Introduction

Manganese (Mn) deficiency occurs in many areas of the world. Soils particularly prone to Mn deficiency include highly leached acidic upland soils, alkaline and calcareous soils with low organic matter status and small amounts of reducible Mn, degraded paddy soils containing large amounts of active Fe, leached sandy soils containing small amounts of Mn, old acid sulfate soils with low base content, alkaline and calcareous organic soils, and highly weathered soils (Dobermann and Fairhurst, 2000). In well-aerated calcareouse soil, the solubility of Mn decreases with increasing levels of both $CaCO_3$ and MnO_2 due to the adsorption of Mn on $CaCO_3$ and its oxidation on MnO_2 surfaces and probably to the precipitation of Mn calcite (Jauregui and Reisenauer, 1982).

Manganese deficiency is uncommon in wetland rice, but can cause yield losses in upland rice and rainfed rice when the soil is not flooded. Solubility of Mn increases under submergence, as Mn^{4+} is reduced to the more plant-available Mn^{2+}. Manganese deficiency can be a problem for rainfed rice on calcareous soils such as Lopburi, Banmi, Saraburi, Takhli soil series in Thailand, when rainfall is insufficient for submergence. It can also be a problem in upland rice on calcareous soils of the highlands that have derived from limestone, e.g. in Pang Ma Pha and Pai district of Mae Hong Son province and some subdistricts of Mae Chaem district, Chiang Mai province. When it occurs in the field, Mn deficiency is usually treated by the addition of maganese fertilizer (usually in the form of $MnSO_4$) applied as a crop spray (Mortvedt, 1994; Chalmers *et al*., 1999). Manganese deficiency can be difficult to

manage as Mn applied to soil is susceptible to rapid oxidation and two or more fertilizer applications are usually required during the growing season. Therefore, the cultivation of selected cultivars with tolerance to a low Mn supply, or with a relatively high Mn efficiency, could have potentail advantages in terms of cost-effectiveness while also representing a more sustainable approach to crop production.

Manganese efficient cultivars are defined as those that display significantly better performance in low Mn supply compared with standard varieties (Graham 1988). The existence of genotypic variation in Mn use efficiency has been identified previousely in oat, wheats, barley, triticale and rye cultivars (Brown and Jones, 1987; Ohki *et al*., 1980; Graham *et al*., 1983; Marcar and Graham, 1987a; Bansal *et al*., 1992; Fang *et al.*, 2000; Rengel, Pedler and Graham, 1994; Pearson and Rangel, 1997, Krahmer and satterlmacher, 2001). Jiang and Ireland (2005) reported Mn use efficiency of a wheat cultivar (cv. Maris Butler) was related to superior internal utilization of Mn. The other mechanisms of tolerance to Mn deficiency may rely on greater excretion of various substances into the rhizosphere by plant roots (Rengel, 2000).

Even though, genotypic variation in Mn efficiency has been found in many plant species, but no information is yet available in rice. While, the precise mechanism underlying Mn efficiency in crop cultivars remains unclear. On the other hand, productive upland and rainfed rice cropping systems based on local varieties have been found soils prone to Mn deficiency in Thailand. So, observations indicate that a certain degree of tolerance to low Mn supply may be present in some rice genotypes. Therefore, finding rice genotypes tolerlant to Mn deficiency and understanding the mechanism of Mn efficiency may improve rice productivity both

upland and lowland rice condition and provide a potentially useful source of parential material for future crop improvement programmes designed to produce rice lines tolerant to depleted Mn supply. The present study has four objectives. First is to evaluate Thai rice genotypes for Mn deficiency. Second is to examine the mechanism of Mn acquisition in Mn efficiency and inefficiency in rice genotypes by the possibility of the presence of root exudate. Third is to evaluate rice genotypes for Mn uptake efficiency of rice genotypes at different levels of external Mn supply. Fourth is to examine Mn utilization efficiency in rice genotypes in dry matter production and partitioning of carbohydrates between root and shoot of rice grown and grain yield.

Chapter 1

Literature review

1.1 Manganese in soil

Manganese (Mn) occurs in various primary rocks and particularly in ferromagnesian materials. The Mn released from these rocks by weathering forms a number of secondary minerals the most prominent being pyrolusite (MnO2), braunite (Mn_2O_3), hausmannite (Mn_3O_4), and manganite (MnO(OH)). Total Mn level may differ considerably between soil. Mn contents between 200 and 300 ppm are most common (Swaine, 1955). Mn can exist in the oxidation states 0, II, III, IV, VI and VII. In biological systems, however, it mainly occurs in oxidation states II, III, and IV with Mn (II) and Mn (IV) being fairly stable and Mn (III) unstable (Hughes and Williams, 1988). In plants, Mn (II) is by far the dominant form, but it can readily be oxidized to Mn (III) and Mn (IV). Mn therefore plays an important role in redox processes. In Figure 1.1 are presented the relationships between the Mn^{2+} and the Mn oxides. This so-called Mn cycle in the soil (Dion and Mann, 1946) shows that the equilibrium between the various Mn forms is governed by oxidation-reduction processes. The easily-reducible Mn contributes to plant supply and Mn^{2+} is also the most important fraction in plant nutrition. Therefore, easily-reducible Mn and Mn^{2+} are called active Mn". The level of Mn^{2+} in the soil depends on oxidation-reduction reactions, all factors inducing these processes have an impact on Mn availability. Mn (II) forms only relatively weak bounds with organic ligands. The ionic radius of Mn^{2+} (0.075

