CHAPTER

4

Cloning and Characterization of *OsGTL1*

4.1). Introduction

GSH is required for normal plant growth and development as discussed in **section-3.1.** The transport and compartmentalization of GSH is essential to perform of its functions. The transporters that could transport peptides including GSH and GSH conjugates have been placed in three groups (reviewed by Stacey et al., 2002a). The ATP binding cassette (ABC family of transporters) uses ATP hydrolysis for transport of small peptides. On the other hand, peptide transporters (PTR) family uses the proton motif force to drive transport (Pallsen & Skurray, 1994). The third group, oligopeptide transporters (OPT) also exploit proton motive force, however in contrast to PTR transporters which only recognize di-and tripeptides, OPT transporters preferentially recognize tetra and pentapeptides (reviewed by Stacey et al., 2002a). Members of OPT family were first characterized in yeast (Lubkowitz et al., 1997; 1998). Although the uptake of GSH and specific transport systems have been studied in different organisms, like bacteria (Sherrill and Fahey, 1998), yeasts (Lubkowitz et al., 1998), and mammals (Iantomasi et al., 1997); the isolation and characterization of a GSH transporter from yeast (*Saccharomyces cerevisiae*) (Bourbouloux et al., 2000) was a significant advancement to understand GSH uptake. This transporter, called ScHGT1 exhibits high affinity for GSH and GSH conjugates. ScHGT1 is a member of OPT family hence also named OPT1 (Miyake et al., 2002). ScOPT2, a close homolog of HGT1 in yeast, did not show GSH transport activity (Lubkowitz et al., 1998). Arabidopsis OPT family members were identified that share 49-53% sequence similarity to

ScOPT1 (Koh et al., 2002). The plant OPTs form a distinct subfamily compared to the fungal OPTs (Yen et al., 2001; Koh et al., 2002; Zhang et al., 2004). Recently the functional GSH transporters were also cloned form *Brassica juncea* (*BjGT1*; Bogs et al., 2003) and rice (*OsGT1*; Zhang et al., 2004). The expression of *BjGT1* was regulated by heavy metal exposure (Bogs et al., 2003). On the other hand the expression of *OsGT1* was not detected under normal growth conditions and there is no available information about the expression of *OsGT1* in response to any environmental stress. OsGT1 and BjGT1 also belong to OPT family which is well characterized in Arabidopsis (Koh et al., 2002; Stacey et al., 2002a; Stacey et al., 2002b; Stacey et al., 2006). On the other hand this family is poorly characterized in rice with the exception of OsGT1 and yellow stripe like (YSL) transporters. In rice a number of putative members of YSL family exist and some of them are regulated by Fe. Koike et al., (2004) reported the existence of 18 putative YSL genes (*OsYSLs*) in the rice genome that exhibited 36–76% sequence similarity to maize Fe(III)-MA transporter YS1. Among these OsYSLs the OsYSL2 is a metal-nicotianamine transporter responsible for the phloem transport of Fe and manganese (Koike et al., 2004). However the YSL subfamily of OPT transporters is phylogenetically distinct from AtOPT transporters (Yen et al., 2001) and the same seems true for rice. In the present studies, a novel GSH transporter (*OsGTL1*) was cloned from rice, the expression of which is strongly induced under Fe-deficient conditions in root and shoots tissue. *OsGTL1* is located on rice chromosome 3 and was predicted to encode a polypeptide of 757 amino acids split in 12 transmembrane domains. In

contrary to all characterized members of OPT family in plants the expression of *OsGTL1* is strongly induced in root tips and root hairs giving the clue that it may have some role in uptake of nutrients from soil.

4.2). Experimental procedures

4.2.1). Cloning of *OsGTL1*

Data obtained by a rice 22K microarray analysis performed to compare expression of genes in response to Fe-deficiency (Ishimaru et al., 2005) was explored to identify *OsGTL1* as a putative member of OPT family upregulated under Fe-deficient conditions. Full-length rice cDNA clone for *OsGTL1* (AK102404) was acquired from the Rice Full-Length cDNA Database (KOME; http://cdna01.dna.affrc.go.jp/cDNA/). The information about genomic structure of this clone was collected from TIGR rice genome database (http://www.tigr.org/tdb/e2k1/osa1/index.shtml). The presence of NPG domain (NPGPFxxKEH; Koh et al., 2002; Stacey et al., 2002a) and KP domain (KLGHYMKIPPR; Stacey et al., 2002a) was confirmed. The predicted membrane-spanning structure of OsGTL1 was generated by the computer topology prediction program SOSUI (Hirokawa et al., 1998). The paralogs of OsGTL1 were identified in rice through BLAST (http://www.ncbi.nlm.nih.gov/BLAST/), screened for the presence of NPG and KP domains as described above and phylogenetic tree was constructed using CLUSTAL W Neighbor–Joining method and the tree was visualized with TreeView.

4.2.2). Subcellular localization of OsGTL1

In silico analysis were performed using computer program pSORT (http://psort.ims.u-tokyo.ac.jp/) to determine the subcellular localization of OsGTL1.

Moreover, the ORF of *OsGTL1* was amplified with forward and reverse primers as 5'-ATGGCGTCGTTGAAGTCGCCGGTGGCG-3' and 5'-GAACACCGGGCATCCCTTGACGGCGCTC-3' respectively and the amplified fragment containing the *OsGTL1* coding sequence was subcloned into pENTR/D-TOPO (Invitrogen, Carlsbad, CA). This pENTR/D-TOPO entry vector containing the *OsGTL1* coding sequence was designated pENTR-*OsGTL1*. The plasmid pDEST35S-sGFP (Ishimaru et al., 2005) was used as destination vector. A subsequent attL substrate and attR substrate recombination reaction (Invitrogen) between the destination and pENTR-*OsGTL1* entry vectors generated an expression clone containing the gene encoding 35S-OsGTL1-sGFP. Onion epidermal cells were transformed using the Biolistic PDS-1000/He Particle Delivery System (Bio-Rad, Tokyo, Japan), and the subcellular localization of 35S-OsGTL-sGFP was visualized as described by Mizuno et al. (2003).

4.2.3). Transport assay

A 2274-bp fragment of *OsGTL1* produced by digestion with *Eco*RI and *Xba*I was inserted into the *Eco*RI, *Xba*I site of the pGEM-3zf(t) vector. The resulting plasmid, pGEMOsGTL1T, was linearized by *Xba*I digestion. Capped complementary RNA (cRNA) was synthesized in vitro with a MEGAscript SP6 kit (Ambion, Austin, TX, USA). The oocytes were prepared as described (Igarashi et al., 2000), and injected with 10 ng of *OsGTL1* cRNA. The injected oocytes were incubated for 2 days and subjected to electrophysiological measurements at pH 7.5. The oocytes were positioned in a 1.0 ml recording chamber perfused with bath solution containing 96

mM NaCl, 2 mM KCl, 5 mM HEPES-NaOH solution (pH 7.5). Membrane currents were measured with the two-microelectrode voltage-clamp method by using an automated Hitachi system containing TEV-200 system (Dagan, Minneapolis, MN, USA). The oocytes were clamped at -80 mV, and the steady-state currents in response to the addition of metal–chelate complex and GSH were obtained. The currents were continuously monitored and analyzed with a Mac Lab system (Adinstruments, Sydney, NSW, Australia). Three independent oocytes injected with *OsGTL1* were used to measure the currents. Substrate induced currents were also measured in three independent water injected oocytes as controls. Transport activity was measured at Hitachi, Ltd, Life Science Group.

4.2.4). Rice transformation and growth conditions

The 1.8 kb 5'-upstream region of the *OsGTL1* gene was amplified by PCR using genomic DNA as a template. The primers used were the forward primer 5'-gagaaagcttTGGCACCACCACCTGCATGCCTCAG-3' and the reverse primer 5'-gagaaagcttCTCCCTAGCCTCGATCTCCTTCCTC-3', which contain *Hin*dIII restriction site. The amplified fragment was fused into the pBluescript II SK+ vector, and its sequence was confirmed. The *OsGTL1* promoter was digested with *Xho*I and *Bgl*II and the digested 1.8 kb fragment was subcloned upstream of the *uid*A ORF, which encodes β-glucuronidase (GUS), in the pIG121Hm vector (Hiei et al., 1994). An *Agrobacterium tumefaciens* strain C58, carrying the above construct was used to transform rice (*Oryza sativa* L. cv. Tsukinohikari) as described (Higuchi et al., 2001). Three transgenic rice lines carrying the *OsGTL1* promoter-*GUS* fusion

were obtained. T_1 seeds were germinated on MS medium containing 50 mg L^{-1} hygromycin B. After four weeks, the plants were shifted to Fe-sufficient or Fe-deficient medium for two weeks and three independent lines were analyzed for GUS expression as described (Inoue et al., 2003).

