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THESIS

IRON CONTENT IN PADDY FIELDS AND CORRELATION OF PHYTIC ACID WITH IRON IN RICE GRAINS: IMPLICATION FOR HUMAN HEALTH IN KHAO YOI DISTRICT, PHETCHABURI PROVINCE

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Iron deficiency is a major nutritional constraint for people in developing countries throughout the world. Rice is the most important staple food for Asia where people usually receive 50% of dietary Fe from rice. In Thailand, it has been found that rice has the lowest Fe concentration comparing to other cereals. Subsequently, anemia disease increasing in people is diagnosed. This study aimed to investigate influence of regional geography on the concentration of Fe in rice plants and human population. An initial field survey was carried out in Khao Yoi District, Phetchaburi Province. Soil samples, rice samples and human hairs of populations living in the Khao Yoi area were collected. The survey indicated that 71% of sampling populations from Khao Yoi area tended to be Fe deficiency. Rice cultivars of Chainat 1 and Suphanburi 1 were the most planted in this area. Both cultivars were low in Fe contents and Fe mole to phytic acid ratio as 1:1.8 and 1:2.4 of polished rice of Chainat 1 and Suphanburi 1, respectively. From the data mentioned above, they showed the nutrition status which presented the relationship between soil, plant and human. It was suggested that the biofortification was suitable for selecting rice cultivar which contributed higher Fe in rice grains.

Therefore, five rice cultivars consisting of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3, were selected to investigate appropriate Fe level by cultivating in hydroponics culture experiment for 28 days at Kasetsart University Greenhouse. The experimental design was on 5x4 in completely randomized design (CRD) with three replicates. The result showed that the appropriate Fe level of Chainat 1 and Pathumthani 1 was Fe-EDTA supply at 100 μmol L^{-1} whereas Fe- EDTA supply in the range of more than 50 μmol L^{-1} to less than 100 μmol L^{-1} was appropriate Fe level for RD 23, Suphanburi 1 and Suphanburi 3. Subsequently, selecting the suitable rate of Fe concentration and rice cultivars, the rice cultivation in hydroponics culture experiment was conducted at 45 days. The experimental design was on 5x5 factorials in completely randomized design (CRD) with three replicates. The Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 cultivars were cultivated in the Fe-EDTA solution at 5 different levels (50, 75, 100, 125 and 150 μmol L^{-1}). The result showed that the Chainat 1 cultivar had the greatest Fe content compared to other rice cultivars when Fe-EDTA was applied at 100 μmol L^{-1}. Furthermore, the Fe content in Chainat 1 was significantly increased by increasing Fe application rate compared to other rice cultivars.

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Nisanath Churthong

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LIST OF ABBREVIATIONS

Al	=	aluminium
°C	=	degree Celsius
Ca	=	calcium
Cd	=	cadmium
Cm	=	centimeter
Co	=	cobalt
Cr	=	chromium
Cu	=	copper
Fe	=	iron
Fe-EDTA	=	ethylnediaminetetraacetic acid iron (III) – sodium salt hydrate
g	=	gram
g dL^{-1}	=	gram per deciliter
IDA	=	Iron Deficiency Anemia
kcal	=	kilocalories
km	=	kilometer
m^2	=	square meter
Mn	=	manganese
Mo	=	molybdenum
Mg	=	magnesium
mg	=	milligram
mg g^{-1}	=	milligram per gram
mg / 100g	=	milligram per 100 gram
ml	=	millilitre
mm	=	millimeter
mM	=	millimolar
mmol L^{-1}	=	millimole per liter
Na	=	sodium
Ni	=	nickel
P	=	phosphorus
Pb	=	lead

LIST OF ABBREVIATIONS (Continued)

Se	=	selenium
Si	=	silicon
Zn	=	zinc
µg	=	microgram
µg L^{-1}	=	microgram per liter
µg day^{-1}	=	microgram per day
µM	=	micromolar
µmol L^{-1}	=	micromole per liter

IRON CONTENT IN PADDY FIELDS AND CORRELATION OF PHYTIC ACID WITH IRON IN RICE GRAINS: IMPLICATION FOR HUMAN HEALTH IN KHAO YOI DISTRICT, PHETCHABURI PROVINCE

INTRODUCTION

Rice is the most important staple food crop for most people in Asia as well as in Thailand. Rice is also the major energy source of carbohydrate and even protein (Juliano, 1995; Gregorio *et al*., 1999). Unfortunately, rice has the reputation for lack of many life - supporting nutrients especially the essential micronutrients which is combined with poor bioavailability of some micronutrients. Iron and manganese are important essential micronutrients for plants and human. Normally, the iron deficiency in plants is manifested as leaf chlorosis which could lead to yield loss or complete crop failure. Deficiency of these micronutrients in human body will result in a series of severe adverse consequences, such as anemia, low immunity function, skin disease, children response slowness and intelligence stunt, etc (Smuts *et al*., 2007). Thus, people who cannot afford a varied diet suffer from multiple micronutrient deficiencies and a rice-based diet becomes the primary cause of micronutrient malnutrition throughout much of the developing world. Currently, one third of world population are facing malnutrition problem especially the iron deficiency which is the most prevalent nutritional deficiency in the world affecting an estimated 3.5 billion people (Haas *et al.,* 2005).

Mostly, rice has the lowest iron concentration in grain among the cereals. Previous studies have shown that grain iron content varies widely among rice genotypes. Most of the commonly eaten rice genotypes in Asia contain only about 10 mg Fe kg^{-1} in brown rice, but some genotypes contain about 15 mg Fe kg^{-1} or more have been found (Prom-u-thai *et al.,* 2003). Moreover, rice seeds are characterized by a very low content of iron (between 0.2 mg and 2.8 mg/100 g rice) and very low bioavailability (Lucca *et al.,* 2002). Interactions between mineral elements in soil are one of the most important factors affecting to the nutrient balance of plant. There is

certain competition between some nutrients, whereas an excess of a nutrient can affect to the availability and uptake by plants and distribution in plant tissue. Therefore, the plant growth may be affected negatively (Bergman, 1992; Marschner, 1995). Fe deficiency is possible in soils with high concentrations of Cu, Mn and Zn and in soils with low organic matter. Excess concentrations of Cu, Mn and Zn in soil solution cause Fe deficiency. Similarly, high levels of Fe in soil can reduce plant uptake of Cu, Mn and Zn (Erdal *et al.,* 2004). Accordingly, the problem about micronutrient deficiency can be solved economically and sustainable by increasing microelements contents in rice grain and improving their bioavailability to increase iron intake and reduce the incidence of Fe deficiency anemia. Several researches are investigated in the approaches to improve and solve this problem such as medication, food fortification and supplementation but they play only a temporal role to malnutrition and use the high costs. However, several studies have shown that the micronutrient biofortification of staple food crop is an effective to solve this problem (Hao *et al.,* 2007).

The purpose of this study is to enhance iron content and bioavailability in rice seeds in order to increase the absorption and utilization of iron within the human body and also improve the iron deficiencies. Therefore, five rice cultivars which have been cultivated and consumed in Thailand are selected to investigate the variations of iron content in different parts of rice under five iron application levels, aiming to develop and enhance the iron content of rice cultivars as well as determining iron efficiency of selected rice cultivars.