nm) lies between Mg^{2+} (0.065 nm) and Ca^{2+} (0.099 nm) and it can therefore substitute or compete, in various reactions involving either of these ions. The binding strength of all three ions for ligands based on oxygen donors are roughly the same (Hughes and Williams, 1988) or higher for Mn^{2+}, as for example, by a factor of about four in case of ATP (Burnell, 1988).

Under waterlogged conditions as for example in paddy soil, reducing processes dominate and thus provide a high level of Mn availability which may even result in Mn toxicity (Tanaka and Yoshida, 1970). After submergence and almost parallel with the disappearance of O_2, the level of soluble Mn^{2+} may easily attain toxic level, while in calcareous or sodic soil the Mn level dose not rise much after flooding. On these soil Mn deficiency can even occur in rice under submergence conditions (Randhawa *et al*., 1978). The effect of anaerobic soil conditions (flooding) and of liming on Mn availability as reflected in Mn content of Lucerne growth on the soil (Graven *et al*., 1965).

Mn availability is also higher in acid soils due to the higher solubility of Mn compounds under low pH condition. The soluble Mn^{2+} decreases 100 fold for each unit increase in pH (Linsay, 1972). Under high soil pH condition Mn availability can thus be inadequate to meet plant demand. An increase in pH also enhances the formation of Mn soil organic matter complexes which also render Mn less available (Page, 1962). From the above discussion it is clear that soil of high pH with large organic matter reserves are particularly prone to Mn deficiency.

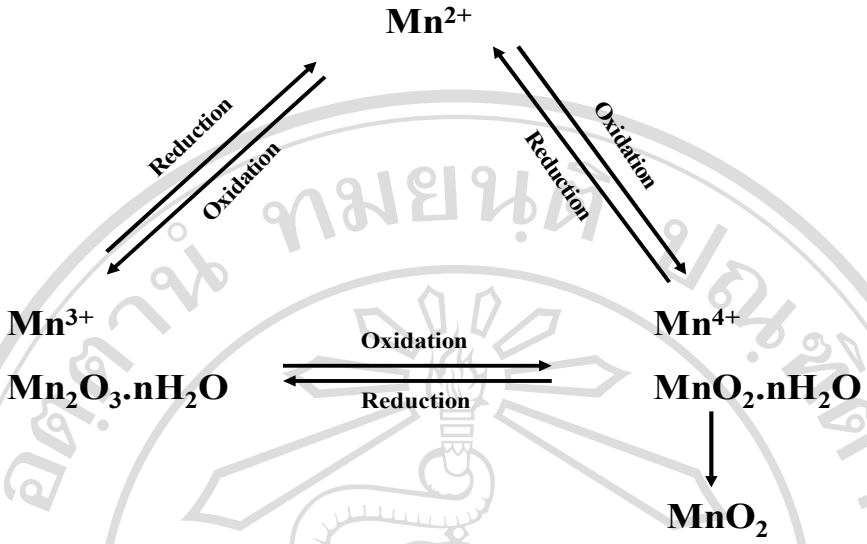


Figure. 1.1 Mn oxidation-reduction cycle in the soil (Dion and Mann (1946)).

1.2 Manganese uptake and translocation in the rice plant

1.2.1 Short distance transport

The regulation of Mn uptake at a cellular level it is necessary to follow the pathway of Mn from the external solution through the cell wall and the plasma membrane into the cytoplasm and vacuole (Marschner, 1995).

Mn is absorbed into the plant mainly as the free Mn^{2+} ion, but it exist in soils as different reduced forms including Mn (III) and Mn (IV) oxides. Movement of Mn solute from the external solution into the cell walls of individual cell of roots (the apoplast or free space) is a non metabolic, passive process, driven by diffusion or mass flow (Marschner, 1995). However, the cell walls can interact with solutes and thus may facilitate or restrict further movement to the uptake sites of the plasma membrane of individual cells of roots (Marschner, 1995). A network of cellulose, hemicellulose (including pectins) and glycoproteins are primary cell walls (Cassab and Varner, 1988). This network contains interfibrillar and intermicellar spaces, which differ in size. Maximum values for the pores in plant cell walls are in the range of 5.0 nm (Carpita *et al*., 1979). The pores themselves would thus not be expected to offer any restriction to movement of Mn in the free space. In contrast to Mn and low-molecular-weight organic solutes, high-molecular weight solutes (e.g., Mn chelates, fulvic acid, and toxins) or virus and other pathogens are either severely restricted or prevented by the diameter of pores from entering the free space of root cells (Marschner, 1995).