4.2.5). Northern blot analysis

Seeds of rice (*Oryza sativa* L. cv. Nipponbare), barley (*Hordeum vulgare* L. cv. Ehimehadaka no. 1), wheat (*Triticum aestivum* L. cv. Chinese spring) and maize (*Zea mays* cv. Alice), were germinated on wet filter paper and cultured as described (Kanazawa et al., 1994). For Fe-deficiency treatments, plants were transferred to culture solution lacking Fe. For Cu, Mn and Zn deficiency treatments, rice plants were transferred to culture solution lacking Cu, Mn and Zn respectively. Roots and leaves were harvested after two weeks, frozen in liquid nitrogen, and stored at -80°C until use. Total RNA was extracted from roots and shoots, and 10 µg per lane were electrophoresed in 1.2% (w/v) agarose gels containing 0.66 M formaldehyde and transferred to Hybond-N+ membrane (Amersham, USA). The *OsGTL1* ORF was labeled with digoxigenin (DIG) by PCR and incubated with the membrane at 68°C and processed as described (Engler Blum et al., 1993; Yoshihara et al., 2003).

4.3). Results

4.3.1). Cloning of *OsGTL1*

The *OsGTL1* was identified through microarray analysis as a putative OPT whose expression is significantly upregulated in response to Fe-deficiency. The expression of *OsGTL1* was highly upregulated in response to Fe-deficiency in new and old leaf, stem and root tissue (**Table-5**). *OsGTL1* was cloned and further characterized for substrate specificity and expression patterns. *OsGTL1* is located on rice chromosome 3 and split in 4 exons (**Fig-4.1**). The size of the gene is 4.12 kb while ORF consists of 2.274 kb and was predicted to encode a polypeptide of 757 amino acids. Seven paralogs of OsGTL1 were identified through blast search including previously identified GSH transporter *OsGT1* (Zhang et al., 2004). OsGTL1 has 50% homology to OsGT1 located on rice chromosome 6. On the other hand OsGTL1 is highly homologous to AtOPT3 and BjGT1 (**Fig-4.1b**). The BjGT1 is a functional GSH transporter while the substrate specificity for AtOPT3 has to be identified yet. The expression of none of the rice GTLs including the previously identified *OsGT1* is regulated by Fe with the exception of *OsGTL1* (Data not shown).

The predicted membrane-spanning structure of OsGTL1 was generated by the computer topology prediction program SOSUI **(Fig-4.2)**. OsGTL1 was predicted to be composed of 12 transmembrane domains. Other members of OPT family are predicted to have 12-14 transmembrane domain (Koh et al., 2002).

Table-5: Microarray Analysis of *OsGTL1*

Expression ratios (-Fe/+Fe)

New Leaf	Old leaf	Stem	Root
22	21	7.0	21

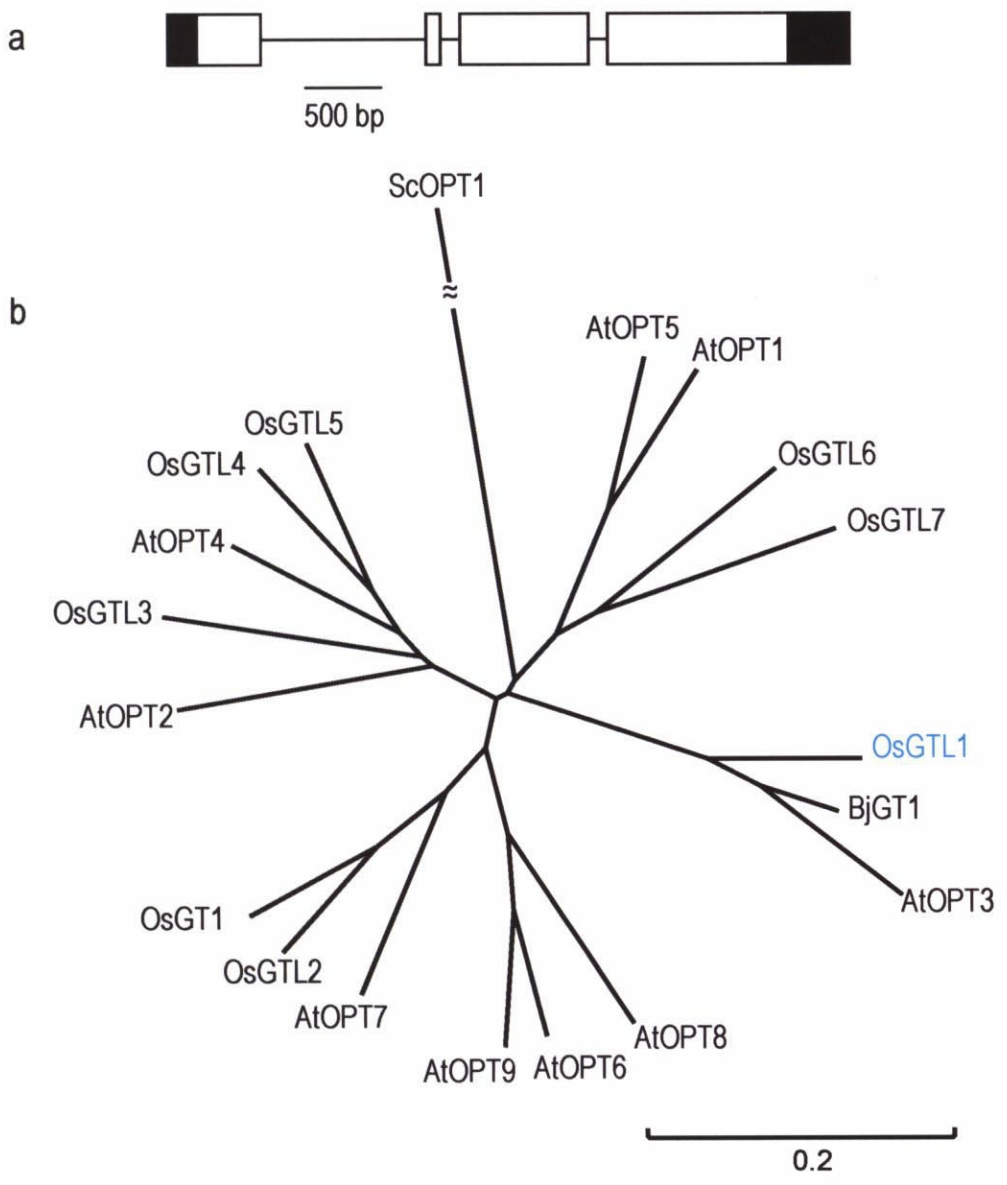

Fig-4.1: Structural and Phylogenetic Characterization of OsGTL1

a) Genomic structure of OsGTL1. OsGTL1 is composed of four exons (boxes) and three introns. Black boxes indicate 5'- and 3'-UTRs.

b) Unrooted phylogenetic tree of the OPT family. The accession numbers of paralogs of OsGTL1 are
OsGTL1: AK102404; OsGTL2: AK072617; OsGTL3: AK070801; OsGTL4: DAB08158; OsGTL5: BAB89477; OsGTL6:AK100814; OsGTL7: AK072608; OsGT1: AK067671.

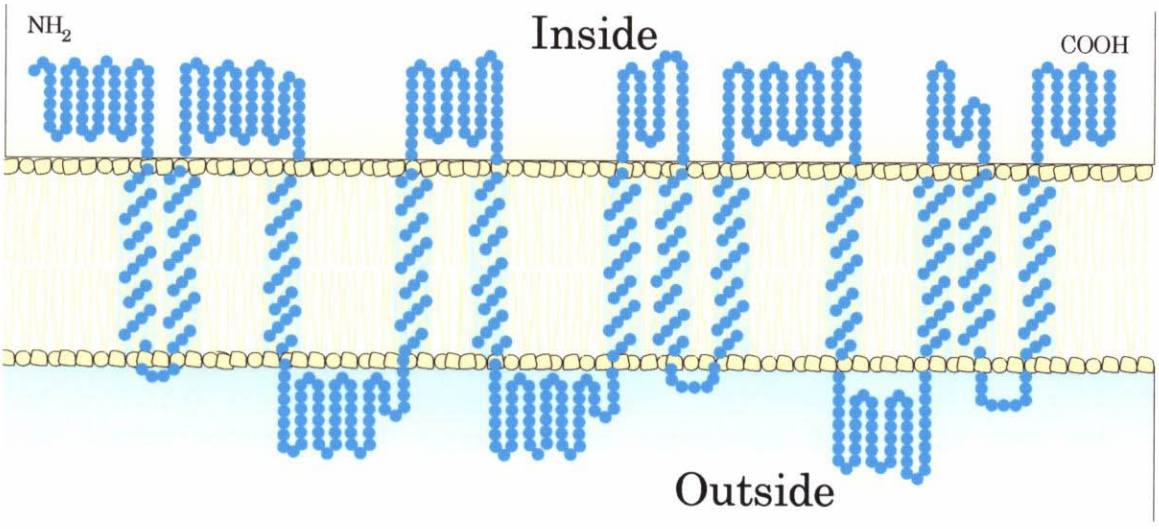

Fig-4.2: Predicted Membrane Spanning Structure of OsGTL1

Predicted membrane-spanning structure of OsGTL1 generated by the computer topology prediction program SOSUI (Hirokawa et al., 1998). Each solid circle indicates one amino acid residue.

4.3.2). Substrate specificity

OsGTL1, when expressed in *Xenopus leivis* oocytes transported GSH confirming that OsGTL1 is a functional GSH transporter (**Fig-4.3**). As OsGTL1 is a member of OPT family so at first it seemed that OsGTL1 could also transport MA-Fe or NA-Fe but in *Xenopus leivis* oocytes OsGTL1 did not transport MA or NA bound with Fe or other metals (data not shown).

