization. The sulfate transporters are classified into four distinct groups viz. group 1 (high affinity), group 2 (low affinity), group 3 (chloroplastic SO^{2-}_4) and group 4 (vacuolar SO^{2-}_4 transport) (26). First analogue/orthologue of sulphate transporter was identified in yeast cells. Later first analogue of sulphate transporters was found in Arabidopsis plant and its functional characterization was performed to reveal its biological function and role in plant growth development. Sulphate transporters not only have affinity for sulphate as a substrate but also these proteins transport other anions such as Se in the form of selenate, arsenate and molybdate (27).

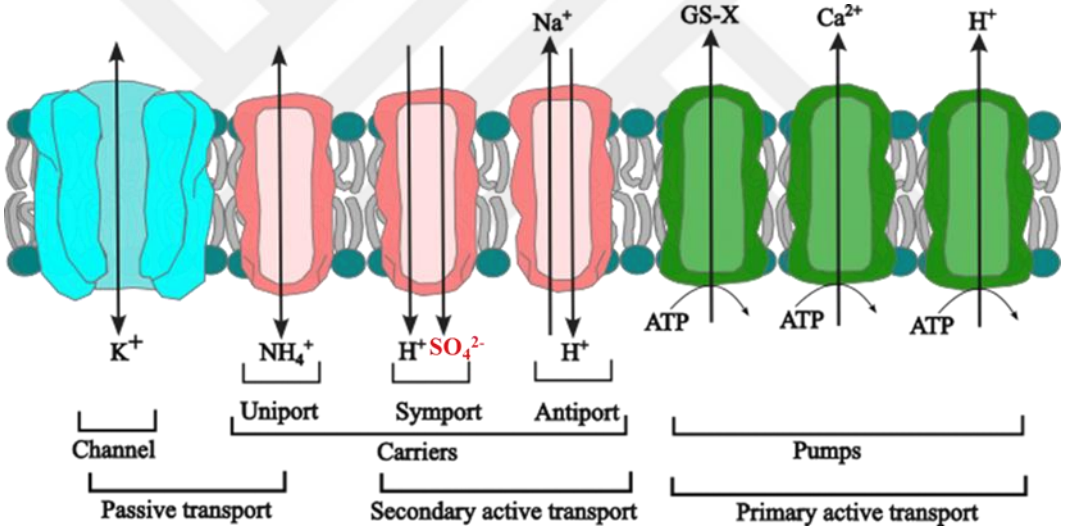

Figure 1.1 Classification of transport membrane proteins for the uptake and transport of various nutrients in the root system. The figure is adapted and modified from (28). Sulfate ions uptake by secondary active transport mechanism, like SO_4^{2-}/H^+ symporter. The figure is adapted and modified from (28) and following (29) for the sulfate uptake via secondary active transport system.

Mineral ions are uptake (influx) or moved out of the cells (efflux) by well co-ordinated transport system which is localized at plasma-membrane, vacuolar membrane (tonoplast) or other organelle.

Sulfate ions existed in apoplastic region taken up (influx) generally by secondary active transport system mediated by sulfate transporters (30) which is mainly evident by due to the formation of proton gradient across the plasma-membrane

(31, 32). Further, more specifically, two members of high-affinity sulfate transporters SULTR1;1 and SULTR1;2 are also part of active transport system for the influx of sulfate ions at the root interface (30).

In addition, selenate uptake facilitated by AtSULTR1;1 and AtSULTR1;2 also follows the pathway of active transport system (15). Thus, high affinity sulfate transporters also follow the secondary active transport pathway for sulfate uptake at root interface. AtSULTR1;1 and AtSULTR1;2 are the major transporters responsible for sulfate assimilation and are enhanced under sulfur-deficient situations at transcriptional and protein levels (33).

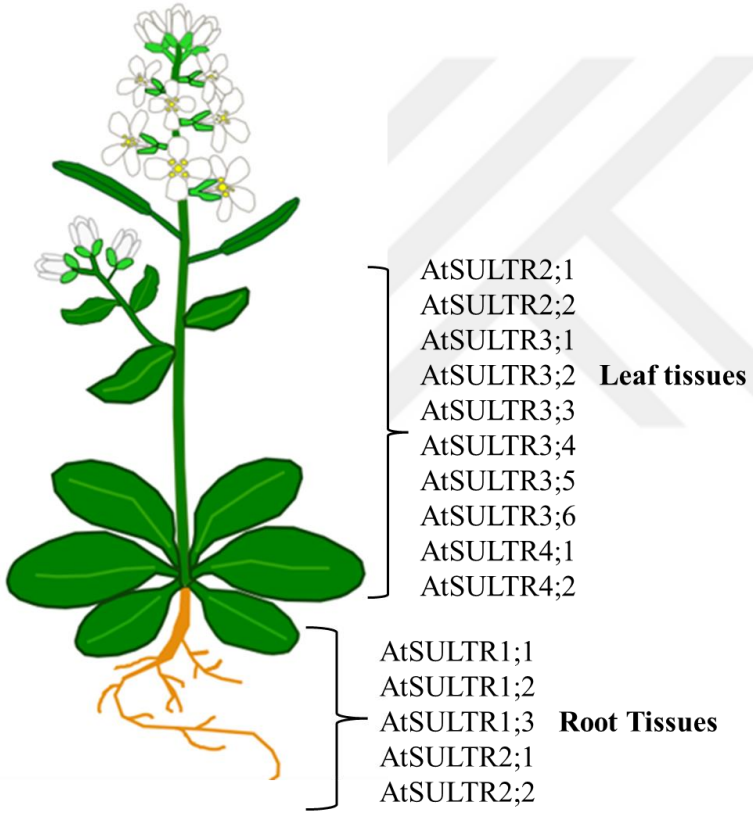

Figure 1.2 Sulfate transporters in Arabidopsis for the uptake and translocation of sulfate. The information of genes for tissue specificity adapted from (34) and (35).

1.3.1 Regulation of Sulfate Transporters by Se Applications

Plant response to Se concentration in soil differently depending on the plant species, while some plants are sensitive and others are hyperaccumulator (12). *Stanleya pinnata* is a hyperaccumulator of selenium which accumulates 0.5% of Se to its dry weight. Further it also exhibits a higher ratio of Se to Sulfur (S) in root and shoot tissues. Normally S inhibits or lowers the uptake of Se as a competition but this hyper accumulator Se plants competes Se over S level. These high level of

Se uptake is attributed possibly due to higher expression of SULTR1;2 and SULTR2;1. On the other hand, non-hyperaccumulator *Stanleya elata* performed vice versa showing low level accumulation of Se than S and as well as lower expression of the sulfate transporter genes (36). SULTR1;1 is highly selective to uptake selenate and dependent under sulphate deficiency only (37). Hyperaccumulator plants exhibit different expression patterns of SULTR1;1 and SULTR1;2 under sulfate and selenate condition. In hyperaccumulator S. pinnata under sulfate deficiency SULTR1;1 induced but reduced to negligible expression under S and Se application. However, SULTR1;2 expression was comparable under S and Se and high expression level was observed.

Unlike regular rice, red rice rich in Se is more susceptible to damage from high Se applications. Interestingly, these Se-rich rice plants can take up more Se from their roots in environments with low levels of selenate. However, this increased uptake is reversed at high selenate concentrations. Furthermore, excessive selenate can harm these red rice plants by causing several issues: yellowing leaves, stunted growth, reduced levels of antioxidants, and worsened oxidative stress.

The study also found that selenate exposure increases the activity of genes responsible for encoding transporters for phosphate (OsPT2) and sulfate (OsSultr1;2). These transporters likely play a role in Se uptake by the rice plants. Finally, the research suggests that a selenium concentration of 20 micromolar (μM) is ideal for the root environment of Se-rich red rice grown in a hydroponics. (38). These findings also point out that the rice cultivar or genotype having higher capacity of Se accumulation is not necessarily correlated with high and toxic level of Se application.

1.3.2 Regulation of High Affinity Sulfate Transporters Under Stresses

Exposure to chromium (Cr) stunted the growth of wheat seedlings. This appears to be linked to a decrease in the activity of the ascorbate-glutathione cycle (important for antioxidant defense) and a down-regulation of a gene (SULTR1;2) responsible for high-affinity sulfate transport (39). Interestingly, adding Se to the Cr treatment had a different effect. In this case, the expression of SULTR1;2 remained similar to the control group (CK). However, when selenium was combined with the plant hormone ABA (abscisic acid), the expression of SULTR1;2 significantly increased compared to CK. The addition of Se, with or

without ABA, also stimulated the expression of another sulfate transporter gene (SULTR1;3). Interestingly, ABA treatment alone also significantly increased the expression of both SULTR1;2 and SULTR1;3 compared to CK. These findings suggest that combining Se and ABA treatment triggered a stronger response in the wheat seedlings, potentially leading to greater absorption and transport of S and/or Se into the chloroplasts (40).

1.3.3 SULTR1;1 Expression in Se Hyperaccumulator

In other Se hyper accumulator expression level of SULTR1;1 was higher in both Sulfate and Se application than in *S. pinata* (41). These results show that Se hyperaccumulators have different tendencies for employing diverse sulfate transporters and are independent or dependent on S application.

It was shown that how plants take up S and Se in two Astragalus groups: hyperaccumulators (high Se uptake) and non-accumulators. They found that low sulfur levels led to increased Se accumulation, while high Se supply boosted sulfur uptake in both root and shoot tissues. This suggests a potential link between the uptake pathways for these two elements. Unlike other plants studied so far, researchers found high levels of activity for genes related to sulfate transporters in both Se-hyperaccumulating and non-accumulating Astragalus species. This high expression was consistent regardless of whether the plants were low on sulfur or selenium. Additionally, the amount of these gene transcripts in Se-hyperaccumulators was similar to what's seen in other plants when they're starved for sulfur. This suggests that Astragalus species might have a different way of regulating these transporters, potentially contributing to their ability to take up and move selenium around, especially in Se-hyperaccumulators (41).

Beside Se transport via sulfate transporters, other membrane transporters such as phosphate transporters also reported to be involved in Se (as a selenite form) uptake and transport in plants.

A study investigated genes involved in sulfur transport in a plant called S. pinnata. They found that two genes, *SULTR1;2* and *SULTR2;1*, were constantly turned on at higher levels than normal. These genes are responsible for bringing sulfur into the roots (*SULTR1;2*) and moving it around within the plant (*SULTR2;1*). Interestingly, another gene (*SULTR1;1*) involved in sulfur uptake from the roots was found to be less active. While this gene can also take up

selenium, it seems to only be important when the plant is deficient in sulfur (37). S. pinnata (hyperaccumulator) plant has very high levels of *Sultr1;2* but negligible levels of *Sultr1;1*, even under sulfur deficiency (when *Sultr1;1* usually increases in other plants). Both *Sultr1;1* and *Sultr1;2* are expressed at significant levels, with *Sultr1;1* being particularly abundant in S. elata (non-hyperaccumulator) and both transporters being similar in *Brassica juncea*. The downregulation of *Sultr1;1* (*S. pinnata*), which can take up Se, suggests it might not be a major player in Se uptake for this hyperaccumulator. This raises the possibility that *S. pinnata*'s high reliance on *Sultr1;2*, which might be more selective for selenate compared to sulfate, contributes to its high Se accumulation. The presence of both *Sultr1;1* and *Sultr1;2* likely allows non-hyperaccumulators to take up both S and Se more readily. Overall, the study suggests that the unique expression patterns of sulfate transporter genes, particularly the downregulation of *Sultr1;1*, might be a key factor in how *S. pinnata* hyperaccumulates Se (36).

1.3.4 Transgene Expression of SULTRs

Two genes (SULTR1;1 and SULTR1;2) important for sulfur uptake in plants were investigated. Mutant plants were developed missing both of these genes and found it had stunted growth and couldn't take up sulfate effectively. To test the function of these genes, also created modified versions with a special tag that allowed them to be tracked. These modified genes, under the control of a strong promoter, were introduced into the mutant plant. Interestingly, the mutant plant recovered its ability to take up sulfate and grow normally when it had either of the modified genes. The researchers also found that both modified genes were mainly active in the roots and became more active when the plant was low on sulfur. This suggests that these genes play a crucial role in root sulfur uptake, and their activity increases when sulfur is scarce (33). Findings suggest that *Sultr1;2* is a key transporter for chromium (Cr) uptake in plants like Arabidopsis. Over-expressor plants took up significantly more Cr, with levels in the shoots being 1.6 to 2 times higher compared to normal plants. In contrast, *Sultr1;1* appears to play a minor role in Cr uptake. Similar study in a single-celled green algae (*Chlamydomonas reinhardtii*) showed that increasing the activity of a different low-affinity sulfate transporter (SULTR2) also led to higher Cr accumulation. These findings highlights the potential role of sulfate transporters in chromium uptake by plants and algae.

(42). Members of *SULTR3s* are chloroplast localized and they play role in chloroplast sulfate uptake and affect ABA biosynthesis and the stress response (43). loss of function mutation in *SULTR3;1* significantly decreases the sulfate uptake of the chloroplast. However, complementation assay confirmed that the *AtSULTR3;1* is involved in chloroplast sulfate transport (44). Under S deficieny, vacuolar transporters *SULTR4;1* and *SULTR4;2* efflux sulfate to short and long distance (30, 45). In heterologous experession system of yeast, sulfate uptake was negligible when expressed alone *SULT3;5*, however, remarkable amount was detected when co-expressed along with *SULTR2;1* indicating protein-protein interaction among sulfate transporters (46). Deletion of phloem companion cells localized *AtSULTR1;3* inhibited sulfate translocation from cotyledons to root and shoot tissues (47). Sulfate transporters also involved in transporting other anions like molybdate other than selenate so also these anions compete with sulfate ions for the transport system. For example, high affinity sulfate transporter *AtSULTR1;2* and *MOT1* (*AtSULTR5;2*) (48) are reported to uptake and transport molybdate. Although the uptake level of molybdate was not high in case of *AtSULTR1;2* (48).

1.4 Se Uptake by Aquaporin and Phosphate Transporters

In plants selenate is up taken by sulfate transporters but selenite uptake is mediated by few diverse transporters including OsNIP2;1 (49) and OsPT2 (50). In rice plants, selenite – a common form of Se found in flooded paddies – enters the plant through a transporter called Lsi1 (OsNIP2;1), which usually carries silicon. When this transporter was malfunctioning in rice mutants, the amount of Se in the shoots and xylem sap significantly decreased after exposure to selenite. Interestingly, this effect wasn't observed with selenate. An additional experiment showed that selenite uptake by rice increased significantly at the lower pH (49). Rice plants engineered with OX of OsPT2 transporters absorbed significantly more selenite. Conversely, mutant plants of OsPT2 showed decreased selenite uptake. This suggests OsPT2 plays a key role in selenite uptake. Rice plants with OX of OsPT2 also had higher levels of Se in their grains, further supporting the link between OsPT2 and selenite uptake. These findings indicate Pi, and Si transporters likely share similar transport mechanisms in selenite uptake in rice. OsPT2 (50) and OsNIP2;1 (49) provides a potential strategy for breeding Se-enriched rice varieties. In addition, *Taesultr4.1* in wheat was highly expressed under selenite application

in Se-tolerant wheat genotype Puelche, suggesting possibility of the efficient vacuolar accumulation of Se in this geotype compared to Se sensitive wheat genotypes (51).

1.5 Rice Sulfate Transporters and Their Functional Characterization

Few rice sufate transporters have been characterized so far, however, the functional characterization of other sulfate transporters remains to be elusive. High affinity sulfate transporter *OsSULTR1;1* is root tissue specific and inducible under S deficient condition. In addition, *OsSULTR1;1* upregulated during As(V) and Cd stresses but not under Pb and Cr. *OsSULTR1;1* also induced under cold and drought stresses. The expression of *OsSULTR1;1* also upregulated due to phytohormones such as IAA and *trans*-zeatin. Overexpression of *OsSULTR1;1* in Arabidopsis exhibited metal and abiotic stress toleranance such as arsenite As(III), arsenate As(V), chromium, cadmium and mannitol (52). OsSultr3;3, is involved in sulfate homeostasis and metabolism which is localized to in the endoplasmic reticulum (53) and responsible for low phytic acid upon function mutation (54). SULTR-like phosphorus distribution transporter (SPDT) homolog of *OsSULTR3;4* which is responsible for decreased level of phosphorus accumulation in rice grains with loss of function mutation in the node (55).

However, todate, rice sulfate transporters are not functionally characterized for their Se uptake and accumulation in plant tissues including shoot and seeds. The goal of this study is to explore the sulfate transporter genes based on the expression level under Se level in Basmati rice. The selecting the highly expressed *SULTR* gene for its ectopic expression and functional characterization in transgenic developed *Arabidopsis thaliana* plants.

2. AIM AND SCOPE OF THE STUDY

Selenium in the form of selenate is highly accumulated in Basmati rice (aromatic, fine rice). Se is also beneficial for human health. However, the genes responsible for the uptake of Se and their regulation and subcellular localization remain elusive in rice. In addition, the functional characterization of the gene responsible for accumulation of Se into seeds and tissue of plant needs to be determined. In order to address these unanswered questions, the following aims were planned and executed.

The study was partitioned into two parts.

2.1 Identification of Se Regulated Sulfate Transporters in Rice

The aim of the first part was to identify the sulfate transporters inducible due to Se application in Basmati rice. Then subsequent studies were aimed at cloning and subcellular localization of the rice Se inducible sulfate transporter gene were aimed. In addition, the poteantial affinity for Se uptake and accumulated Se content determination in aromatic rice under vegetative and reproductive stage as well as pH dependency was focused.

2.2 Functional Characterization of *OsSULTR1;1* in Transgenic Arabidopsis Plants

The second aim of this study was the genetic transformation of Se inducible sulfate transporter gene into *Arabidopsis thaliana* for the transgenic plants' development for its functional characterization. The functional characterization was aimed to learn Se uptake and accumulation at vegetative (seedling stage) and reproductive stage (seed and shoot tissues) of the transgenic plants. In addition, the effect of Se inducible sulfate transporter on phenotype of transgenic plants and as well as synergistic effect of Se on the accumulation patterns of other mineral nutrients in seeds and shoots was planned. The purpose of this part was to determine the capacity of rice sulfate transporter gene for the accumulation of Se in shoot and seeds due to its ectopic expression. In addition, abiotic stresses mitigation effect of the transgene was aimed to determine its potential impact under S deficiency and minimal Se presence.

The scope of the study is to pave the way for the better understanding of rice high affinity sulfate transporter and also Se biofortification of food crops or forage

crops utilizing sulfate transporters in breeding programs or via transgenic or genome editing approaches. This study will help out not only to understand Se accumulation patterns but also synergisitically other nutrients as well via sulfate transport system. The understanding of sulfate transport system in Se accumulation in tissues especially edible parts in other cereal or legume crops for Se biofortification will be facilitated.

3. MATERIALS AND METHODS

3.1 First Part of the Study

3.1.1 Sequences and Phylogenetic Tree of Sulphate Transporters

Sequences of the genes encoding rice sulphate transporter proteins were retrieved from NCBI (56) by using accession numbers which were reported previously (29, 35). Sequences of *Arabidopsis thaliana* sulphate transporters were retrieved from Phytozome database (57) using accession numbers as mentioned already (35). Phylogenetic tree (Figure 3.1) was developed by using protein sequences of *Arabidopsis thaliana* and rice sulphate transporters by neighbor joining method using MEGA software (58).

3.1.2 Plant Materials and Growth Conditions

Rice (*Oryza sativa* L. indica) CV. Super Basmati was used in this study. Growth conditions were followed as described previously (50) with slight modifications. Rice seeds were surfaced-sterilized, rinsed with sterile deionized water, and germinated on filter papers in an incubator at 28°C in darkness. Once the rootlets reached 2 centimeters (Figure 3.1), the seedlings were placed in a diluted Kimura B nutrient solution for pre-conditioning (59) in a growth chamber, with a light: dark cycle of 16: 8 h (24: 18°C). The light intensity and humidity were c. 300 µmol m^{-2s-1} and 65% respectively. After three days, the seedlings were moved to a full-strength nutrient solution for further growth over twelve days and similar size seedlings were transferred to 5 L containers. To maintain pH 5.6, every day 1 mM HCl or 1 mM NaOH was used. The solution was refreshed every four days.

Figure 3.1 Seeds of Super Basmati rice variety germinating on floating net.

3.1.3 Se Uptake Experiment

Rice seedlings were subjected to Se uptake at two different growth stages. First, at vegetative (30 d) (Figure 3.2) and for the reproductive stage (70 d) with respect to days after transplant (DAT) of seedlings to hydroponic medium, the seedlings were continued to growth from vegetative (Figure 3.3) to seed development stage (Figure 3.4). After a thorough rinsing with distilled water, the seedlings were placed in either a regular nutrient solution or one

Figure 3.2 30-day-old Basmati rice seedlings used for Se uptake experiments.

specifically lacking in sulfur (S-deficient). This modified solution was based on the Kimura B nutrient. For comparison, a separate group of seedlings received the standard, unmodified Kimura B solution. In the S-deficient solution$MgSO_4$, $ZnSO_4$, and $CuSO_4$ were substituted by an equimolar amount of corresponding chloride salts. For vegetative growth stage after 3 d, the seedlings were transferred to normal (0.0 µM Na_2SeO_4) or S-deficient medium containing 0.25, 2 and 4 µM Na_2SeO_4 for another 3 d, and then the tissues (roots, shoots) were rinsed, dried at 70°C for 2 d and analyzed for Se content. However, Se treatment in case of developing seeds, 2 µM selenate was supplied throughout the treatment period. The developing seeds (Figure 3.5 and 3.6) at days

Figure 3.3 Vegetative growth stages of rice plants growing in ½ Kimura B solution.

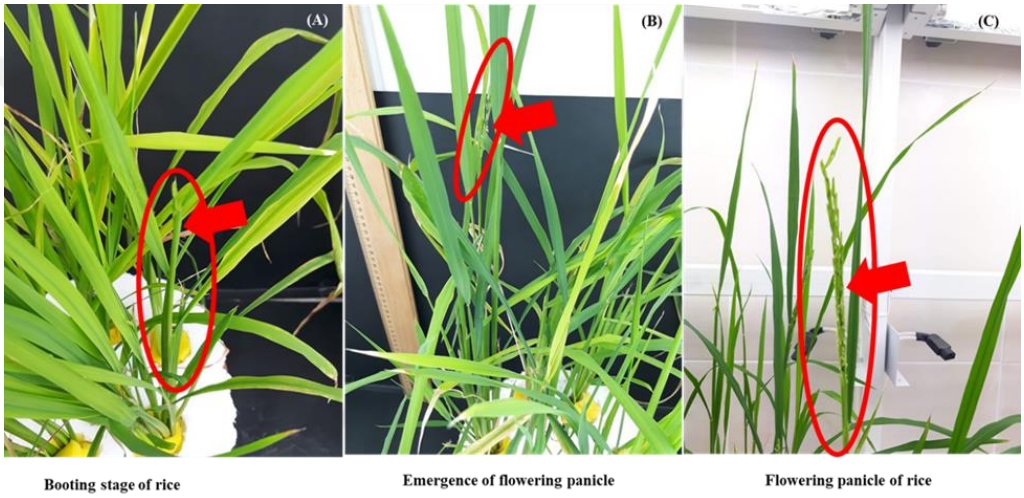

Figure 3.4 Rice plants at the reproductive stage. (A) shows the boot stage of rice plants, approximately 70-80 days after germination. (B) shows the emergence of inflorescences of rice plants approximately 80-90 days after germination. (C) shows the flowering panicle stage of rice plants approximately 90–100 days after germination.

10 (milky stage), 17 (soft dough seed stage) and 25 days (hard dough seed stage) were harvested for Se analysis. The rice tissues from the same treatments were also frozen in liquid nitrogen and stored at -80°C for RNA isolation and gene expression studies.

Figure 3.5 Seed development stage of rice plants approximately 100-120 days after germination.

Figure 3.6 Rice harvested at different growth and development stages used for determination of Se content and gene expression studies. 10 days = milky stage, 17 days = soft dough stage, 25 days = hard dough stage.

3.1.4 Effect of pH on Selenate Uptake in Rice

pH dependent selenate uptake experiment was performed as described previously (49) with slight modification. To determine the effect of pH on selenate uptake in rice, a short duration (60-minute) experiment was conducted. Fourteen-day-old seedlings (Figure 3.7) were exposed to a nutrient solution with a fixed selenate concentration (2.5 µM) but varying pH levels (3.5, 4.5, 5.5, 6.5, or 7.5) maintained by a buffer (5 mM MES). After one hour, the roots were meticulously rinsed to remove any surface solution, then separated from the shoots and dried.

Notably, the solution's pH remained stable throughout the experiment. The amount of selenium accumulated in the roots was measured using a method as described below.

Figure 3.7 14-day-old Super Basmati rice seedlings used for pH-dependent selenate uptake. The pH values used were 3.5, 4.5, 5.5, 6.5 and 7.5, and they were adjusted with 5 mM MES buffer.

3.1.5 Expression Analysis Using qRT-PCR

20 days old rice seedlings were used for this study (Figure 3.8). The selenium application is same as described under the heading of **3.1.3 Se Uptake Experiment.** RNA (total) from root, shoot and developing rice grains was isolated using the GeneJET RNA Purification Kit (Thermo Scientific, USA) following the manufacturer's instructions, followed by treatment with RNase-free DNase (Thermo Scientific, USA) to remove any genomic DNA contamination as reported earlier (60, 61). The integrity of RNA was determined by gel electrophoresis on 1.2% agarose gel. Real time qRT-PCR was performed in 20 µL reaction volume including 2X Fast SYBR® Green Master Mix by using StepOnePlus™ Real-Time PCR System (Thermo Scientific, USA). The list of selected genes and primers employed in the study shown in Table 1. The rice *Actin* (62) and *Histone H3* (63) was used as a reference gene to normalize the transcript level in root, shoot and developing seeds respectively. This is Two-step PCR. The real time PCR reaction conditions were; Step 1: initial denaturation at 95°C for 20 seconds and Step 2: denaturation at 95°C for 3 seconds and primer annealing temperature was 60°C for 30 seconds for 40 cycles. The melting curve reaction conditions were followed; denaturation at 95°C for 15 seconds, primer annealing temperature 60°C for 60 seconds and final denaturation at 95°C for 15 seconds was performed following manufacturer's instructions. The quantification of threshold cycle (CT) value analysis was calculated by the $2^{(-\Delta\Delta CT)}$ method (64).

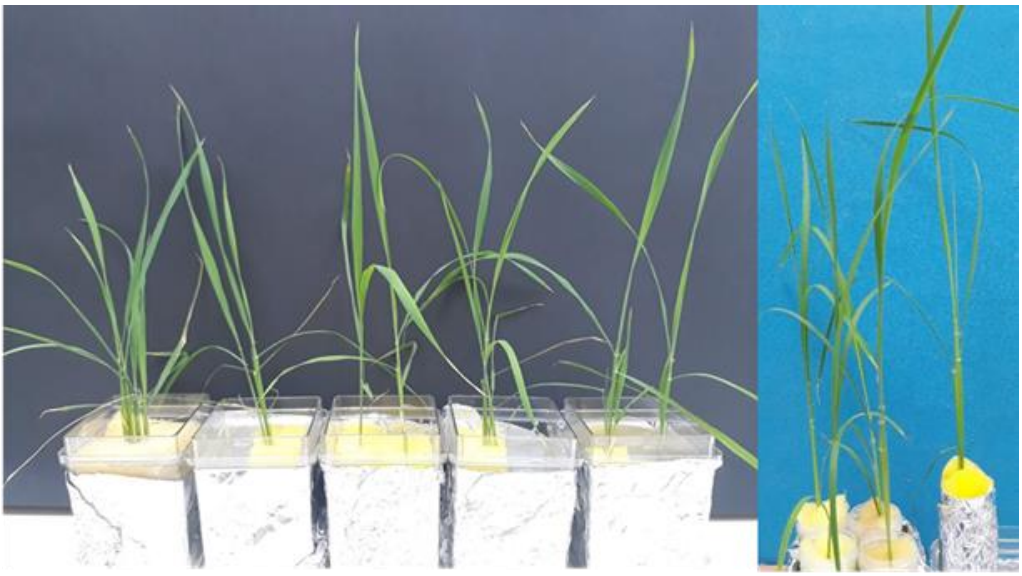

Figure 3.8 20-day-old Basmati rice seedlings used for gene expression studies.