The apoplast consist of carboxylic groups in the cell wall, composing thos associated with polygalacturonic acid in the middle lamella, act as cations exchangers. Therefore, in root cations from the external solution can accumulate in a

nonmetabolic step in the free space, whereas anions are repelled. Because of these negative charges the apoplasm does not provide a free space for the movement of charged solutes. Hope and Stevens (1952) introduced the terms apparent free space (AFS), which comprises the water free space (WFS), that is freely accessible to ions and charged and uncharged molecules, and the Donnan free space (DFS), where cation exchange and anion repulsion take place. Ion distribution within the DFS is characterized by the typical Donan distribution which occurs in soils at the surfaces of negatively charged clay particles. Divalent cations are therefore preferentially bound to these cation-exchange sites. Plant species differ considerably in cation-exchange capacity (CEC), that is, in the number of cation-exchange site, located in cell wall (Marschner, 1995).

The main sites of selectivity in the uptake of cations and anions as well as solutes in general are located in the plasma membrane of individual cells. The plasma membrane is an effective barrier against the diffusion of solutes either from the apoplasm into the cytoplasm (influx) or from the cytoplasm into apoplasm and the external solution (efflux). The plasma membrane is also the principal site of active transport in either direction. The other main barrier to diffusion is the tonoplast (vacuolar membrane). In most mature plant cells the vacuole comprises more than 80-90% of the cell volume (Leigh and Wyn Jones, 1986; Wink, 1993) acting as central storage compartment for Mn and other ions, but also for other solutes (e.g. sugars, secondary metabolites). Nevertheless the plasma membrane and tonoplast are the main biomembranes directly involved in solute uptake and transport in root, it must be borne in mind that compartmentation by biomembrane (Leigh and Wyn

Jones, 1986). Solute tansport into organelles by membranes which separates organelles from the surrounding cytoplasm (Marschner, 1995)

Mn is absorbed into the plant mainly as the free Mn^{2+} ion. The membrane transport of divalent cations can be mediated by proteinaceous ion channels. Based on the thermodynamic considerations. Cu^{2+} uptake could be facilitated by a carrier-type protein (transporter), which across the plasma membrane (Graham, 1981). Welch (1995) speculated that the uptake of Mn cation into the root cells of grasses might be in a similar way as Fe (III)MAs through metal-phytometallophores transporters across the plasma membrane. The term of phytosiderophores is based on broad meaning "plant metal bearer" or "plant claw", including phytosiderophores, phytochelatins, and low molecular metal chelators. Mn can form complexes in the rhizophere with organic legends of plant and microbial origin, which may increase the mobility of Mn in the rhizosphere (facilitating diffusion up to the root-cell plasma membrane). It has been reported that Mn absorption can be enhanced by phytosiderophores produced in response to Fe deficiency (Marschner, 1988; Marschner *et al*., 1989). However, Mn deficiency did not induce phytosiderophore release in root of Mn efficient barley genotypes (Webb, 1994), more reductants of microorganism source were found to exist in the rhizosphere of Mn efficient wheat genotype (Rengel, 1997). Cation micronutrient uptake by grass might follow similar model as that for Fe uptake by grass. Whereas, there is no direct evidence showing M(nI)-chelate complexes transporter proteins or genes except for some evidence showing the presence of Fe(III)-MAs transporters in root plasma membranes.

1.2.2 Long distance transport

1.2.2.1 Xylem transport

Long distance transport form the roots to the shoots occur predominantly in the nonliving xylem vessels. Xylem principal water-conducting tissue consists of several different types of cells, living and nonliving (Esau, 1898). Xylem transport is driven by the gradient in hydrostatic pressure (root pressure) and by the gradient in the water potential (Burgess *et al*., 2000), created by water loss at the surface by evapotranspiration. Accordingly, values for water potential are usually negative. The gradient in the water potential between roots and shoots is quite steep particularly during the day when the stomata are open. Values become less negative in the following sequence: atmosphere>> leaf cell> xylem sap> root cell> external solution. Thus, solute flow in the xylem from the roots to shoots is unidirectional (Lang and Thorp, 1989). Xylem plays the dominant role in the flow of water through the plant and in nutrient delivery from the root to the shoot (Schurr, 1998).

Solutes can also be absorbed from the xylem (apoplast) into the symplast along the pathway of the xylem sap from the roots to the leaves. Reabsorption from the xylem can result either from transient or permanent storage in the xylem parenchyma and other stem tissue, or from xylem-phloem transfer, mediated by transfer cells. In some species, the reabsorption of certain mineral element from the xylem sap is very pronounced and can have important consequences for the mineral nutrition of these plants (Marschner, 1995).