4.3.3). Northern blot analysis

Northern blot analysis was performed using Full length ORF of *OsGTL1* as a probe. Under normal growth conditions the expression of *OsGTL1* was not observed in root and shoot tissue, and the expression of *OsGTL1* was observed only under Fe-deficient conditions in root and shoot tissue. The expression is stronger in shoot tissue as compared with root tissue. Northern blot analysis was also done to confirm the presence of *OsGTL1* orthologs in barley, wheat and maize and it was revealed that *OsGTL1* orthologs exist in all crops examined. The same pattern of expression was observed in *OsGTL1* orthologs in barley, wheat and maize where expression was induced under Fe-deficiency (**Fig-4.4**). The expression of *AtOPT3*, a homolog of *OsGTL1* in Arabidopsis, is also regulated by Fe-deficiency.

Northern blot analysis was also performed to check whether the expression of *OsGTL1* is also regulated by the Zn, Mn or Cu deficiency. Northern blot analysis

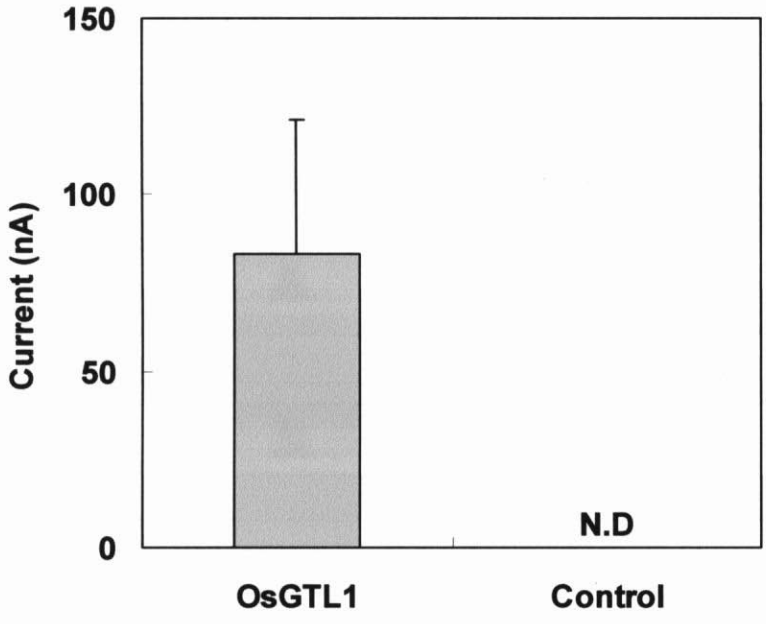

Fig-4.3: GSH Transport Activity of OsGTL1 Analyzed in *Xenopus laevis* Oocytes

The transport activity for GSH was measured using the two electrode voltage-clamp method. The oocytes were clamped at -80 mV, and the steady-state current in response to the addition of GSH was obtained. The data represents mean ± SD of three independent oocytes injected with *OsGTL1*. The same number of water-injected oocytes was used as a control.
N.D.: Not detected.

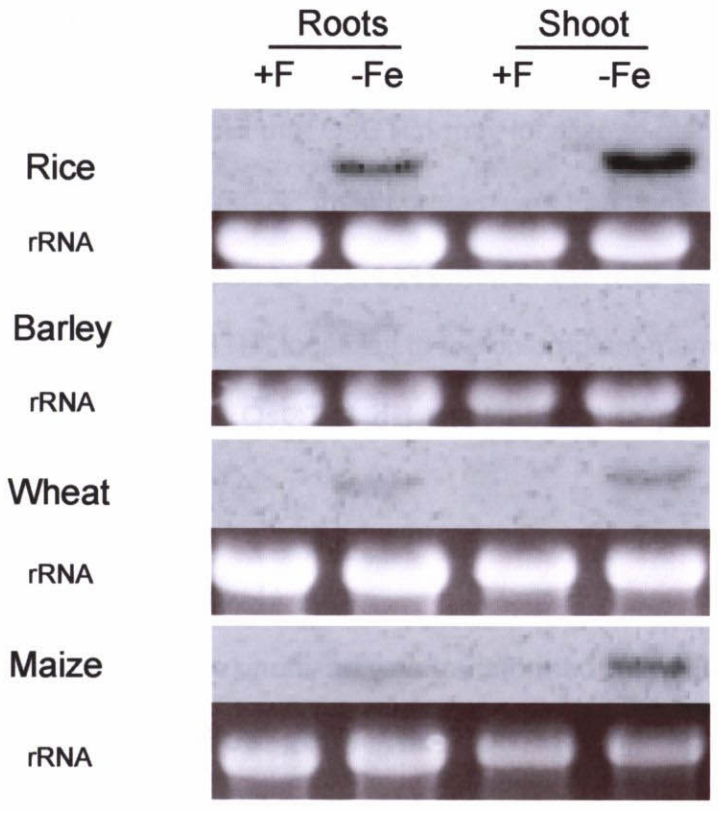

Fig-4.4: Northern Blot Analysis of *OsGTL1* in Response to Fe-deficiency

Full length of *OsGTL1* was used as probe.

revealed that the expression of *OsGTL1* is specifically induced in response to Fe-deficiency and only slight induction was observed in response to Zn, Mn and Cu deficiency (**Fig-4.5**).

4.3.4). Subcellular localization of OsGTL1

In silico analysis predicted that OsGTL1 may localize to chloroplast membrane. On the other hand OsGTL1:sGFP fusion protein was transiently expressed in onion epidermal cells and was localized to the cell membrane (**Fig-4.6**). Although *in silico* analysis indicated that OsGTL1 localizes to the chloroplast membrane in plant cells, at least in onion epidermal OsGTL1 did not localized to plastids after transient expression.

4.3.5). Promoter GUS analysis

Oryza sativa cultivar Tsukinohikari was transformed with 1.8kb promoter region of *OsGTL1* fused with GUS gene. The GUS staining was not observed in Fe-sufficient roots (**Fig-4.6a-b**). Similarly no GUS staining was observed in longitudinal section of the roots harvested from plants grown under Fe-sufficient conditions (Data not shown). On the other hand strong GUS staining was observed in Fe-deficient roots confirming that expression of *OsGTL1* is induced under Fe-deficient conditions (**Fig-4.7c-h**). Strong GUS staining was observed near root tips including root hairs (**Fig-4.7c, d-e**). The GUS staining became weak as the distance from the root tip increased (**Fig-4.7c, f-h**). Near root tips the GUS staining was observed in all tissue with particularly strong staining in epidermis and vascular

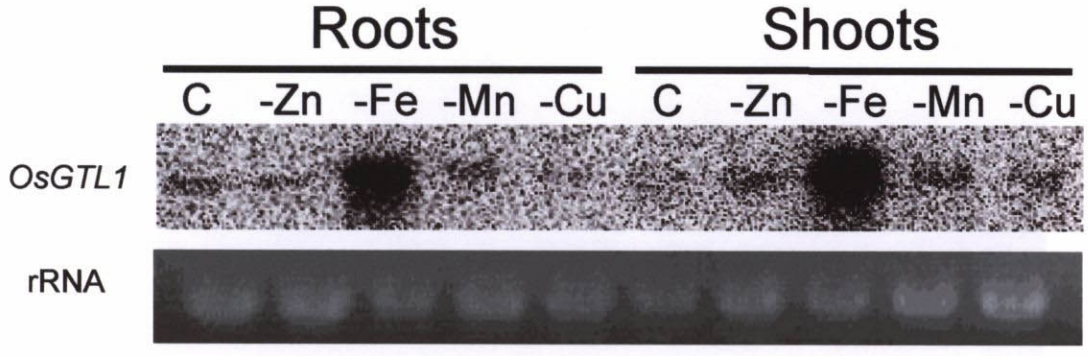

Fig-4.5: Northern Blot Analysis of *OsGTL1*

Full length of *OsGTL1* was used as probe.

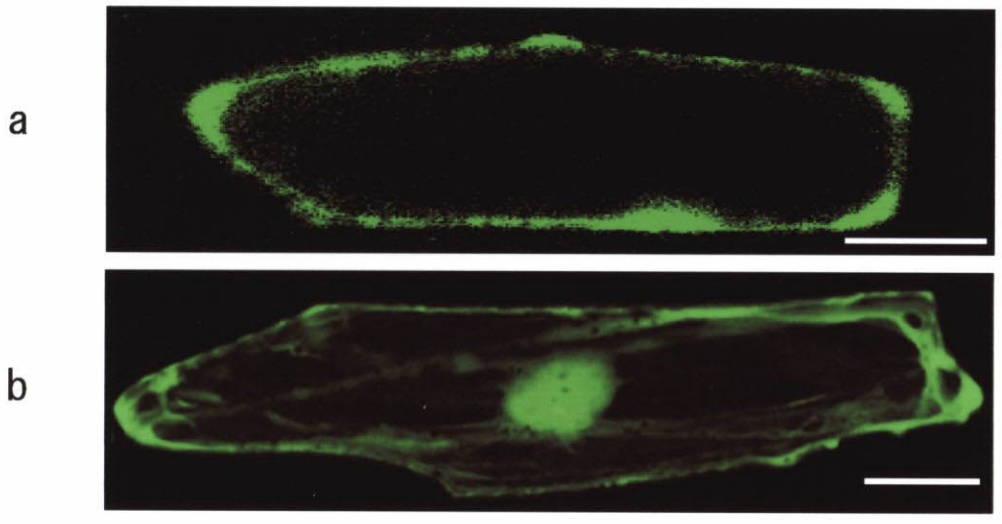

Fig-4.6: Subcellular Localization of OsGTL1 in Onion Epidermal Cells

a) Localization of 35S-OsGTL1-sGFP.
b) Localization of 35S-sGFP
Scale: 100 μm

tissue, and the expression decreased as the distance from root tip increased. At a distance of ~1cm from root tip (**Fig4.7g**), the staining was observed in epidermis, part of cortex and vascular bundle. As the distance further increased, GUS expression was only observed in epidermis and vascular tissue (**Fig-4.7h**).