3.1.6 Subcellular Localization of Sulphate Transporters

The sulphate transporter gene with the highest expression in root especially under selenate application was selected for subcellular localization. The selected rice sulphate transporter protein was monitored by transient expression using GFP protein as a reporter system. The open reading frames (ORFs) of selected sulpahate transporter without a stop codon was amplified by using the gene specific primer. To determine subcellular localization, procedure was carried out as described previously (61). Gene of interest (GOI) *OsSULTR1;1* was cloned using gene specific forward and reverse primers (Table 1) including Gateway cloning specific nucleotide sequences.

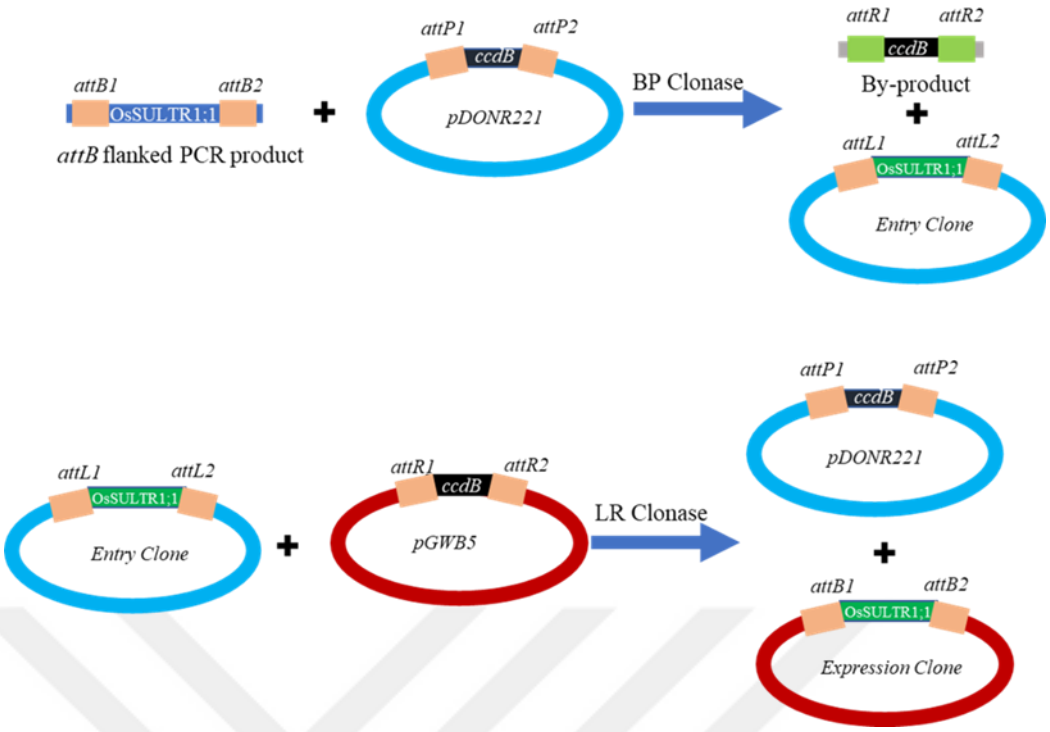

Figure 3.9 Illustration for the development of expression construct of the *OsSULTR1;1*. *OsSULTR1;1* gene specific primer pair containing attB1 and attB2 flanking sequences used to clone the gene, purified PCR products were recombined with pDONR221 plasmid using BP clonase reaction. The resultant entry clone of OsSULTR1;1 was obtained. Then entry clone was recombined with expression plasmid pGWB5 via LR clonase reaction. The end product obtained was expression clone of pGWB5::OsSULTR1;1.

The entry clones and expression clones were developed by Gateway cloning procedure Figure (3.9). The sequence of the *OsSULTR1;1* was verified by Sanger sequencing and the sequenced was deposited in GenBank under accession PP697850.1. Expression construct was transformed into *Agrobacterium tumefaciens* C58C1 strain then infiltration of *Agrobacterium* was carried out following the procedure (65) into onion epidermal cells (Figure 3.10). Transient expression of protein in onion epidermal cells was monitored using the confocal laser microscope system C2si (Nikon, Japan).

Figure 3.10 Infiltration of bacterial cells into onion epidermal cells. Single colonies of *Agrobacterium tumefaciens* C58C1 transformed with plasmid construct of

pGWB5::OsSULTR1;1 or only empty plasmid pGWB5 was suspended in YEB liquid media. The collected pellete of bacterial cells were resuspended in transformation media and injected into onion epidermal cells. Then onion was wrapped with aluminium foil and palced under dark at 25°C for 3 days.

3.2 The Second Part of the Study

The selected rice sulfate transporter *OsSULTR1;1* gene was transformed into *Arabidopsis thaliana* plants for the functional characterization of the gene. The Se uptake and accumulation in wild type (WT) and transgenic Arabidopsis plants was determined in vegetative and reproductive phase. The aim of this study was to determine the capacity of rice sulfate transporter gene for the uptake of Se and accumulation at seedling stage, and also at reproductive stage in shoot and seeds by over-expression in transgenic plants.

3.2.1 Transformation of *OsULTR1;1* into *Arabidopsis thaliana* for Functional Analysis

Arabidopsis thaliana (Col-0) plants were grown to flowering stage at 25°C day/23°C night, and floral dip transformation (Figure 3.11) was performed as described earlier (66). *Agrobacterium tumefaciens* strain C58C1 harboring the binary plasmid pGWB5::OsSULTR1;1 used to develop transgenic plants. Bacteria grown in LB liquid culture (10 g tryptone, 5 g yeast extract, 5 g NaCl per liter water) at 28°C, 200 r.p.m. containing kanamycin (50 μg mL^{-1}). Cells were collected by centrifugation for 20 min at 5500 g and then resuspended in floral dip medium to a final OD600 of approximately 0.80 prior to use. The medium was composed of 5.0% sucrose and 0.05% (i.e. 500 μl L^{-1}) Silwet L-77 (OSi Specialties, Inc., Danbury, CT, USA). To confirm the transformants, putative transgenic seeds were surface sterilized and screened on MS agar media using 50 μg/mL hygromycin.

3.2.2 Se Uptake Kinetics in WT and Transgenic Arabidopsis Plants

Se uptake kinetics was performed as concentration and time dependent following the method mentioned previously (50). For concentration dependency of Se uptake, three seedlings were pretreated in Se-free solution (100 μM KH_2PO_4, 100 μM $Ca(NO_3)_2$ and 100 μM $MgCl_2$, pH 5.5) for 0.5 h, then transferred to the absorption solution containing 5 mM MES (2 morpholinoethanesulphonic acid), 0.5 mM $Ca(NO_3)_2$ and different Se concentrations (0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, and 9.6 μM Na_2SeO_4, pH 5.5). After 3 h treatment, the roots were rinsed with

Figure 3.11 Floral dip transformation in *Arabidopsis thaliana*. *Arabidopsis thaliana* wild type plants ready for floral dip transformation (upper panel). Floral shoots of Arabidopsis were dipped into transformation infilteration media containing Agrobacterium tumefaciens which harbor expression construct.

desorption solution (5 mM MES, 0.5 mM Ca $(NO_3)_2$ and 0.5 mM K_2SO_4 (pH 5.5)) and then soaked in the same solution for 15 min to remove external Se. The seedlings were blotted and used for Se analysis. For time dependency experiment, roots of three individual plants were pretreated in Se-free solution, then moved to treatment solution containing 2.5 μM Na_2SeO_4 for different time points (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 h). After the Se treatment, the roots were washed and dried for Se analysis.

3.2.3 Se Accumulation in Tissues of Transgenic Arabidopsis Plants

For the Se content at vegetative stage of the plants were determined by following the conditions as described earlier (67) with major modifications. Both types of seeds (WT and transgenic *Arabidopsis thaliana*) were placed on Kimura B growth medium and kept in the dark at 4°C for 3 days. Afterward, they were transferred to a growth chamber with specific conditions: 60% humidity, 16 hours of light per day with light (250 $\mu mol\, m^{-2}\ s^{-1}$), and a temperature of 25°C. To determine the response of WT and OX plants, seeds were germinated in a modified Kimura B medium low in sulfate (15 μM) and added varying amounts of sodium selenate (Na_2SeO_4) to represent different selenate concentrations (0, 2.5, 10, 25, and 50 μM). Following germination, phenotypic responses of both WT and transgenic seedlings were evaluated. The lengths of root, shoot, and plant height were measured using a ruler and number of leaves and lateral roots were counted manually. To determine the toxic level of Se, chlorophyll content was determined as described earlier (61). To determine the Se content in seeds of WT and transgenic Arabidopsis plants were grown in the peat soil as described earlier (66). The plants were supplied with Na_2SeO_4 at 2.5 uM concentration alongwith 15 μM sulfate every week until the seed maturation. The shoots and mature seeds of the WT and transgenic Arabidopsis were harvested, and Se content determined as mentioned below.

3.2.4 Analysis of total Se and Other Minerals

Se contents were determined as described previously with modifications (50) using seeds, shoots and seedlings of WT and OX plants. Briefly, the glass vessels were immersed in 10% HCl to remove Se contamination, and wshed with ddH2O (> 18 MΩ) and oven dried. Dried and homogenized samples (c. 200 mg) were weighed and put into 100 ml digestion tubes (TFM vessels). Then 6 mL of HNO3 (65%) and 2 mL of 30% H2O2 was added to wet the plant tissues. The samples were digested using microwave oven (Milestone Stard D, Sorisole Bergamo, Italy) following the manufacturer instructions. Once the samples cooled down, they were diluted with ddH2O to a final volume of 20 mL. Total Se was analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher Scientific, USA). In addition to Se, this study also analyzed the concentrations of essential mineral elements sulfur (S), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), boron (B), copper (Cu), Manganese (Mn), nickel (Ni),

molybdenum (Mo), and zinc (Zn) in the seeds and shoots of both wild-type (WT) and overexpressing (OX) plants. The mineral elements were quantified by Atomic Absorption Spectrophotometer (Shimadzu AA-7000) to detect the mineral contents of K, Mg, Fe, Ca, Cu, Mn, Ni and Zn. The concentration of Mo, S, and B was determined by ICS-3000 Ion Chromatography System (Thermo Fisher Scientific, USA). In addition, the P content of the shoots and seeds were determined calorimetrically at 660 nm using the spectrophotometer (68).

3.2.5 Root Acidification Assay

Bromocresol purple which is pH sensitive indicator was used for this assay following the procedure as reported earlier (69) with minor modifications. Briefly, kimura B solution was used to prepare 1% (w/v) agarose containing 15 µM $MgSO_4.7H_2O$ while the sulfate salts were replaced with chloride salts. Seeds of WT and OX were germinated on the bromocresol purple plates but also 7 days old seedlings were also placed for the assay.

3.2.6 Phenotypic Measurements

For the phenotypic analysis, seeds of WT, OX-1 and OX-2 were germinated in sterile peat media. The peat media from Klassman TS 1 was used for growing Arabidopsis plants. The nutrients added by the company were nitrogen (mg N/l): 140, phosphorus (mg P_2O_5/l): 160, potassium (mg K_2O/l): 180, magnesium (mg Mg/l): 100 including micronutrients. (accessed on 15 December 2023) https://www.agrohoum.gr/datafiles/files/TS1%20FINE.pdf. The growing conditions were 16 h light/8 h dark cycle and 25ºC, light intensity c. 250 µmol m^{-2s-1}. The humidity was controlled at 65%.

Water was supplied once a week. The plants were grown for the period of one month and data was collected. For rosette area, ImageJ was used (70). Plant height was measured using a ruler. The number of branches, pods, leaves, rosette, and seeds were recorded by manual counting. The pod weight was measured using analytical weigh balance.

3.2.7 Metal Stresses

3.2.7.1 Aluminium (Al) Stress

Al treatment was done as described earlier (71) with minor modifications. Brifely, 7 days old seedlings were exposed to without Al (control) and with Al (treatment) 50 µM at pH 4.5 adjsuted with 1N HCl using 100 mM $CaCl_2$ as a medium (Figure 3.12). $AlCl_3.6H_2O$ was used as source of Al. Se application (2 µM

Na_2SeO_4) was performed to the Al exposed seedlings to determine its mitigating impact. The seedlings assembly holder was prepared for this experiment especially. The seedlings were treated under control and Al treatments for 24 hours. The root lengths were measured before and after treatment.

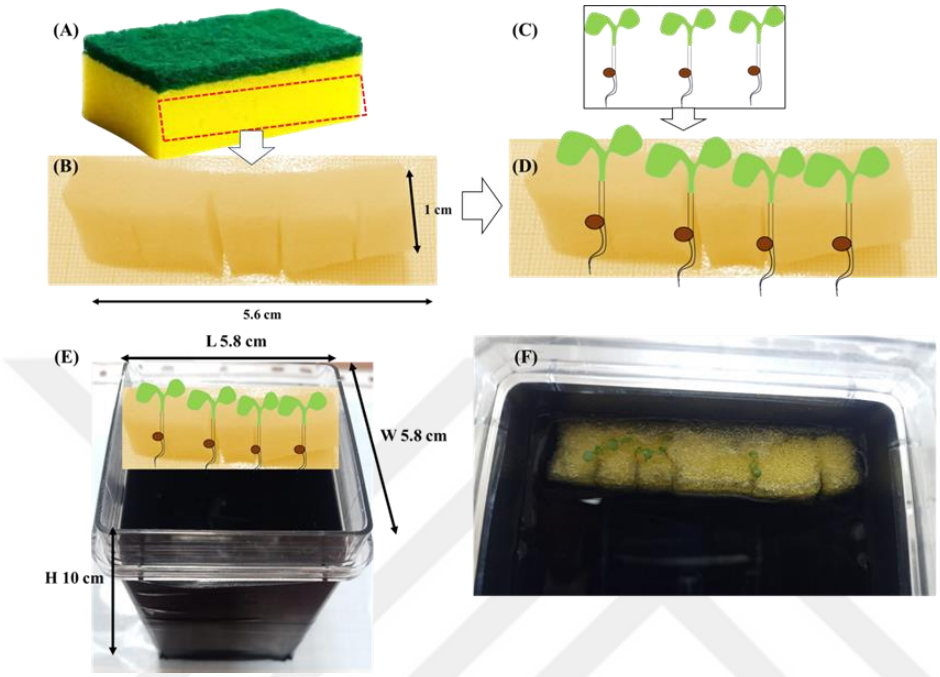

Figure 3.12 Experimental layout for the aluminium stress experiment for WT and OX *Arabidopsis thaliana*. (A) Cut the piece of dish sponge at a dimension of (B) 1 cm width and 5.6 cm long. Make slits of 0.5 cm with help of a pair of scissors and place 7 days seedlings (C). Then the (D) assembly of sponge and seedlings (E) placed over the surface of hydroponic media. (F) Representation of original sponge and seedling assembly floating in hydroponic solution.

3.2.7.2 Cadmium (Cd) Stress

For Cd stress, 35 µM $CdCl_2$ was supplied to Kimura B agar media containing 2 µM Se in the presence of 15 µM sulphate in the form of $MgSO_4.7H_2O$ following the method with modification as described (67). The Arabidopsis WT and OX seedligns were grown for the period of one month and data were collected.

3.2.7.3 Lead (Pb) Stress

Pb stress was performed following the method described previously (72) with majör modifications. For Pb stress, four days old seedlings were placed on Kimura B media containing 300 µM Pb acetate as a source of Pb and also 2 µM Se in the presence of 15 µM sulphate in the form of $MgSO_4.7H_2O$. The sulfate salts of Kimura B were replaced with equimolar solutions of their chloride salts.

Anthocyanin was determined following the method reported earlier (73). Briefly, at the end of treatment period (30 days) the leaf tissues were harvested and ground in liquid nitrogen. The extraction was performed using extraction buffer which was consisted of 1% HCl, 18% propanol and 81% water. The spectrophotometric observsations were made at 535 and 650 nm.

3.2.8 Heat Stress

Seeds of WT and OX were germinated, and seedlings were developed using autoclaved peat media. The plant pots were transferred to growth chamber which was set at 32ºC. chlorophyll content was determined as described earlier (61). The other growth conditions were the same as mentioned under the heading of 3.2.3 Se accumulation in tissues of transgenic Arabidopsis plants.

3.2.9 Statistical Analyses

The data generated both from Rice and transgenic Arabidopsis plants were evaluated by single ANOVA or Student's t test with three biological replicates. Each experiment was repeated at least twice to get reproducibility of the results. XL-stat software was used to calculate ANOVA or t test and comparison test (HSD) among the treatments for each experiment. Morphological traits were measured with ruler or ImageJ software using photographs of the WT and transgenic plants.

Table 1. List of primers used in this study.

Sr. No.	Sulphate transporters	Gene specific primer sequences	Reference
1	*OsSultr1;1*	5’-AGAACATTCGCTGGCCTGAA-3’ 5’-AAAGGATGGCATTGGGCGTA-3’	This study
2	*SULTR1;2*	5’-TCACCATGGGGCAATGGAAC-3’ 5’-TGAGCAAGTATGGGCCAGTG-3’	This study
3	*OsSultr1;3*	5’-ATCTGAAGAACCGCGTGTGT-3’ 5’-CCCGATATCCTGGGGAATGC-3’	This study
4	*OsSultr2;1*	5’-TGGTTACTGTGGTTACCAGGC-3’ 5’-GTGGACAGGATGACGGACAG-3’	This study
5	*OsSultr2;2*	5’-CTCTCGCCCTTGTCCTGAAA-3’ 5’-ACCGTGAGGAAGAACCATCC-3’	This study
6	*OsSultr3;1*	5’-ATCATCGACTACAAGGCCGC-3’ 5’-GCCGTGCGATGAACAATAGG-3’	This study
7	*OsSultr3;2*	5’-GTCGGGATCTCCATCCTTCG-3’ 5’-CGATCCTCTCACGCAGGTAG-3’	This study
8	*OsSultr3;3*	5’-GGATCTGTCAGCTGTCCCTG-3’ 5’-CACTGCTTCCCCAGTGGTTA-3’	This study
9	*OsSultr3;4*	5’-ATGTACCTCGGCGAGAGGAT-3’ 5’-GCTGCAACAGCACTCATGTC-3’	This study
10	*OsSultr3;5*	5’-TCTTCACCGAGCAAGTGAGG-3’ 5’-ATGCCACGCTTCAGAGTACC-3’	This study
11	*OsSultr3;6*	5’-GACGTGAAGCAATACCCCCA-3’ 5’-CGACAGAAGTGACACCACCA-3’	This study
12	*OsSultr4;1*	5’-GTGCCTTGGCTGCAATTGTT-3’ 5’-TTGCAAAGTGCAGTCCTGGT-3’	This study
13	*OsSultr5;1*	5’-TGCTCTGCTTCGTTCGTGAT-3’ 5’-GAAATTCATGAGCCCCACGC-3’	This study
14	*OsSultr5;2*	5’-GTCAGCAGCAGCAACAGTG-3’ 5’-GATGAACCCTTGCTTCCACG-3’	This study
15	*OsActin*	5’-GTATCCATGAGACTACATACAACT-3’ 5’-TACTCAGCCTTGGCAATCCACA-3’	(62)
16	*HistoneH3*	5′-GGTCAACTTGTTGATTCCCCTCT-3′ 5′-AACCGCAAAATCCAAAGAACG-3′	(63)
17	*AtActin2*	5'-AGGTCCAGGAATCGTTCACAGA-3' 5'-CCCCAGCTTTTTAAGCCTTTGA-3'	(74)
18	OsSULT1;1 Subcellular localization	5′GGGGACAAGTTTGTACAAAAAAGCAGGCTTA***ATGCCGCGAGCCGTGT***-3′ 5′GGGGACCACTTTGTACAAGAAAGCTGGGTT***GACATTCTCTACTGCCTTAGGAGCA***-3′	This study
19	For transgene presence of *OsSULTR1;1*	5'-GATGGACCCCCACCCACG-3' 5'- GGGTCGTCGGCGAAGAACG -3'	This study Product length 382 bp

	or colony PCR		
2	For transgene expression of Os*SULTR1;1*	5'-CGCGAGCCGTGTCCGAC-3' 5'-TACTGCCGCAACGGGTCG-3'	This study Product length 203 bp

4. RESULTS

4.1 Selenium Accumulation in Shoot and Root Tissues under Various Selenate Treatments

Selenium content was determined in shoot and root of 30 days old seedlings which were treated with 0.00, 0.25, 0.75, 2.00 and 4.00 µM sodium selenate for 3 days. The minute Se content in control application in shoot and root tissue also appeared that could be due to impurity of the nutrient salts used for hydroponic media. In shoot tissue 10 mg/kg Se content was found under 0.25 µM sodium selenate. At 0.75 µM selenate application, Se content was accumulated to double (20 mg/kg). However, remarkable enhancement in Se content (160 mg/kg) was observed at 2.00 and 4.00 µM sodium selenate application. In root tissues, Se content was found nearly 5 mg/kg and double (10 mg/kg) at 0.75 µM sodium selenate application. the highest Se content was found also in root tissues at 2.00 and 4.00 µM selenate application nearly 130 mg/kg and 140 mg/kg respectively. These results suggest that basmati rice cultivar has a very good capacity of Se accumulation both in shoot and root when supplied as sodium selenate (Figure 4.1).

4.2 Selenium Accumulation in Seed During Growth and Development Stages

Se content was negligible under control treatment possibly due to impurities in nutrient salts of hydroponic. However, at milky stage of Basmati rice the Se content initiated to accumulate in seed tissues. At the soft dough stage (yumuşak hamur aşaması) 0.14 mg/kg Se content was found. However, at the hard dough stage the Se content was enhanced to 0.33 mg/Kg. The results suggest that Basmati rice accumulates Se content during seed growth and developmental stage when sodium selenate is applied (Figure 4.2).

4.3 Selenium Uptake in Basmati Rice Seedlings at Different pHs

In order to determine pH dependency of the selenate uptake in Basmati rice, we have conducted selenate uptake at various pH levels adjusted by 5 mM MES buffer. The highest Se uptake was found at the lowest pH 3.5 and then decreased with the increase in pH. The Se uptake decreased greatly at the pH 7.5. the results suggest that under alkaline condition Basmati rice would be able to take little portion of Se content than at the mild acidic conditions pH5.5 and 6.5 (Figure 4.3).

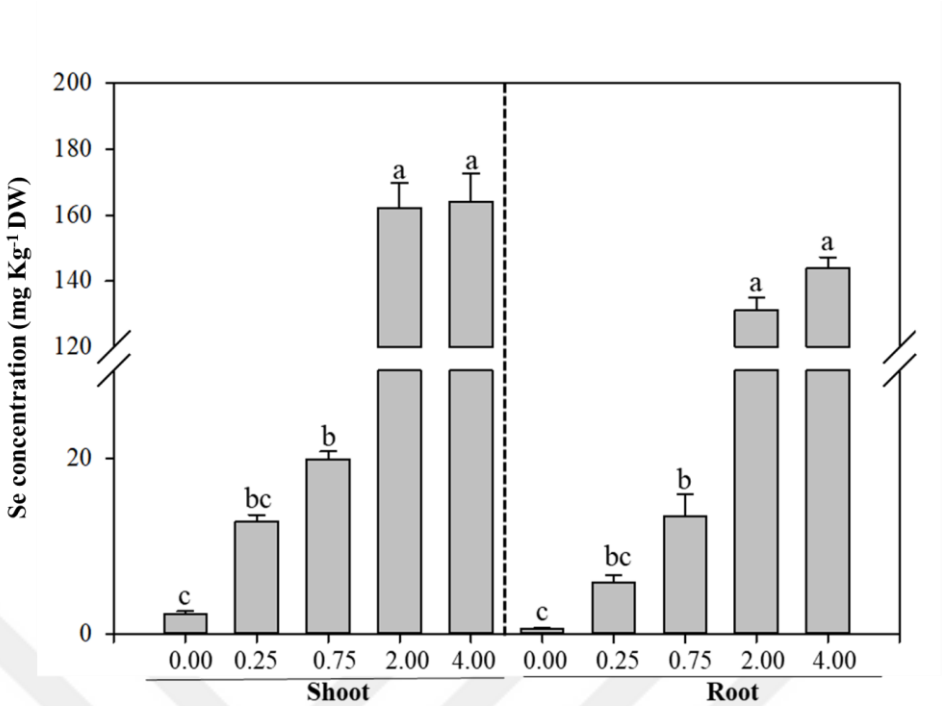

Figure 4.1 Selenium content in shoot and root samples of 30 days old rice seedlings. The selenate application (0.00 µM, 0.25 µM, 0.75 µM, 2.0 µM and 4.0 µM) was continued for 3 days under hydroponic condition. The bars represent the mean ± standard error (SE) of three samples from three independent experiments.

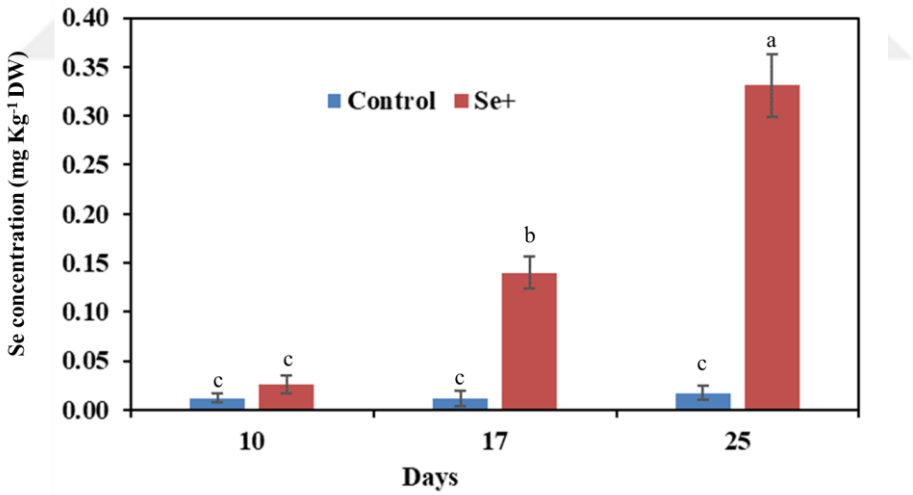

Figure 4.2 Selenium content in developing grains (10 days, 17 days and 25 days) of Basmati rice. The selenate application (2.5 µM) during the reproductive stage was continued under hydroponic condition. The bars represent the mean ± standard error (SE) of three samples from three independent experiments.