The concentration of Mn ions and organic solutes in the xylem sap depends on factors such as plant species, mineral element supply to the roots, assimilation of mineral nutrients in the roots and nutrient recycling. Moreover, concentration of solutes is also strongly influenced by the degree of dilution by water and is therefore dependent on the transpiration rate and the time of day. Composition and

concentration of xylem sap also change typically during the ontogenesis of plants. Polyvalent heavy metal cations in the xylem sap exist mainly in organic form complexed with organic acids, amino acids and peptides in perennial species (White *et al*., 1981a, b). In annual species, both number and distribution of the complexes vary with plant age (Cataldo *et al*., 1988).

1.2.2.2 Phloem transport

Long distance transport in the phloem takes place in living cells, the sieve tubes. The principles of the transport mechanism in the phloem was proposed as early as 1930 by Munch in a pressure flow hypothesis based on the principle of the osmometer. Munch suggested that solutes such as sucrose are concentrated in the phloem of leaves and water enters the phloem due to osmotic potential, creating a positive internal pressure. This pressure induces a mass flow in the phloem to the site of lower positive pressure caused by removal of solutes from the phloem. Flow rate and direction of flow are therefore closely related to the release or unloading at the sink. This type of pressure driven mass flow in the phloem differs from that in the xylem in three important ways (Marschner, 1995): a) organic compounds are the dominant solutes in the phloem sap, b) transport take place in living cells, and c) the unloading of solutes at the sink plays an important role.

For mineral nutrients such as Mn, the main sites (sources) for phloem loading are located in the stem and the leaves as components of either mineral nutrient supply to growth sinks (shoot apices, fruits, roots) or of nutrient recycling.

Within the phloem, the sieve tube elements are associated with companion and storage parenchyma cell. The individual sieve tube elements are stretched end to end in a long series, forming sieve tubes which are interconnected by conspicuous pores

called sieve plate pores. The sieve tubes are highly specialized vascular systems for the long distance transport of solutes. The sieve tube cell contains a thin layer of cytoplasm, which forms transcellular filaments that pass through the sieve plate pore. The anatomical features of long distance transport in the sieve tube across the sieve plate pores are similar to those of short distance transport in the symplasm across the plasmodesmata (Eschrich, 1976).

1.2.3 Mobility in the phloem

All mineral nutrients have been found in the phloem sap, except for molybdenum and nikel where no data are available so far. On the basis of such studies and in condiseration of the data on phloem sap composition, mineral nutrients can be classified depending on their phloem mobility.

Table 1.1 Characteristic differences in mobility of mineral nutrients in the phloem

High Mobility	Intermediate Mobility	Low Mobility
Potassium	Iron	Calcium
Magnesium	Zinc	Manganese
Phosphorus	Copper	
Sulphur	Boron	
Nitrogen (amino-N)	Molybdenum	
Chlorine		
(Sodium)		

The classification in Table 1.1 from Marschner (1995) is only a first approximation as certain factors are ignored, for example, genotypical differences or

the nutritional status of plants. However, for the micronutrients, it is at least intermediate phloem mobility with the exception of Mn (Wood *et al*., 1986). Mn has low mobility in the phloem.

1.2.4 Transport between the xylem and phloem

In the vascular bundles, phloem and xylem are separated by only a few cell. In the regulation of long distance transport, exchange of solutes between the two conducting systems is very important. It is evident that a transfer from phloem to xylem can occur downhill, through the plasma membrane of the sieve tubes, if an adequate concentration gradient exists. In contrast, for most organic and inorganic solutes a transfer from xylem to phloem is usually and uphill transport against a steep concentration gradient between the apoplasm and the symplasm of the surrounding xylem parenchyma cells and the cells of phloem. The xylem-to-phloem transfer is of particular importance for the mineral nutrition of plants, because xylem transport is directed mainly to the sites of highest transpiration, which are usually not the sites of highest demand for mineral nutrients. This transfer of organic and inorganic solutes can take place all along the pathway from root to shoot, and the stem plays an important role in this respect (McNeil, 1980; Van Bel, 1984) most likely via transfer cells (Kuo *et al*., 1980; Jeschke and Pate, 1991). In stem, site of intensive xylem-to-phloem transfer are the node, which function in cereals (Marschner, 1995). These mineral nutrients are subsequently transported in the xylem into the ears (Martin, 1982).

1.2.5 Manganese remobilization

Remobilization is base on a range of different physiological and biochemical processes: Utilization of mineral nutrients stored in vacuoles, breakdown of storage proteins, or finally, breakdown of cell structures (e.g. chloroplast) and enzyme proteins thereby transforming structurally bound mineral nutrients (e.g. magnesium in chlorophyll, micronutrient in enzymes) into a mobile form. Remobilization of mineral nutrients is important during the ontogenesis of a plant at the following stages (Marschner, 1995): seed germination, vegetative stage and reproductive stage.

Seed germination

During the germination of seeds mineral nutrients are remobilized within the seed tissue and translocated in the phloem or xylem, or both, to the developing roots or shoots. As a role, seedling will grow for at least several days without an external supply for mineral nutrients. In seeds many mineral nutrients are usually bound to phytic acid as phytate; thus, remobilization of these mineral nutrients and also of phosphorus is correlated to the phytate activity.