In contrast to Northern blot analysis slight GUS expression was observed in Fe-sufficient leaves. The expression was weak and was only observed in part of vascular tissue (**Fig-4.8**). However, under Fe-deficient conditions GUS expression was observed in all parts of leaf with strong staining in vascular tissue.

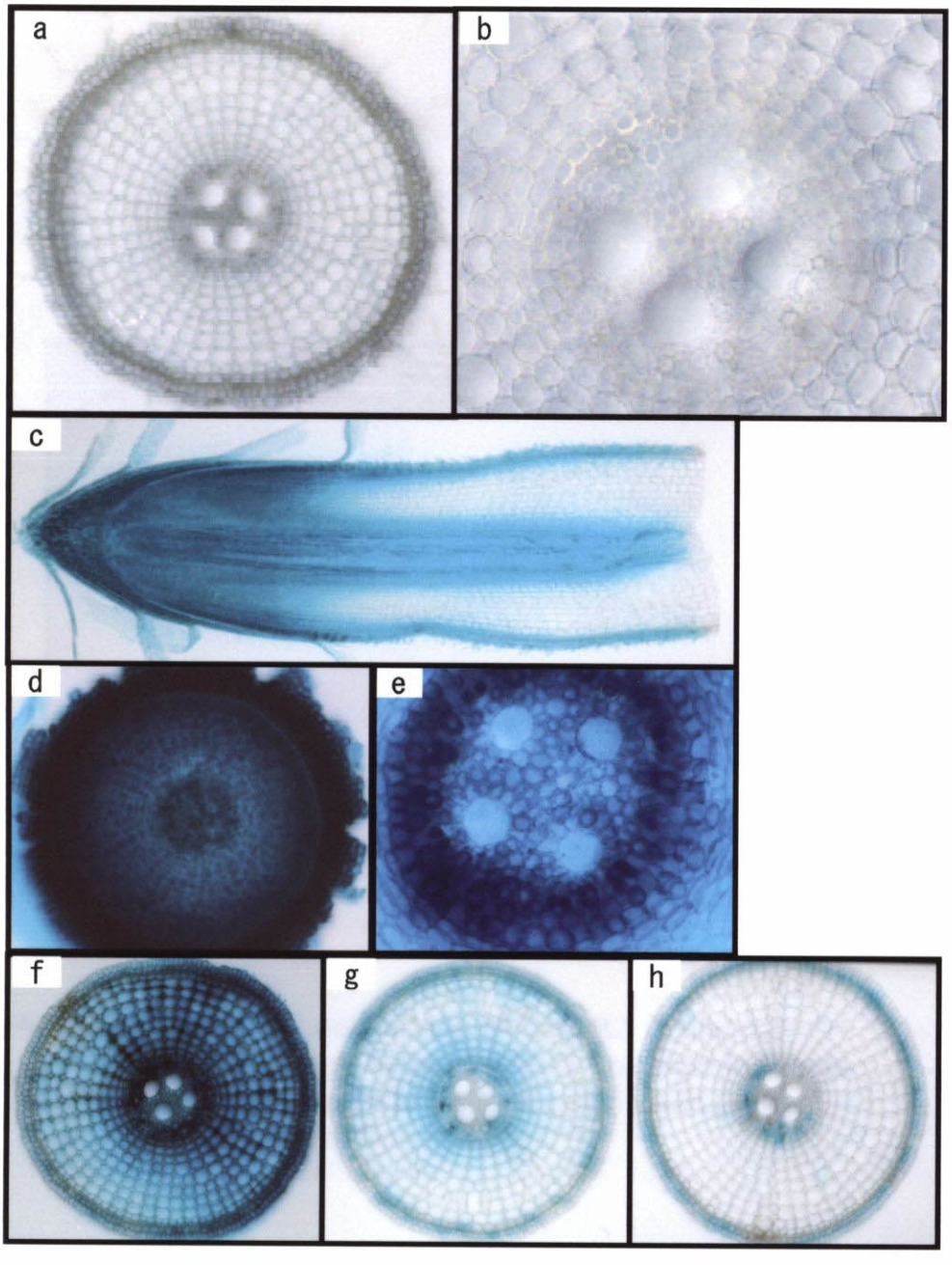

Fig-4.7: Histochemical Localization of OsGTL1 Promoter Activity in Roots of Promoter-GUS Transgenic Plants Cultured Hydroponically Under Fe-sufficient (+Fe) or Fe-deficient (-Fe) Conditions.

a (+Fe), d,f-h (-Fe): root transverse section; b (+Fe), d (-Fe): enlarged part of stele; c) (-Fe): root longitudinal section.

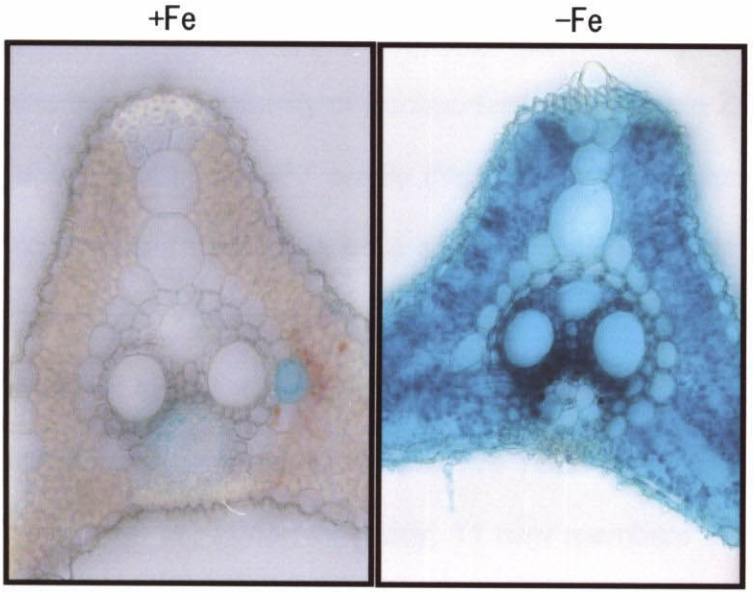

Fig-4.8: Histochemical Localization of OsGTL1 Promoter Activity in Shoots of Promoter-GUS Transgenic Plants Cultured Hydroponically Under Fe-sufficient (+Fe) or Fe-deficient (-Fe) Conditions.

4.4). Discussion

Among the three protein families, transporting small peptides including GSH (Stacey et al., 2002a), PTR and OPT transporters are proton symporter, although no significant sequence similarity is found between the two families (Koh et al., 2002). The OPT family of transporters is a large and diverse family of transporters. It also contains the YSL subfamily of transporters, for example ZmYS1, an Fe-MA transporter is a member of OPT family (Yen et al., 2001), functions as proton coupled symporter and transports MAs and NA chelated metals (Schaaf et al., 2004). YS1 orthologs YSLs (YS like) identified in Arabidopsis (Didonato et al., 2004), rice (Koike et al., 2004), and barley (Murata et al., 2006) are supposed to be members of OPT family. Previously, 18 putative YSLs were reported in rice (Koike et al., 2004; Gross et al., 2003). Recently, 11 new members of OPT family were reported in rice (Wiles et al., 2006). However in the present studies, only eight members of OPT family were identified in rice excluding previously reported YSLs. There are eight full length YSLs (AtYSLs) genes in the Arabidopsis genome (DiDonato et al. 2004), forming a cluster phylogenetically distinct from the AtOPTs. On the other hand, nine OPT members (AtOPT1-9) were reported in Arabidopsis (Koh et al., 2002; Stacey et al., 2002a; Stacey et al., 2006). Although YSLs are member of OPT family they are phylogenetically different from other OPT members. Moreover, in YSLs the OPT motif LNPGP{F/W/Y}{N/S/T}XKE{H/Q} is replaced by X_4PFTRQEX (Wiles et al., 2006). Another line of conflict lies in substrate specificity for YSLs and OPTs. OPT proteins can transport diverse substrates of

peptide-containing compounds or peptide derivatives (Bourbouloux et al., 2000; Hauser et al., 2000; Hauser et al., 2001; Lubkowitz et al., 1997; Lubkowitz et al., 1998; Osawa et al., 2006) while the YSLs are only reported to transport tripeptides (MAs or NA) bound to different metals. The nomenclature used in this thesis is GT like genes (OsGT1 and OsGTL1-7) instead of OPTs, as in the present studies originally the homologs of OsGTL1 were searched.