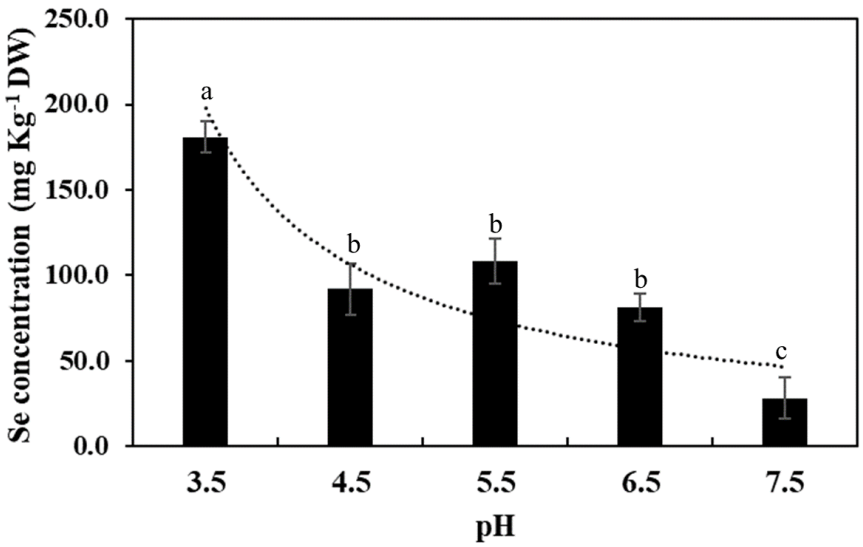

Figure 4.3 Selenium content in 20 days old seedlings of Basmati rice. The selenate application (2.5 µM) was performed for 24 hours under hydroponic condition. The bars represent selenium content in shoots and the mean ± standard error (SE) of three samples from three independent experiments.

4.4 Expression Pattern of Sulfate Transporters under Selenium Application in Root and Shoot Tissues

To determine the tissue specificity of the 13 sulfate transporter genes (Figure 4.4) 20 days old rice seedlings were exposed to without (control) or with sodium selenate (Na_2SO_4) for three days. The gene expression of the sulfate transporters was determined while using the *OsActin* as a reference gene. The expression of the *OsSULTR1;1* was highly root tissue specific and also dependent on Se application. In control root or shoot the expression was negligible (Figure 4.5). The results suggest that the *OsSULTR1;1* has root tissue specificity and also induces by selenate. The expression of the *OsSULTR1;2* was very negligible even under Se application and only confined to shoot tissues under 0.75 µM and 4.0 µM selenate (Figure 4.6).

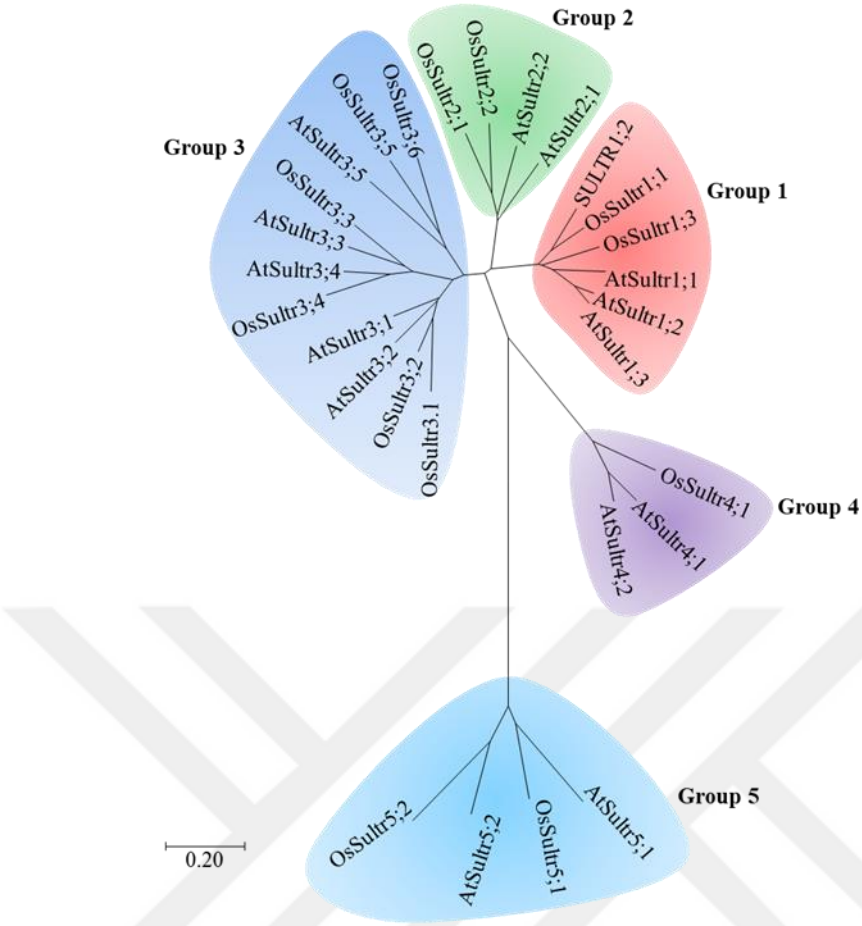

Figure 4.4 Phylogenetic tree of rice and Arabidopsis sulfate transporters. To retrieve protein sequences from NCBI or Phytozome the same accession numbers were used as mentioned previously (29, 35). The protein sequences were aligned by ClustalW (75) and the phylogenetic tree was created by the neighbor-joining method using MEGA software (58).

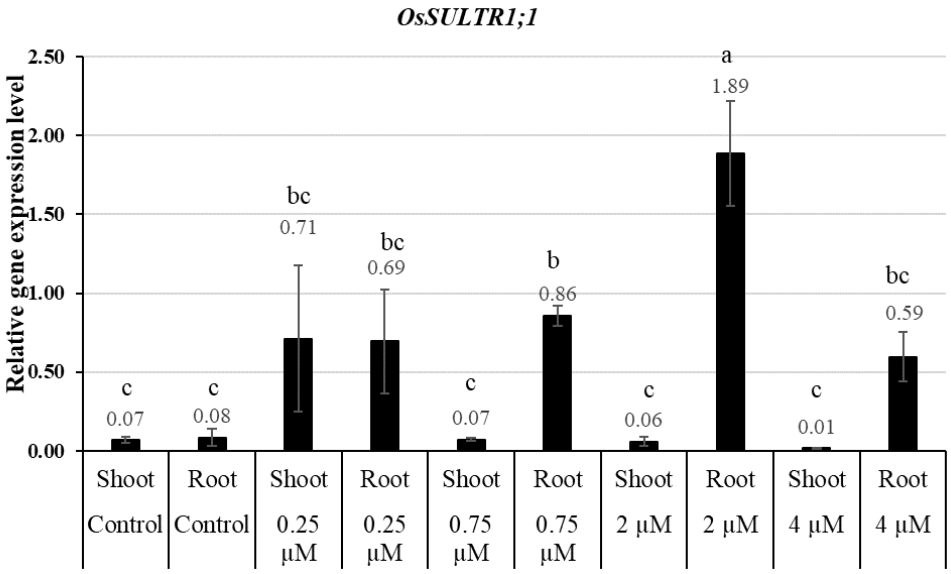

Figure 4.5 Expression pattern of the *OsSULTR1;1* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

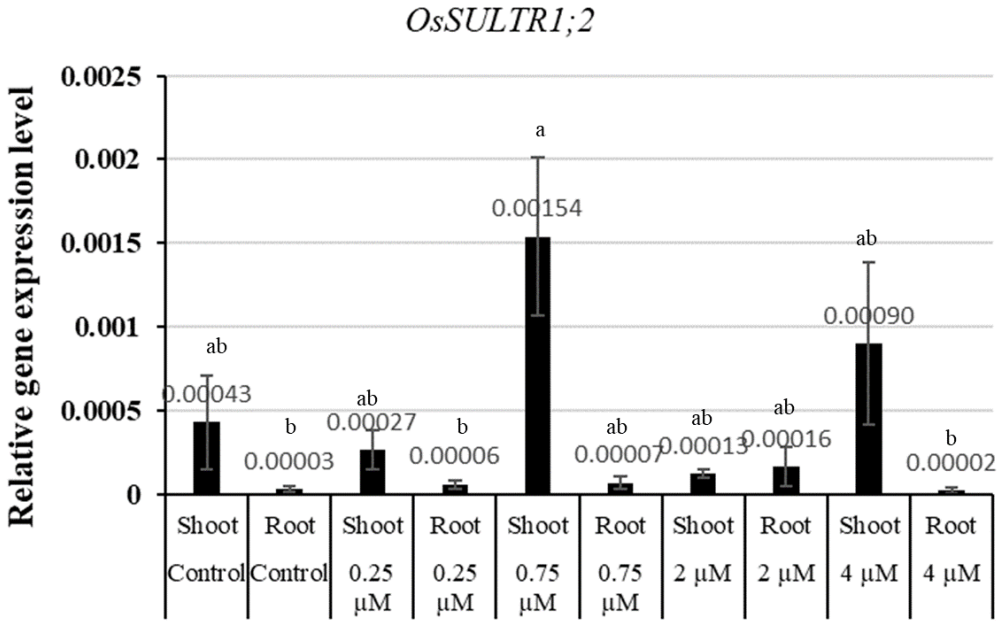

Figure 4.6 Expression pattern of the *OsSULTR1;2* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

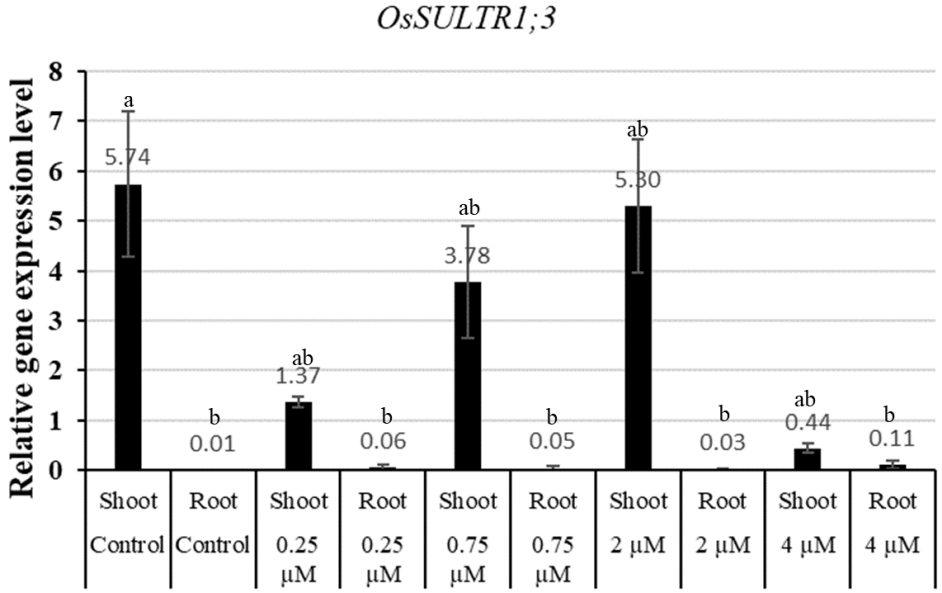

Figure 4.7 Expression pattern of the *OsSULTR1;3* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

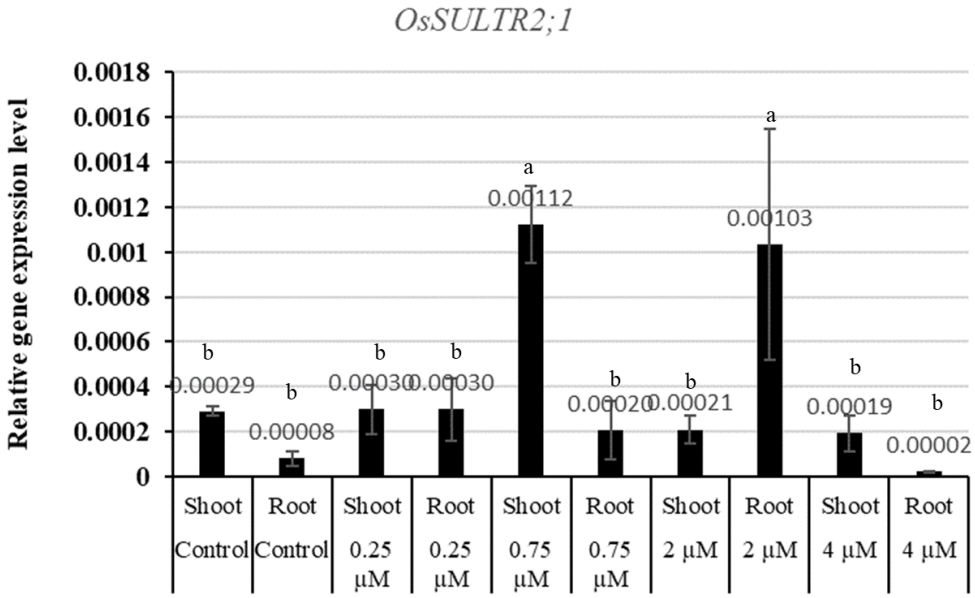

Figure 4.8 Expression pattern of the *OsSULTR2;1* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

The expression of the *OsSULTR1;3* was limited to shoot tissues and absent in root tissues. The expression was highest in control shoots and also under 0.25 µM, 0.75 µM and 2.0 µM selente treated shoots. The expression was suppressed at 4.0 µM in

shoot. The results suggest the gene has shoot tissue specificity but having low concentration Se dependency expression (Figure 4.7). The expression of the *OsSULTR2;1* was inhibited but only minute expression was detected in shoot (0.75 µM Se) and root (2 µM Se) tissues (Figure 4.8). The results suggest that the gene expression was regulated negligible under Se application. The *OsSULTR 2;2* also followed a similar expression pattern as did the *OsSULTR2;1* (Figure 4.8 and Figure 4.9). The genes *OsSULTR2;1* and *OsSULTR2;2* belong to low affinity group (the genes which express under high nutrient condiction) therefore, it could be possible their expression under low concentration of selenate could not induce.

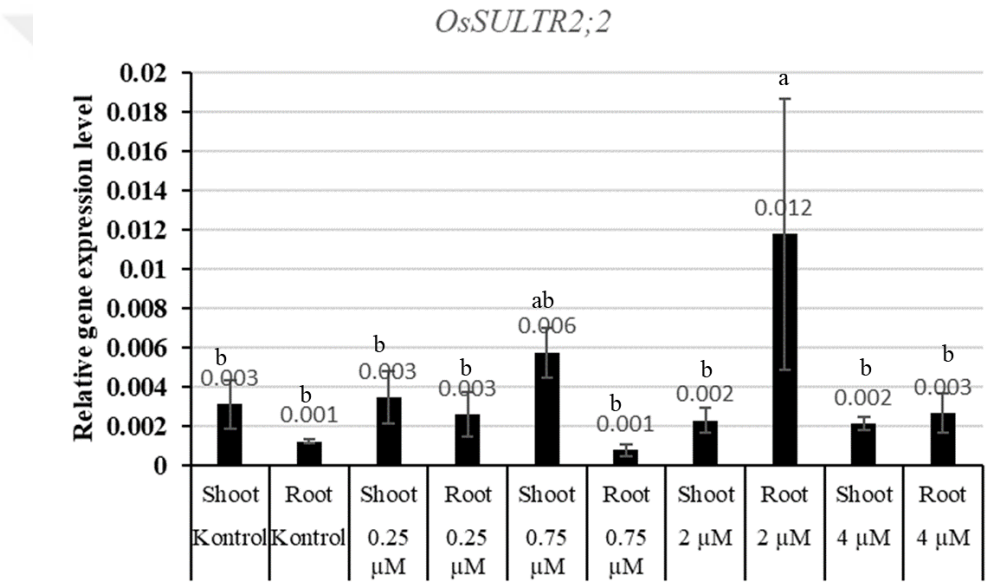

Figure 4.9 Expression pattern of the *OsSULTR2;2* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

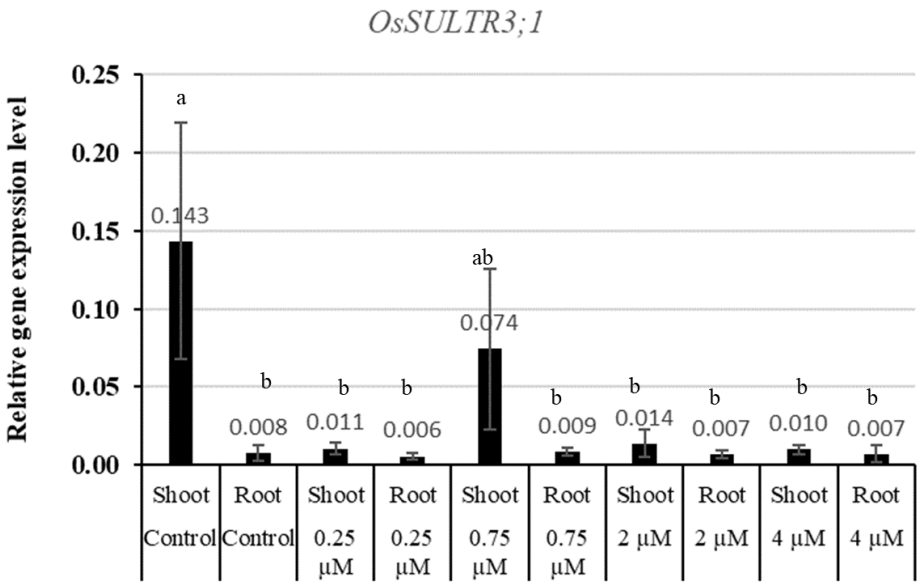

Figure 4.10 Expression pattern of the *OsSULTR3;1* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

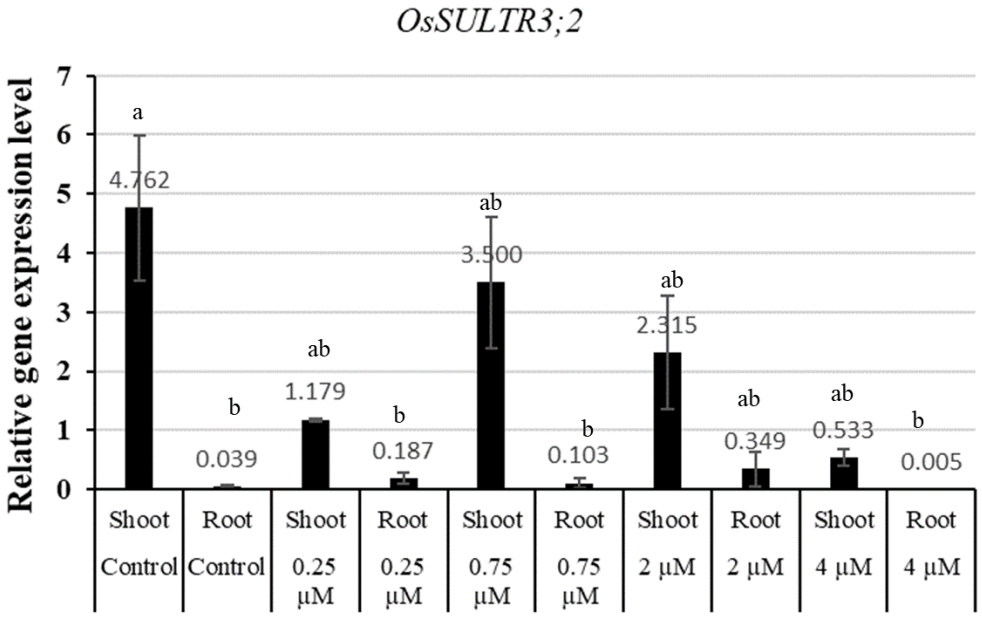

Figure 4.11 Expression pattern of the *OsSULTR3;2* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

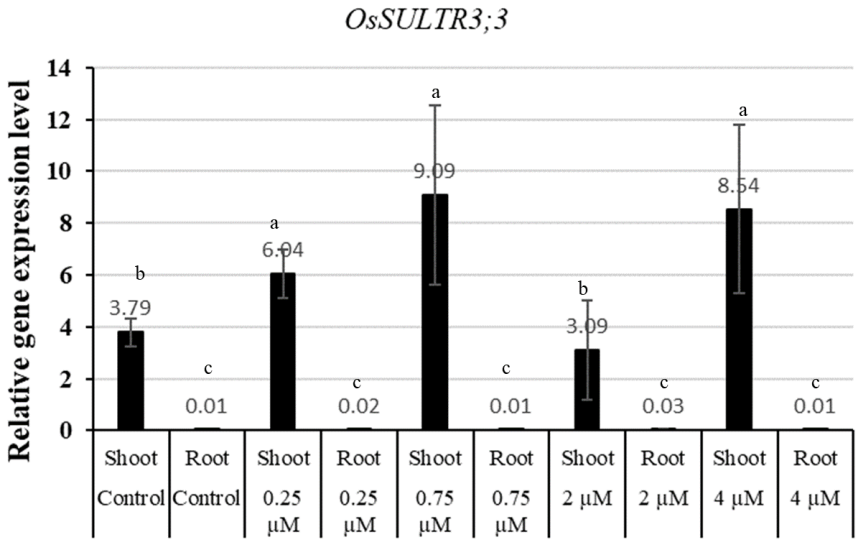

Figure 4.12 Expression pattern of the *OsSULTR3;3* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

The expression of the *OsSULTR3;1* was limited to shoot tissues only but in minute amount in control and 0.75 µM Se shoots and absent in root tissues. The results suggest the gene has probably shoot tissue specificity (Figure 4.10). The expression of the *OsSULTR3;2* was also confined to shoot tissues and absent in root tissues. In control shoot it was the highest but also expressed under 0.25 µM, 0.75 µM and 2.0 µM Se treated shoot tissues. However, expression was inhibited under 4.0 µM Se shoot tissues (Figure 4.11). The expression of the *OsSULTR3;3* was also limited to shoot tissues and absent in root tissues. The gene expressed under control and Se treated shoo tissues. However, under 0.25 µM, 0.75 µM and 4.0 µM Se was the expression was high compared to control (Figure 4.12).

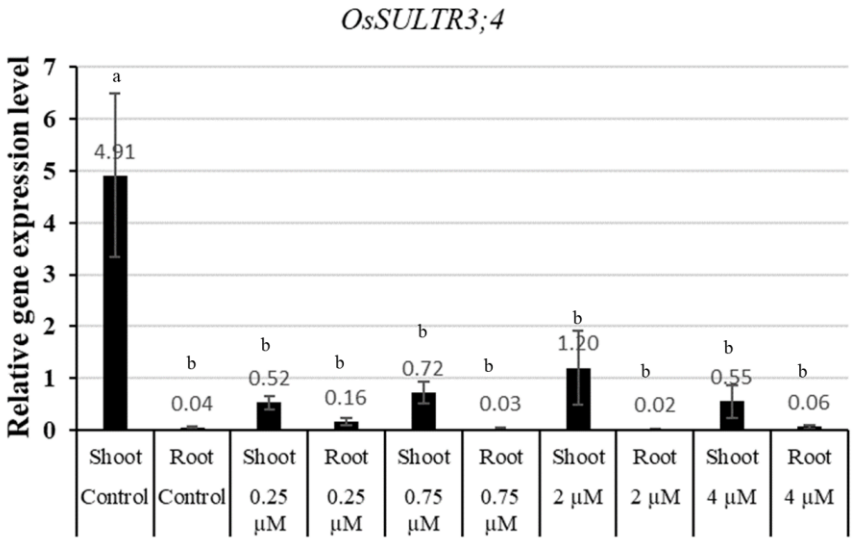

Figure 4.13 Expression pattern of the *OsSULTR3;4* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

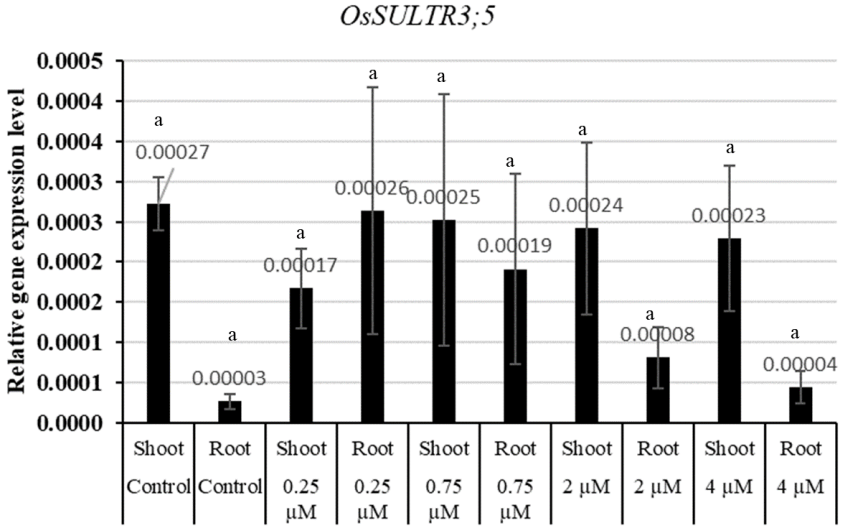

Figure 4.14 Expression pattern of the *OsSULTR3;5* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

The expression of the *OsSULTR3;4* was also limited to shoot tissues and absent in root tissues. Under control the expression was the highest compared to Se treatments. However, minuted expression was observed in Se treated shoots (Figure 4.13). The expression of *OsSULTR3;5* was inhibited totally in all treatments (Figure 4.14). The expression of *OsSULTR3;6* was expressed in minute amount. The gene

expression was observed in shoot and root both tissues but not in all treatments. The expression was found in control root, 0.25 µM Se shoot and root, 0.75 µM Se shoot, 2.0 µM, 4.0 µM Se shoot and root tissues. The expression was downregulated in control shoot and 0.75 µM Se treated root (Figure 4.15).

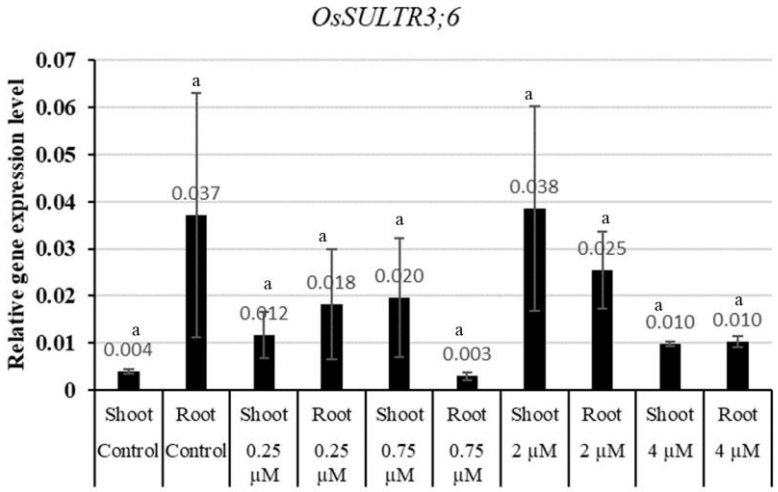

Figure 4.15 Expression pattern of the *OsSULTR3;6* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

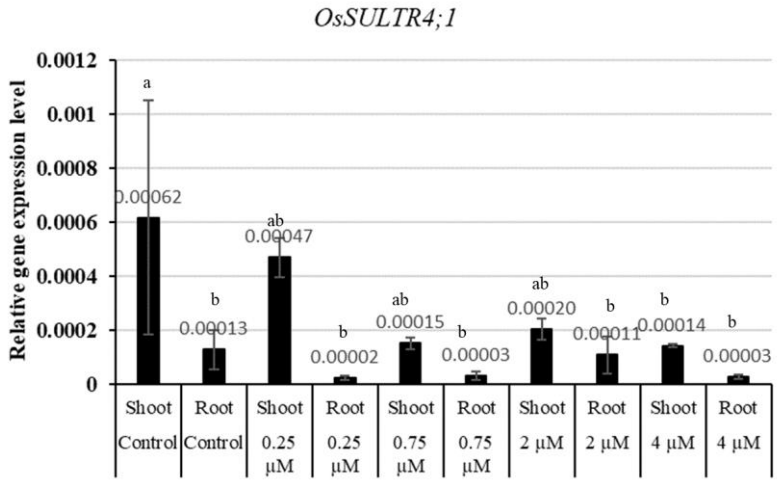

Figure 4.16 Expression pattern of the *OsSULTR4;1* in tissues (root and shoot) of three samples from three independent experiments. Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

The results suggest that the genes from group 3 (*OsSULTR3;1*, *OsSULTR3;2*, *OsSULTR3;3*, *OsSULTR3;4*, *OsSULTR3;5*, *OsSULTR3;6*) sulfate transporters are mainly shoot specific and to some extent induced due to selenate application

especially in shoot tissues. The expression of *OsSULTR 4;1* was downregulated in all treatments (Figure 4.16).