Vegetative stage

In vegetative growth, nutrient supply to the root is often either permanently insufficient as the case of low soil nutrient content. Remobilization of mineral nutrient from mature leaves to areas of new growth is thus of key importance for the completion of the life cycle of plant under these experimental conditions. The extent to which remobilization takes place, however, also differs between mineral nutrients and this is reasonably well reflected in the distribution of visible deficiency symptoms in plants. Deficiency symptoms which predominantly occur in young leaves and

apical meristems reflect insufficient or only a relatively small fraction of the mineral nutrients can be transformed into a mobile form in the fully expanded older leaves.

Reproductive stage

Remobilization of mineral nutrients is particularly important during reproductive growth when seeds, fruit, and storage organs are formed. At this growth stage root activity and nutrient uptake generally decrease, mainly as a result of decreasing carbohydrate supply to the roots. Therefore, the mineral nutrient contents of vegetative parts quite often decline sharply during the reproductive stage.

The extent of this remobilization depends on various factors, including (a) the specific requirement of seeds and fruits for a given mineral nutrient; (b) the mineral nutrient status of the vegetative parts; (c) the ratio between vegetative mass (source size) and number and size of seeds and fruits (sink size); and (d) the nutrient uptake rate by the roots during the reproductive stage.

Remobilization is highly selective for mineral nutrients. This selectivity and the corresponding discrimination against mineral elements which are either not essential or required only at very low levels is quite impressive. During the reproductive stage the degree of remobilization of micronutrient is often astonishingly high compared with that during vegetative growth (Marschner, 1995).

1.3 Manganese deficiency

1.3.1 Function of manganese in plant

Manganese is involved in oxidation-reduction reactions in the electron transport system O_2 evolution in photosynthesis, containing enzymes, activates certain enzymes (e.g., oxidase, peroxidase, dehydrogenase, decarboxylase, kinase), protein,

carbohydrate and lipid synthesis, and cell division and extension. Mn is required for the following processes:

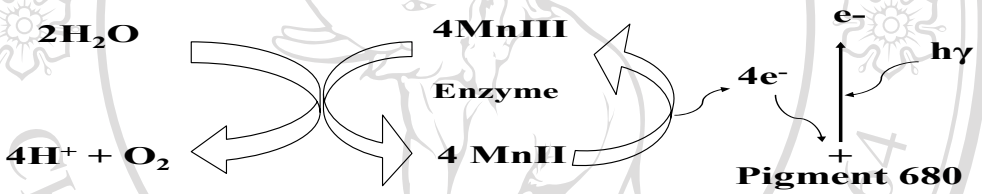
Photosynthesis and oxygen evolution

The particular role of Mn in photosynthesis was discovered in green algae (Pirson, 1937). In higher plants photosynthesis in general and photosynthetic O_2 evolution in photosystem II (PS II), in particular, are the processes which respond most sensitively to Mn deficiency. A decrease in Mn content of young leaves has only a small effect on chlorophyll content or leaf dry weight (Nable *et al*., 1984) but photosynthetic O_2 evolution drops by more than 50%. Resupplying Mn^{2+} to deficient leaves restores photosinthetiv O_2 evolution within one day to the levels in leaves adequately supplied with Mn. Mn deficiency-induced alteration in O_2 evolution are correlated with changes in the ulterstucture of thylakoid membranes, namely the loss of certain particles (PS II function unit) associated with the stacked areas of thylakoid membranes. Resupplying Mn restores the number of the particles in the thylakoid membranes (Simpson and Robinson, 1984). When Mn deficiency becomes more severe, the chlorophyll content also decreases and the ultrastryture of the thylakoids is drastically changed. These ultrastructural alterations are either very difficult to restore, or irreversible and are presumably caused by inhibition of biosynthesis of lipid and carotenoids. They are not brought about by enhanced photooxidation (lipid peroxidation) of the thylakoids and chlorophyll (Polle *et al*., 1992).

Manganese containing Enzymes

Although a relatively large number of enzymes are activated by Mn^{2+}, to date the existence of only two Mn-containing enzymes is well established, namely the Mn-protein in PH II and the Mn-containing superoxide dismutase (MnSOD). Former

reports on a Mn-containing acid phosphatase (Uehara *et al*., 1974) have not been confirmed. This enzyme contains two atoms of iron per molecule and requires iron but not Mn for its activity (Hefler and Averill, 1987). Beside, the functioning of the Mn atoms in both transientelectron storing and electron transmitting is coupled with fluctuations in the oxidation state of Mn between Mn (II) and Mn (IV) (Rutherford, 1989). In photosynthesizing cells this role in PS II is the most sensitive function of Mn to be impaired by Mn deficiency (Marschner, 1995).