Here the cloning of a functional GSH transporter from rice is reported. OsGTL1 is located on rice chromosome 3 and split in 4 exons. The size of the gene is 4.12 kb while ORF consists of 2.274 kb and predicted to encode a poly peptide of 757 a.a. OsGTL1 was predicted to be composed of 12 transmembrane domains. The AtOPTs were predicted to encode 12-14 transmembrane domains (Koh et al., 2002). Seven paralogs of OsGTL1 were identified in rice including previously characterized OsGT1 (Zhang et al., 2004). Although in Pfam database (http://www.sanger.ac.uk/Software/Pfam/) 45 members of OPT family are reported, most of them belong to YSL family, are too short (Less than 500 a.a) or seems truncations of the same loci. The designed criteria to screen the members of OPT family in rice is that is the clone is at least 600 a.a. and contain NPG and KP domains previously reported in AtOPTs. The OsGTL1 showed high homology with AtOPT3 and BjGT1. BjGT1 is a functional GSH transporter while the substrate for AtOPT3 has not been reported yet (Stacey et al., 2006). OsGTL1 showed 50% homology to OsGT1, a functional GSH transporter (Zhang et al., 2004). OsGT1 transports GSH, GSSG, GS conjugates and other peptides with wide substrate

specificity. However its expression was not observed under normal growth conditions (Zhang et al., 2004) and no information about its expression in rice is available to date in response to any environmental stress. Micro array analysis showed that expression of this gene is not regulated by Fe (Data not shown). In fact in Arabidopsis and rice only the expression of AtOPT3 (Stacey et al., 2006) and OsGTL1 is regulated by Fe. Like *OsGT1*, the expression of *OsGTL1* was not observed under normal growth conditions (+Fe); however expression was observed under Fe-deficient conditions (**Fig-4.4**). Although *OsGTL1* orthologs were not found through blast in barely, wheat or maize, Northern blot analysis showed that its orthologs exist in all crops examined and that its expression is also regulated by Fe-deficiency in these crops.

In onion epidermal cells the OsGTL1 localized to cell membrane; however the *in silico* analysis did not confirm these results and raised the possibility that OsGTL1 may localize to chloroplastic membrane. The subcellular localization of close homologs of OsGTL1, AtOPT3 and BjGT1 has not been reported. Further experiments may clarify the situation. *OsGTL1* driven promoter GUS expression was upregulated in response to Fe-deficiency and was observed in roots tips and in Fe-deficient leaves. Although expression of AtOPT3, a close orthologs of OsGTL1 is also up- regulated by Fe-deficiency (Stacey et al., 2006) no expression was observed in root tips. Previously, characterization of *AtOPT3* knock out plant revealed that *AtOPT3* is crucial for normal embryogenesis, as indicated by the embryo-lethal phenotype of plants homozygous for the *opt3* mutant allele.

Consistent with the embryo-lethal phenotype associated with *opt3*, *AtOPT3* expression was localized in the endosperm tissues, integuments and, most notably, in the embryos of developing seeds (Stacey et al., 2002b). OPTs are redundant in function and to date, only two plant OPT members have been reported to show a mutant phenotype, *AtOPT3* and the maize *ZmYS1*, where mutants exhibited a chlorotic phenotype due to a defect in Fe uptake (Curie et al. 2001; Stacey et al., 2002b; von Wirén et al., 1994). It is important to mention that the expression of both these genes is regulated by Fe-deficiency. The lack of observable phenotypes in loss of function mutants of other *AtOPTs* may be the result of functional redundancy among the family members. Among all OPTs in rice, only the expression of *OsGTL1* is regulated by Fe raising the possibility for a phenotype for *OsGTL1* mutant making the functional characterization of *OsGTL1* mutant essential to understand its role.

These results raised the possibility that OsGTL1 has a role in Fe-deficiency induced stress; however it is difficult to predict the exact role it plays under Fe-deficiency stress. As GSH is required for cell division, the expression of *OsGTL1* in root tips may be essential to maintain the cell division in roots. It is important to mention that number of root hair increase in response to Fe-deficiency showing that mitotic activity increases in Fe-deficient root tips. GSH is also a part of systematic signal transduction system sensing the sulfur status of plants and mediating inter-organ regulation of sulfur nutrition (Lappartient and Touraine, 1996) the possibility that increased transport of GSH to root tips as a source of reduced

sulfur for MAs biosynthesis cannot be ruled out. It is already reported that Fe-deficiency modulates redistribution of sulphur in barley suggesting that Fe-deficiency affects the portioning of reduced sulphur pool from the shoot to root with in plants (Astolfi et al., 2006b), and that sulfur deficiency affects the MAs secretion (Astolfi et al., 2006a). The expression in root tips also raised the possibility that OsGTL1 may be involved in absorption of small peptides from soil. If it is true then it makes the *OsGTL1* more important as previously, none of *AtOPT*s expresses in roots tips (Stacey et al., 2006) and one difference between the PTR family and OPT was thought to be that PTR express in root tips and involved in acquisition of small peptides from soil. It is still difficult to predict the role of OsGTL1 in shoot tissue. In shoot it may be involved in Fe-homeostasis under Fe-deficiency. The possible role of GSH in Fe homeostasis is already discussed in **section-3.4**.

In conclusion, *OsGTL1* is a functional GSH transporter regulated by Fe-deficiency and may have a role in mitigating Fe-deficiency stress in plants. The exact role for OsGTL1 is difficult to predict without experimental evidence and characterization of plants lacking/overexpressing *OsGTL1* may help to understand its role.

CHAPTER

5

References

5). References

1). Alfenito, M., Souer, E., Goodman, C.D., Buell, R., Mol, J., Koes, R. and Walbot, V. (1998). Functional complementation of anthocyanin sequestration in the vacuole by widely divergent glutathione S-transferases. Plant Cell. 10, 1135–1149

2). Anonymous. (2003). Concept Paper, International year of rice 2004. International Rice Commission Newsletter, FAO, Rome, 52 (Sp. Edition), 19-27

3). Apel, K. and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373–399

4). Astolfi, S., Cesco, S., Zuchi, S., Neumann, G., and Roemheld, V. (2006). Sulfur starvation reduces phytosiderophores release by Fe-deficient barley plants. Soil Sci. Plant Nutr. 52, 80–85

5). Astolfi, S., Zuchi, S., Cesco, S., Toppi, L. S., Pirazzi, D., Badiani, M., Varanini, Z., and Pinton, R. (2006). Iron deficiency induces sulfate uptake and modulates redistribution of reduced sulfur pool in barley plants. Functional Plant Biol. 33, 1055-1061

6). Bar-Ness, E., Hadar, Y., Chen, Y., Romheld, V. and Marschner, H. (1992). Short-term effects of rhizosphere microorganisms on Fe uptake from microbial siderophores by maize and oat. Plant Physiol. 100, 451-456

7). Bogs, J., Bourbouloux, A., Cagnac, O., Wachter, A., Rausch, T. and Delrot, S. (2003). Functional characterization and expression analysis of a glutathione transporter, BjGT1, from Brassica juncea: evidence for regulation by heavy metal exposure. Plant Cell Environ. 26, 1703–1711

8). Bourbouloux, A., Shahi, P., Chakladar, A., Delrot, S. and Bakhawat, A.K. (2000). Hgt1p, a high affinity glutathione transporter from the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. 275, 13259–13265

9). Bradford, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal. Chem. 72, 248-254

10). Briat, J.F., Fobis-Loisy, I., Grignon, N., Lobreaux, S., Pascal, N., Savino, G., Thoiron, S., von Wirén, N. and van Wuytswinkel, O. (1995). Cellular and molecular aspects of iron metabolism in plants. Biol. Cell. 84, 69-81

11). Brunold, C. and Rennenberg, H. (1997). Regulation of sulfur metabolism in plants: first molecular approaches. Prog. Bot. 58, 164–186

12). Cakmak, I. and Marschner, H. (1988). Enhanced superoxide radical production in roots of zinc-deficient plants. J. Exp. Bot. 39, 1449–1460

13). Chen, Y. and Barak, P. (1982). Iron nutrition of plants in calcareous soils. Adv. Agron. 35, 217-240.