OBJECTIVES

1. Determining the concentration of Fe in hair of selected popultion living in Khao Yoi District, Phetchaburi Province

2. Determining nutrition status in paddy soils and rice plants at Khao Yoi District, Phetchaburi Province

3. Selecting rice cultivar with high Fe availability and low phytic acid

4. Investigating Fe level in hydroponic for rice growth

5. Selecting the appropriate rice cultivar in high Fe uptake

LITERATURE REVIEW

1. Rice

1.1 Importance of Rice

Rice is a monocarpic annual plant which grows to 1-1.8 m by depending on the variety and soil fertility. The seed is a grain 5 - 12 mm long and 2 - 3 mm thick. The ovule after fertilization develops into the seed with its coats completely fused together with the developing ovary wall or pericarp. The rice grain has the following structures: Pericarp, Seed Coats, Aleurone Layer and Endosperm (Figure 1). It has long, slender leaves 50-100 cm long and 2-2.5 cm broad. The small wind-pollinated flowers are produced in a branch arching to pendulous inflorescence 30 - 50 cm long. Rice (*Oryza sativa*) cultivation areas have increased due to the productivity, reliability, and profitability (Welch and Graham, 1999). Rice is the staple food for half of the world's population especially in East, South and Southeast Asia, and provides 35 - 59 % of the energy consumed by the 3 billion people in Asia. Rice also contributes to 69 % of proteins consumed in South Asia and 51 % in South East Asia (Juliano, 1995; Gregorio *et al*., 1999). Moreover, rice is revered today for its nutritional value and plays an important role in the diets and economics of nations around the world. Rice is a central part of many cultures and some countries even credit rice cultivation with the development of their civilization. It is remarkable that almost every culture has its own way of harvesting, processing and eating rice and these different traditions are, in fact, part of the world's cultural heritage.

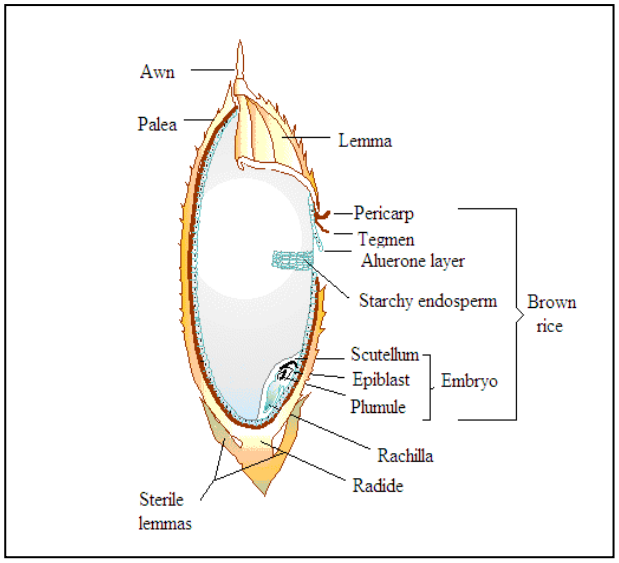

Figure 1 Structure of rice seed

Source: Anonymous (2007)

1.2 Rice Economy in Thailand

Rice plays at least three major roles in the Thai economy: (i) it engages over 50% of the total cultivable area and labour force; (ii) it is the main staple food crop; and (iii) it is one of the major foreign-exchange earning sectors. Rice research has helped the efficient performance of these roles. Changes in the role of rice in the economy in the future will also change the demand for rice research (Evenson *et al.*,1996). Thailand's success in the international rice trade is due to its high quality, long-grain white rice, which has a substantial price advantage over lower grades. This emphasis on grain quality is also the main reason for Thai farmers' limited adoption of modern, high-yielding rice cultivars (Office of Agricultural Economics, 2007). At the end of rice production year 2005-2006, world's rice production is about 415.5 million tons, rice consumption is about 413.2 million tons and the trading of world's rice is 27.6 million tons. It is assumed that the countries where report a large number of rice are Thailand (7.4 million tons, 27%), India (5.0 million tons, 18%) and Pakistan (3.0 million tons, 11%).

1.3 Characteristics of Rice Cultivars

1.3.1 RD 23 cultivar

RD 23 is a rice cultivar derived from cross fertilization between RD 7, IR 32, and RD 1. Photoperiod insensitivity is very specific characteristic for this variety. It can be grown throughout all the regions of irrigated rice fields. For general performance, this cultivar has fair tillering with 115-120 cm plant height and needs approximately 125 days to complete maturity. High yield productivity leads to about 800 kilogram grain yield per rai in average. Additionally, this cultivar is resistant to some major diseases such as Bacterial Leaf Blight and Ragged Stunt Virus. For grain quality, this variety has straw color hull, slight chalkiness, and 25-30% amylose content. Suphanburi Rice Experiment Station, therefore, submitted SPRLR76002-168-1-4 line to the Research and Development Committee, Department of Agriculture to be a newly recommended cultivar, named "RD 23" on June 17, 1981 (Rice Department, 2006).

1.3.2 Pathumthani 1 cultivar

Pathumthani 1 is a rice cultivar derived from cross fertilization between BKNA6-18-3-2 and PTT85061-86-3-2-1. Photoperiod insensitivity is very specific characteristic for this cultivar. It can be grown throughout at Central Region of irrigated rice fields. For general performance, this variety has fair tillering with 104-133 cm plant height and needs approximately 104 - 126 days to complete maturity. High yield productivity leads to about 650 - 774 kilogram grain yield per rai in average. This cultivar is resistant to some major insect pests such as Brown Planthopper and diseases such as Bacterial Leaf Blight. For grain quality, it has straw color hull, good milling quality, and 15 - 19% amylose content. Pathumthani Rice Research Center, therefore, submitted PTT90071-93-8-1-1 line to the Research and Development Committee, Department of Agriculture to be a newly recommended cultivar, named "Pathumthani 1" on May 30, 2000 (Rice Department, 2006).

1.3.3 Chainat 1 cultivar

Chainat 1 is a rice cultivar is derived from cross fertilization between IR13146-158-1, IR15314-43-2-3-3 and BKN6995-16-1-1-2. Photoperiod insensitivity is very specific characteristic. It can be grown throughout all the regions of irrigated rice fields. This cultivar has fair tillering with 104 - 133 cm plant height and needs approximately 113 days to complete maturity. High yield productivity leads to about 740 kilogram grain yield per rai in average. This variety is resistant to some major insect pests such as Brown Planthopper and diseases such as Ragged Stunt Virus. It has straw color hull, slight chalkiness and 26 - 27% amylose content. Chainat Rice Experiment Station, therefore, submitted CNTBR82075-43-2-1 line to the Research and Development Committee, Department of Agriculture to be a newly recommended cultivar, named “Chainat 1” on September 9, 1993 (Rice Department, 2006).

1.3.4 Suphanburi 1 cultivar

Suphanburi 1 is a rice cultivar derived from cross fertilization between IR25393-57-2-3, RD 23, IR27316-96-3-2-2, SPRLR77205-3-2-1-1 and SPRLR79134-51-2-2. Photoperiod insensitivity is very specific characteristic. It can be grown throughout all the regions of irrigated rice fields. It has fair tillering with 125 cm plant height and needs approximately 120 days to complete maturity. High yield productivity leads to about 806 kilogram grain yield per rai in average. This cultivar is resistant to some major insect pests such as Brown Planthopper and diseases such as Ragged Stunt Virus. It has straw color hull, 29% amylose content, fertilization response, and high productivity. Suphanburi Rice Experiment Station, therefore, submitted SPRLR85163-5-1-1-2 line to the Research and Development Committee, Department of Agriculture to be a newly recommended variety, named “Suphanburi 1” on October 28, 1994 (Rice Department, 2006).

2. Iron (Fe)

Iron (Fe) is the fourth abundant element in lithosphere and constitutes about 5% of the Earth's crust. It is among the very first elements identified as essential nutrient for plants. Solubility of iron in soil depends on pH and greatly decreases with increasing in pH (Osotsapar, 2003). Fe is an essential micronutrient for all living organisms on earth. It plays several important roles in different metabolic processes of organisms. It is a component of the proteins hemoglobin in red blood cells and myoglobin in muscle cells. Both the hemoglobin and the myoglobin molecules contain iron as a carrier of oxygen. Among the enzymes, the iron is vital to the processes by which cell generate energy. Fe is also needed to make new cells, amino acids, hormones and neurotransmitters.