Manganese dependent or activated enzymes

Mn act as a cofactor, activating about 35 different enzymes (Burnell, 1988). Most of these enzymes catalyze oxidation-reduction, decarboxylation, and hydrolytic reactions. Mn has a primary role in the tricarboxylic acid cycle (TCA) in oxidative and nonoxidative decarboxylation reactions, as for example, the NADPH specific decarboxylating malate dehydrogenase, malic enzyme, and isocitrat dehydrogenase.

Malic enzyme catalyzing the reaction:

$$\text{Malate} + \text{NADP} \xrightarrow[Mn^{2+},\, Mg^{2+}]{} \text{Pyruvate} + \text{NADPH} + H^{+} + CO_2$$

Isocitrate dehydrogenase catalyzing the reaction:

$$\text{Isocitrate} + NADP^{+} \xrightarrow[Mn^{2+},\ Mg^{2+}]{} \text{Oxalosuccinate} + NADPH$$

An absolute requirement of Mn^{2+} occurs in the bundle sheath chloroplasts of those C4 plants in which oxaloacetate acts as the carbon shuttle and where the decarboxylation is catalyzed by PEP carboxykinase. This enzyme has an absolute requirement for Mn^{2+} which cannot be replaced by Mg^{2+}. Mn activates several enzymes of shikimic acid pathway, and subsequent pathways, leading to the biosynthesis of aromatic amino acids, such as tyrosin, and various secondary products, such as lignin, flavonoids, as well as IAA (Burnell, 1988; Hughes and Williams, 1988). In Mn deficiency leaves the IAA oxidase activity is exceptionally high, as is also the case for leaves suffering from Mn toxicity (Morgan *et al.*, 1976). The role of Mn in IAA oxidase activity is still obscure (Horst. 1988). In the biosynthetic patway of isoprenoids producing carotenoids, sterols and GA, Mn-dependent enzymes have also been found, as for example, a phytoene synthetase (Wilkinson and Ohki, 1988).

Proteins, Carbohydates and lipids

Alther Mn^{2+} activates RNA polymerlase (Ness and Woolhouse, 1980), protein systhesis is obviously not specifically impaired in Mn-deficient tissues. The protein content of deficient plants is either similar to or somewhat higher than that of plants adequately supplied with Mn (Lerrer and Bar-Akiva, 1976). The accumulation of soluble nitrogen is a reflection of a shortage in reducing equivalents and

carbohydrates for nitrate reduction, as well as a low demand for reduced nitrogen. Mn deficiency has the most severe effect on the content of nonstructural carbohydrate. The role of Mn in lipid metabolisms is more complex. In Mn-deficient leaves not only the chlorophyll content is lower but even more so, the content of typical thylakoid membrance constituents such as glycolipids and polyunsaturated fatty acids. These are depressed in content by up to 50% (Constanttopoulus, 1970). This depression in lipid content in chloroplast can be attributed to the role of Mn^{2+} in biosynthesis of fatty acids and of carotenoids and related compounds (Wilson *et al*., 1982). In Mn deficient, the Mn content of leaves, seed yield and oil content are lower. The lower oil content in seed of deficient plants probably results mainly from rates of photosynthesis and thus a decreased supply of carbon skeletons for fatty acid synthesis (Wilson *et al*., 1982). Lower lignin contents in the Mn deficient plants are reflection of the requirement for Mn in various steps of lignin biosynthesis (Brown *et al*., 1984). The decrease is particularly evident in the root, and an important factor responsible for the lower resistance of Mn deficient plants to root infecting pathogens (Marschner, 1995).

Cell division and extension

Inhibition of root growth in Mn deficient plants is caused by shortage in carbohydrates as well as by a direct requirement for growth (Campbell and Nable, 1988). The rate of elongation seems to respond more rapidly to Mn deficiency than dose the rate of cell division (Marschner, 1995).

1.3.2 Response by rice plant to low manganese in soil

Mn deficiency is mainly a problem in rice grown in upland and organic soil with low Mn status (Snyder and Jones, 1988) and soil pH 7.0 (Snyder *et al*., 1990). Mn deficiency is uncommon in lowland rice because the solubility of Mn increases under submerged condition (Dobermann and Fairhurst, 2000). However, in rainfed rice Mn deficiency may occur in prolonged non-submerged. Therefore, the availability of Mn is low and may be inadequate for rice that were grown in these conditions. Leaves are affected by interveinal chlorosis starting at the tip of younger leaves. Pale grayish green interveinal chlorosis spreads from the tip of the leaf to the leaf base. Necrotic brown sports develop later, and the leaf becomes dark brown. Newly emerging leaves are short, narrow, and light green. At tillering, deficient plants are shorter, have fewer leaves, weigh less, and have a smaller root system than plants supplied with sufficient Mn. Plants are stunted but tillering is not affected. Affected plants are more susceptible to brown spot (caused by *Helminthosporium oryzae*). A critical value of 40 mg Mn/Kg for the rice Y-leaf is suggested during both the tillering and panicle differentiation growth stages and 20 mg Mn/kg for the rice shoot is suggested the tillering stages (Bell and Kovar, 2000).