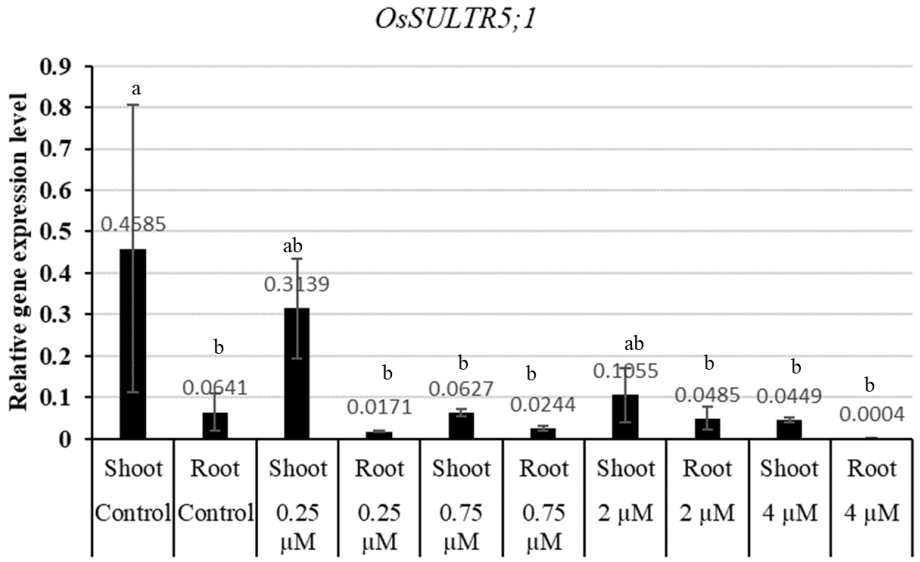

Figure 4.17 Expression pattern of the *OsSULTR5;1* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

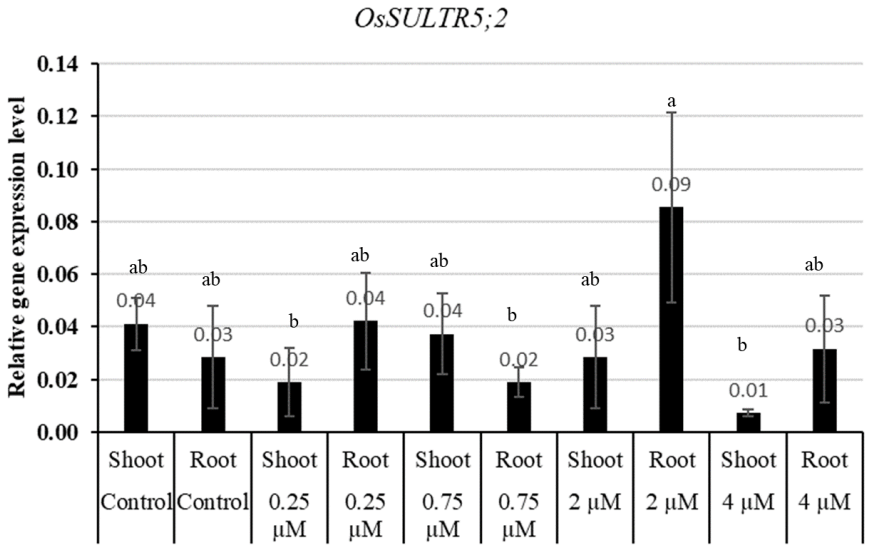

Figure 4.18 Expression pattern of the *OsSULTR5;2* in tissues (root and shoot) of Basmati rice under control and selenate application. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

The expression of the *OsSULTR5;1* was expressed in low amount in control shoot and 0.25 µM Se treated sügün. However, in the rest of the treatment and samples expression was downregulated (Figure 4.17). In general, the expression of

OsSULTR5;2 was also in lower amount in all the tissues and the treatments but slightly highly expressed in root under 2 µM Se treatment. Expression of the gene was downregulated in 4 µM Se shoot (Figure 4.18).

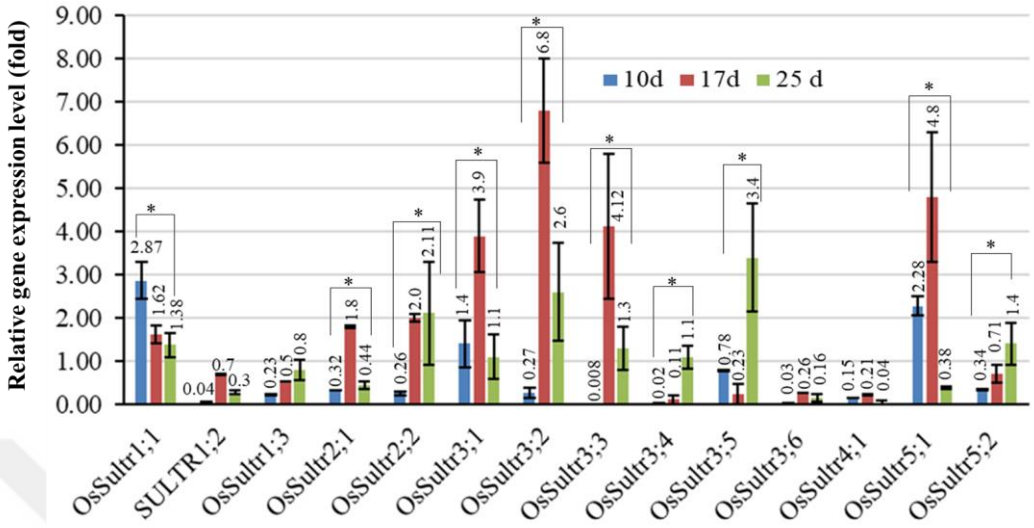

Figure 4.19 Expression pattern of the sulfate transporter genes in developing grains (10 days, 17 days and 25 days) of Basmati rice under selenate (2.5 µM) application. The selenate application was continued during the reproductive stages. The expression of the genes was calculated relative to control treatment. The bars represent the mean and standard error (SE) of three samples from three biological experiments.

4.5 Expression Pattern of Sulfate Transporters under Selenium Application in Seed During Growth and Development Stages

In order to determine the grain tissue specificity of sulfate transporters under selenate treatment, gene expression was determined during grain development and growth stages (Figure 4.19). The expression of the *OsSULTR1;1* was the highest (3-fold than control) during early seed development stage (10 günler) under selenate treatment. However, the expression was slightly reduced during 17 and 25 days. The expression of the *OsSULTR1;2* and *OsSULTR1;3* was downregulated in the seed during growth and developmental stages. The expression of the *OsSULTR2;1* was only slightly induced (2-fold of control) during seed development at 17 days. The expression of *OsSULTR2;2* was inhibited at 10 days and induced (2-fold of control) at 17 and 25 days of seed development period. The expression of *OsSULTR3;1* was 1-fold during 10 and 25 days of seed development. However, the highest expression (4 fold) was found at 17 days. The expression of *OsSULTR3;2* was downregulated at days 10. However, at days 17 the highest expression (7 fold)

was observed among all the genes and then again reduced to (3 fold) at days 25. The expression of *OsSULTR3;3* was absent at days 10 and expressed 4-fold at 17 days. Then again downregulated at 25 days of seed growing period. The expression of *OsSULTR3;4* was downregulated at 10 and 17 days. But slightly induced to 1 fold at days. The expression of *OsSULTR3;5* was downregulated at 10 and 17 days. But a 3-fold expression was found at 25 days. The expression of *OsSULTR3;6* and OsSULTR4;1 was completely downregulated. The expression *OsSULTR5;1* was found at 2-fold level for 10 days and 5-fold at 17 days. However, downregulated at 25 days. *OsSULTR5;2* was downregulated at 10 and 17 days. The 1.5-fold expression was found at 25 days of seed growth and developmental stage Figure 4.19. Taken together, results suggest that all the sulfate transporters have great variation in expression during seed growth and development stage under selentate application in Basmati rice. However, the expression *OsSULTR1:1* seems to be promising to be selected as candidate gene as it shows induction by selenate treatment at the earlier stage (10 days) of seed development (Figure 4.19). The expression was retained more than 1-fold during 17 and 25 days of seed development. Thus, the gene was selected as a possible candidate gene for subcellular localization study.

4.6 Vector Development for Nuclear Genetic Transformation

The expression vector was constructed by Gateway® cloning (Figure 3.9). The full length open reading frame (ORF) of the *OsSULTR1;1* (Figure 4.20) was cloned into pDONR221 by BP Clonase reaction and transformed into *E.coli* cells to get single colonies of entry clones (Figure 4.21). The isolated entry clones were recombined with destination plasmid pGWB5 using LR reaction and transformed into *E. coli* cells. The single colonies of expression construct pGWB5::OsSULTR1;1 were obtained (Figure 4.21). The expression construct was isolated and transformed into *Agrobacterium tumefaciens* cells and single colonies obtained (Figure 4.22). The final expression vector pGWB5::OsSULTR1;1 was confirmed by gel electrophoresis (Figure 4.23) and PCR (Figure 4.24). The detailed and simplified schematic map of the construct is shown in Figures 4.25 and 4.26 respectively. For the enhacned expression of the gene, the *OsSULTR1;1* was placed under the control of the constituve promoter CaMV 35S without stop codon at the C terminus fused with GFP (Figure 4.26). The expression construct contained the

gene *OsSULTR1;1* cloned from Super Basmati aromatic rice was sequenced and deposited to Genbank (accession number; PP697850.1). The difference between the *OsSULTR1;1* reported here (accession number; PP697850.1) and the reported earlier (accession number; AF493792.1) from non-aromatic indica rice IR-64 was 1 SNP which was revealed by sanger sequencing. The one SNP "C" at nucleotide 1732 was replaced by "G" and the resultant amino acid at position L578 (Leucine 578) was replaced by V578 (Valine 578) as shown in Figure 4.27.

4.7 Subcellular Localization of OsSULTR1;1

To predict and better understanding of gene function of the *OsSULTR1;1* we have performed subcellular localization in onion epidermal cells. The expression of control GFP was observed in whole onion cell including nucleus, cytoplasm and the peripehy of the cell. On the other hand, the expression of OsSULTR1;1::GFP was limited to the periphery of the cell and was absent in nucleus or cytoplasm (Figure 4.28). Therefore, the result suggests that OsSULTR1;1 is a plasma-membrane localized sulfate transporter.

4.8 Development of Transgenic *Arabidopsis thaliana* Plants by Floral Dip Transformation

The Floral dip transformation method (Figure 3.11) produced enough number of putative transgenic seedlings (Figures 4.29 and 4.30) which were screened by using hygromycin as a selection agent. The seedlings with true leves were selected and transplated into autoclaved peat media (Figure 4.30) and grown to vegetaive (Figure 4.31A) and reproductive (Figure 4.31B) stage to obtain mature seeds. The T_2 seeds were germinated in peat media and PCR was performed to screen the confirmed lines. Lines 2 to 5 were confirmed as transgenic lines (Figure 4.32). To determine the expression of line 3 (OX-1) and 4 (OX-2) real time PCR was performed. The expression of the transgenic lines OX-1 and OX-2 were significantly higher than the WT (Figure 4.33). Therefore, these two lines were used for the phenotypic traits analyses (Figures 4.34-4.39).

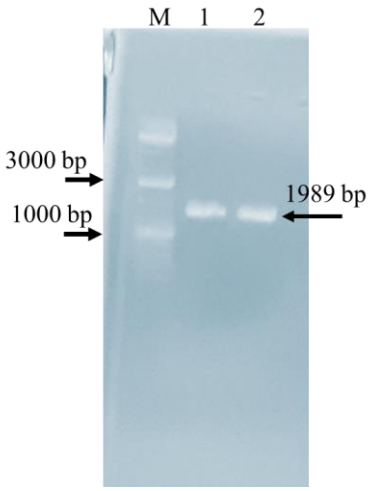

Figure 4.20 PCR amplification of the *OsSULTR1;1* from cDNA by using full length open reading frame (ORF) primer set. Lane M represents ladder and 1-2 ORF bands of the *OsSULTR1;1*.

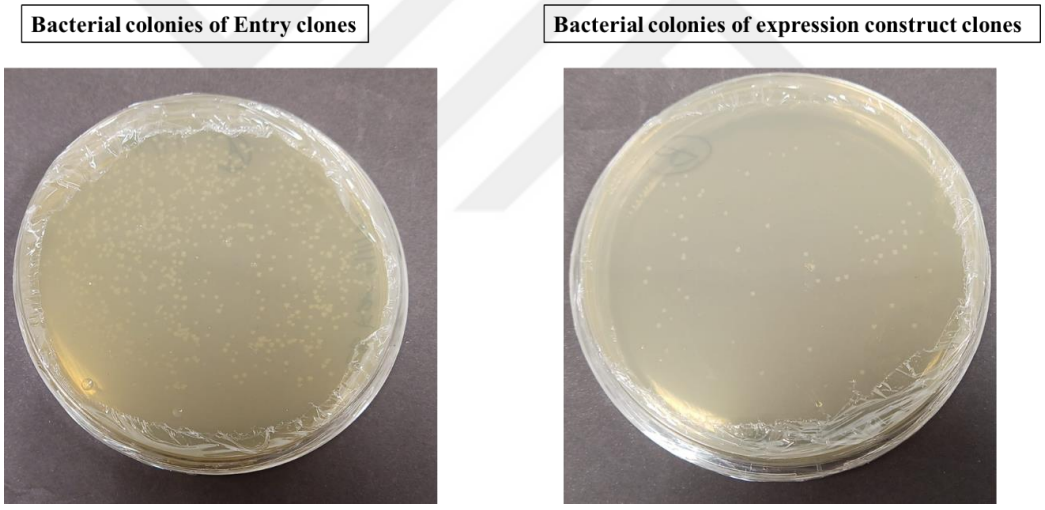

Figure 4.21 Bacterial colonies of entry and expression clones. Left side plate shows entry clones and right-side plate represent expression construct colonies.

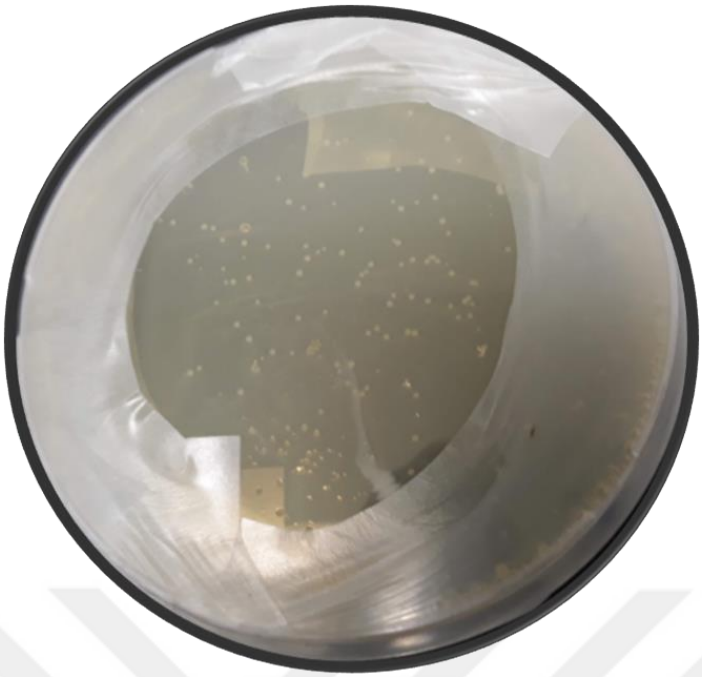

Figure 4.**22** *Agrobacterium tumefaciens* C58C1 transformed with plasmid construct of pGWB5::OsSULTR1;1 for subcellular localization experiment. Single colony was grown in liquid YEB media collected for onion epidermal cells transformation.

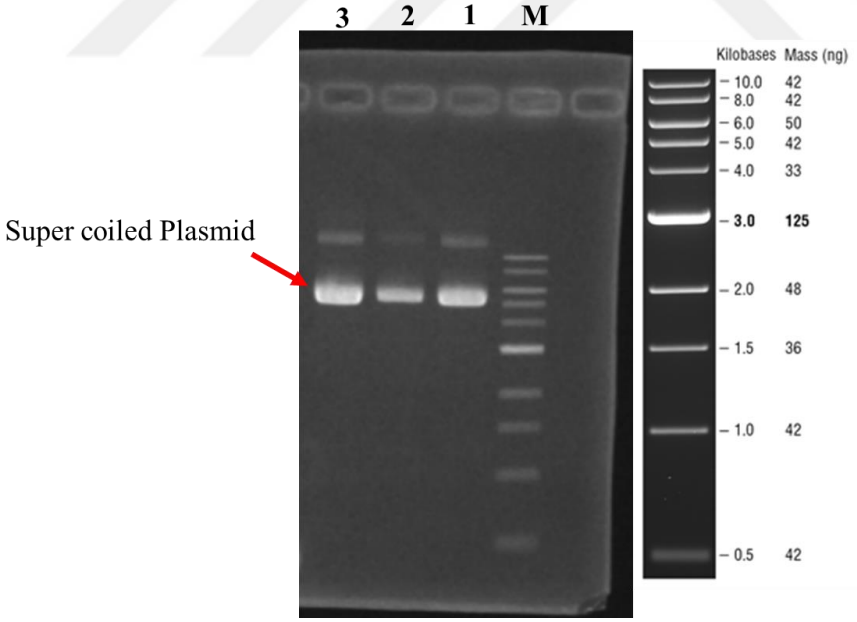

Figure 4.23 Expression construct pGWB5::OsSULTR1;1 gel electrophoresed, 1% agarose gel was used.

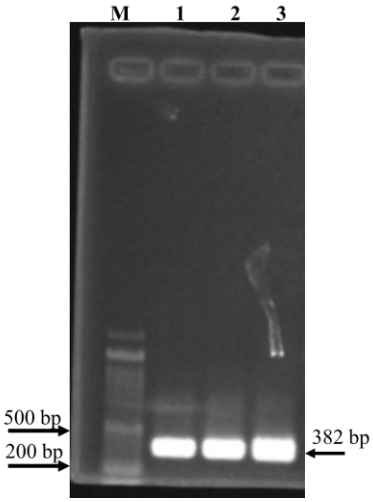

Figure 4.24 Colony PCR of the expression construct pGWB5::OsSULTR1;1 gel electrophoresed. 2% agarose gel was used. M=100 bo ladder, Lane 1 to 3 represent independent bacterial colonies confirmed by colony PCR.

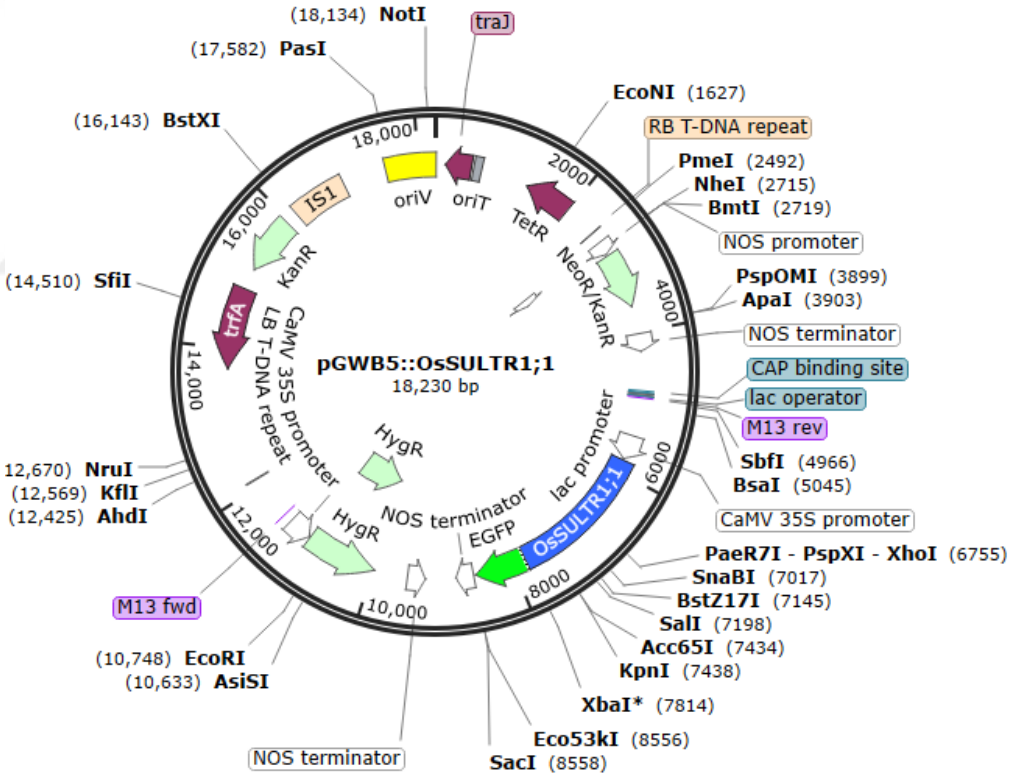

Figure 4.25 Detailed illustration of expression construct of pGWB5::OsSULTR1;1.

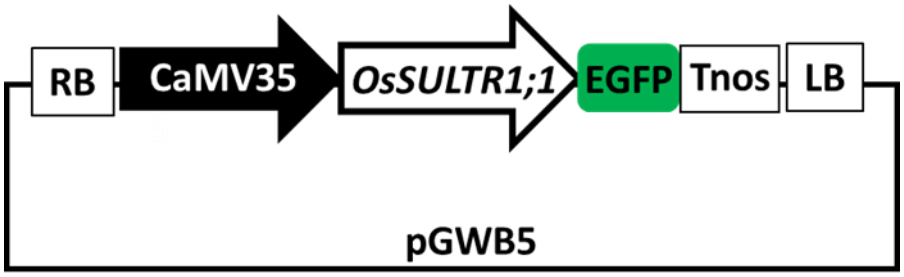

Figure 4.26 Simplified components of pGWB5::OsSULTR1;1 expression construct.

Figure 4.27 Pairwise alignment of protein sequence of the two OsSULTR1;1 originating indica rice. The difference between single amino acid is depicted by red rectangle. AF493792

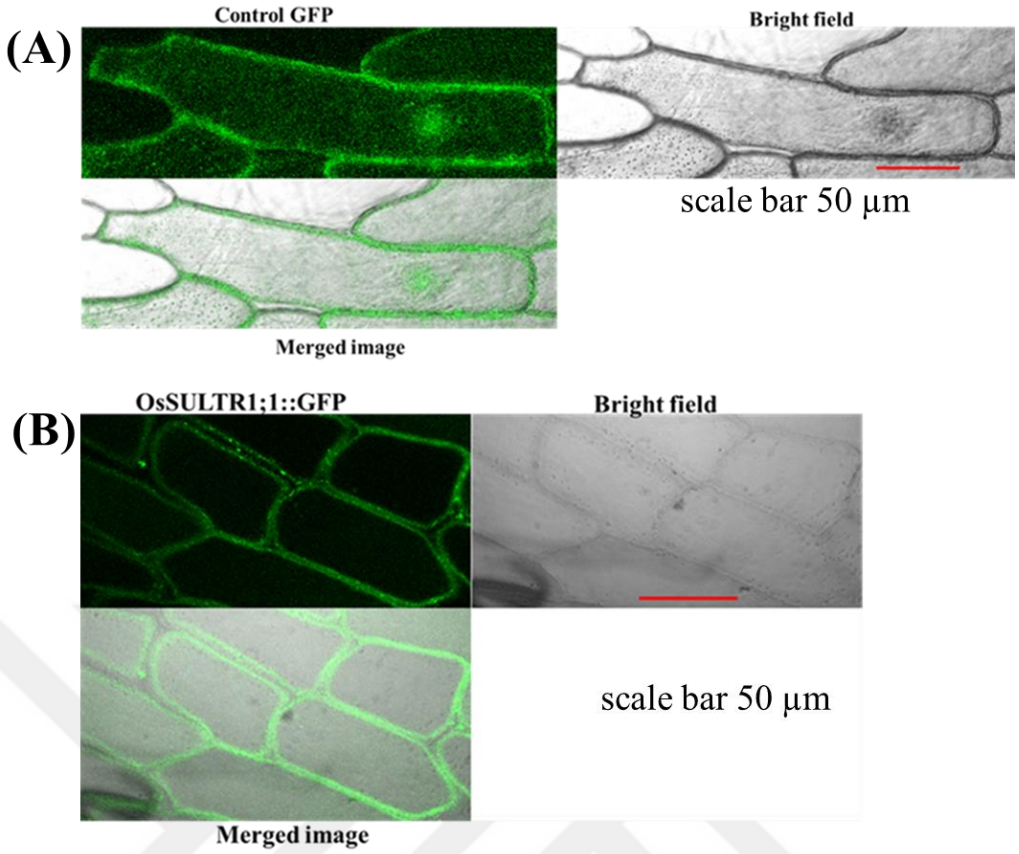

Figure 4.28 The control (empty plasmid pGWB5) and OsULTR1;1::GFP was observed under confocal laser scanning microscope. The control cells showed normal distribution of GFP expression in cell including nucleus, cytoplasm, and plasma membrane. On the other hand, the expression of OSULTR1;1 was limited to only periphery of the cell i.e plasma membrane. Upper panel shows confocal imaging of control GFP in onion cells with scale bar 100 µm. Lower panel shows confocal imaging of OsSULTR1;1::GFP in nion cells with scale bar 50 µm.

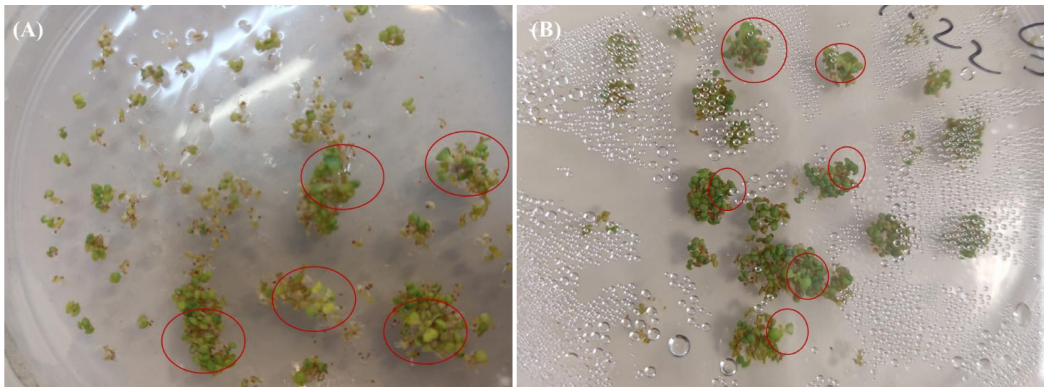

Figure 4.29 Screening of putative transformed seeds of Arabidopsis on ½ MS agar media containing hygromycin as a selection agent. Red circle shows the presence of seedlings with third and fourth emerging leaves.