Human needs the iron for the synthesis of the oxygen transport proteins haemoglobin and myglobin, including the formation of haem enzymes and other Fe-containing enzymes. That is important for energy production, immune defense, and thyroid functions. It is critical for meristematic tissues due to its composition of protein such as Fe-S proteins, ferredoxins and heme protein. The inadequate Fe absorption will lead to lower haemoglobin levels or anaemia disease. The Fe deficiency anaemia affects about one billion people worldwide and is most prevalent in infants, children, and women of childbearing age in developing countries. Fe deficiency anaemia can decrease mental and psychomotor development in children (Lucca *et al.,* 2002). It also decrease work performance and decrease infection resistance.

2.1 Fe distribution in the soils

Iron solubility and the Fe^{2+}/Fe^{3+} concentration is strongly dependent on the oxidation-reduction conditions of the soils. There are significant spatial variations in redox potential and thus, Fe^{2+} concentration in paddy soils (Howler and Bouldin, 1971). The horizontal changes of Eh and reduced Fe in the profile of paddy soils has been described by Ratering and Schnell (2000). Liesack *et al*. (2000) defined three regions differing in redox potential in paddy soils that were included a thin oxic surface layer, the reduced puddle bulk soil and the oxic rhizosphere and rhizoplane. The extent of the oxic surface layer varies between 2 and 10 mm and is partially determined by a nitrate-

dependent microbial re-oxidation of Fe^{2+}. The highest Fe^{2+} concentrations are found at 2-15 cm soil depth. In subsurface layers with less organic matter, Fe^{2+} concentrations can be lower. In rhizosphere, oxygen is released from rice roots and caused a decrease in Fe^{2+} (Yamanouchi *et al*., 1989). This is determined by the formation of aerenchyma (oxidation power of the rice root) and the root density (Frenzel and Bosse, 1999). The rhizosphere of rice is an effective site in which Fe^{2+} oxidation occurs; although Fe reduction can also happen in this part. Facultative anaerobic chemo-organotrophic microorganisms (e.g.,*Geobacter* sp., *Pseudomonas* sp., *Clostridium* sp., or *Bacillus* sp.) play a role in the reduction and mobilization of Fe-oxides (Munch and Ottow, 1977). Some fungi are also hypothesized to be capable of reducing and mobilizing Fe-oxides (Munch and Ottow, 1977; Trolldenier, 1988). Oxidized Fe used as an alternative electron acceptor for respiration requires energy-rich electron donors (e.g., easily mineralizable organic root exudates). Then, the abundance of iron-reducing microorganisms is higher in the rhizosphere than in the bulk soil (Benckiser *et al*., 1983; Wang and Liu, 1992). Nevertheless, processes of iron re-oxidation dominate over the Fe reduction in the rhizosphere of most Indica-type rice, which leads to a considerable Fe^{3+} accumulation and as a result, the formation of iron plague around rice roots (Kirk *et al,* 1990).

2.2 Fe in the plant

Rice plants tend to absorb more iron than most other plant species. Moreover, the ferrous ion is quite abundant, and even very much abundant in paddy soils. Since the reduced iron is easily absorbed, iron oxide (Fe^{3+}) uptake mechanisms are probably less significant in flooded environments (Mengel, 1988). After absorption in the root cortex, the reduced iron (Fe^{2+}) can reach the xylem by symplastic passage through the Casparian band. The greater portion of the absorbed ferrous ions can reach the xylem directly through apoplastic pathway. It can be evidenced that damage to rice roots as a result of pulling up and transplanting the rice seedling. In the xylem, ferrous ion transport follows the acropetal transpiration flow. The Fe absorption and transportation are not the same for cultivated plants under aerobic (rainfed rice) or anaerobic conditions. In those cases iron transport is dominated by the ferric ion (Fe^{3+}) complexed by citrate or carbon hydrate peptidic components (Clark *et al*., 1973; Schmidt, 1999). When it

reaches the apoplastic zone of the leaf, the iron gets back into the symplasm. The exact mechanism which the iron is absorbed by foliar cells is not yet fully known. Rice roots absorb Fe as reduced form (Fe^{2+}) because it is the physiologically active form of iron in the cell. The excessive quantities of iron in the cell can catalyze the generation of active oxygen species such as superoxide, hydroxyl-radical and H_2O_2 (Marschner, 1995). Toxic concentration of Fe alone or in combination with fatty acids can be related to formation of highly reactive perferryl-radicals (Halliwell and Gutteridge, 1984).

Free radicals are responsible for the damage caused by iron toxicity (Thongbai and Goodman, 2000). They irreversibly damage membrane lipids (Thompson and Legge, 1987), proteins (Miyao *et al*., 1995) and nucleic acids (Elstner, 1982) and affect the membrane charge (Vladimirov, 1980). Iron toxicity increases activity of phenol oxidases and as a result, oxidized polyphenols can accumulate (Yamauchi and Peng, 1993). On the contrary, the free radicals can oxidize the chlorophyll and subsequently leads to a decrease in chlorophyll content (Monteiro and Winterbourn, 1988).

2.3 Fe in human

Iron is required as a trace element by terrestrial living organisms. It is the most abundant metal in human with healthy adults processing some 3-4 g. Most of this occurs in the oxygen, which carries pigment hemoglobin found in red blood cells and myoglobin in muscle cells. Iron is essential to all cells. Functions of iron include involvement in energy metabolism, gene regulation, cell growth and differentiation, oxygen binding and transport, muscle oxygen use and storage, enzyme reactions, neurotransmitter synthesis and protein synthesis. The special provisions the body makes for iron's handling indicated that it is body's gold, a precious mineral to be tightly hoarded.

Normally, only about 10 to 30 % of dietary iron is adsorbed; but the body's supply is diminished or the need increases for any reason, absorption increases. The body makes several provisions for absorbing iron. A special protein in the intestinal cells captures iron and holds it in reserve for release into the body as needed. The blood

protein (transferrin) carries the iron to tissues throughout the body. When more iron is needed, higher levels of these special proteins are produced so that more than the usual amount of iron can be absorbed and carried. If there is a surplus of iron, special storage proteins in the liver, bone marrow and other organs will store it (Plumepunya and Kamperayos, n.d.).

3. Effect of Iron Deficiency

In fact, the oxygen that is breathed into our body will not be just stopped at the lungs and pumped through the arterial blood system to nourish the brain, organs and tissues that allow the body to function. Oxygen travels to these organs through the bloodstream especially in the red blood cells transporter of oxygen. Blood is actually a liquid made up of several different cell types and one of the most important and most numerous cell types is the red blood cells. Usually, the blood cell, especially red blood cells that disperses within the blood, gives the red color to blood and has an important substance contained within called Hemoglobin.

Hemoglobin is a substance that consists of a central iron atom called “heme”, hooked to a clump of protein called “globin”. It is the most efficient oxygen carrier which has the unique property of combining reversibly with oxygen and is also the medium which oxygen is transported throughout the body. It takes up oxygen as blood passes through the lungs and releases it as blood passes through the tissues. Therefore, to form enough hemoglobin, the body needs to have plenty of iron. The average human contains about 4 g of iron, a lot of which circulates as hemoglobin. If the diet does not contain the 6 mg of iron needed each day, anemia will be eventually developed.

3.1 Anemia

Anemia or anaemia from the Greek meaning "without blood", is a deficiency of red blood cells (RBCs) and/or hemoglobin. This results in a reduced ability of blood to transfer oxygen to the tissues, causing tissue hypoxia. Since all human cells depend on oxygen for survival, so the varying degrees of anemia can have a wide range of clinical consequences. Hemoglobin (the oxygen-carrying protein in the

red blood cells) has to be presented to ensure adequate oxygenation of all body tissues and organs.

Anemia, one of the more common blood disorders, occurs when the level of healthy red blood cells (RBCs) in the body is lower than normal level. Such a condition is caused by a deficient number of erythrocytes (red blood cells), an abnormally low level of hemoglobin in the individual cells, or both these conditions simultaneously. This can lead to health problems because RBCs contain hemoglobin**,** which carries oxygen to the body's tissues. Anemia can cause a variety of complications, including fatigue and stress on bodily organs.