Uptake efficiency of metals in graminaceous plants, is based on the release of phytosiderophores in the rhizospere and specific uptake systems on the root surface. Phytosiderophore from chelates not only with Fe but also with Mn, Zn and Cu and therefore able to mobilize other micronutrients in case of deficiency (Treeby *et al*., 1989; Takagi *et al*., 1984). Phytosiderophores such as mugineic acid from complex with Mn (II), Zn (II), Cu (II) and highly stable complexes with Fe (III). As a second component of grasses, a highly specific constitutive transport system is present in the

plasma membrane of root cells of grasses (Römheld and Marschner, 1990) which transfers the Fe (III), Mn (II), Zn (II) and Cu (II) phytosiderophores into the cytoplasm (Figure 1.2). Although phytosiderophores form complexes also with Mn, Zn and Cu, the translocator in the plasma membrane has only a low affinity to the corresponding complexes (Marschner *et al*., 1989; Ma *et al*., 1993).

Beside, the mechanism of plant is respond to unavailable (Mn^{4+}) for plant uptake. Uren (1981) clearly established that substance originating from sunflower (*Helianthus annuus* L.) roots could reduce insoluble Mn (IV) oxides. Linehan *et al*., (1985) reported a fifteen-fold increase in concentration of Mn in soil solution during early stages of barley development. Plants are able to increase the availability of nutrients by exuding various organic substances into the rhizosphere. Exuded compounds can alter micronutrient availability both directly, by influencing the solubility and equilibria of nutrient chemical forms, and indirectly, by altering the composition and prevalence of rhizosphere microflora populations capable of nutrient chemical transformations (Darrash, 1993; Uren, 1998; Uren and Reisenauer, 1988). The ability to exude such substances is a key strategy in an ability of plants to tolerate the Mn-fixing conditions in alkaline substrates (Marschner, 1995).

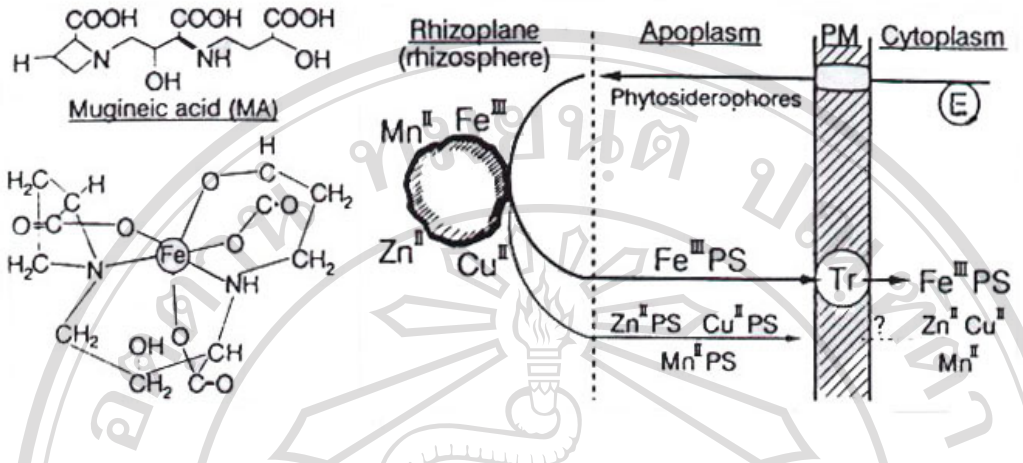


Figure 1.2 Model for root responses to iron deficiency in graminaceous species; Strategy II: (E) enhanced synthesis and release of phytosiderophores; (TR) translocator for Fe (III) phytosiderophores in the plasma membrane: structure of the phytosiderophore muginic acid and its corresponding Fe (III) chelate. (Marschner, 1995)

1.4 Nutrient efficiency

Definition of nutrient efficiency by Graham (1984) 'The nutrient efficiency of genotype (for each element separately) as the ability to produce a high yield in a soil that is limiting in that element for a standard genotype'. Similarly, Mn efficiency has been defined as a genotype's ability to produce high yield in a soil whose Mn content is limiting for a standard genotype (Ascher-Ellis *et al*., 2001). According, Mn efficient genotypes absorb more Mn from soils and thus can survive and yield better in a soil with low Mn availability than do Mn inefficient genotypes (Bansal *et al*., 1991; Graham, 1988; Huang and Graham, 1997). Such as Mn efficient barley

genotype took up more Mn independent of Mn supply levels both in solution (Huang *et al*., 1994) and in the soil (Huang and Graham, 1997), suggesting that efficient Mn uptake in barley is a constitutive system.

Moreover nutrient efficiency was differing in genotype. Nutrient efficiency may have a higher rate of uptake and translocation nutrient. For example, uptake efficiency has higher nutrient uptake per dry weight or root length and translocation efficiency of mineral nutrient may show high rates of partitioning from root to shoot, seed and storage organs. Beside, utilization efficiency was produce high dry weight per nutrient unit (Marschner, 1995).