14). Cobbett, C.S. (2000). Phytochelatins and their roles in heavy metal detoxification. Plant Physiol. 123, 825–832

15). Creissen, G., Edwards, E.A., Enard, C., Wellburn, A. and Mullineaux, P. (1992). Molecular characterization of glutathione reductase cDNAs from pea (*Pisum sativum* L.). Plant J. 2, 129–131

16). Curie, C., Panaviene, Z., Loulergue, C., Dellaporta, S. L., Briat, J. F., and Walker, E. L. (2001). Maize yellow stripe 1 encodes a membrane protein directly involved in Fe(III) uptake. Nature. 409, 346–349

17). DiDonato, Jr, R. J., Roberts, L. A., Sanderson, T., Eisley, R. B. and Walker, E. L. (2004). Arabidopsis Yellow Stripe-Like2 (YSL2): a metal-regulated gene encoding a plasma membrane transporter of nicotianamine-metal complexes. Plant J. 39, 403–414

18). Edwards, E., Rawsthorne, S. and Mullineaux, P. (1990). Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (*Pisum sativum* L.). Planta. 180, 278–284

19). Engler-Blum, G., Meier, M., Frank, J. and Muller, G.A. (1993). Reduction of background problems in nonradioactive northern and Southern blot analyses enables higher sensitivity than 32P-based hybridizations. Anal. Biochem. 210, 235-244

20). Food and Agriculture Organization of the United Nations. (1995). FAO quarterly bulletin of statistics. 8, 1-2

21). Foyer, C.H., Lelandais, M., Galap, C. and Kunert, K-J. (1991). Effects of elevated cystosolic glutathione reductase activity on the cellular

glutathione pool and photosynthesis in leaves under normal and stress conditions. Plant Physiol. 97, 863–872

22). Gavidia, I., Perez-Bermudez, P., and Seitz, H. U. (2002). Cloning and expression of two novel aldo-keto reductases from *Digitalis purpurea* leaves. Eur. J. Biochem. 269, 2842–2850

23). Graziano, M., Beligni, M.V. and Lamattina, L. (2002). Nitric oxide improves internal iron availability in plants. Plant Physiol. 130, 1852–1859

24). Graziano, M. and Lamattina, L. (2005). Nitric oxide and iron in plants: an emerging and converging story. Trends Plant Sci. 10, 4–8

25). Greer, S. and Perham, R.N. (1986). Glutathione reductase from *Escherichia coli*: cloning and sequence analysis of the gene and relationship to other flavoprotein disulfide oxidoreductases. Biochem. 25, 2736–2742

26). Grotz, N. and Guerinot, M. L. (2002). Limiting nutrients: an old problem with new solutions? Curr. Opin. Plant Biol. 5, 158-163

27). Grusak, M.A. (2002). Enhancing mineral content in plant food products. J. Am. Coll. Nutr. 21, 178S-183S

28). Guerinot, M.L. (2001). Improving rice yields-ironing out the details. Nat. Biotechnol. 19, 417-418

29). Guerinot, M.L. and Yi, Y. (1994). Iron: nutritious, noxious, and not readily available. Plant Physiol. 104, 815-820

30). Halliwell, B. and Gutteridge, J.M. (1986). Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch. Biochem. Biophys. 246, 501–514

31). Harmer, S. L., Hogenesch, J. B., Straume, M, Chang, H. S., Han, B., Zhu, T., Wang, X., Kreps, J. A. and Kay, S. A. (2000). Orchestrated Transcription of Key Pathways in Arabidopsis by the Circadian Clock. Science. 290, 2110-2113

32). Hauser, M., Donhardt, A.M., Barnes, D., Naider, F. and Becker, J.M. (2000). Enkephalins are transported by a novel eukaryotic peptide uptake system. J. Biol. Chem. 275, 3037–3041

33). Hauser, M., Narita, V., Donhardt, A.M., Naider, F. and Becker, J.M. (2001). Multiplicity and regulation of genes encoding peptide transporters in *Saccharomyces cerevisiae*. Mol. Memb. Biol. 18,105–112

34). Herbik, A., Koch, G., Mock, H. P., Dushkov, D., Czihal, A., Thielmann, J., Stephan, U. W., and Bäumlein, H. (1999). Isolation, characterization and cDNA cloning of nicotianamine synthase from barley. Eur. J. Biochem. 265, 231-239

35). Hiei, Y., Ohta, S., Komari, T., and Kumashiro, T. (1994). Efficient transformation of rice (*Oryza sativa* L.) mediated by agrobacterium and sequence analysis of the boundaries of the T-DNA. Plant J. 6, 271-282

36). Higuchi, K., Nishizawa, N. K., Yamaguchi, H., Römheld, V., Marschner, H., and Mori, S. (1995). Response of nicotianamine synthase activity to Fe deficiency in tobacco plants as compared with barley. J. Exp. Bot. 46,1061–1063

37). Higuchi, K., Kanazawa, K., Nishizawa, N. K., Chino, M., and Mori, S. (1994). Purification and characterization of nicotianamine synthase from Fe-deficient barley roots. Plant Soil. 165(2), 173-179

38). Higuchi, K., Suzuki, K., Nakanishi, H., Yamaguchi, H., Nishizawa, N. K., and Mori, S. (1999). Cloning of nicotianamine synthase, novel genes involved in the biosynthesis of phytosiderophores. Plant Physiol. 119, 471–480

39). Higuchi, K., Watanabe, S., Takahashi, M., Kawasaki, S., Nakanishi, H., Nishizawa, N.K. and Mori, S. (2001). Nicotianamine synthase gene expression differs in barley and rice under Fe-deficient conditions. Plant J. 25, 159–167

40). Hirokawa, T., Boon-Chieng, S. and Mitaku, S. (1998). SOSUI: Classification and secondary structure prediction system for membrane proteins. Bioinformatics. 14, 378–379

41). Hyndman, D., Bauman, D. R., Heredia, V. V., and Penning, T. M. (2003). The aldo-keto reductase superfamily homepage. Chem. Biol. Interact. 143, 621–631

42). Iantomasi, T., Favilli, F., Marraccini, P., Magaldi, T., Bruni, P. and Vincenzini, M.T. (1997). Glutathione transport system in human small intestine epithelial cells. Biochem. Biophy. Acta. 1330, 274-283

43). Inoue, H., Higuchi, K., Takahashi, M., Nakanishi, H., Mori, S., and Nishizawa, N. K. (2003). Three rice nicotianamine synthase genes, *OsNAS1*, *OsNAS2*, and *OsNAS3* are expressed in cells involved in long-distance transport of iron and differentially regulated by iron. Plant J. 36, 366–381

44). Igarashi, Y., Yoshiba, Y., Takeshita, T., Nomura, S., Otomo, J., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2000). Molecular cloning and characterization of a cDNA encoding proline transporter in rice. Plant Cell Physiol. 41, 750–756

45). Ishikawa, T., Madhusudhan, R. and Shigeoka, S. (2003). Effect of iron on the expression of ascorbate peroxidase in *Euglena gracilis*. Plant Sci. 165, 1363–1367

46). Ishimaru, Y., Suzuki, M., Kobayashi, T., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N.K. (2005). *OsZIP4*, a novel zinc regulated zinc transporter in rice. J. Exp. Bot. 56, 3207–3214

47). Iturbe-Ormaetxe, I., Moran, J.F., Arrese-Igor, C., Gogorcena, Y., Klucas, R.V. and Becana, M. (1995). Activated oxygen and antioxidant defences in iron-deficient pea plants. Plant Cell Environ. 18, 421–429

48). Jez, J. M., Bennett, M. J., Schlegel, B. P., Lewis, M., and Penning, T. M. (1997). Comparative anatomy of the aldo-keto reductase superfamily. Biochem J. 326(3), 625–636

49). Jung, R., Nam. Y.W., Saalbach, I., Muntz, K., Nielsen, N.C. (1997). Role of the sulfhydryl redox state and disulfide bonds in processing and assembly of 11S seed globulins. Plant Cell. 9, 2037–2050

50). Kaminaka, H., Morita, S., Nakajima, M., Masumura, T. and Tanaka, K. (1998). Gene cloning ans expression of cytosolic glutathione reductase in rice (*Oryza sativa* L.). Plant Cell Physiol. 39, 1269–1280

51). Kanazawa, K., Higuchi, K., Nishizawa, N. K., Fushiya, S., and Mori, S. (1995). Detection of two distinct isozymes of nicotianamine aminotransferase in Fe-deficient barley roots. J. Exp. Bot. 46, 1241-1244

52). Kanazawa, K., Higuchi, K., Nishizawa, N. K., Fushiya, S., Chino, M., and Mori, S. (1994). Nicotianamine aminotransferase activities are

correlated to the phytosiderophore secretions under Fe-deficient conditions in Gramineae. J. Exp. Bot. 45,1903-1906

53). Kawai, S., Takagi, S., and Sato, Y. (1988). Mugineic acid family phytosiderophores in root-secretions of barley, corn and sorghum varieties. J. Plant Nutr. 11, 633-642

54). Khush, G.S. and Toenniessen, G.H. eds (1991). Rice Biotechnology: International Rice Research Institute. 6, 7-8

55). Kim, S.Y., Lim, J-H., Park, M.R., Kim, Y.J., Park, T.I., Seo, Y.W., Choi, K.G., Yun, S.J. (2005). Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under saline stress. J. Biochem. Mol. Biol. 38, 218–224

56). Kobayashi, T., Nakanishi, H., Takahashi, M., Kawasaki, S., Nishizawa, N. K., and Mori, S. (2001). In vivo evidence that *Ids3* from *Hordeum vulgare* encodes a dioxygenase that converts 2'-deoxymugineic acid to mugineic acid in transgenic rice. Planta. 212, 864–871

57). Kobayashi, T., Nakayama, Y., Itai, R. N., Nakanishi, H., Yoshihara, T., Mori, S., and Nishizawa, N. K. (2003). Identification of novel cis-acting elements, IDE1 and IDE2, of the barley IDS2 gene promoter conferring iron-deficiency-inducible, root-specific expression in heterogeneous tobacco plants. Plant J. 36, 780–793

58). Kobayashi, T., Suzuki, M., Inoue, H., Itai, R.N., Takahashi, M., Nakanishi, H., Mori, S., and Nishizawa, N. K. (2005). Expression of iron-acquisition-related genes in iron-deficient rice is coordinately induced by partially conserved iron-deficiency-responsive elements. J. Exp. Bot. 56, 1305–1316