3.2 Types of Anemia

3.2.1 Iron deficiency anemia (IDA)

Iron deficiency anemia occurs when the body has insufficient iron to synthesize hemoglobin for carrying oxygen to the whole body. Iron deficiency can happen when the body loses blood from many problems like heavy periods, ulcers, colon polyps, or colon cancer. The diets that do not have enough iron in them can also cause IDA. Moreover, pregnancy can cause IDA if there is not enough iron for the mother and fetus.

3.2.2 Megaloblastic (or vitamin deficiency) anemia

This anemia often occurs when the body has not sufficient in folic acid or vitamin B-12. These vitamins help the body keep healthy blood and a healthy nervous system. With this type of anemia, the red blood cells cannot deliver oxygen to the organs throughout the body.

3.2.3 Underlying diseases

Certain diseases cause the body in lacking of ability to produce red blood cells. For example, people with kidney disease, especially those getting dialysis

are at higher risk for developing anemia. Consequently, the kidney cannot produce enough hormones to form blood cells while the iron is lost in dialysis.

3.2.4 Inherited blood disease

This type of anemia is a higher risk when the family has a blood disease. One type of inherited blood disease is sickle cell anemia. Instead of having normal red blood cells that move through blood vessels easily, sickle cells are hard and have a curved edge. These cells cannot squeeze through small blood vessels and also block the organs from getting blood. Although the body destroys sickle red cells quickly, but it cannot make new red blood cells fast enough; therefore, the anemia is developed. Another inherited blood disease is thalassemia. It happens when the body is missing certain genes or when variant (different from normal) genes are passed down from parents that affect to the formation of hemoglobin of the body.

3.2.5 Aplastic anemia

This rare type of anemia occurs when the body cannot be formed sufficient red blood cells. Since this problem affects to the white blood cells and platelets, so there is a higher risk for infections and bleeding that cannot be stopped.

From the above mentioned types of anemia, iron deficiency anemia is the most common type of anemia and can be found around the world especially the developing countries. Dietary iron in developing countries consists primarily of non-heme iron, whose poor absorption is considered as a major factor in the etiology of iron deficiency anemia. Iron deficiency is the first step towards anemia when the body's storage of iron are reduced. If the body's iron storage cannot be replenished at this point, continuing iron deficiency can cause the body's normal hemoglobin production to slow down. When hemoglobin levels and red blood cell production drop below normal, then a person is determined to have anemia.

3.3 Iron Deficiency Anemia

Iron-deficiency anemia is a common and easily treatable condition that occurs when the body does not have enough iron to synthesize hemoglobin and healthy red blood cells, which means that there is not enough hemoglobin to carry oxygen throughout the body. A lack of iron in the body can occur from bleeding, not eating a proper diet that contain enough iron, or poor absorbing on iron in the digestive system from poor food diets. Normally, the body obtains the iron from the diets. The iron in diets composed of two forms which are ferrous (heme iron) and ferric (non-heme iron). The ferrous or heme iron has been found in red meats, eggs, liver, etc, while the ferric or non-heme iron that combines with protein has been found in vegetables, cereals, grains and beans (Plumepunya and Khampeerayos, n.d.). The most diets which are composed of iron are staple foods for the world's population, so iron deficiency is determined to be one of the most important deficiencies relating to malnutrition.

3.3.1 Risk groups for iron-deficiency anemia

The major risk factors for iron-deficiency anemia are blood loss and the diets which are low in iron. Three of the highest risk groups are women, young children, and adults with intestinal bleeding.

Women, who lose a lot of blood during monthly periods, are at higher risk of developing iron-deficiency anemia which 1 in 5 women of childbearing age has iron-deficiency anemia. Pregnant women need iron two times more for their diets than women who are not pregnant. If a pregnant woman has not get enough iron for herself and the fetus, she can develop iron deficiency anemia will be occurred which half of all pregnant women have this type of anemia.

Infants and toddlers at 6 - 24 months of age need a lot more of iron for growing and development of the body. The iron that full-term infants have been stored in the bodies is used up in the first 4 - 6 months of age. After that, infants need to get iron from foods or supplements. Premature and low-birth-weight babies are even at greater risk for iron deficiency anemia because they do not have enough iron storage in

bodies. The other groups of children at risk for anemia are children with poor nutrition, children with low-income family, children with lead in the blood, infants who are fed with cow's milk before 1 year of age, and breastfed infants who are older than 4 months and are not receiving iron-rich solid foods or iron supplements.

Persons who have bleeding in their intestinal tract are at risk for iron-deficiency anemia. This group includes people who have bleeding ulcers or colon cancer as well as people who use medicines that can cause intestinal bleeding. Other adults who are at risk for iron-deficiency anemia include those who are on kidney dialysis, vegetarians (with improper diets) and older adults who have poor diets.

3.3.2 Signs and symptoms of iron-deficiency anemia

Anemia must take time to develop within the body. At the beginning, the body may not have any signs or only mild symptoms. However, when it gets progress, the following symptoms are shown as: fatigue, weakness, reduced ability and concentration on any work, low body temperature, pale skin, fast heartbeat, shortness of breath, chest pain, dizziness, irritability, numbness or coldness on hands and feet, and headache.

3.3.3 Effects of iron-deficiency anemia on the body

Iron-deficiency anemia can range from slight mild to severe. A slight mild case usually causes no symptoms or problems. However, a severe case can cause extreme fatigue (tiredness) and weakness. Severe iron-deficiency anemia can lead to serious problems for young children and pregnant women that it can affect to the heart. In young children, iron-deficiency anemia can cause a heart murmur and delays in growth and development. It puts the children at greater risk for lead poisoning and infections and it can cause behavior problems. In pregnant women, iron-deficiency anemia can increase the risk of a premature delivery and a low-birth-weight baby. The problem with heart is affected when there is a lack of oxygen in the body. The heart has to work harder to get enough oxygen throughout the body. Over time, this stress on the

heart can lead to a fast or irregular heartbeat, chest pain, an enlarged heart and even heart failure.

3.3.4 Diagnosis

Anemia is diagnosed by using a person's medical history, physical exam, and tests which these methods are used for determining the cause, severity and treatment for the particular type of anemia. Mild to moderate anemia may have no symptoms or very mild symptoms. In fact, anemia is often discovered unexpectedly on blood tests looking for other conditions.

Diagnosis tests and procedures

Usually, the first test used to diagnose anemia is a complete blood count (CBC). The CBC tells a number of things about a person's blood, including:

a. Hemoglobin level: Hemoglobin is the iron-rich protein in red blood cells (RBCs) that carries oxygen through the body. The normal range of hemoglobin levels for the general population is 11-15 g dL^{-1}. A low hemoglobin level means a person has anemia.

b. Hematocrit level: Hematocrit level measures how much of the blood is made up of RBCs. The normal range for hematocrit levels for the general population is 32–43 %. A low hematocrit level is another sign of anemia.

c. Blood test: the blood is tested by measuring the level of protein ferritin, because ferritin helps the body store iron. A low level of ferritin in the blood indicates a low level of iron.

3.3.5 Treatment

The goal of anemia treatment is to increase the oxygen-carrying capacity of the blood. This can be made by increasing the red blood cell (RBC) count

and/or hemoglobin level in the RBCs as close as possible to normal levels. An additional goal is to treat the underlying condition or cause of the anemia. The treatment will depend on the type, cause, and severity of the anemia of each person. Treatment may include dietary supplements, changing in diets, medicines and/or medical procedures such as blood transfusions or surgery.

3.3.6 Prevention of iron-deficiency anemia (IDA)

As mentioned above, it can be determined that the major cause of iron deficiency anemia occurs when the body does not get enough iron to form hemoglobin due to poor nutrition. Therefore, to get adequate amount of iron, some factors can help to prevent some types of anemia. The diets containing high iron can increase the iron amount in the body such as; red meat, fish, chicken, liver, egg, dried fruits (like apricots, prunes, and raisins), lentils and beans and green and leafy vegetables (like spinach, broccoli, and cereal). Moreover, eating and drinking is able to help the body to absorb iron, like orange juice, strawberries, broccoli, other fruits juices and vegetables with high vitamin C. Drinking coffee or tea should be prohibited with meal because cause he difficulty of iron absorbing. High calcium food can also block the process of iron absorption in the body.