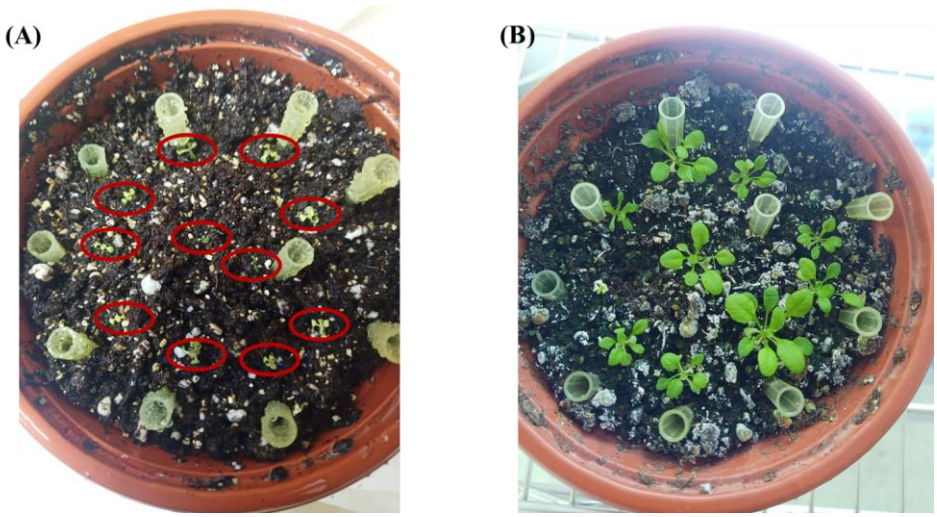

Figure 4.30 Transfer of selected putative transgenic seedlings. Antibiotic selected transgenic seedlings transferred to sterile peat media (A). Two weeks older putative transgenic seedlings growing in peat media (B).

Figure 4.31 Growth of transgenic plants. Transgenic Arabidopsis plants at flowering and pod formation stage (A). Ripening stage of pods started (B).

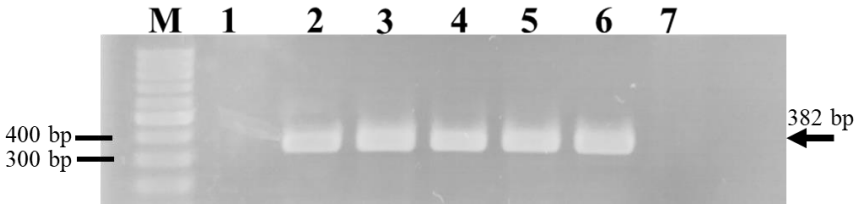

Figure 4.32 Screening of putative transgenic Arabidopsis lines by PCR. Lane M represents ladder, lane 1 wild type (WT) gDNA, 2 to 5 are from putative transgenic Arabidopsis plants gDNA, lane 6 expression construct (pGWB5::OsSULTR1;1) and lane 7 negative control.

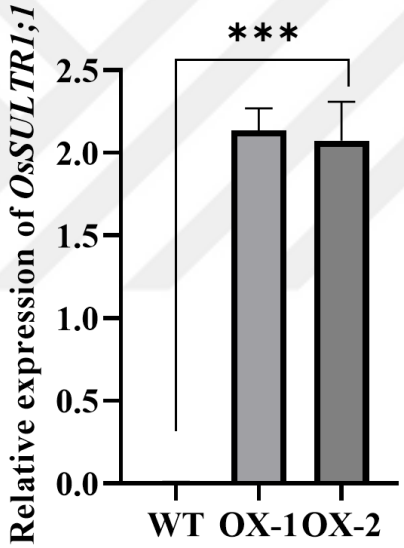

Figure 4.33 Real time PCR expression of transgenic and WT Arabidopsis plants. The expression was normalized to *AtActin2*.

4.9 Phenotypic Traits of Transgenic Arabidopsis Plants due to Overexpression of *OsSULTR1;1*

The rosette area of OX-1 of 10 old seedlings was highly significant than WT (Figures 4.34 and 4.35) suggesting the role of *OsSULTR1;1* in plant growth development. In additon, the plant phenotyping was performed at reproductive stage of WT and OX-1 and OX-2 plants (Figure 4.36). Plant height of OX plants

was highly significant than WT (Figure 4.37A) but non-significant in number of branches (Figure 4.37B). The number of pods on main branch or pods per plant and pod weight were highly significant in OX than WT (Figures 4.37 C, D, E). The number of ripen pods were significantly higher in OX than WT (Figure 4.37F). The number of seeds per pods were also significantly higher in OX than in WT (Figure 4.37G). However, the number of rosettes and leaves per plant was non-significant among WT and OX plants (Figure 4.37 H, I).

4.10 Correlations Matrix among Plant Height and Morphological Traits

There were significant positive correlations between plant height (PH) and all the morphological traits like total leaves (TL), number of rosettes (NR), number of pods (NP), number of branches (NBR), number of pods on main branch (NPMBR), pod weight (PW), number of seeds (NS), number of ripen pods (NRP). Further, significant positive correlation was found among NP and NPMBR, however, significant negative correlation was found among NBR and NS (Figure 4.38).
Similarly, PH of OX plants also exhibited significant positive correlation smong all the other morphological traits like TL, NR, NP, NBR, NPMBR, PW, NS, NRP (Figure 4.39). In addition, significant positive correlation was found among NP and PH, NS and PH, NR and NRP, NS and NPMBR. A significant negative correlation was found among NP and TL (Figure 4.39).

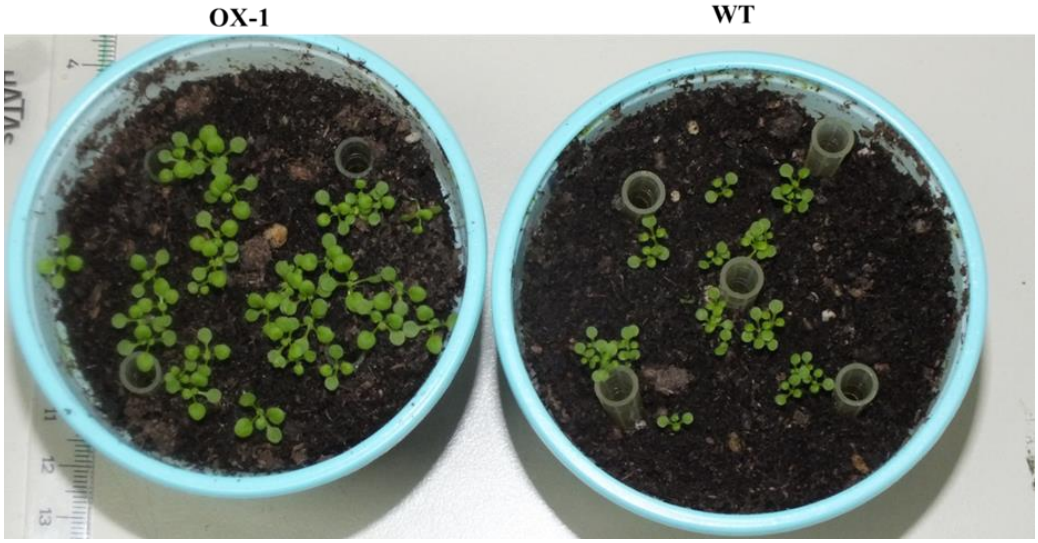

Figure 4.34 Phenotypic assessment was performed at 10 days old seedlings in peat media. The seeds of OX-1 and WT were grown in peat media under 16 h light/8 h dark photoperiod. The rossette area was recorded and photographs were taken at 10 days growth.

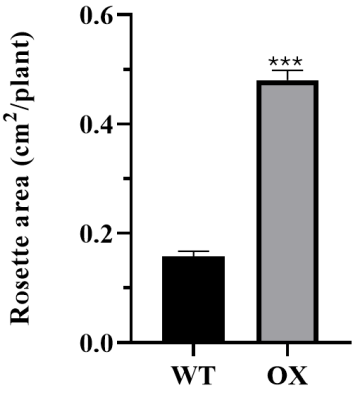

Figure 4.35 Rosette phenotyping at 10 days old seedlings in peat media. The seeds of OX-1 and WT were grown for 10 days, and their rosettes were determined using imageJ software. At least 20 seedlings of each WT and OX line were used to record the phenotypes. Data represented mean±SE of three replications. The asterisks show significance at ***$P \leq 0.001$ as determined by Student's t-test.

Figure 4.36 Phenotypes of WT and OX lines of *Arabidopsis thaliana*. The plants were grown in the peat media for the period of one month at normal growth conditions and the data were taken from WT, OX-1 and OX-2 lines.

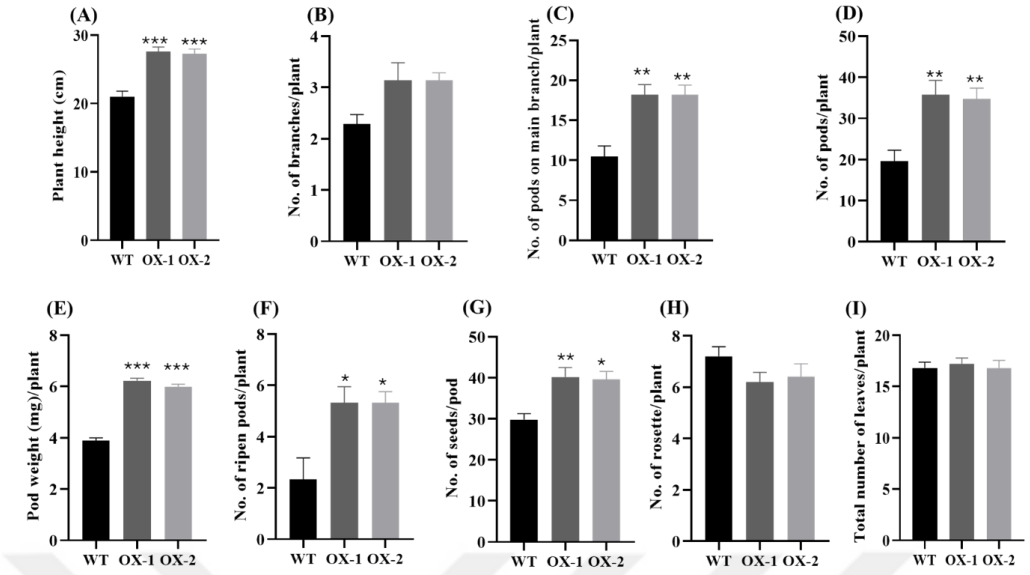

Figure 4.37 Phenotypes of WT and OX lines of *Arabidopsis thaliana* plants grown in peat media. The plants were grown for the period of one month under the normal growth conditions and the data were recorded. At least 5 seedlings of each WT and OX line were used to record the phenotypes and three replications were done. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks show significant differences at *P≤0.05, **P≤0.01, ***P≤0.001.

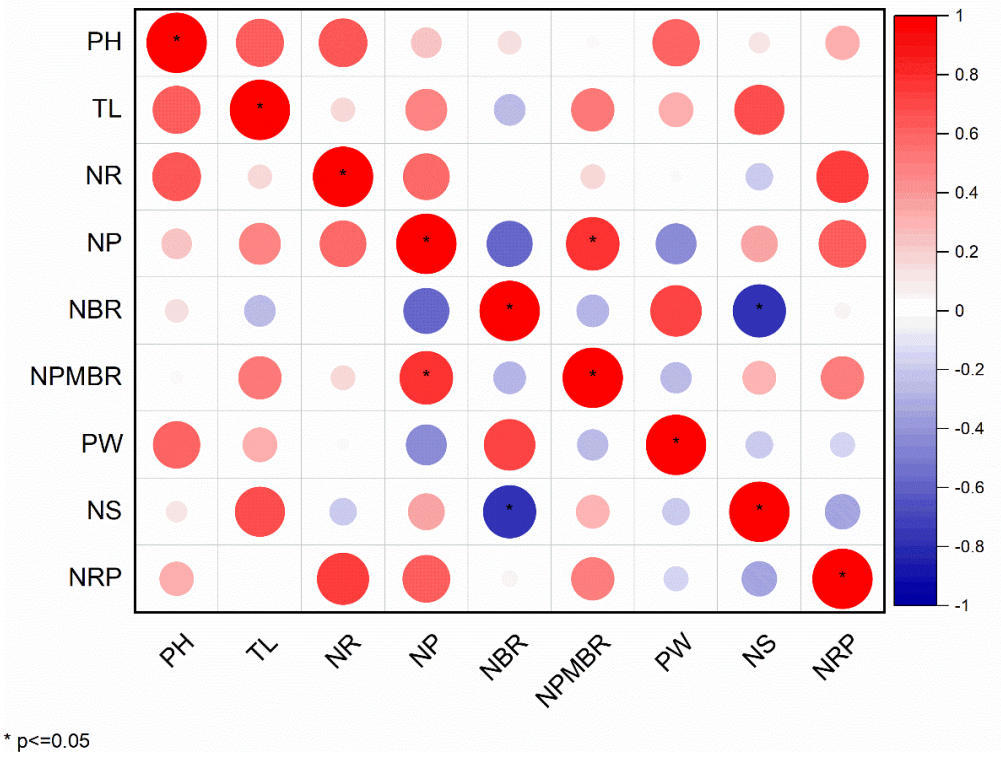

Figure 4.38 Phenotypic correlation matrix of WT plants. Asterisks shows significance at P≤0.05.

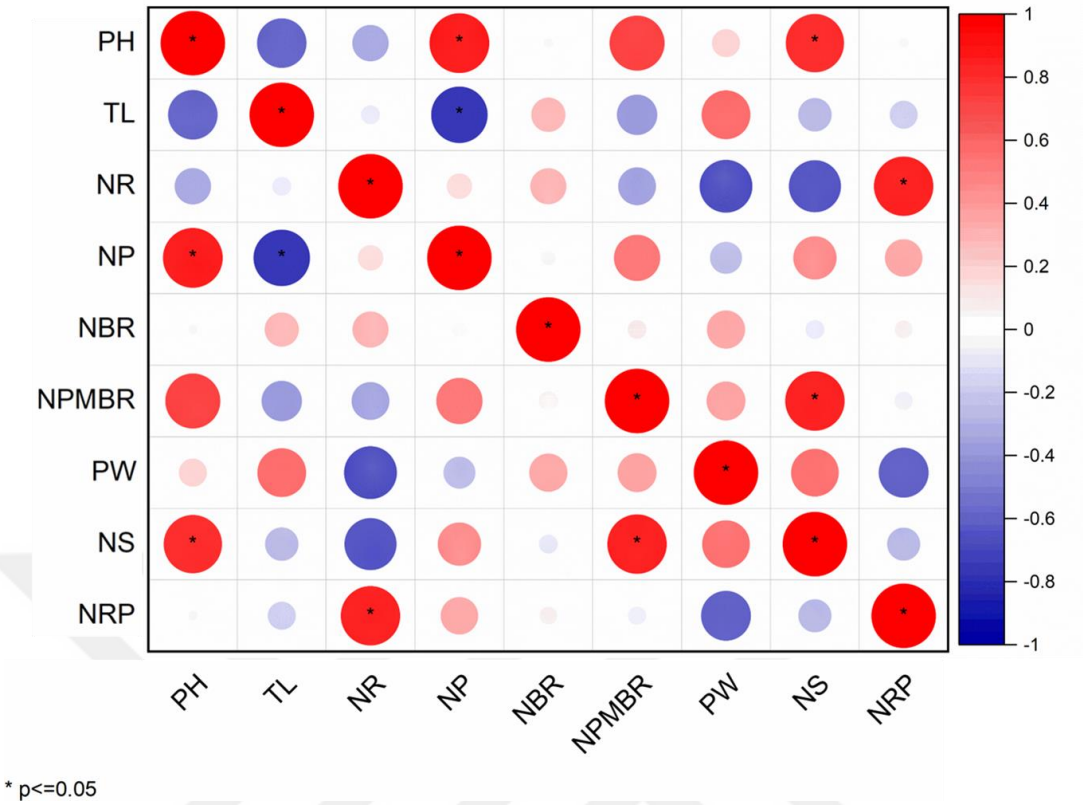

Figure 4.39 Phenotypic correlation matrix of OX plants. Asterisks shows significance at $P \leq 0.05$.

4.11 Root Acidification under Low Sulphate Condition

The rhizosphere acidification was tested among WT and OX during seed germination and at seedlings stage under S deficient (15 µM) condition. The OX exhibited the strong rhizosphere acidification both at seed germination (Figure 4.40A) and at seedling (Figure 4.40B). These results suggest the possible involvment of active H^+ pumping by the plasma-membrane localized H^+ ATPase as the pathway shown in Figure 1.1 for SO_4^{2-}/H^+ uptake or $SeO^{2-}{}_4/H^+$ (Figure 4.41). As $SeO^{2-}{}_4/H^+$ follows the same route of SO_4^{2-}/H^+ therefore, selenate is depicted in the pathway as shown in figure (4.41).

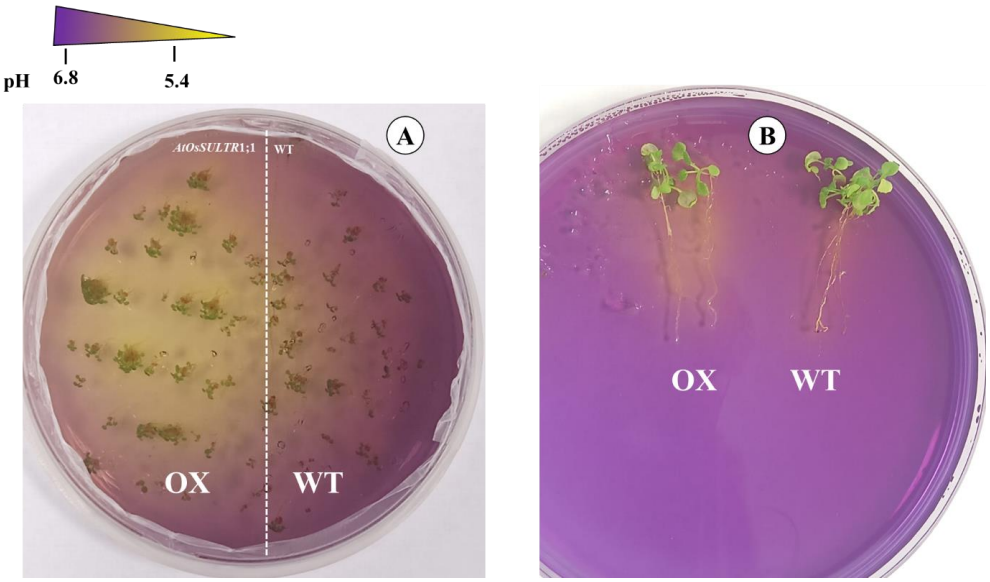

Figure 4.40 Rhizosphere acidification under low sulphate condition. (A) Seeds of WT and OX (OX-1) were germinated on Kimura B agar media containing sulfate supplied at 15 µM. (B) 7 days old seedlings were placed on Kimura B agar media containing sulfate at 15 µM. The plates were prepared with 0.8% w/v agar and 0.006% (w/v) bromocresol purple. The photographs were taken after 3 days (A) and 24 hours (B).

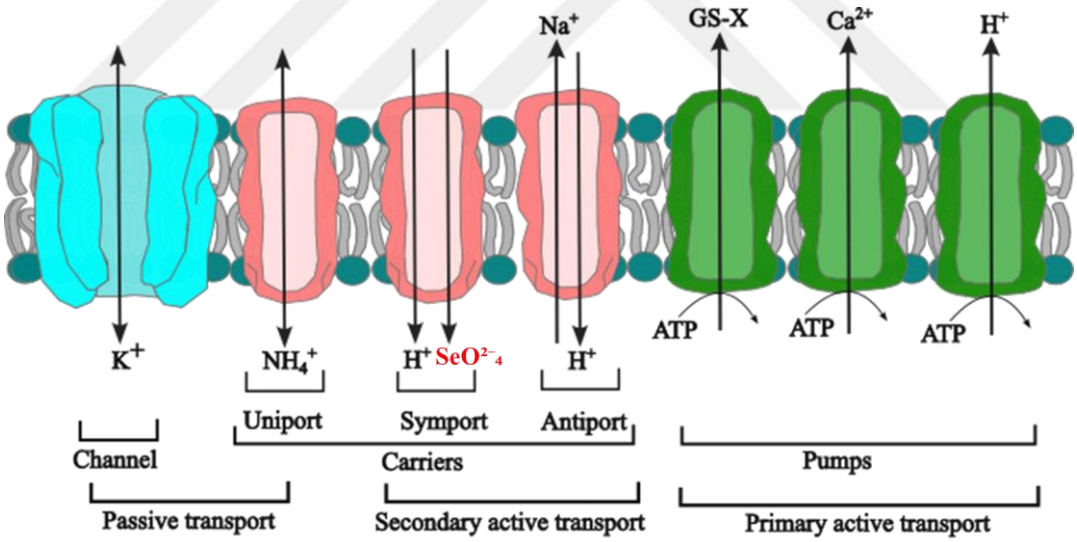

Figure 4.41 Classification of transport membrane proteins. Simplified depiction of primary active transport mechanisms, such as ABC transporters, metal transporters and H+ -ATPase, secondary active transport mechanisms, like SO_4^{2-}/H^+ (Figure 1.1) or $SeO^{2-}{}_4/H^+$ symporter as shown in this figure, and passive transport mechanisms. The figure is adapted and modified from (28) and following (29) for the selenate uptake via secondary active transport system. Therefore, it is proposed that same pathway for selenate uptake as sulfate does.

4.12 Phenotypic Traits of OX and WT under Control and Se Application on Kiumra B Media

4.12.1 Sulfate Deficiency as a Control Treatment

Primary and lateral root lenghts, number of lateral roots and leaves and chlorophyll contents were measured in WT and OX (OX-1) line under S deficient (15 µM) condition (Figure 4.42). Primary and lateral root lengths were highly significant in OX than WT (Figures 4.42, 4.43 A, B) suggesting higher growth rate in OX line. However, the number of lateral roots were same in WT and OX. The number of leaves, chlorophyll content and fresh weight (FW) was found to be highly significant in OX (Figures 4.43 D, E and F). These results suggest that OX of *OsSULTR1;1* promotes and boost plant growth and development under normal growing conditions.

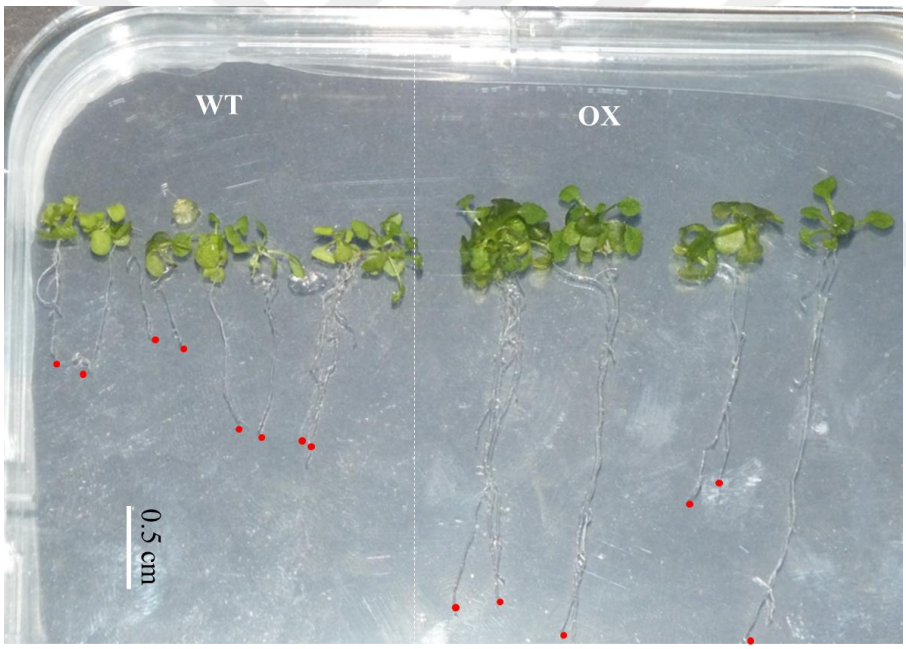

Figure 4.42 WT *Arabidopsis thaliana* and OX-1 transgenic line of *OsSULTR1;1*. The seedlings were grown for a period of three weeks. After three weeks phenotypic observations were recorded. Scale bar 0.5 cm.

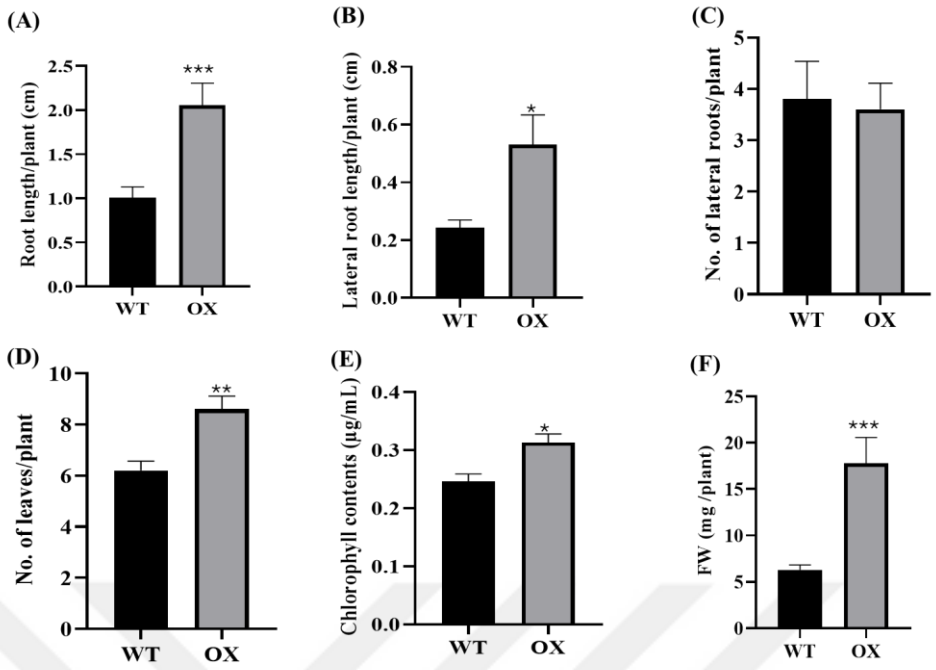

Figure 4.43 Phenotypic observaions of WT *Arabidopsis thaliana* and OX of *OsSULTR1;1*. The plants grown under control treatment (without Se application) for the period of three weeks. At least 5 seedlings of each WT and OX line were used to record the phenotypes and three replications were done. Data represented mean±SE of three replications. The asterisks show **P*≤0.05, ***P*≤0.01, ****P*≤0.001 as determined by Student's t-test.

4.12.2 Effect of 2.5 µM Selenate Application to Morphological and Physiological Traits of WT and OX plants

Under the Se application (Figure 4.44), shoot lengths of both WT and OX seedlings were similar in length (Figure 4.45 A). However, lateral root lengths of OX seedlings were highly significant than WT (Figure 4.45 B). The relative root lenghts measured between two intervals (18 days and 30 days) were non-significant among WT and OX (Figure 4.45 C). Similarly, the number of laeral roots (Figure 4.45 D), leaves (Figure 4.45 E) and chlorophyll contents (Figure 4.45 F) were also non-significant among the plants. These results suggest that under low Se application for extended time (one month), OX of *OSULTR1;1* induces longer lateral roots preferentially. Se uptake by seedlings was significantly high in OX than in WT (Figure 4.45 G) suggesting that *OsSULTR1;1* is efficiently involved in Se uptake at root interface.