59). Koh, S., Wiles, A.M., Sharp, J.S., Naider, F.R., Becker, J.M. and Stacey G. (2002). An oligopeptide transporter gene family in Arabidopsis. Plant Physiol. 128, 21–29

60). Koike, S., Inoue, H., Mizuno, D., Takahashi, M., Nakanish, H., Mori, S., and Nishizawa, N. K. (2004). OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. Plant J. 39, 415-424

61). Lappartient, A. and Touraine, B. (1996). Demand-driven control of root ATP sulfurylase activity and SO42- uptake in intact canola. Plant Physiol. 111, 147–157

62). Lipton, A.J., Johnson, M.A., Macdonald, T., Lieberman, N.W., Gozal, D. and Gaston, B. (2001). S-nitrosylothiols signal ventilatory response to hypoxia. Nature. 413, 171–174

63). Lipton, S.A. (2001). Nitric oxide and respiration. Nature. 413, 118–121

64). Lubkowitz, M.A., Hauser, L., Breslav, M., Naider, F. and Becker, J.M. (1997). An oligopeptide transport gene from *Candida albicans*. Microbiology.143, 387–396

65). Lubkowitz, M.A., Barnes, D., Breslav, M., Burchfield, A., Naider, F. and Becker, J.M. (1998). *Schizosaccharomyces pombe* isp4 encodes a transporter representing a novel family of oligopeptide transporters. Mol. Microbiol. 28, 729–741

66). Ma, J. F. and Nomoto, K. (1993). Two related biosynthetic pathways of mugineic acids in Graminaceous Plants. Plant Physiol. 102, 373-378

67). Ma, J. F., Shinada, T., Matsuda, T., and Nomoto, K. (1995). Biosynthesis of phytosiderophores, mugineic acids, associated with methionine cycling. J. Biol. Chem. 270, 16549-16554

68). Ma, J. F., Taketa, S., Chang, Y-C., Takeda, K., and Matsumoto, H. (1999). Biosynthesis of phytosiderophores in several Triticeae species with different genomes. J. Exp. Bot. 50(334), 723-726

69). Maria, F.D., Pedersen, J.Z., Caccuri, A.M., Antonini, G., Turella, P., Stella, L., Bello, M.L., Federici., G., Ricci, G. (2003). The specific interaction of dinitrosyl-diglutathionyl-iron complex, a natural NO carrier, with the glutathione transferase superfamily. J. Biol. Chem. 278, 42283–42293

70). Marrs, K.A. (1996). The functions and regulation of glutathione S-transferase in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47, 127–158

71). Marschner, H. and Römheld, V. (1994). Strategies of plants for acquisition of iron. Plant Soil. 165, 261-274

72). Marschner, H., Römheld, V., and Kissel, M. (1986). Different strategies in higher plants in mobilization and uptake of iron. J. Plant Nutr. 9, 695–713

73). Meister, A. (1988). Glutathione metabolism and its selective modification. J. Biol. Chem. 263, 17205–17208

74). Miyake, T., Kanayama, M., Sammoto, H. and Ono, B. (2002). A novel cis-acting cysteine-responsive regulatory element of the gene for the high-affinity glutathione transporter of *Saccharomyces cerevisiae*. Mol. Genet. Genomics. 266, 1004–101

75). Mizuno, D., Higuchi, K., Sakamoto, T., Nakanishi, H., Mori, S., and Nishizawa N. K. (2003). Three nicotianamine synthase genes isolated from maize are differentially regulated by iron nutritional status. Plant Physiol. 132, 1989-1997

76). Moog, P.R. and Bruggermann, W. (1994). Iron reductase systems on the plant plasma membrane- a review. Plant Soil. 165:241- 260

77). Mori, S and Nishizawa, N. K. (1989). Identification of barley chromosome no. 4, possible encoder of genes of mugineic acid synthesis from 2'-deoxymugineic acid using wheat-barley addition lines. Plant cell Physiol. 30, 1057-1061

78). Mori, S, Nishizawa, N. K., Hayashi, H., Chino, M., Yoshimura, E., and Ishihara, J. (1991). Why are young rice plants highly susceptible to Fe-deficiency? Plant Soil. 130, 143-156

79). Mori, S., Nishizawa, N. K. (1987). Methionine as a dominant precursor of phytosiderophores in graminaceae plants. Plant Cell Physiol. 28,1081–1092

80). Mullineaux, P.M., Creissen, G.P. (1997). Glutathione reductase: regulation and role in oxidative stress. In: Scandalios JG, ed. Oxidative stress and the molecular biology of antioxidants. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press, 667–713

81). Murray-Kolb, L.E., Takaiwa, F., Goto, .F, Yoshihara, T., Theil, E.C. and Beard. J.L. (2002). Transgenic rice is a source of iron for iron-depleted rats. J. Nutr. 132, 957-960

82). Nakanishi, H., Okumura, N., Umehara, Y., Nishizawa, N. K., Chino, M., and Mori, S. (1993). Expression of a gene specific for iron deficiency (Ids3) in the roots of *Hordeum vulgare*. Plant Cell Physiol. 34, 401–410

83). Nakanishi, H., Yamaguchi, H., Sasakuma, T., Nishizawa, N. K., and Mori, S. (2000). Two dioxygenase genes, *Ids3* and *Ids2*, from *Hordeum*

vulgare are involved in the biosynthesis of mugineic acid family phytosiderophores. Plant Mol Biol. 44, 199–207

84). Negishi, T., Nakanishi, H., Yazaki, J., Kishimoto, N., Fujii, F., Shimbo, K., Yamamoto, K., Sakata, K., Sasaki, T., Kikuchi, S., Mori, S., and Nishizawa, N. K. (2002). cDNA microarray analysis of gene expression during Fe-deficiency stress in barley suggests that polar transport of vesicles is implicated in phytosiderophores secretion in Fe-deficient barley roots. Plant J. 30, 83-94

85). Nelson, M. J., and Snell, E. E. (1986). Enzymes of vitamin B6 degradation. Purification and properties of 5- pyridoxic-acid oxygenase from Arthrobacter sp. J. Biol. Chem. 261, 15115–15120

86). Noctor, G. and Foyer, C.H. (1998). Simultaneous measurement of foliar glutathione γ -glutamylcysteine and amino acids by high-performance liquid chromatography: comparison with two other assay methods for glutathione. Anal. Biochem. 264, 98–110

87). Noctor, G., Gomez, L., Vanacker, H. and Foyer, C.H. (2002). Interactions between biosynthesis, compartmentalization and transport in the control of glutathione homeostasis and signaling. J. Exp. Bot. 53, 1283–1304

88). Nomoto, K., Sugiura, Y., and Takagi, S. (1987). Mugineic acids, studies on phytosiderophores. In Iron Transport in Microbes, Plants and Animals. VCH Publishers, Weinheim, Germany, pp 401-425

89). Nozoye, T., Itai R. N., Nagasaka, S., Takahashi, M., Nakanishi, H., Mori, S., and Nishizawa, N.K. (2004). Diurnal changes in the expression of genes that participate in phytosiderophore synthesis in rice. Soil Sci. Plant Nutr. 50, 1125-1131

90). Ogawa, K., Tasaka, Y., Mino, M., Tanaka, Y., Iwabuchi, M. (2001). Association of glutathione with flowering in *Arabidopsis thaliana*. Plant Physiol .42, 524530

91). Ogawa, K.I., Tasaka, Y., Mino, M., Tanaka, Y. and Iwabuchi, M. (2001). Association of glutathione with flowering in *Arabidopsis thaliana*. Plant Cell Physiol. 42, 524–530

92). Ogo, Y., Itai, R. N., Nakanishi, H., Inoue, H., Kobayashi, T., Suzuki, M., Takahashi, M., Mori, S., and Nishizawa, N. K. (2006). Isolation and characterization of IRO2, a novel iron-regulated bHLH transcription factor in graminaceous plants. J. Exp. Bot. 57, 2867-2878

93). Ohata, T., Kanazawa, K., Mihashi, S., Nishizawa, N. K., Fushiya, S., Nozoe, S., Chino, M., and Mori, S. (1993). Biosynthetic pathway of phytosiderophores in iron-deficient graminaceous plants: Development of an assay system for the detection of nicotianamine aminotransferase activity. Soil Sci. Plant Nutr. 39: 745-749

94). Okumura, N., Nishizawa, N. K., Umehara, Y., Ohata, T., Nakanishi, H., Yamaguchi, T., Chino, M., and Mori, S. (1994). A dioxygenase gene (*Ids2*) expressed under iron deficiency conditions in the roots of *Hordeum vulgare*. Plant Mol. Biol. 25,705–719

95). Osawa, H., Stacey, G. and Gassmann, W. (2006). ScOPT1 and AtOPT4 function as proton-coupled oligopeptide transporters with broad but distinct substrate specificities. Biochem. J. 393: 267–275

96). Pastori, G.M., Mullineaux, P.M. and Foyer, C.H. (2000). Post-transcriptional regulation prevents accumulation of glutathione reductase protein and activity in the bundle sheath cells of maize. Plant Physiol. 122, 667–675

97). Patra, J., Panda, B. (1998). A comparison of biochemical response to oxidative and metal stress in seedlings of barley, *Hordeum vulgare* L. Environ. Pollut. 101, 99–105