3.4 Iron deficiency anemia (IDA) in Thailand

Thailand has addressed nutrition in its national development policy since the mid-1970s and IDA was included in the national goal. Nutritional improvement in Thailand has been implemented as an integral part of primary health care system and community development with the aim of improving food and nutrition in the household. This provides an important infrastructure that has extended beyond government services to include community participation.

3.4.1 Anemia trends in the Thai population

The prevalence of anemia in pregnant women in Thailand has declined substantially between the 1980s and 1990s, based on 3rd and 4th Thailand National

Nutrition Surveys. The prevalence of anemia among lactating women, however, has remained unchanged during the same period. Despite limited data for children less than 5 years of age, comparison of the two national surveys shows a reduction in the prevalence of anemia from 41 to 25%. Recent evidence of a possible high prevalence of anemia in infancy is of concern. Based on World Health Organization (WHO) cut-off value for hemoglobin (<11 g dL^{-1}), the prevalence of anemia in young infants (4-6 month of age) in small survey areas was found to be as high as 32 and 62%, despite fairly proper growth (Winichagoon, 1991). In addition to national survey data, the prevalence of anemia among pregnant women and school-age children has been reported through the routine health information systems since 1988. These reports also indicate declining trends in anemia prevalence in both groups. A small increase was observed in the prevalence among pregnant women around 1998, which coincided with Thailand's economic crisis.

3.4.2 Policy on prevention and control of anemia in Thailand

Based on Thailand's National Food and Nutrition Policy since the 1980s, the first notable goal was to eradicate anemia among pregnant and lactating women. This goal was later modified to include school children. The new goal specified a certain percentage reduction in the prevalence of anemia among both pregnant women and school children. The community-based nutrition program, which has been an integral part of primary health care (PHC), was in charge of implementation of this policy. The specific strategy on IDA was confined almost solely to pregnant women and school children.

4. Manganese (Mn)

4.1 Importance of Manganese

Manganese (Mn) is a naturally occurring substance, which is found in many types of rock, soil, and water. It is ubiquitous in the environment and comprises about 0.1% of the Earth's crust. Crusted rock is a major source of manganese found in the atmosphere (Stokes *et al.,* 1988). Pure manganese is a gray-white silver metallic

element, resemble and rust like iron but not magnetic and harder and more brittle. Manganese does not occur in the environment as the pure metal, rather it is usually combined with other chemicals such as oxygen, sulfur, and chlorine. These compounds are solid that do not evaporate. However, small dust particles of the solid material can become suspended in air. Some manganese compounds can dissolve in water and low level of these compounds are normally present in lake, stream, and the ocean or sea bed. Manganese can change from one compound to another (either by natural processes or by human's activities), but it does not break down or disappear in the environment. Manganese atomic number is 25; atomic weight 54.9; melting point 1,244 °C; boiling point 1,962 °C; specific gravity 7.21 to 7.44.

Due to a natural component in the environment, manganese is always exposed in low level to water, air, soil and food. In drinking water, levels are usually about 0.004 parts manganese per million parts of water (ppm). In the air, levels are usually about 0.02 micrograms manganese per cubic meter of air ($\mu g/m^3$). Levels in the soils usually range from 40 to 900 mg kg^{-1}. Manganese is also a component of plants and animals, so manganese is present in foods. For human, food is the main source of manganese and the usual daily intakes range from 2,000 to 9,000 $\mu g\ day^{-1}$ which the exact amount of intake depends on the diet.

4.2 Manganese in humans

Manganese is an essential micronutrient which has antioxidant, free-radical-fighting properties, that is needed to activate a number of enzymes in digestion system. It is an essential trace mineral that helps the body convert protein and fat to energy. It also promotes normal bone growth, maintains healthy reproductive, nervous, and immune systems, and involves in blood sugar regulation. In addition, manganese involves in blood clotting and cartilage and lubricating fluid in the joints. Manganese is also necessary for normal brain and nerve function. It is linked to decreased manganese superoxide dismutase (MNSOD) activity in white blood cells, which protect the body more vulnerable to the damaging effects of free-radicals.

The large quantities of manganese are found in plants and animals, but very little of this element is found in human tissue. Manganese is predominantly stored in the bones, liver, kidney, and pancreas. It can help the body to reduce fatigue levels, prevent the incidence and severity of osteoporosis, and even improve brain's memory. Moreover, it helps the body to absorb vitamin B1 (thiamin) and vitamin E, and works with all B-complex vitamins to resist depression, anxiety and other nervous disorders, including reduces heavy menstrual flows and improves thyroid function (thyroid function is dependent on a balance of manganese and iodine, and a shortage of either could cause hypothyroidism).

Usually, the recommendation dietary allowance is about 2.5 to 5.0 mg for human age around 11 years and older. Daily intake of a small amount of manganese per day is important to maintain the health. The amount of manganese in a normal diet (about 2,000-9,000 $\mu g\ day^{-1}$) seems to be enough to meet the daily need, and no cases of illness from eating too little manganese have been reported in humans.

However, too much intake of manganese can cause serious illness. Although there are some differences between different kinds of manganese, most manganese compounds seem to cause the same effects. Manganese miners or steel workers long term exposed to high levels of manganese by inhalation may result in central nervous system (CNS) effects. This may have mental and emotional disturbances, and the body movements may become slow and clumsy. The combination of symptoms is a disease called manganism. Usually, the symptoms of manganism have not been developed unless they have been exposed for several months or years. Manganism, occurring from too much intake, can injure some parts of the brain that uses to control the body movements. The manganism symptoms can be reduced by medical treatment, but the brain injury is permanent. Moreover, the low intake of manganese can also cause the health problem. Manganese deficiency has been linked to infertility, bone malformation, weakness, seizures, atherosclerosis, confusion, convulsions, eye problems, hearing problems, heart disorders, high cholesterol levels, hypertension, irritability, memory loss, muscle contractions, pancreatic damage, profuse perspiration, rapid pulse, tooth-grinding, tremors and osteoporosis. In present, however, the amount of manganese that

is absorbed into the body is not yet concerned though its availability seems to be tied in some way to iron absorption.

4.3 Manganese in soil

Manganese is one of the most abundant trace elements in the lithosphere, and its common range in rocks is 350 to 2000 ppm. Its highest concentrations are usually associated with mafic rocks. Manganese forms a number of minerals in which it commonly occurs as the ions Mn^{2+}, Mn^{3+}, or Mn^{4+} and its oxidation stage +2 is most frequent in the rock-forming silicate minerals. The cation Mn^{2+} is known to replace the sites of some divalent cation (Fe^{2+}, Mg^{2+}) in silicates and oxides. The major pool of manganese in soils originates from crusted sources, with other sources including direct atmospheric deposition, wash-off from plant and other surfaces, leaching from plant tissues, and the shedding or excretion of material such as leaves, plant residual and animal excrement. Manganese in soil can migrate as particulate matter to the air or the water, or soluble manganese compounds can be leached from the soil.

During weathering, manganese compounds are oxidized under atmospheric conditions and the released manganese oxides are re-precipitated and readily concentrated in the form of secondary manganese minerals. The behavior of manganese in surface deposits is very complex and is governed by different environmental factors, of which Eh-pH conditions are most important. Moreover, the physical features of manganese oxides and hydroxides, such as small size of crystals and consequently a large surface area, have important geochemical implications. Apparently, this is responsible for the high degree of association of manganese concretions with some heavy metals, in particular with Co, Ni, Cu, Zn and Mo. In addition, the oxidation of As, Cr, V, Se by manganese oxides is likely to control the redox behavior of these elements in soils.