Gerloff (1977) classified plants into 4 responses groups as follows:

a) Efficient responders, plants with produce high yields at low levels of nutrition and which respond to nutrient additions.

b) Inefficient responders, plants with low yield at low levels of nutrition which have a high response to added nutrients.

c) Efficient non-responders, plants with high yield at low levels of nutrition but which do not respond to nutrient addition.

d) Inefficient non-responders, plants with low yield at low levels of nutrition and which do not respond to nutrient addition.

1.5 Genotypic variation of manganese deficiency

Plant species and cultivars within a species differ considerably in susceptibility to Mn deficiency when grown on low Mn soils. For example, oat, wheat, soybean or peaches are very susceptible whereas maize and rye are not susceptible (Reuter *et al*., 1988). Great genotypic differences in Mn efficiency in soil

have been widely recognized since Mn deficiency was first identified in the 1920s, and has been reported in wheat, durum wheat (*Triticum turgidium* L. Var. Durum), oat and barley (Bansal *et al*., 1991; Graham, 1988; Huang and Graham, 1997; Kaur *et al*., 1989; Marcar and Graham, 1987a, b; Nyborg, 1970; Saberi *et al*., 1997). In contrast, the critical deficiency contents of Mn in plants are similar, varying between 10 and 20 mg Mn/kg dry weight in fully expanded leaves, regardless of plant species or cultivar or prevailing environmental conditions. Only *Lupinus angustifolius* has a critical deficiency content which is twice as high as that of other plant species (Hannam and Ohki, 1988). In rice plant, a critical value of 20 mg Mn/kg dry weight for whole shoot is suggested 33-56 day after sowing. A critical value of 13 mg Mn/kg dry weight for whole shoot is suggested 37 day after sowing in wheat and in barley has a critical value of 12.4 Mn/kg dry weight for whole shoot is suggested 30 day after sowing (Reuter *et al*., 1997).

Genotypic differences in Mn efficiency have been reported in range of crop species (Graham, 1988). Mn efficiency cultivars are able to reach their yield potential on Mn deficient soil without Mn supplementation (Graham, 1988). Genotypic variation exists within barley for Mn efficiency genotypes base on grain yields (Hebbern *et al*., 2005). Similarly, Pedas *et al*. (2005) observed differences in high-affinity influx resulted in a higher Mn net uptake of Vanessa (Mn-efficient genotype) compared to Antonia (Mn-inefficiency genotype). In wheat, Pearson and Rengel (1997) found that the Mn-efficient genotype C8MM had a greater shoot fresh weight than the Mn efficient genotype Bayonet under sufficient or deficient Mn supply. In the case of Mn deficiency, carbohydrate production was limited, but partitioning between roots and shoots was not altered. Beside, in Lucerne, Salado (a genotype

tolerant to Mn deficiency) had increase in exudation of all carboxylate than Sirosal (an intolerant genotype) at Mn deficiency condition (Gherardi and Rengel, 2004). Release of various compounds in to the rhizosphere by plant roots may also be a mechanism by which certain species and genotypes are able to tolerate conditions of low Mn availability better than other. There is as yet no report on genotypic variation in Mn efficiency in rice.

1.6 Genetics of Mn efficiency

However, until recently, a little was known about the genetics and molecular backgrounds for Mn efficiency in higher plants. As it is well known that rye is outstandingly Mn efficient in comparison with wheat. The study with rye addition lines, showed that efficiency is carried on the 2R chromosome (Graham, 1988). Recent experiments with barley suggest the involvement of simple mechanisms of Mn efficiency controlled by single, major, dominant gene (Longnecker *et al*., 1990; McCarthy *et al*., 1988). In the study of 72 barley genotypes from a world collection, the pedigree relationships within the most efficient group and within the inefficient group were consistent with single, major-gene inheritance. In the study of the progeny from cross between Mn efficient and Mn inefficient barley genotypes, the distribution of F_2 individual followed a 3:1 ratio characteristic of a single dominant gene for Mn efficiency in barley, with a narrow-sense heritability of 71% (Rengel *et al*., 1994).

Genotypic variation and the mechanism in Mn efficiency have been found in many plant species but in rice is yet to be fully elucidated. So, finding rice genotypes that can yield well in Mn deficiency conditions and understanding the mechanism of

Mn efficiency will facilitate attempts to decrease yield loss from Mn deficiency in rice genotype. Moreover, a wide range of germplasm with wide differences in Mn efficiency is already available which could provide great possibility to improve genetically modern cultivars in Mn efficiency. The objectives of this study are as follows: a) to screen the resistance of manganese deficiency in rice genotypes. b) to examine the response of rice genotypes in manganese deficiency. c) to examine the physiological acclimation of rice roots to manganese deficiency in nutrient solution culture and its implications for manganese acquisition efficiency of roots. d) to evaluate the machanism of uptake, transport and utilization by Mn efficiency and inefficiency in rice genotypes.