98). Paulsen, I.T. and Skurray, R.A. (1994). The POT family of transport proteins. Trends Biochem. Sci. 19, 404

99). Romero-Puertas, M.C., Corpas, F.J., Sandalio, L.M, Marina, L., Rodríguez-Serrano, M., del Río, L.A. and Palma, J.M. (2006). Glutathione reductase from pea leaves: response to abiotic stress and characterization of the peroxisomal isozyme. New Phytologist. 170, 43-52

100). Römheld, V. and Marschner, H. (1983.) Mechanism of iron uptake by peanut plants. I- Fe(III) reduction, chelate splitting, and release of phenolics. Plant Physiol. 71, 949-954

101). Römheld, V., and Marschner, H. (1990). Genotypical differences among graminaceous species in release of phytosiderophores and uptake of iron phytosiderophores. Plant Soil. 123, 147-153

102). Römheld, V., Muller, C. and Marschner, H. (1984). Localization and capacity of proton pumps in roots of intact sunflower plants. Plant Physiol. 76, 603-606

103). Roncarati, R., Salamini, F. and Bartels, D., (1995). An aldose reductase homologous gene from barley: regulation and function. Plant J. 7, 809-822

104). Sanchez-Fernandez, R., Fricker, M., Corben, L.B., White, N.S., Sheard, N., Leaver, C.J., Van, Montagu, M., Inzé, D. and May, M. J. (1997). Cell proliferation and hair tip growth in the Arabidopsis root are under mechanistically different forms of redox control. Proc. Nat. Aca. Sci. USA. 94, 2745–2750

105). Schaaf, G., Ludewig, U., Erenoglu, B. E., Mori, S., Kitahara, K., and von Wirén N. (2004). ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. J. Biol. Chem. 279, 9091-9096

106). Sherrill, C. and Fahey, R.C. (1998). Import and metabolism of glutathione by Streptococcus mutants. J. Bacteriol. 180, 1454–145

107). Shojima, S., Nishizawa, N. K., Fushiya, S., Nozoe, S., Kumashiro, T., Nagata, T., Ohata, T. and Mori, S. (1989). Biosynthesis of nicotianamine in the suspension-cultured cells of tobacco (*Nicotiana megalosiphon*). Biol. metals. 2, 142-145.

108). Shojima, S., Nishizawa, N. K., Fushiya. S., Nozoe, S., Irifune, T., and Mori, S. (1990). Biosynthesis of phytosiderophores. In vitro biosynthesis of 2'-deoxymugineic acid from L-methionine and nicotianamine. Plant physiol. 93,1497-1503

109). Singh, K., Chino, M., Nishizawa, N. K., Ohata, T., and Mori, S. (1993) Genetic aspects of plant mineral nutrition (Randall, R.,J., Delhaize, E., Richards, R.,A., and Munns, R, eds) Kluwer academic. 335-339

110). Stacey, G., Koh, S., Granger, G. and Becker, J.M. (2002a). Peptide transport in plants. Trends Plant Science. 7, 257–263.

111). Stacey, M. G., Koh, S., Becker, J.M. and Stacey, G. (2002b). AtOPT3, a member of the oligopeptide transporter family, is essential for embryo development in Arabidopsis. Plant Cell. 14, 2799–2811

112). Stacey, M. G., Osawa, H., Patel, A., Gassmann, G. and Stacey, G. (2006). Expression analysis of Arabidopsis oligopeptide transporters during seed germination, vegetative growth and reproduction. Planta. 223, 291–305

113). Stevens, G.R., Creissen, G.P. and Mullineaux, P.M. (1997). Cloning and characterization of a cytosolic glutathione reductase cDNA from pea (*Pisum sativum* L.) and its expression in response to stress. Plant Mol. Biol. 35, 641–654

114). Takagi, S. (1976). Naturally occurring iron-chelating compounds in oat- and rice-root washing. I. Activity measurement and preliminary characterization. Soil Sci. Plant Nutr. 22, 423–433

115). Takagi, S., Nomoto, K., Takemoto, S. (1984). Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. J. Plant Nutr. 7, 469-477

116). Takahashi, M. (2003). Overcoming Fe deficiency by a transgenic approach in rice. Plant Cell Tiss. Organ Cult. 72, 211–220

117). Takahashi, M., Nakanishi, H., Kawasaki, S., Nishizawa, N. K., and Mori, S. (2001). Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. Nat. Biotechnol. 19, 466–469

118). Takahashi, M., Yamaguchi, H., Nakanishi, H., Shioiri, T., Nishizawa, N. K., and Mori, S. (1999). Cloning two genes for nicotianamine aminotransferase, a critical enzyme in iron acquisition (Strategy II) in graminaceous plants. Plant Physiol. 121, 947–956

119). Takizawa, R., Nishizawa, N. K., Nakanishi, H. and Mori, S. (1996). Effect of iron deficiency on S-adenosylmethionine synthetase in barley roots. J. Plant Nutr. 19, 1189–1200

120). Tewari, R.K., Kumar, P., Neetu and Sharma, P.N. (2005). Signs of oxidative stress in the chlorotic leaves of iron starved plants. Plant sci. 169, 1037-1045.

121). Turella, P., Pedersen, J.Z., Caccuri, A.M., Maria, F.D., Mastroberardino, P., Bello, M.L., Federici, G. and Ricci, G. (2003). Glutathione transferase superfamily behaves like storage proteins for dinitrosyl-diglutathionyl-iron complex in heterogeneous systems. J. Biol. Chem. 278, 42294–42299

122). Ueda, A., Shi, W., Nakamura, T., Takabe, T. (2002). Analysis of salt-inducible genes in barley roots by differential display. J. Plant Res. 115, 119–130

123). Ueno, D. Iwashita, T., Nomoto, K., Rombola A. D. and Ma, F. J. (2004). Identification of two new phytosiderophores secreted from perennial grasses. XII international symposium on iron nutrition and interactions in plants, April 11-15, 2004, Tokyo Japan. 102

124). Unterlinner, B., Lenz, R., and Kutchan, T. M. (1999). Molecular cloning and functional expression of codeinone reductase: the penultimate enzyme in morphine biosynthesis in the opium poppy *Papaver somniferum*. Plant J. 18, 465–475

125). Vanin, A.F. (1991). Endothelium-derived relaxing factor is a nitrosyl iron complex with thiol ligands. FEBS Lett. 289, 1–3

126). Vernoux, T., Wilson, R.C., Seeley, K.A., Richheld, J.P., Muroy, S., Brown, S., Maughan, S.C., Cobbett, C.S., VanMontagu, M., Inze´, D., May, M.J. and Sung, Z.R. (2000). The root meristemless1/cadmium sensitive2 gene defines a glutathione dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. Plant Cell. 12, 97–110

127). von Wirén, N, Mori, S., Masrchner, H. and Römheld, V. (1994) Iron inefficiency in maize mutant ys1 (*Zea mays* L. cv Yellow-Stripe) is caused by a defect in uptake of iron phytosiderophores. Plant Physiol. 106, 71–77

128). Watt,s R.N., Richardson, D.R. (2002). The mechanism of nitrogen monoxide (NO)-mediated iron mobilization from cells. Eur. J. Biochem. 269, 3383–3392

129). Watts, R.N. and Richardson, D. R. (2001). Nitrogen monoxide (NO) and glucose: unexpected links between energy metabolism and NO-mediated iron mobilization from cells. J. Biol Chem. 276, 4724–4732

130). Wiles, A.M., Naider, F. and Becker, J.M. (2006). Transmembrane domain prediction and consensus sequence identification of the oligopeptide transport family. Res. Microbiol. 157, 395-406

131). World Health Organization. (2002). Nutrition. Reduction of iron deficiency anaemia. http://www.who.int/nut/ida.htm, 09/12/2002.

132). Yen, M.R., Tseng, Y.H. and Saier, M. H. Jr. (2001). Maize Yellow Stripe1, an iron-phytosiderophore uptake transporter, is a member of the oligopeptide transporter (OPT) family. Microbiology. 147, 281-83

133). Yokochi, N., Yoshikane, Y., Trongpanich, Y., Ohnishi, K. and Yagi, T. (2004). Molecular cloning, expression, and properties of an unusual aldo-keto reductase family enzyme, pyridoxal 4-dehydrogenase, that catalyzes irreversible oxidation of pyridoxal. J. Biol. Chem. 279,37373-37384

134). Yoshihara, T., Kobayashi, T., Goto, F., Masuda, T., Higuchi, K., Nakanishi. H., Nishizawa, N.K. and Mori, S. (2003). Regulation of Fe-deficiency responsive gene, Ids2 of barley in tobbaco. Plant Biotech. 20, 33–41

135). Zaharieva, T.B. and Abadía, J. (2003). Iron deficiency enhances the levels of ascorbate, glutathione, and related enzymes in sugar beet roots. Protoplasma. 221, 269–275

136). Zaharieva, T.B., Gogorcena, Y. and Abadía, J. (2004). Dynamics of metabolic responses to iron deficiency in sugar beet roots. Plant Sci. 166, 1045–1050

137). Zhang, M. Y., Bourbouloux, A., Cagnac, O., Srikanth, C. V., Rentsch, D., Bachhawat, A. K. and Delrot, S. (2004). A novel family of transporters mediating the transport of glutathione derivatives in plants. Plant Physiol, 134, 482–491