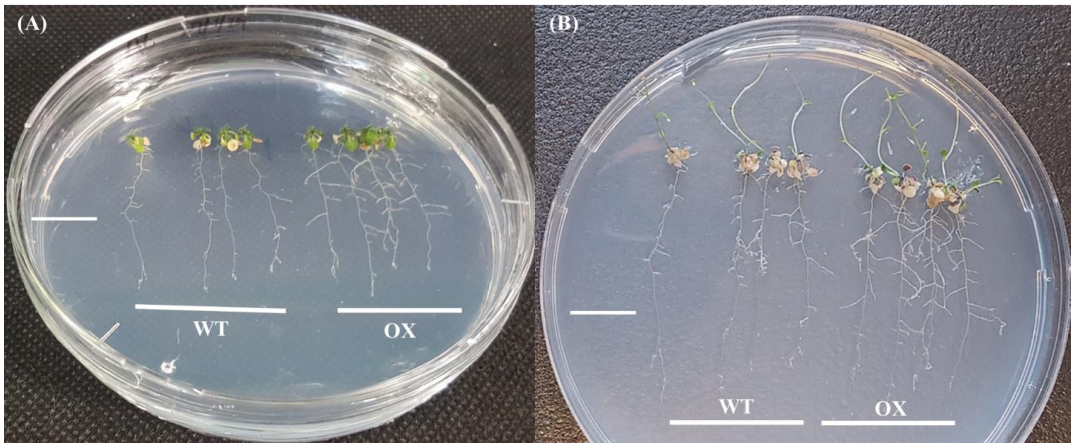

Figure 4.44 WT *Arabidopsis thaliana* and OX transgenic line of *OsSULTR1;1*. The seedlings were grown for the period of one month in the presence of 2.5 µM selenium as a sodium selenate. Phenotypic observations were recorded at 18 days and 30 days of the treatments. 18 days old seedlings (A) and 30 days old seedlings (B) were used for relative root length measuurements. Scale bar 1 cm.

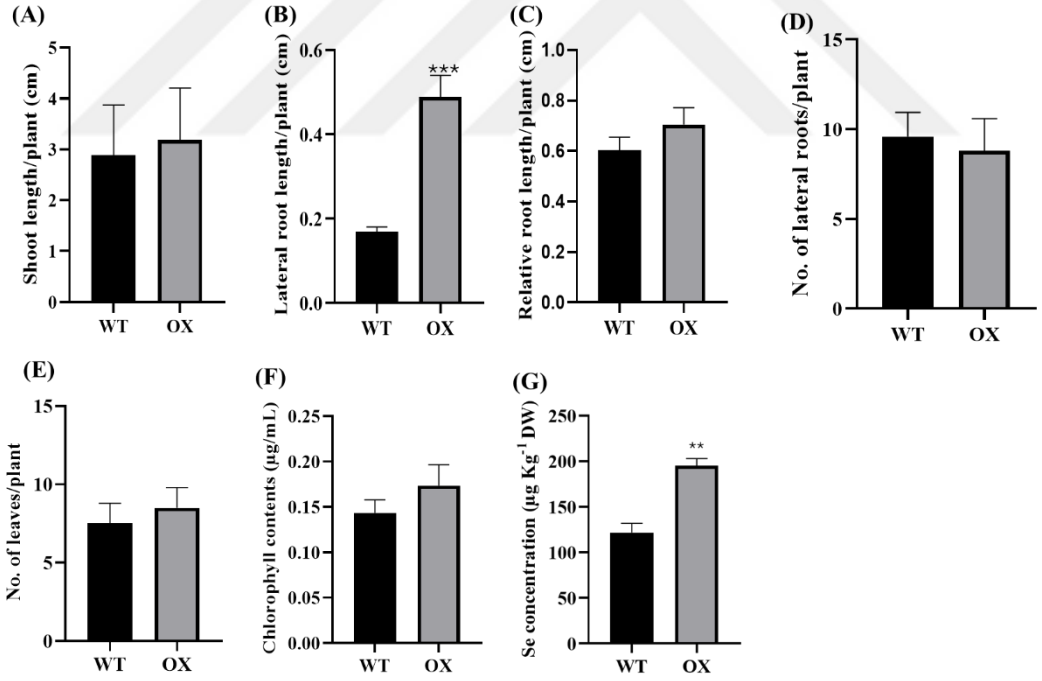

Figure 4.45 Phenotypic observaions of WT *Arabidopsis thaliana* and OX of *OsSULTR1;1*. The plants grown under Se treatment (2.5 µM Se application) for the period of four weeks. At least 4 seedlings of each WT and OX line were used to record the phenotypes and three replications were done. Shoot length here refers to the floral shoot (A) which were recorded after 30 days. Laterla root lenghts were measured after 30 days of treatment (B). Relative root lenghts were measured by the difference in roots of 18 days old seedlings and 30 days old seedlings (C). The number of lateral roots and leaves were measured at the end of the treatment.

Chlorophyll content was measured at 18 days of the treatment. Data represented mean±SE of three replications. The asterisks show ***$P \leq 0.001$ as determined by Student's t-test.

4.12.3 Effect of 10 µM Selenate Application to Morphological and Physiological Traits of WT and OX Plants

Under the Se application of 10 µM (Figure 4.46), the few morphological traits could be recorded due to strong inhibitory effects of Se at slightly higher concentration (10 µM) than 2.5 µM. Therefore, observeable traits like root length and number of rosettes leves could be recorded. The relative root lenghts measured between two intervals (18 days and 30 days) was significant among WT and OX (Figure 4.47 A). However, rosette leaves (Figure 4.47 B) and chlorophyll contents (Figure 4.47 C) were non-significant among the plants. These results suggest that under 10 µM Se application for extended time (one month), OX of *OSULTR1;1* could retain higher root lengths. At this Se concentration, Se uptake was significantly high in OX than WT (Figure 4.47 D) indicating that OX of *OsSULTR1;1* involved in Se uptake.

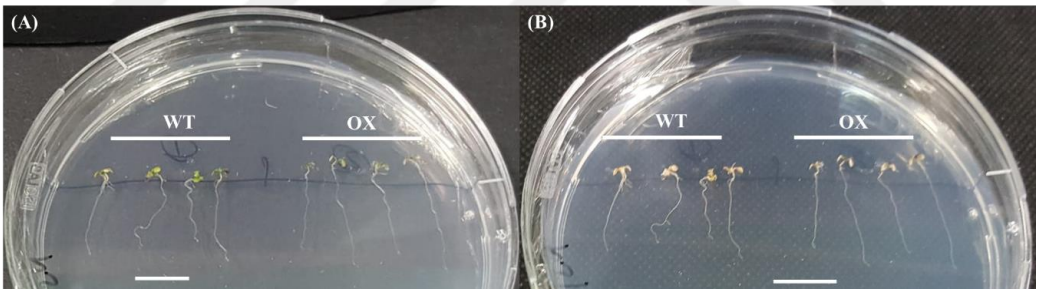

Figure 4.46 WT *Arabidopsis thaliana* and OX transgenic line of *OsSULTR1;1*. The seedlings were grown for the period of one month in the presence of 10 µM selenium as a sodium selenate. Phenotypic observations were recorded at 18 days and 30 days of the treatments. 18 days old seedlings (A) and 30 days old seedlings (B) were used for relative root length measuurements. Scale bar 1 cm.

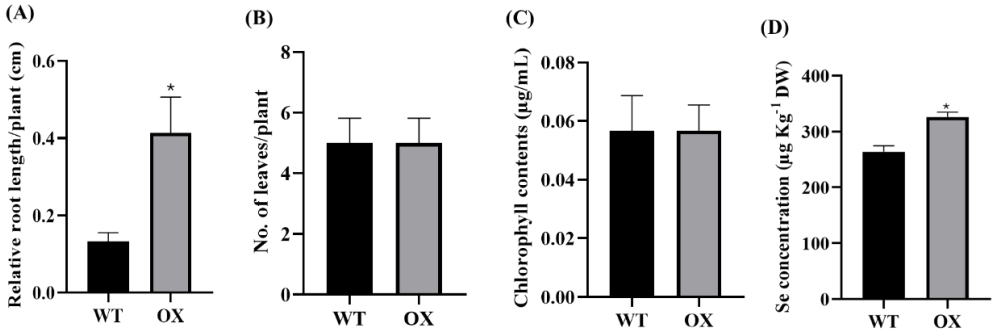

Figure 4.47 Phenotypic observations of WT *Arabidopsis thaliana* and OX of *OsSULTR1;1*. The plants grown under Se treatment (10 µM Se application) for the period of four weeks. At least 4 seedlings of each WT and OX line were used to record the phenotypes and three replications were done. Relative root lenghts were measured by the difference in roots of 18 days old seedlings and 30 days old seedlings (A). Number of lateral roots and leaves were measured at the end of the treatment (B). Chlorophyll content was measured at 18 days of the treatment (C). Data represented mean±SE of three replications. The asterisks show *$P \leq 0.05$ as determined by Student's t-test.

4.12.4 Effect of 25 µM Selenate Application to Morphological and Physiological Traits of WT and OX Plants

Under the Se application of 25 µM due to inhibitory impact of Se application few morphological traits could be recorded (Figure 4.48). Therefore, traits like root length, number of rosettes leves and chlorophyll contents could record. The relative root lenghts measured between two intervals (18 days and 30 days) were non-significant among WT and OX (Figures 4.49 A). Likewise, rosette leaves (Figure 4.49 B) and chlorophyll contents (Figure 4.49 C) were non-significant among WT and OX plants. These results suggest that 25 µM Se application has severe growth inhibitory effect for both OX of

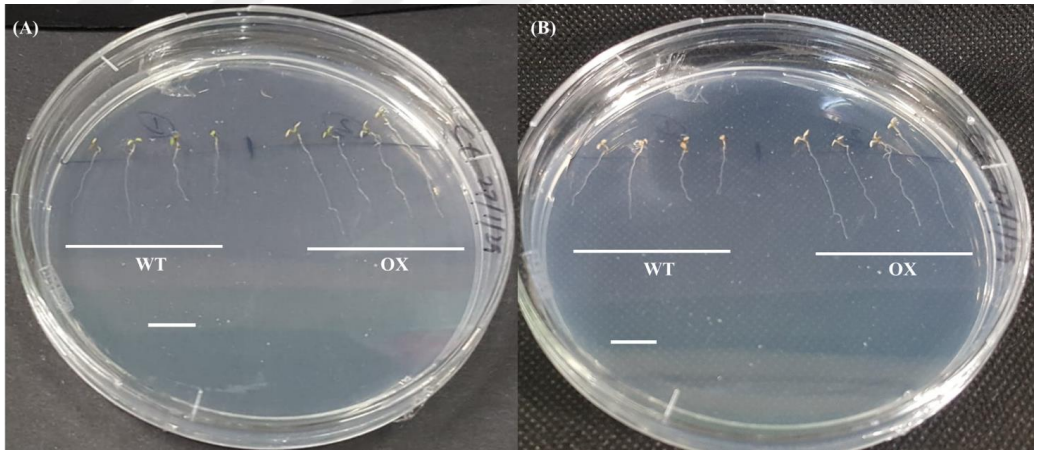

Figure 4.48 WT *Arabidopsis thaliana* and OX transgenic line of *OsSULTR1;1*. The seedlings were grown for the period of one month in the presence of 25 µM selenium as a sodium selenate. Phenotypic observations were recorded at 18 days and 30 days of the treatments. 18 days old seedlings (A) and 30 days old seedlings (B) were used for relative root length measuurements. Scale bar 1 cm.

OSULTR1;1 and WT plants. However, the growth inhitory effect is more pronounced in OX due to significant high Se uptake (Figure 4.49 D) as Se is toxic

at high concentration. The growth inhibitory effect due to Se in OX is much clear when comparing Figure 4.47 A to Figure 4.49 A.

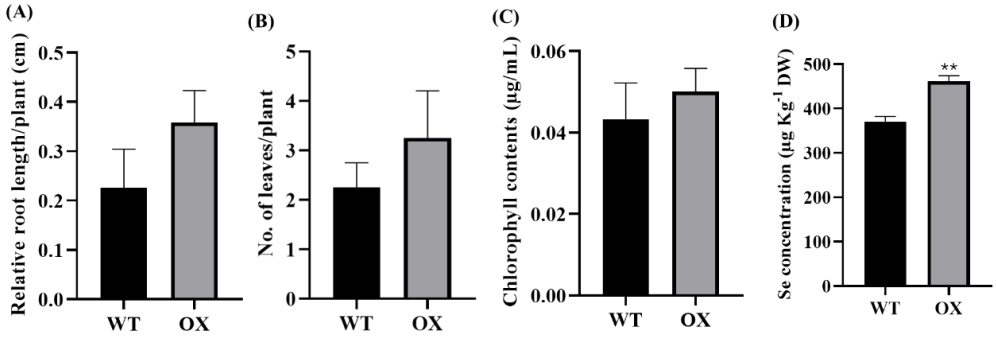

Figure 4.49 Phenotypic observaions of WT *Arabidopsis thaliana* and OX of *OsSULTR1;1*. The plants grown under Se treatment (25 µM Se application) for the period of four weeks. At least 4 seedlings of each WT and OX line were used to record the phenotypes and three replications were done. Relative root lenghts were measured by the difference in roots of 18 days old seedlings and 30 days old seedlings (A). The number of lateral roots and leaves were measured at the end of the treatment. Chlorophyll content was measured at 18 days of the treatment. Data represented mean±SE of three replications.

4.12.5 Effect of 50 µM Selenate Application to Morphological and Physiological traits of WT and OX Plants

Under the Se application of 50 µM due to inhibitory impact of Se application few morphological traits could be recorded (Figure 4.50). Therefore, traits like root length, number of rosettes leves and chlorophyll contents could record. The relative root lenghts measured between two intervals (18 days and 30 days) were non-significant among WT and OX (Figures 4.51 A). Likewise, rosette leaves (Figure 4.51 B) and chlorophyll contents (Figure 4.51 C) were non-significant among WT and OX plants. These results suggest that 50 µM Se application has severe inhibitory effect for both OX of *OSULTR1;1* and WT plants. Also, the Se uptake is significantly higher in OX than in WT (Figure 4.51 D) following the same pattern as described for Figure 4.49 D.

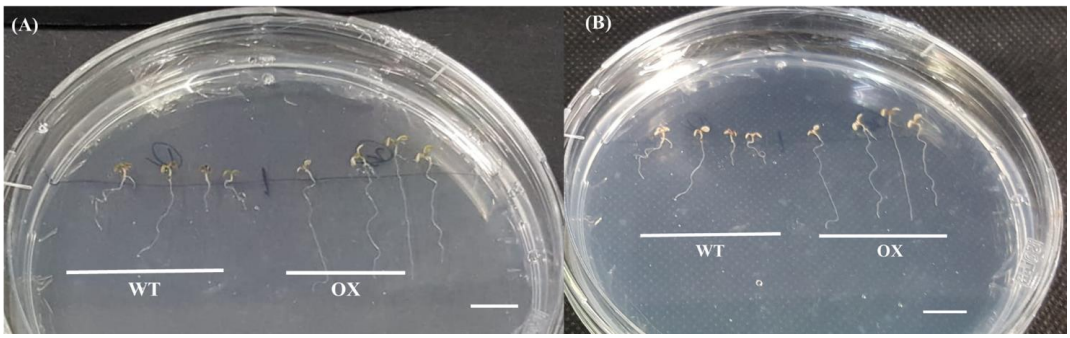

Figure 4.50 WT *Arabidopsis thaliana* and OX transgenic line of *OsSULTR1;1*. The seedlings were grown for the period of one month in the presence of 50 µM selenium as a sodium selenate. Phenotypic observations were recorded at 18 days and 30 days of the treatments. 18 days old seedlings (A) and 30 days old seedlings (B) were used for relative root length measuurements. Scale bar 1 cm.

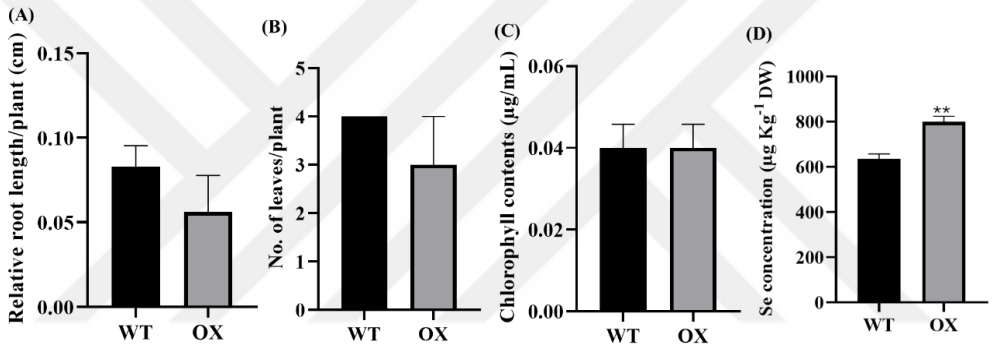

Figure 4.51 Phenotypic observaions of WT *Arabidopsis thaliana* and OX of *OsSULTR1;1*. The plants grown under Se treatment (50 µM Se application) for the period of four weeks. At least 4 seedlings of each WT and OX line were used to record the phenotypes and three replications were done. Relative root lenghts were measured by the difference in roots of 18 days old seedlings and 30 days old seedlings (A). The number of lateral roots and leaves were measured at the end of the treatment. Chlorophyll content was measured at 18 days of the treatment. Data represented mean±SE of three replications.

4.13 Selenate Uptake is Increased in the OX of *OsSULTR1;1*

Both time (Figure 4.52) and concentration-dependent (Figure 4.53) selenate uptake experiments were conducted to determine whether OX of *OsSULTR1;1* has a higher activity to uptake selenate than the wild-type *Arabidopsis thaliana* seedlings.

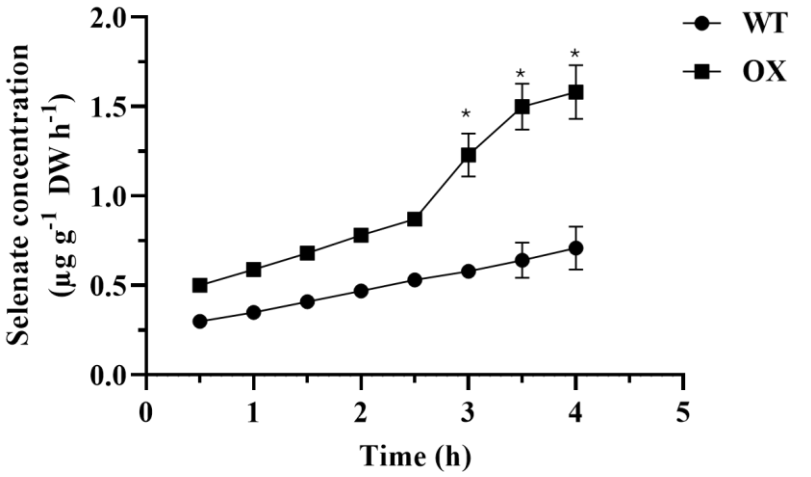

Figure 4.52 Time dependent selenate uptake activity of WT and OX of *OsSULTR1;1* in *Arabidopsis thaliana* seedlings. Data represented mean±SE of three replications. The asterisks show significance at *$P \leq 0.05$ as determined by Student's t-test.

Time-dependent selenate uptake activity showed that *OsSULTR1;1* exhibited higher selenate concentrations than the wild-type at all selenate-treated time-points (Figure 4.52) but significantly higher at 2.5 h. After 2 h exposure, Se concentrations in OX roots exhibited sudden enhanced uptake rate than in WT. These results manifestd that OX of *OsSULTR1;1* supported the acccumulation of selenate over the time period, suggesting that efficient employment of sulphate uptake pathway for the uptake of Se.

4.14 Concentration Dependent Se Uptake in WT and OX of *OsSULTR1;1* Seedlings of *Arabidopsis thaliana*

The concentration dependent experiment revealed that selenate uptake was increased by OX of *OsSULTR1;1* than the WT of Arabidopsis thaliana suggesting its positive role in concentration dependent Se uptake. Se concentration uptake activity manifested by OX line at logarithmic pace, while WT exhibited near saturation pahse at the higher concentration of selenate (Figure 4.53). Se uptake rate was significantly increased at 6 µM and highly enhanced at 9 µM Se applcation. Taken together, it is suggested that Se uptake in time and concentration dependent manner is facilitated by the overexpression of *OsSULTR1;1* in Arabidopsis seedlings.

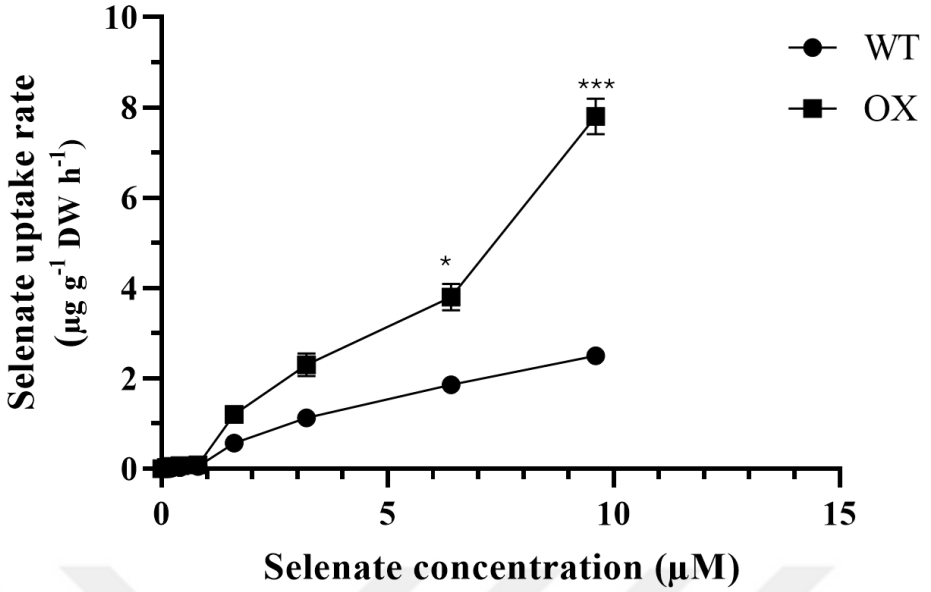

Figure 4.53 Concentration dependent selenate uptake activity of WT and OX of the *OsSULTR1;1* in *Arabidopsis thaliana* seedlings. Data represented mean±SE of three replications. The asterisks show *$P \leq 0.05$, and ***$P \leq 0.001$ as determined by Student's t-test.

4.15 Selenium Accumulation in Tissues of WT and OX-1 Line of Arabidopsis

4.15.1 Selenium Accumulation in Seeds of WT and OX Lines of Arabidopsis

Se accumulated highly significant in seeds of OX than in WT suggesting the functional role of *OsSULTR1;1* in Se accumulation (Figure 4.54) suggests that the ectopic expression of *OsSULTR1;1* facilitates significantly higher uptake of Se in seeds.

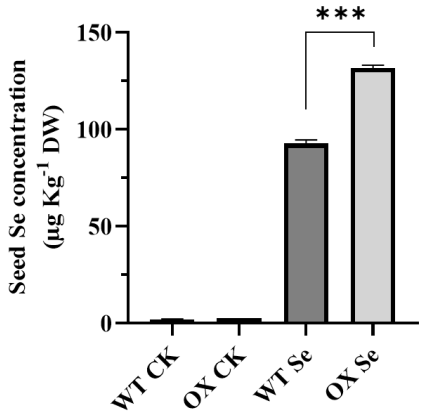

Figure 4.54 Selenium accumulation into seeds of WT and OX lines. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds were

harvested, and Se content was analyzed by ICP-MS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks represent significant differences at $P \leq 0.001$.

4.15.2 Selenium Accumulation in Shoots of WT and OX-1 Line of Arabidopsis

Se accumulation was also recorded in shoots of OX seedlings than in WT as a highly significant due to functiona of *OsSULTR1;1* (Figure 4.55). This again suggests that *OsSULTR1;1* is responsible for Se accumulation in plant shoot tissues.

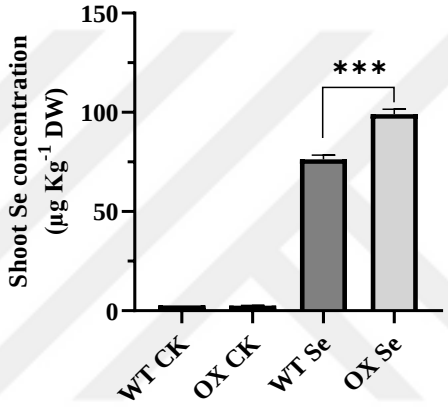

Figure 4.55 Selenium accumulation into shoot of WT and OX lines. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds were harvested, and Se content was analyzed by ICP-MS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks represent significant differences at $P \leq 0.001$.

4.16 Iononomics of WT and OX Lines under Se Application

4.16.1 Sulphate Concentration Status in WT and OX Plants due to Se Application

Besides Se accumullation in plant tissues, other ions were also explored in WT and OX seedlings to determine their accumulation patterns due to application of selenate. S was enhanced in seeds of OX plants highly significant than in WT under control treatment. However, S was reduced from highly significant to significant increment in OX under Se application than in WT (Figure 4.56 A). S was also observed highly significant accumulated in shoot tissues of OX than in WT under control treatment. However, under Se treatment, S was decreased dramatically highly significantly in OX than in WT (Figure 4.56 B). These resulsts

suggest that S accumulation was exhibited significantly in seeds and shoot due to the function of *OsSULTR1;1* and application of Se decreased the S accumulation than in control treatments as Se and S compete for the same transport pathway (Figures 1.1 and 4.41).

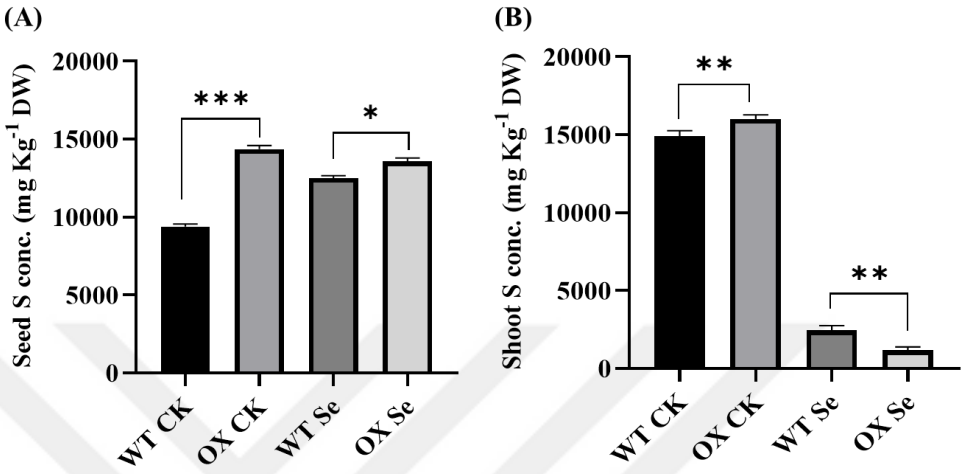

Figure 4.56 Sulphate concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested, and S conc. was analyzed by ICP-OES. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks indicate significant differences at *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

4.16.2 Calcium Concentration Status in WT and OX Plants due to Se Application

OX of *OsSULTR1;1* also exhibited significant Ca accumulation without or with Se application. However, a highly significant pronounced effect of OX for Ca accumulation was observed under Se treatment in seeds (Figure 4.57 A). Similar pattern of Ca accumulation was observed under control or Se treatment in WT and OX plants Figure 4.57 B. These results suggest that Se could enhance Ca accumulation in seed and shoot tissues due to OX of *OsSULTR1;1*.

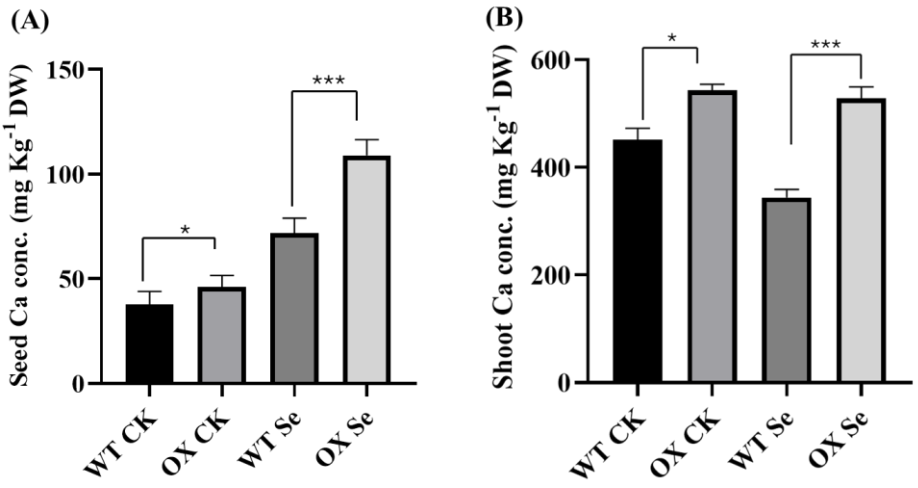

Figure 4.57 Calcium concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested, and Ca conc. was analyzed by AAS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks indicate significant differences at $*P \leq 0.05$ $***P \leq 0.001$.