Bartlett (1986) reported that the manganese is likely to occur in soils as oxides and hydroxides compound by coating on other soil particles and forming different diameters of nodules. The nodules often exhibit a concentric layering that is suggestive

of seasonal growth. The manganese concretions in soils are reported to accumulate Fe and several trace elements.

All manganese compounds are very important to soil constituents because this element is an essentially plant nutrition and its control effecting on the behaviors of other several micronutrients. It also has a considerable effect on some properties of soil, and in particular on soil pH. The manganese compounds are known for their rapid oxidation and reduction under variable soil environments. Thus, the oxidizing conditions may greatly reduce the availability of manganese and associated micronutrients, whereas reducing conditions may lead to the availability of these elements even up to the toxic range. The reduction of manganese oxides has dual effects on soil cation exchange. The solubility of manganese in soils depends on the pH and redox potential. Therefore, the most common reactions occurring in soils are oxidation-reduction and hydrolysis. Water-soluble manganese in soils is directly proportional to pH, with oxidation state being another major determinant of manganese solubility. The low oxidation state of Mn (II) predominates in reducing conditions. The result of Mn (II) effects higher concentrations in flooded soils or other reducing situations (Stokes *et al.,* 1988). This is normally reflected in higher manganese bioavailability in flooded soils. There are two main mechanisms involving in the retention of manganese by soil. Firstly, through cation exchange reactions, manganese ions and the charged surface of soil particles form manganese oxides, hydroxides and oxyhydroxides, which, in turn, form adsorption sites for other metals. Secondly, manganese can be adsorbed to other oxides, hydroxides, and oxyhydroxides through ligand exchange reactions (Evans, 1989).

Due to the low solubility of manganese compounds in oxidizing systems at pH levels near neutrality, small shifts in the Eh-pH conditions can be very important in the manganese content of the soil solution. The abundance of soluble species of manganese in the soil solution is reported to range from 25 to 8000 $\mu g\ L^{-1}$. For neutral solutions and acid soils, the manganese range is reported to vary from 1 to 100 $\mu m\ L^{-1}$.

Hodgson *et al.,* (1966) reported that the soluble manganese in soil solutions is mainly involved in organic complexity. In surrounding of plants roots, the reduction of

MnO_2 and complexity of manganese by root exudates are apparently a significant factor controlling the manganese mobility.

The solubility of soil manganese is significant since the manganese supply for plant depends mainly on the soluble manganese pool in the soil. In well-drained soils, the solubility of manganese always increases with the increase of soil acidity. However, the manganese that forms the anionic complexes and complex with organic ligands may contribute to increase manganese solubility in the alkaline pH range. Several extractants have been widely investigated for soil testing analysis. The best correlation with manganese uptake by plants was usually obtained for the water-soluble, the exchangeable, and the reducible fractions of soil manganese. It appears that all extractants are used to extracted the easily reducible manganese (the fraction extractable with hydroquinone), but the effects vary widely. The availability of manganese supply to crop plants is growing concern for some soils. In soils that have been limed for structural reasons, the availability of manganese is limited. On the other hand, the soils with an increased mobility of manganese (e.g., well drained soils at pH levels below 5.5, poorly aerated soils at pH of about 6.0 or higher), manganese toxicity can occur. Highly alkaline soils can cause of producing manganese toxicity.

Manganese is not distributed uniformly in soil substrata, in addition to various nodules, and is known to be also concentrated at certain spots which are usually enriched in several other trace elements. The variation of manganese content in surface soils rarely seems to be correlated with soil typology, but is positively associated with clay contents. However, higher manganese levels are often reported in ultramafic soils, soils rich in Fe and/or organic matter and soils from arid or semiarid regions. Although manganese can be concentrated in various soil horizons, particularly in those enriched in Fe oxides or hydroxides; usually, this element is also accumulated in top soils as result of its fixation by organic matter.

4.4 Manganese in plants

Manganese occurs in plant fluids and extracts mainly as free cationic form. It appears that manganese is likely to be transported as Mn^{2+}, and also forms complex

molecules with 1000 to 5000 mol wt of organic molecules which have been found in phloem exudates. Van Goor (1974) reported that the manganese concentration in phloem exudates is lower than in leaf tissue and concluded that the transportation of manganese in the phloem vessels is responsible for the low manganese concentration, it occurs in fruits, seeds, and storage roots. Manganese is transported to tissues, thus its concentration is mostly observed in young expanding tissue. Heenan and Campbell (1980) found that the manganese supply in leaves is correlated with plants age. Therefore, manganese appeared to have a low mobility when the supply concentration for the plant was limited. The manganese concentration fluctuates greatly within the plant parts and the vegetative period.

Manganese is an essential nutrient for plant growth as a constituent of metalloenzymes that occupy key roles in metabolism (Clarkson and Hanson, 1980; Woolhouse, 1983; Burnell, 1988). Nutritional manganese requirements for terrestrial plants are around 10 - 50 mg kg^{-1} tissue (Hannam and Ohki, 1988; Reisenauer, 1988). Critical nutritional levels vary widely between species and among cultivars of a species (Reisenauer, 1988). Calcareous soils, especially poor drainage and high organic matter, are the manganese-deficiency soil.

The terrestrial plant species vary a great deal in their ability to accumulate the manganese. The total concentration of manganese in soils is generally less important to plants than the availability of manganese, which is determined by pH, cation exchange capacity, other cations concentration, organic content, temperature and microbial activity. Plants take up manganese from soil primarily in the divalent state. Differences in plant uptake can be explained with the differences in the ability of plants to bring about the dissolution of oxidized manganese (Stokes *et al.,* 1988). The application of chelating manganese concentrations of 500 and 1000 mg kg^{-1} agents significantly reduced the manganese uptake in roots, stems and leaves of okra (*Abelmoschus esculentus*).

Concentrations of manganese in terrestrial plants tend to range from 20 to 500 mg kg^{-1}. Members of the Ericaceae family, which includes blueberry, cranberry and

huckleberry are regarded as manganese accumulators. There are numerous reports of foliar manganese levels in excess of 2000 - 4000 mg kg^{-1}.

However, the manganese content of plants is not only an effect of plant characteristics, but also of the pool of available manganese which is highly controlled by soil properties. Generally, the most readily available manganese is in acid and flooded soil. The increase more than a tenfold in the manganese content of lucerne (alfalfa) was observed in plants grown on flooded soil, compared to background values. Therefore, the reducing ability of root exudates and bacteria in the rhizosphere apparently is direct importance in manganese nutrition of plants. Manganese seems to be easily taken up by plants when it occurs in soluble forms in soils, so the manganese content of plants should be a direct function of the soluble manganese pool in soils. Indeed, manganese concentration in plants shows a negative relationship with increasing soil pH and a positive relationship with soil organic matter.

All plants have a specific requirement for manganese and apparently the most important manganese function is related to the oxidation-reduction process. Mn^{2+} is known to be a specific component of two enzymes, arginase and phosphotransferase, but this metal can also substitute for Mg in other enzymes. The mechanism by which Mn^{2+} activates several oxidases is not yet known precisely, but it appears to be related to the valency change between Mn^{3+} and Mn^{2+}.