4.16.3 Phosphate Concentration Status in WT and OX Plants due to Se Application

Phosphate concentration in seed tissues was enhanced significantly both in WT and OX under control treatment while decreased under Se application (Figure 4.58 A). The result suggests that Se application influence negatively the accumulation of phosphate in seed tissues irrespective of WT or OX plants. On contrary, shoot phosphate concentration was decreased under control treatment both in WT and OX plants. However, highly significant concentration was observed in WT under Se treatment than in OX (Figure 4.58 B). The results suggest that Se treatment decreased shoot phosphate accumulation in OX due to antagonistic effect of Se and P.

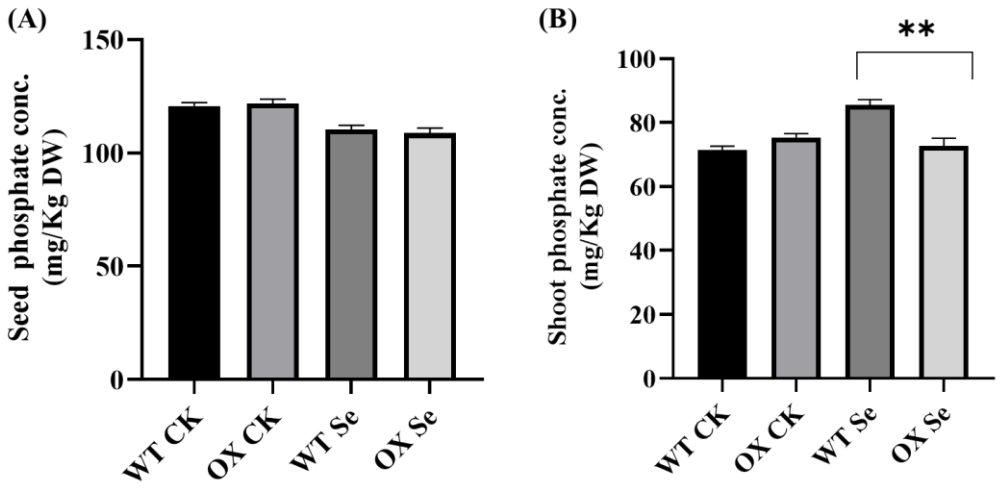

Figure 4.58 Phosphate concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested, and phosphate conc. was analyzed by UV-spectrophotometer. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks indicate significant differences at $*P \leq 0.05$ $**P \leq 0.01$.

4.16.4 Potassium (K) Concentration Status in WT and OX Plants due to Se Application

Potassium accumulation was significantly decreased in OX than in WT under control treatment. However, Se treatment enhnaced potassium accumulation in OX significantly than in WT (Figure 4.59 A). The results suggest that Se application mediates potassium accumulation via OX of *OsSULTR1;1* in seeds. Potassium level was non-significant in both WT and OX plants under control treatment in shoot tissues. However, under Se application, potassium concentration was accumulated significantly in OX than in WT suggesting synergistic role of Se in potassium accumulation (Figure 4.59 B).

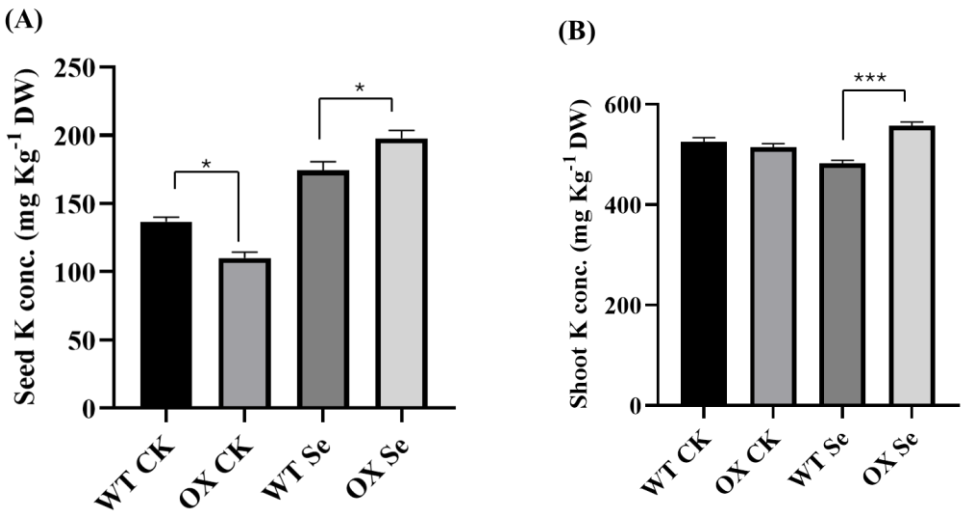

Figure 4.59 Potassium concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested, and potassium conc. was analyzed by AAS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks indicate significant differences at *$P \leq 0.05$ ***$P \leq 0.001$.

4.16.5 Magnesium (Mg) Concentration Status in WT and OX Plants due to Se Application

Magnesium accumulation was not significant in seeds under control or Se treatment irrespective of WT or OX plants suggesting no effect of Se on magensium concentration (Figure 4.60 A). However, a highly significant level of Mg was found in OX than in WT under Se treatment indicating synergisitic effect of Se in OX for the Mg level (Figure 4.60 B).

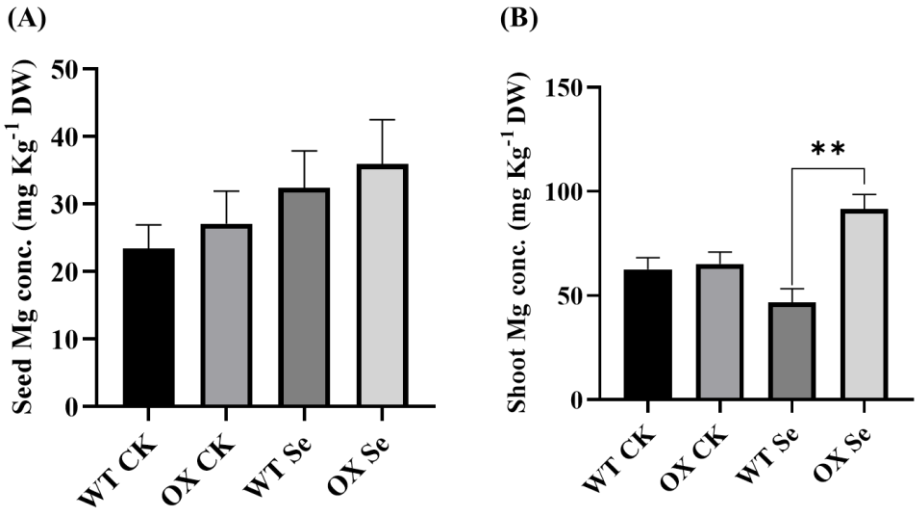

Figure 4.60 Magnesium concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested, and magnesium conc. was analyzed by AAS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks indicate significant differences at $^{**}P \leq 0.01$.

4.16.6 Copper Concentration Status in WT and OX Plants due to Se Application

Cu concentration in seeds of both WT and OX plants was non-significant under control as well as Se application (Figure 4.61 A). Similarly, in shoot tissues, Cu concentration was non-significant under congtrol and Se treatment both in WT and OX plants (Figure 4.61 B) suggesting no impact of Se absence or presence on accumulation pattern in seeds.

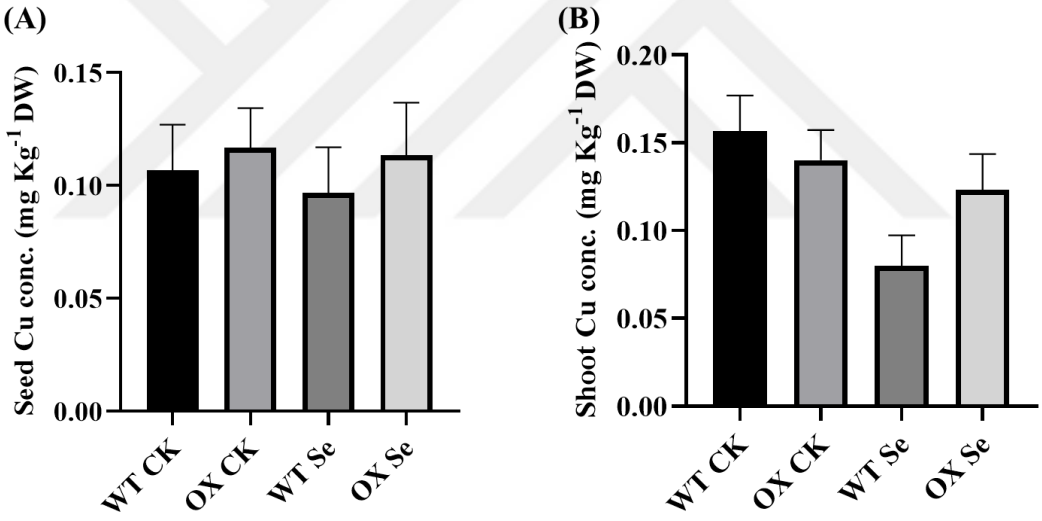

Figure 4.61 Copper concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested, and copper conc. was analyzed by AAS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test.

4.16.7 Iron (Fe) Concentration Status in WT and OX Plants due to Se Application

Iron (Fe) concentration in seeds was found to be highly significant in OX than in WT under control treatment. Again, under Se treatment, Fe concentration was highly significant in OX than in WT under control treatment (Figure 4.62 A)

suggesting synergisitic role of *OsSULTR1;1* in enhancing Fe in seeds. Fe accumulation was non-significant in WT and OX plants both under control and Se treatment in shoot tissues (Figure 4.62 B).

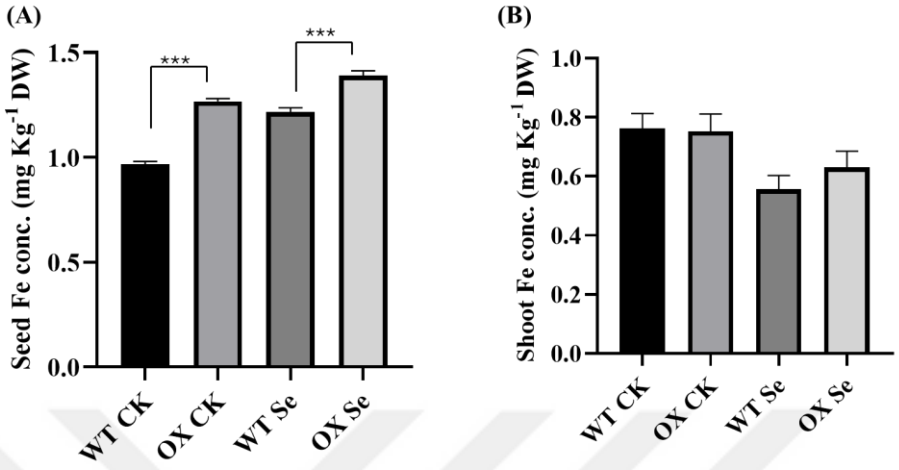

Figure 4.62 Iron concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested, and iron conc. was analyzed by AAS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks indicate significant differences at ***$P \leq 0.001$.

4.16.8 Boron (B) Concentration Status in WT and OX Plants due to Se Application

Boron (B) concentration was found to be non-significant in WT and OX plants under control treatment. However, under Se application B concentration was enhanced in OX significantly than in WT (Figure 4.63 A) suggesting synergistic effect of *OsSULTR1;1* for the accumulation in seeds. B concentration was found to be highly significant in shoots of OX than in WT under control treatment suggesting positive role of *OsUSLTR1;1* for B accumulation. However, B was decreased under Se treatment both in WT and OX but significantly decreased in OX (Figure 4.63 B) suggesting antgaonistic effect of Se on B accumulation via *OsSULTR1;1*.

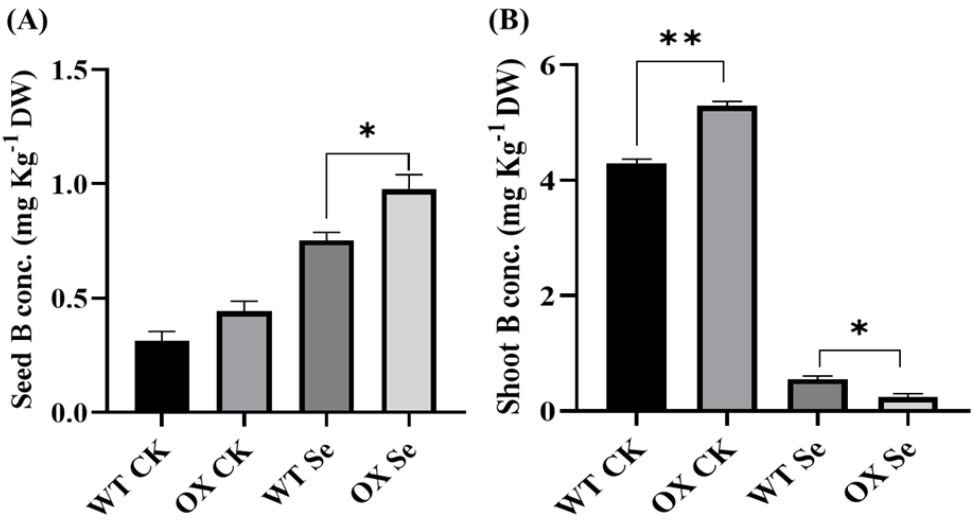

Figure 4.63 Boron (B) concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested, and B conc. was analyzed by AAS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks represent significant differences at *P≤0.05 ***P≤0.001.

4.16.9 Molybdenum (Mo) Concentration Status in WT and OX Plants due to Se Application

Molybdenum (Mo) concentration was found non-significant in WT and OX plants under control treatment. However, under Se application Mo accumulation was enhanced in OX significantly than in WT (Figure 4.64 A) suggesting positive effect of Se in Mo enhancement via *OsSULTR1;1* in seeds. Mo concentration was found to be highly significant in shoots of OX than in WT under control treatment suggesting positive role of *OsUSLTR1;1* for Mo accumulation. However, Mo was decreased under Se treatment both in WT and OX (Figure 4.64 B) suggesting antgaonistic effect of Se on Mo accumulation.

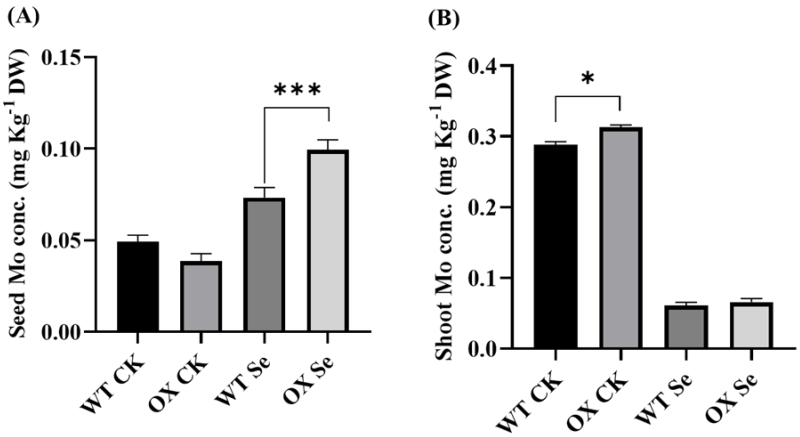

Figure 4.64 Molybdenum (Mo) concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested, and Mo conc. was analyzed by AAS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks represent significant differences at *$P \leq 0.05$ ***$P \leq 0.001$.

4.16.10 Manganese (Mn) Concentration Status in WT and OX Plants due to Se Application

Manganese (Mn) accumulation was found to be non-significant under control and Se treatment both in WT and OX plants suggesting no effect of Se or *OsSULTR1;1* in Mn accumulation in seeds (Figure 4.65 A). In shoot tissues of WT and OX plants, under control treatment, Mn was enhanced highly significant in OX suggesting positive impact of *OsSULTR1;1* in Mn enhancement. Under Se treatment although Mn concentration was decreased both in WT and OX but still highly significant maintained in OX (Figure 4.65 B) suggesting synergisitic effect of Se via *OsSULTR1;1*.

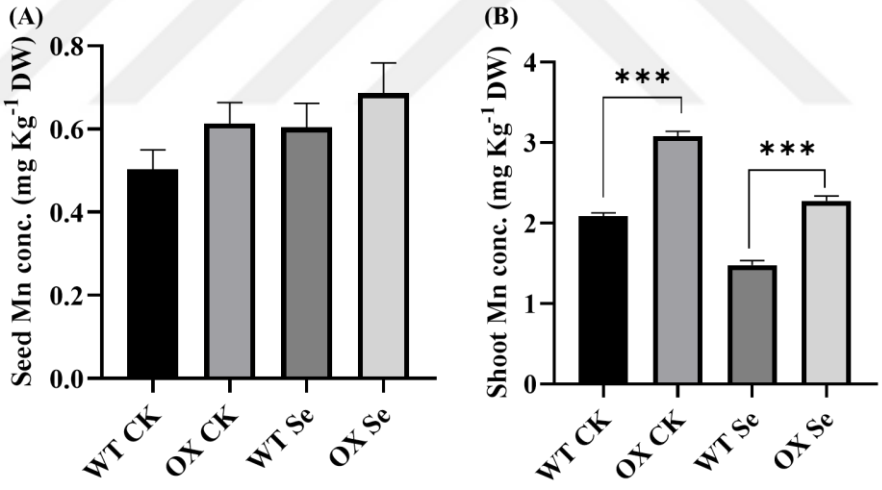

Figure 4.65 Manganese (Mn) concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested, and manganese conc. was analyzed by AAS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks represent significant differences at ***$P \leq 0.001$.

4.16.11 Nickel (Ni) Concentration Status in WT and OX Plants due to Se Application

Nickel (Ni) concentration was found significantly higher in WT under control treatment than in OX suggesting that *OsSULTR1;1* negatively regulates the accumulaiton of Ni in seeds. However, under Se application, the level of Ni was non-significant in WT and OX suggesting the synergistic effect of Se in OX (Figure 4.66 A). on the other hand, Ni was found non-significant in WT and OX under control treatment. Under Se treatment, Ni was accumulated highly significant in shoots of OX (Figure 4.66 B) suggesting synergistic effect of *OsSULTR1;1*.

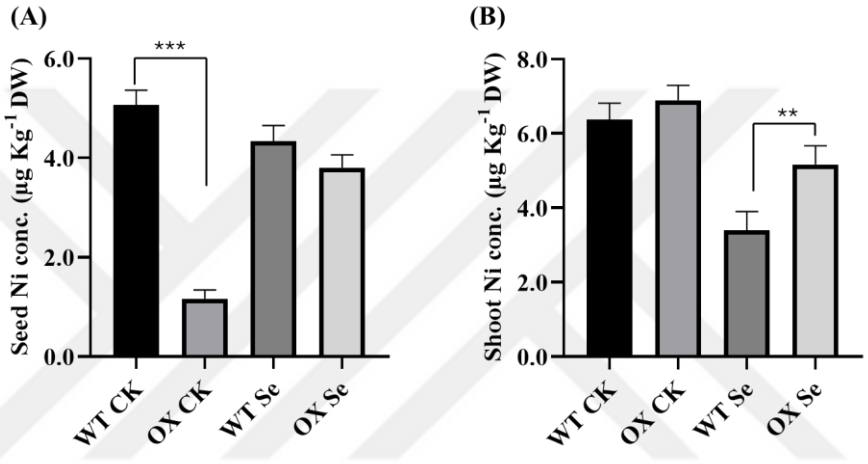

Figure 4.66 Nickel concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested and nickle conc. was analyzed by AAS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks represent significant differences at $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$.

4.16.12 Zinc (Zn) Concentration Status in WT and OX Plants due to Se Application

Zinc (Zn) concentration was found to be highly significant in seeds of OX under control and Se application suggesting ubiquitous funcioning of *OsSULTR1;1* to maintain high Zn level in seeds (Figure 4.67 A). However, Zn was found non-significant in shoots of WT and OX under control treatment. On the other hand, Zn was found to be highly significant in shoots of OX plants under Se treatment suggesting positively Zn regulation of *OsSULTR1;1* (Figure 4.67 B).

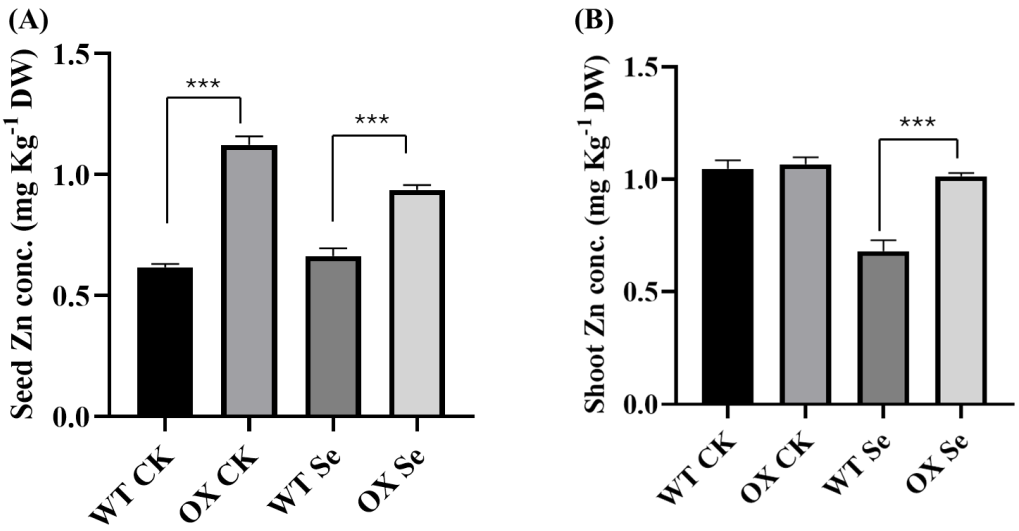

Figure 4.67 Zinc concentration in seeds and shoots of WT and OX line mediated by Se application. The selenate was supplied at 2.5 µM concentration for the period of 5 weeks. The seeds (A) and shoots (B) were harvested, and zinc conc. was analyzed by AAS. Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks represent significant differences at ***$P \leq 0.001$.

4.17 Abiotic Stresses

4.17.1 Growth Sttributes of Arabidopsis Plants Positively Enhanced by Se Application in WT and OX Line under Heat Stress

4.17.2 Heat Stress Tolerance

Under high growing temperature of 32ºC for the period of one month, imposed negative impact profoundly on WT and OX in the absence of Se (control) in terms of dgreening (early onset of senescence) and shorter pod length (Figure

4.68 and Figure 4.70) respectively. On the other hand, every week application of 2.5 µM Se maintained highly significant pod length and chlorophyll content in WT and more profoundly in OX (Figure 4.69 and Figure 4.70) suggesting Se alleviate heat stress and improves pod length contributing to yield componenet. Furthermore, Se also delayed senescence in OX and WT (Figure 4.69).

Figure 4.68 Phenotype of OX and WT Arabidopsis plants grown in the absence of Se application for 35 days at temperature 32℃. Please note the complete senescence of leaves in OX and WT plants.

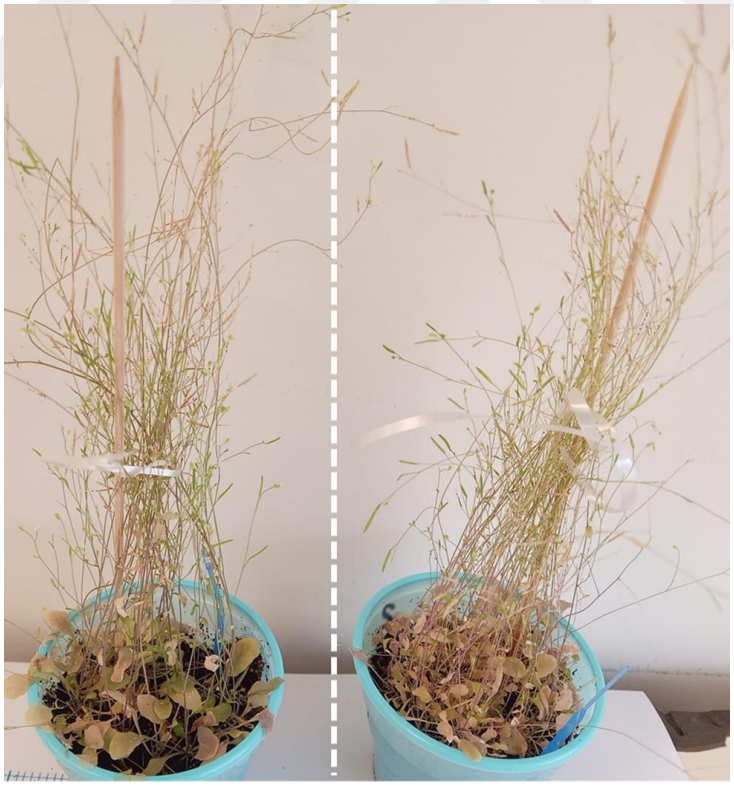

Figure4.69 Phenotype of OX and WT Arabidopsis plants grown in the presence of Se application (2.5 μM) for 35 days at temperature 32℃. The Se was supplied every 7 days. Please note the onset of senescence in leaves in OX and partial senescence in WT plants.

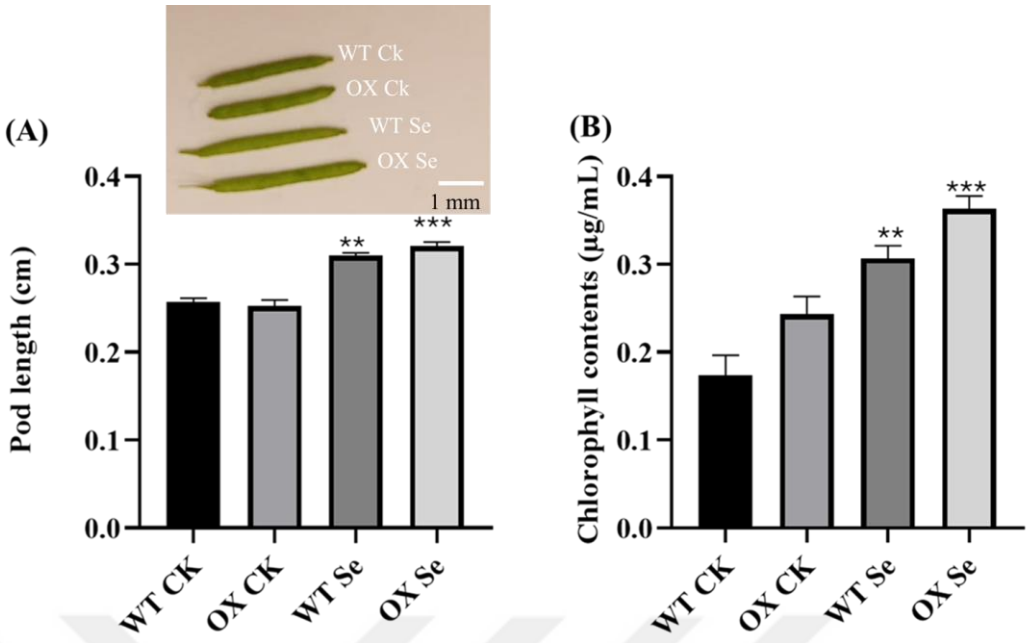

Figure 4.70 The growth attributes of Arabidopsis plants without (control) and with Se (2.5 µM) application at high temperature (32℃). Data represented mean±SE of three replications. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks represent significant differences at $^{**}P \leq 00.01$ and $^{***}P \leq 0.001$.