Adequate levels of available manganese are necessary in plants nutrition. Chloroplasts are the most sensitive of all cell components to manganese deficiency and react by showing structural impairment. The deficiency symptoms occur first in younger leaves as interveinal chlorosis. At further stages, necrotic spots on leaves and browning of roots would appear. Plants deficient in manganese apparently are less frost hardy. The growth of manganese deficient plants is retarded, the turgor is reduced, and the affected leaves break. Crops and plant species differ in their susceptibility to manganese deficiency. There are numerous examples of manganese deficiency, especially among crop plants. Manganese deficiency of peanut (*Arachis hypogaea*) is a common problem on some soils of the coastal plain region of the southern USA. Manganese deficiency occurred at pH levels of 6.8; maintaining soil pH at 6 provided a desirable medium for

plant growth without the need for manganese fertilizer. Critical manganese levels ranged between 12 and 15 mg kg^{-1} in leaves (Parker and Walker, 1986). Application of manganese sulfate (15 mg Mn kg^{-1}) to highly calcareous soils enhanced the growth of soybean (*Glycine max*) plants (Ahangar *et al.,* 1995). Critical limits for a variety of plant species have been calculated: for example, for corn (*Zea mays*), 10.6 mg Mn kg^{-1} in the ear leaf and 4.9 mg Mn kg^{-1} in the grain (Uribe *et al.,* 1988); for oats (*Avena sativa*), 4.5 mg diethylenetriaminepentacetic acid (DTPA)-extractable Mn kg^{-1} soil and 19 mg Mn kg^{-1} dry matter for mature leaf blades; and for cowpea (*Vigna unguiculata*), 2.4 mg DTPA-extractable Mn kg^{-1} soil and 41 mg Mn kg^{-1} dry matter for leaf blades (Bansal and Nayyar, 1998).

Symptoms of manganese toxicity to terrestrial plants vary widely with species and include marginal chloroses, necrotic lesions, and distorted development of the leaves (Woolhouse, 1983). Manganese is the cause of recognizable disorders in some crops, such as crinkle leaf in cotton (Adams and Wear, 1957) and stem streak necrosis in potato (Robinson and Hodgson, 1961). In such instances, induced deficiencies of other mineral nutrients, such as Fe, Mg and Ca, are involved to various degrees. Toxic manganese concentrations in crop plant tissues vary widely, with critical values ranging from 100 to 5000 mg kg^{-1} (Hannam and Ohki, 1988).

Toxicity of manganese had been found in some field crops might be expected on acid soils at pH around 5.5 or lower and with a high manganese level. However, the critical manganese content and unfavorable soil pH range depend upon other several environmental factors. Manganese toxicity is also known to occur at higher pH levels in poorly drained soils. However, if acid soils are very low in total manganese, the plants are not subjected to manganese toxicity. The most common symptom of manganese toxicity is Fe chlorosis. Leaf puckering, necrotic brown spots and an uneven distribution of chlorophyll in older leaves manganese toxicity symptoms. In severely injured plants, the roots become brown. Plants resistant to manganese excess have an ability to accumulate the manganese in root tissues or to precipitate MnO_2, which is deposited mainly within the epidermis. Moreover, an increased Fe uptake by these plants has been observed. Symptoms of manganese toxicity are more shown in warm and hot weather.

4.5 Interactions between Manganese (Mn) and Iron (Fe)

Manganese and Iron antagonism is widely known and is observed mainly in acidic soils that contain large amounts of available manganese. In general, Fe and Mn are interrelated in their metabolic functions and their appropriate proportion (the Fe and Mn ratio should range from 1.5 to 2.5) is necessary for the healthy plant. Alvarez-Tinaut *et al*., (1980) reported that both deficient and normal manganese levels antagonize the iron absorption, but the reverse influence occurs when manganese reached toxic concentration in plants. In certain field and crop conditions, both manganese or iron toxicity can be remedied by the iron and manganese application.

A species which demonstrates the interaction between manganese and iron is the algae. Manganese can induce iron deficiency in some algae, notably blue-green algae, and this can lead to inhibition of chlorophyll synthesis (Csatorday *et al.,* 1984). The mechanism is thought to be competition for an active site where iron is necessary for functional integrity. Csatorday *et al.,* (1984) found that in the algae *Anacystis nidulans*, manganese blocks access of iron ions to some functional site involved in the magnesium branch of the tetrapyrrole synthesis pathway in the synthesis of the pigment phycobili protein. The site of action was the step after the insertion of magnesium into the protoporphyrin ring.

5. Phytic Acid

Phytic acid is the hexaphosphoric ester of cyclohexane (inositol hexaphosphoric acid, IP6). It is usually found as a complex with essential minerals and/or proteins (Widdowson and Mccance, 1942; Mccance *et al.,* 1943; Cheryan, 1980; Fox and Tao, 1989). It is a natural plant compound that is a simple ringed carbohydrate with six phosphate groups attached to each carbon. In a pH range of 0.5-9.0, it adopts the sterically stable one phosphate at carbon position, two in the axial position, and five phosphates in the equatorial position while the pH range over 9.5 sterically hindered five phosphates in the axial and one phosphate in equatorial position. This unique structure, with 12 replaceable protons and high density of negatively charged phosphate groups, is

responsible for its characteristic properties which allow it to form very stable complexes with multivalent cations (Dost and Tokul, 2005).

Phytic acid is a common constitution and organic compound which naturally found in mature cereal grains, some vegetables and fruits (Oberleas, 1973). It originates in most cereal grains, legumes, nuts, oilseeds, tubers, pollen, spores and organic soils. It is found in high concentrations in seeds of grains, pluses and oleaginous products and in lesser amounts in tubers and garden produce (Alabaster *et al.,* 1996). Moreover, the phytic acid is also the major phosphorous storage compound in plant seed and can storage up to 80% of total phosphorus in seed. In grain, it often occurs as phytin (a mixed calcium - magnesium salt of the phytic acid) and can represent 60 - 90% of total phosphorous (Lolas and Markakis, 1975). The levels can vary according to growing conditions, maturity (Makower, 1970), type, variety, and mill fraction of the grain. In cereals, the approximately 1 - 2% of the seed weight is the phytic acid and sometimes it could reach to 3 - 6% (Febles *et al.*, 2001). Generally, in many plant species, the 90% of the phytic acid is localized in the aleurone layer and only 10% in the embryo which there are many factors, such as genetics, environmental fluctuations, locations, irrigation conditions, type of soils, time and fertilizer application that can effect the phytic acid content and phosphorous availability in cereal grains. During the germination, the phytate salt is degraded by the action of phytases enzymes, which provides the growing seedling with phosphate.

In general, the presence of the phytic acid does not imply the existence of acute toxicity problems. However, the phytic acid has been considered as an anti-nutrient because of its ability to complex multi-charged metal ions, especially Zn (II), Ca (II), and Fe (III) (Harland and Oberleas, 1987). Excessive amounts of the phytic acid in the diet can cause a negative effect on mineral balance because the phytic acid forms insoluble complexes with Cu^{2+}, Zn^{2+}, Fe^{3+} and Ca^{2+} at physiological pH values (Graf, 1986; Nolan *et al.*, 1987) and consequently, reduces the bioavailability of these minerals (Morris 1986). Therefore, the consumption of great quantities of food which contains the high phytic acid levels could produce a deficit in the absorption of some dietary minerals.

However, the adverse effect of the phytic acid could be defeated by controlling the consumption of the phytic acid rich cereal products. The antinutritive effect can be eliminated by the partial dephosphorylation of the phytic acid (IP6) to myo-inositol tetrakisphosphate (IP4) or the lower esters. Additionally, processing (Han and Wilfred, 1988) and fermentation during dough making (Nayini and Markakis, 1983) can dephosphorylate the phytic acid to the lower phosphate esters. Regarding human health, the phytic acid is important to prevent against cancer, heart diseases and formation of renal stones (Vucenik *et al.*, 1998).

At present, the determination of the phytic acid level in dietary whole products becomes a very important task. Several methods are available for determining the phytic acid concentrations in cereal products, biological and urine samples.

Dost and Tokul, (2005) used a sensitive method for accurate determination of phytic acid in wheat and wheat products. The method was based on metal replacement reaction of the phytic acid from colored complex (iron (III) - thiocyanate), separation and monitoring any decrease in concentration of colored complex. The samples were analyzed by using the developed procedure with High Performance Liquid Chromatography/Ultraviolet Visible (HPLC/UV-vis) method and the spectroscopic method that was used to check accuracy of the developed procedure. The content of the phytic acid in the samples was calculated by using the calibration curve obtained from the standard solution of phytic acid and iron (III) - thiocyanate solutions containing 100 $\mu g\ ml^{-1}$ iron (III) ion. The result demonstrated that the temperature played an important role in wheat products. The content of phytic acid is 11.9 $mg\ g^{-1}$ in raw wheat flour while it is 4.9 $mg\ g^{-1}$ in bread that was made from the raw wheat flour. The reason for that is phytic acid decompose during baking processes. Fermentation also changed the phytic acid content but its effect is not as effective as baking. This study could be a guide for consumption of wheat and wheat products to prevent kidney stone crystallization for public.