4.17.3 Cadmium Stress Alleviation

Cadmium (Cd) stress exposure for the period of one month imposed negative effect on growht on WT in terms of primary root and shoot lengths. The lengths of primary root and shoot significantly enhanced in OX than in WT suggesting Cd stress amiloration effect in OX due to the Se application (Figure 4.71 and Figures 4.72 A and C). However, the lateral lenghts were non-significant among WT and OX (Figure 4.72 B).

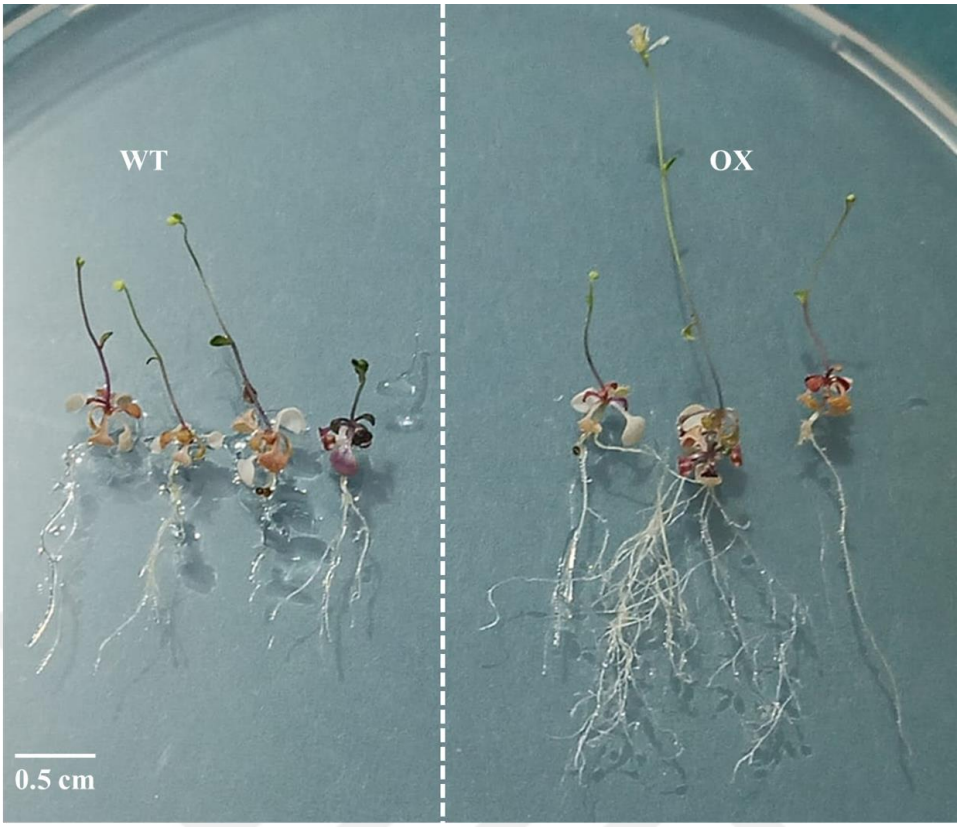

Figure 4.71 Cd stress (35 µM $CdCl_2$) alleviated by Se application in OX line. The Arabidopsis WT and OX seedligns were grown on Kimura B media supplemented with 2 µM sodium selenate and 15 µM sulphate in the form of $MgSO_4.7H_2O$. The plants were grown for the period of one month and data was collected. Representative figure of the plants shown here.

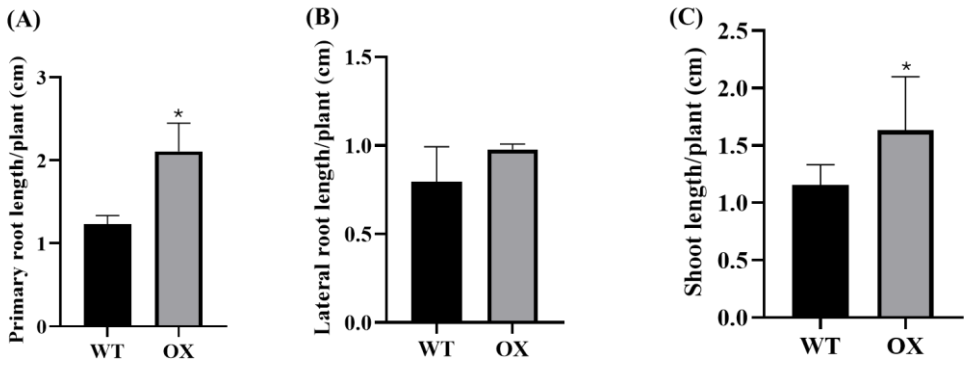

Figure 4.72 Growth attributes of WT and OX seedlings under Cd stress in the presence of 2 µM Se for the period of 4 weeks. Data represented mean±SE of three replications. The asterisks show *$P \leq 0.05$ as determined by Student's t-test.

4.17.4 Pb Stress

Primary root length of WT and OX seedlings was found non-significant under Pb stress in Se treatment. However, Se application (2 µM) alleviated the harmful effects of Pb on OX seedlings by promoting highly significant level of anthocynanin production in young and newly emerging leaves (Figure 4.73 and Figure 4.74 B). Although primary root growth did not differ among WT and OX (Figure 4.74 A) but on the otherhand anthocynanin could not accumulate in WT seedlings and no emergence of new leaves (Figure 4.73 and Figure 4.74 B) suggesting that Se ameliorating imapct on Pb stress alleviation is specifically mediated by *OsSULTR1;1*. The number of leaves also significantly increased in OX than in WT (Figure 4.74 C).

Figure 4.73 Phenotypes of WT and OX Arabidopsis plants under Pb stress in the presence of Se (2 µM). Four days old seedlings were placed on Kimura B media containing 300 µM Pb acetate as a source of Pb. Treatment continued for the period of one month and then data were recorded.

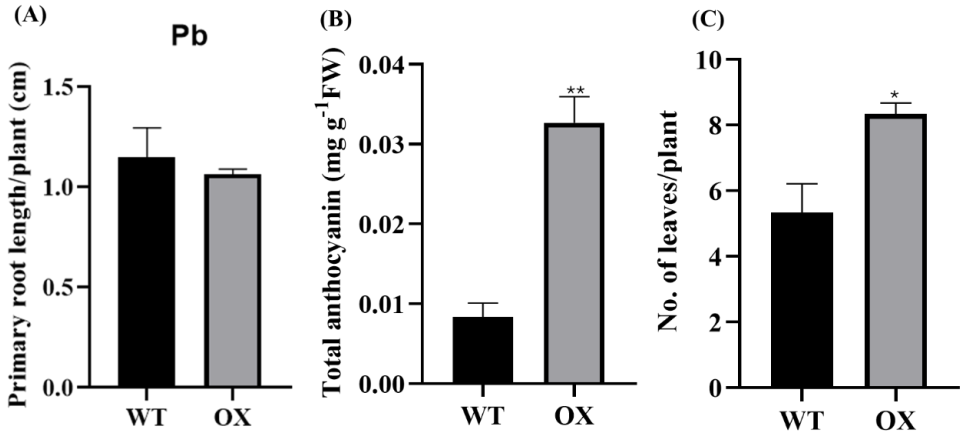

Figure 4.74 Growth attributes of WT and OX seedlings under Pb stress (300 µM) in the presence of 2 µM Se for the period of 4 weeks. Data represented mean±SE

of three replications. The asterisks show *$P \leq 0.05$ **$P \leq 0.001$ as determined by Student's t-test.

4.17.5 Al Stress Alleviated by Application of Se in OX and WT

WT and OX plants were exposed to 50 µM Al stress for 24 h. Al severely inhibited root length in WT in the absence of Se which was highly significant. However, under Se application, root length was inhibited but significantly than in control (Figure 4.75 A). The results suggest that Se application partially resuced root inhibition in WT. On the other hand, in OX seedlings, root length was inhibited significantly in the absence of Se but in Se application, root length of OX was mainatained that was non-significant to the control suggesting that Se treatment in OX positively alleviated the harmful effect of Al (Figure 4.75 B). These results suggest that over-expression of *OsSULTR1;1* in the presence of Se minimizes the degree of root inhibition due to Al stress.

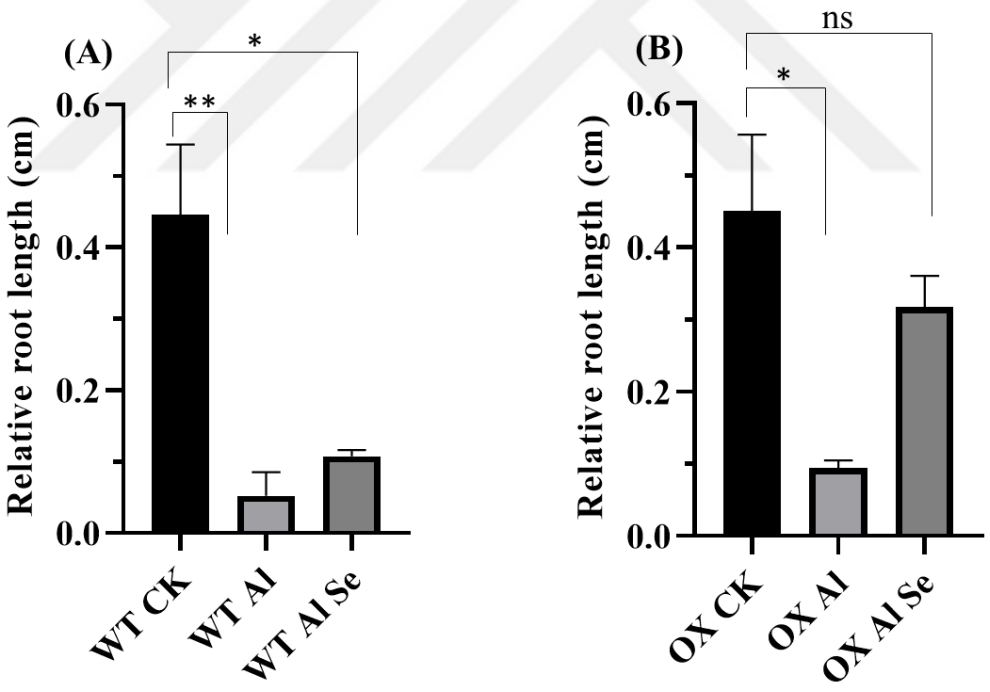

Figure 4.75 Impact of aluminum (Al) on the root length inhibition of WT and OX line in the absence and presence of Se application. Al treatment was performed for 24 h at pH 4.5 and the media was consisted of 100 mM $CaCl_2$ including 50 µM Al. The root length was measured before and after the treatments. The data was analyzed by one way ANOVA and significant differences followed by Tukey's test. The asterisks show significant differences at *$P \leq 0.05$ **$P \leq 0.01$ and ns represent non-significant differences.

5. DISCUSSION

There are five groups of sulfate transporters which are reported for Arabidopsis and rice (8). The group of sulfate transporters 1 (SULTR1) is considered as high affinity transporter, SULTR2 as low affinity and SULTR 3 as interplay of low affinity sulfate transporter, group of SULTR4 and SULTR5 as tonoplast localized sulfate transporters. Since, in literature there is no study related to sulfate transporters especially in context to selenate uptake and the regulation in Basmati cultivar which is an aromatic indica rice. Therefore, the present study was focused on sulfate transporters regulation and expression under selenate application. We found that among high affinity sulfate transporters, *OsSULTR1;1* is mainly expressed in root tissues under selenate application at seedling stage Figure 4.5. The transporters localized in root tissues play a major role in nutrient uptake from rhizosphere or media. The comprehensive expression and loss of function-mutant analysis have verified the high-affinity sulfate transporters as the basic step for the entrance of sulphate into the plant (14, 47, 76). Because sulfate transporters are known to bring in sulfate, it turns out they can do the same for selenate. This is because selenate is chemically very similar to sulfate (14, 15). Scientists were able to identify a particular sulfate transporter, *SULTR1;2,* by looking at Arabidopsis plants that can resist selenate. This transporter plays a key role in bringing enough sulfate into the roots. (9). Therefore, we have investigated the expression of *OsSULTR* genes under selenate in shoot, root, and grain. The expression of the *OsSULTR1;1* in root interface as well in developing seeds could have role in Se uptake in Basmati rice (Figures 4.5 and 4.19). Other high affinity transporters *OsSULTR1;2* and *OsSULTR1;3* mainly expressed in shoot tissues only. The low affinity transporters (*OsSULTR2;1* and *OsSULTR2;2*) expressed at negligible level. The group of sulfate transporters 3 expressed mainly in shoot tissue but absent in root tissues. Group 4 and 5 sulfate transporters also expressed at low level. All this experimental evidence favors *OsSULTR1;1* as the key gene for Se uptake from selenate in Basmati rice in contrary to *OsSULTR1;2* which was found responsible for selenate uptake in Se- rich red rice of japonica origin (10). pH dependcy of the Basmati rice (Figure 4.3) shows similar pattern of selenate uptake as reported earlier for selenite uptake in japonica rice (11). As group 3 sulfate transporters express at the highest level in seed during seed development and growth stages and in shoot

in case of Basmati rice. Similar expression patterns were also reported for sulfate transporter of group 3. The gene similar to *AtSultr3.1* in chickpea is active in various parts of the plant, including pods, testa (seed coat), and developing embryos. This suggests it plays a role in sulfate transport across many cell types in chickpea. Another gene, similar to *AtSultr3.3*, is found in nearly all chickpea organs, with high levels specifically in developing embryos (77). In contrast, the Arabidopsis genes *AtSultr3.1*, *3.2*, and *3.3* seem to be active only in the leaves. Overall, the researchers observed broader activity of sulfate transporter genes in chickpea compared to Arabidopsis (78). Therefore, present findings highly corroborate with previous reported results that sulfate transporters of group 3 are shoot and specific and chloroplast localized transporters (34, 43, 44).

OsSULTR1;1 is localized to plasma-membrane of the cell implicating its possible role in nutrient uptake through root cells which is depicted by its tissue specificity in root tissues (Figure 4.5) and expressed in developing seeds (Figure 4.19). The genes which express in root cells for the nutrient uptake also show liability of nutrient accumulation in rice seeds. For example, rice phosphate transporter OsPT2 which also expressed in root and plasma-membrane localized could enhance Se content in rice grains (12, 13).

In conclusion, *OsSULTR1;1* could be a potential gene for active uptake of Se and subsequently high accumulation in grains of Basmati indica rice. Further, detailed functional characterization of the gene by developing over-expressed or knock-out mutants could revelae the detailed functions of the gene. Therefore, by utilizing approaches of forward genetics in the current study to explore the detailed functions of the gene.

Sulfate is a preferred source of sulfur which is taken up by plants. In soil sulfate uptake is mediated by secondary active transport system of sulfate transporters (Figure 1.1). Like other nutrients, sulfate influx also follows the Michelis-Mentem equation and this phenomenon strongy indicates the involvement of transport protein which is specific for sulfate influx. Takahashi (29) proposed the secondary active transport mechanism of sulfate uptake via sulfate transport system at plasma-membrane interface. Since sulfate has a negative charge, transport of ions is mediated by protons.

Similarly, proton-based transport of phosphate and iron also well established earier (79, 80). Therefore, the release of proton and change in rhizosphere

acidification (Figure 4.40) strongly point to the secondary active transport system as shown in Figure 1.1.

Root functions primarily in obtaining sulfur sources from the soil environment. As a result, the pace at which the root absorbs its first sulfate influences the fluxes of sulfur that are produced by internal transport and metabolic conversion. Since it may use a proton as a motive force to pump up sulfate to the cytosol, a secondary active transport system can be proposed as a membrane-bound facilitator for the inward rectifying flow of sulfate across the plasmamembranes (29).

OsSULTR1;1 is a high affinity sulphate transporter which is primarily responsible for S transport from root especially under limiting condition (52, 67). The ectopic expression of the transporter in *Arabidopsis thaliana* also improves growth of plant, root length and germination under limiting S but also protects from hramful effect of heavy metals like As(III), As(V), Cd and Cr (67). The improved growth of the transgenic plants under heavy metals stress could be due to ehnanced antioxidants since heavy metals induce oxidative stress and generation of reactive oxygen species.

However, the functional characterization of *OsSULTR1;1* under Se treatment remained to be elusive. Therefore, transgenic *Arabidopsis thaliana* plants transformed with *OsSULTR1;1* were generated, and the gene was cloned from Super basmati rice cultivar. The transgenic Arabidopsis plants exhibited higher growth in terms of primary and lateral root length, leave numbers and chlorophyll content under limited S (15 μM) condition (Figures 4.42 and 4.43). These results shed light on the role of *OsSULTR1;1* plant growth and development in details than reported earlier where seed germination and root length was reported (67).

High concentration (for long term application) of Se is toxic to plant health and poses severe phytotoxicity (81). To determine suitable concentration of Se, a range of Se applications were applied to WT and OX Arabidopsis seedlings. In the current findings only 2.5 μM Se application proved to be beneficial in terms of plant health and growth (Figures 4.44 and 4.45). However, other high concentration applications (10, 25 and 50 μM) posed severe growth inhibition (Figures 4.46-4.51) corroborating with previous finding when functional mutant of *AtSultr1;2* (*Sel1*) was used (14, 15). Similarl findings have been reported earlier for the Se beneficial and harmful effects on plant growth and development respectively. Concentration of Se more than 0.75 and 0.25 mg/L for tomato and basil respectively found to be

phytotoxic and yield determinant therefore lower concentrations of Se than the aforementioned were proposed to avoid yield reduction and possible phytotoxic (82). Likewise, higher concentration treatment to *C. violifolia* posed negative effect on growht even plants were died (83).

Hartikainen points out that selenium has a double-edged effect. In small amounts, it acts like a shield for plants, protecting them from damage and even helping them grow better. But at higher levels, it becomes a pro-oxidant, disrupting the metabolic processes and lowering the yield (84). Although under control, primary and lateral root length was enhanced significantly in OX but also found enhanced chlorophyll content, FW, and number of leaves (Figure 4.43). However, under Se treatment at 2.5 μM concentration did not pose negative impact on WT and OX plants except OX exhibited significantly higher number of lateral roots. Higher concentrations of Se at 10, 25 and 50 μM found to be deterimental for the growth of both OX and WT seedlings (Figures 4.46-4.60). Althogh, at 10 μM Se treatment, relative root length was found significant in OX but other growth attribute like number of leaves or chlorophyll content were totally inhibited. Several studies witnessed about the low concentration of Se as a beneficial agent for plant growth and improves tolerance agianst various stresses (84). Therefore, low dose of 2.5 μM Se application was applied in the subsequent experiment without posing negative impact of Se on plant health.

For short term treatment of Se, increased concentration in media enhanced the uptake of Se but more profoundly in OX than WT (Figure 4.53). Similarly Se content was increased in plants of *Cardamine violifolia* with increasing Se concentration (83). Although rice plant has high affinity to uptake for selenite than selenate (50, 85) but the pattern of increasing selenate was time dependent and enhanced over time corroborating with our findings (Figure 4.52).

Super Basmati rice exhibited higher capacity of Se accumulation when applied as foliar (86). In another study, selenate improved Se content in rice crop (19). Arabidopsis shoots accumulated 1.7-fold high Se when selenate was applied to seedlings (87). Ectopic expression of selenocysteine methyltransferase (SMT) gene in Arabidopsis enhanced Se uptake in overexpressed plants than WT (88). These studies demonstrate that plant organs like shoots or seeds can accumulate significant amounts of Se when selenate is supplied. In the present investigation, Se

was enhanced in shoot and seeds of OX plants significantly than in WT indicate that Se is utilizing S transport system Figures 4.41, 4.54 and 4.55.

Se application is beneficial for plant growth and development not only enhances antioxidants (89, 90) but also upregulate the accumulation of mineral nutrients like Ca, Mg, K, Zn, Fe, B, Mo, Mn, Ni (Figures4.57, 4.59, 4.60, 4.62, 4.63, 4.64, 4.65, 4.66 and 4.67). Minerals contents were compared among seeds and shoots of WT and OX without (control) or with Se application. In the present findings, S was found to be highly significant in OX under control and significantly under Se treatment in the seeds. However, in shoot, under controto be nonas found significantly high in OX but highly significan decreased in OX than WT under Se. phopsphate was found to be non-significant in seeds in OX under control or Se treatment. However, in shoot under Se treatment P decreased significantly in OX than in WT.

Se application enhacned Ca level highly significant in seeds and shoots of OX. K level was significant in seeds of OX but highly significant in shoots of OX. There was no significant change for Mg content in seeds of OX, but a highly significant level found in shoots of OX. Fe content was highly significant in seeds of OX control or under Se application, however, significant difference was not observed in shoot of OX. B was found significant than WT under Se application in OX seeds than WT but no difference in control. In shoot tissues under control, B was highly significant in OX than WT and strikingly under Se application, B was decreased significantly lower than WT. Mo concentration in OX seeds was highly significant than WT under Se application. Mo was found significant in OX under control. In shoot under control and Se application, Mn was found to be more significant than WT. In WT seeds under control Ni was enhanced significantly than OX. On the other hand, Ni was found to be highly significant in OX under Se. Under control treatment, Zn was highly significant in OX seeds. Similarly, Zn was also found significantly higher in OX seeds under Se treatment. However, in shoot under Se treatment, Zn was found highly significant in OX. These findings highly corroborate with the previous reports where Se enhanced the minerals in different tissues. Previous studies also reported that Se-nano particle application could enhance Ca, Mg, and K in corriander plants (91). In another study, Se treatment enhanced Zn, leaf Cu, stem/root Fe, stem/root Ca, and stem/root Mg (92). Se treatment enhanced Ca, Mg, Mn and K in fruits of strawberry (93) also in other

study the application of Se or Se-nanoparticle could enhance P, K, Ca, Zn and Fe in leaf of pomegranate. However Cu content was non-significant among WT and OX (Figure 4.61) following the same pattern as reported earlier where Cu concentration was unchanged under the treatment of Se (94). S concentration in seeds was accumulated highly significant in OX than WT under control treatment. However, the level of S reduced in OX under Se treatment due to competing effect or antagonistic effect of Se (Figure 4.56). Likwise findings previously reported in several plant species (95-97). The reason why S and Se have competing effect in plant uptake is due to their analogous nature in physical and chemical properties (96, 97) as well as sulfate transport system is used by both elements (14, 15).

P concentration was non-significant in control or Se treatment in WT and OX seeds but significantly reduced due to Se treatment in OX shoot (Figure 4.58). Similar findings reported earlier that the growth of algae was hampered due to inhibitory effect of selenate on phosphate uptake (98, 99).

Previous finidings corroborate with our investigations for the enhancement of Ca, Mg, K, Mn, Fe, Zn. However, B, Mo, and Ni could also enhance in the present finidings in OX seedlings either in seed or shoot tissues. These results arise could be due to influence of the over-expression of *OsSUTR1;1* on the genes of the minerals transporters (due to co-expression) in *Arabidopsis thaliana*.

Heat stress not only damages photosynthetic apparatus, but also reduces both photosynthetic rate and duration of the assimilate supply (100). In addition high temperatrature stress also triggers reactive oxygen species (ROS) in chloroplast which ultimately decreases antioxidant activity (101). Xue, Hartikainen (102) found that plants exposed to high temperatures experienced less stress-related damage and kept their leaves green for a longer time when treated with selenium. The previous reports are closely related to our findings where Se application not only delayed senescence by maintaining chlorophyll content and enhanced pod length both in WT and OX plants (Figures 4.69 and 4.70). Therefore, Se application is beneficial for plants to alleviate heat stress especially more profound effect was seen in OX of *OsSULTR1;1* plants.

A study carried out by (103) demonstrated that 2 μM Se application effectively played protective role against Cd stress in rape seedlings. In their findings, Cd strongly reduced fresh weight of hypocotyl, cotyledons as well as root. Green synthesized selenium nano-particle positively inlfuenced the root and shoot

length under Cd stress in tomato elaborating the growth promoting effect of Se (104). In an other study of Se application to purple flowering stalks under Cd stress, enhanced fresh weight as well as lengths of root and shoot (105) again confirming positive and protective role of Se in Cd stress. These studies closely corroborate with our finding where OX seedlings under Cd stress in presence of Se ameliorated the toxic effect of the metal and enhanced root and shoot significantly (Figure 4.72 A, C).

Pb causes oxidative damadge to plant tissues via ROS system (106) and also causes phytotoxicity and limited the plant growth development as well as metabolism and physiological processes (107). Se application has protective roles against heavy metals which limits ROS production and improves antioxidant system at low concentration. The application of Se to cucumber improved plant growht and yield performance (108). In wheat, Pb exposure reduced plant height, shoot fresh weight and pigment concentration. Supplementation of Se at low dose improved not only antioxidants but also improved plant growth and development (109). Se application enhanced higher level of anthocyanin in wheat grains of cv. 202w17 (94) along with higher level of Se accumulation and low level of Pb. In our finding, OX leaves exhibited significantly higher levels of anthocyanin (Figures 4.73 and 4.74 B) possibly due to enhanced level of Se and low level of Pb.

Our findings corroborate with the previous reports for the ameliorating effect of Se application to improve plant growth by increasing significant number of leaves and pigment enhancement (anthocyanin) in OX of *OsSULTR1;1* (Figures 4.73 and 4.74 B).

Aluminum (Al) inhibits root growth thereby limiting nutrient and water uptake therefore, impacting on plant growth and development (60). Low concentration of Al inhibits root growth in plants (71) even during short term exposure. In ryegrass Se application at low concentration (2 μM) improved root growth compared to non-Se treated plants under Al stress (110). In our results, also Se application improved root growth in the presence of Al more profoundly and significantly in OX of *OsSULTR1;1* (Figure 4.75). These findings indicate that Over-expression of rice sulfate transporter (OsSULTR1;1) is responsible for improving root growth under Al stress in the presence of low Se dose.

6. CONCLUSIONS AND RECOMMENDATIONS

Super Basmati cultivar exhibited enhanced Se uptake in vegetative tissues, seed developmental stages and pH dependency. Detailed expression analysis of the 13 sulafte transporters in Super Basmti rice under Se treatments was performed. The selected *OsSULTR1;1* Se inducible root tissue specific transporter was employed for the comprehensive functional characterization and subcellular localization. OsSULTR1;1 has amino acid difference having leucine in Super Basmati rice (aromatic indica rice) and valine in IR-64 (non-aromatic indica rice) could have important functional implementations. Subcellular localization showed that OsSULTR1;1 is a plasma-membrane localized sulfate transporter. The OsSULTR1;1 exhibited enhanced selenate uptake in the tissues than in WT Arabidopsis plants. The findings show that OsSULTR1;1 is responsible for Se uptake and accumulation as well as having synergistic effect in accumulating other mineral nutrients thereby enhancing plant growth and development than in WT.

1. OsSULTR1;1 induced under selenate along with S deficiency.
2. In future, *OsSULTR3* group of sulfate transporters which is shoot tissue specific could be studied in detail regarding functional characterization.
3. Transgenic Arabidopsis plants could uptake Se and accumulated significantly in seeds and shoots could be used for Se biofortification studies in future.
4. OX of *OsSULTR1;1* also improved uptake of other plant essential minerals therefore improving plant growth and development.
5. OX of *OsSULTR1;1* also improved abiotic stress tolerance such as heat, Cd, Pb and Al. However, further studies of physiological and biochemical should be done to explore the tolerance mechanisms in depth.

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