Febles *et al.*, (2001) determined the phytic acid content in the infant flour commonly consumed among the infant population in the Canary Islands. The four hundread samples of the eight varieties of infant flour were analyzed. The method

proposed by Garcia-Villanova, Garcia-Villanova and Ruiz de Lope (1982) was used for determination of phytic acid content in cereal flours by complexometric titration of excess of iron (III). The result demonstrated that most of the samples showed a phytic acid content higher than 20 mg g^{-1}. Significant differences were observed for the different flour types. Gluten-free flour had the content lower than the rest. The nine cereal flour had a phytate concentration lower than the other flours tested but higher than gluten-free flour. Among wheat samples, phytate values were lower than in the varieties muesli-chocolate, 7-cereal, 8-cereal, multicereal and cereal-biscuit. Moreover, the multicereal flour had a lower content than muesli-chocolate.

6. Hydroponics

Hydroponics or soil-less culture is a simple technology for growing plants in nutrient solutions that supply all nutrient elements needed for optimum plant growth with or without the use of an inert medium such as gravel, vermiculite, rockwool, peat moss, saw dust, coir dust, coconut fibre, etc. to provide mechanical support. With the hydroponics method, the water is enriched with the essential nutrients that is perfectly balanced and does not harm to the environment. Therefore, the plants are able to uptake nutrients easily and directly from the nutrient solutions. In this system, the energy is usually used to develop long roots that have a correlation with vegetative growth. Moreover, very little water is lost in evaporation of hydroponics system due to its application in drought stricken areas. The problems with pest infestation, funguses and diseases also decrease (Thongaram, 2004). Thus, any kind of plants can be grown with hydroponics method because the plants can reach to the exact nutrients they need. They can respond by growing more rapidly and producing bigger yields. Field tests have shown that the products grown from hydroponics method have gained more vitamins and minerals than from soil.

Most growers utilize hydroponics indoors by creating a suitable environment for plant growth. Having the right combination of light, temperature, water, CO_2, oxygen, pH and nutrients are essential. The good combination of medium's selection of hydroponics can help plant to achieve the maximum results.

6.1 Classification of Hydroponics

The term hydroponics originally meant nutrient solution culture with no supporting medium. However, plant growing in solid media for anchorage using nutrient solution is also included in hydroponics. This technique is called aggregate system. Hydroponics systems are further categorized as open (i.e., once the nutrient solution is delivered to the plant roots, it is not reused) or closed (i.e., surplus solution is recovered, replenished and recycled). Current hydroponics systems of cultivation can be classified according to the techniques employed. A hydroponics technique refers to the method of applying nutrient solution to the plant roots (Department of Agriculture, 2007).

6.1.1 The Nutrient Film Technique or NFT is a true hydroponics system where the plant roots are directly exposed to nutrient solution. A thin film (0.5 mm) of nutrient solution flows through channels. The channel is made of flexible sheet. The growing medium absorbs nutrient solution for young plants and the roots form a mat in the channels when the plants grow. The nutrient solution is pumped to the higher end of each channel and flows by gravity to the lower end wetting the root mat. This technique provides enough support for tall growing plants.

6.1.2 The Nutrient Flow Technique or NFLT is a hydroponics system where allows the nutrient solution to flow directly to the plant roots through the channel. The plants are grown in a constant flow of nutrient enriched water. This system is similar to the Nutrient Film Technique which the nutrient solution flow slowly over the root system of the plant but the nutrient solution of this system is spread out so as to flow in approximately 10 - 15 mm of depth over a flat surface.

6.1.3 The Dynamic Root Floating Technique or DRFT is passive liquid systems. It can be done without electrical pumps or specialized instruments. The main feature of this system is the placement of plants on a panel fitted onto a bed or box filled with a shallow layer of hydroponics nutrient solution with a space between the panel and solution. In DRF, crops are grown above a ridged culture bed containing the solution,

with the roots dangling freely into the solution. The bottom roots are immersed in the solution and the top roots are exposed to humid air inside the chamber. Roots suspended above the solution specialize in absorbing oxygen (aeroroots), while the lower roots dangling in the solution specialize in water and nutrient uptake (nutriroots).

6.1.4 The Deep Flow Technique or DFT is the system which the 2-3 cm deep nutrient solution flows through 10 cm diameter PVC pipes that the plastic net pots with plants are fitted. The plastic pots contain planting materials and their bottoms touch the nutrient solution that flows in the pipes. The PVC pipes may be arranged in one plane or zig zag shape depending on the types of crops grown. The zig zag system utilizes the space efficiently but it is suitable for low growing crops. The single plane system is suitable for both tall and short crops. Plants are established in plastic net pots and fixed to the holes made in the PVC pipes. Old coir dust or carbonized rice husk or mixture of both may be used as planting material to fill the net pots. A small piece of net as a lining is placed in the net pots to prevent the planting material falling into the nutrient solution. Small plastic cups with holes on the sides and bottom may be used instead of net pots. When the recycled solution falls into the solution in the stock tank, the nutrient solution gets aerated. The PVC pipes must have a slope of drop of 1 in 30-40 to facilitate the flow of nutrient solution. The PVC pipes should be painted in white color to help in reducing the heating up of nutrient solution. This system can be established in the open space or in protected structures as part of CEA.

6.2 Nutrient Solution for Hydroponics

Plants require 17 essential elements for their growth and development. The plants cannot complete their life cycles without the nutrients. Moreover, the roles of the nutrients in plant growth can not be replaced by any other elements. These 17 essential elements are divided into macro elements (required in relatively large quantities) and micro or trace elements (required in considerably small quantities) (Department of Agriculture, 2007).

The macro elements are carbon (C), hydrogen (H), Oxygen (O), nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S). The

micro elements are iron (Fe), chlorine (Cl), boron (B), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo) and nickel (Ni). All essential nutrients are supplied to hydroponics plants in the form of nutrient solution, which consists of fertilizers salts dissolved in water. The hydroponics grower must have a good knowledge of the plant nutrients, as management of plant nutrition through management of nutrient solution is the key to success in hydroponics gardening.

The hydroponics methods enable growers to control the availability of essential elements by adjusting or changing the nutrient solution to suit the plant growth stage and to provide them in balanced amounts. As the nutrients are present in ionic forms in the nutrient solution, the hydroponics plants reach maturity much sooner. Optimization of plant nutrition is easily achieved in hydroponics than in soil.

6.3 pH Level

In simple terms, pH is a measure of acidity or alkalinity on a scale of 1 to 14. In a nutrient solution, pH determines the availability of essential plant elements (Figure 3). A solution is considered to be neutral at pH 7.0. For pH values above 7.5, the iron, manganese, copper, zinc and boron becomes less available to plants. The optimum pH range for hydroponics nutrient solution is between 5.8 and 6.5 (Department of Agriculture, 2007).

6.4 Electrical Conductivity (EC)

The electrical conductivity indicates the strength of nutrient solution, as measured by an EC meter. The unit for measuring EC is dSm^{-1}. A limitation of EC is that it indicates only the total concentration of the solution and not the individual nutrient components. The ideal EC range for hydroponics is between 1.5 and 2.5 $dS\ m^{-1}$. The higher EC will prevent nutrient absorption due to osmotic pressure and the lower EC severely affect plant health and yield.

The EC of the solution changes when the plants take up the nutrients and water from the solution. If the EC is higher than the recommended range, the fresh water

must be added to reduce it. If it is lower, the nutrients will be added to raise it as shown in Figure 2.

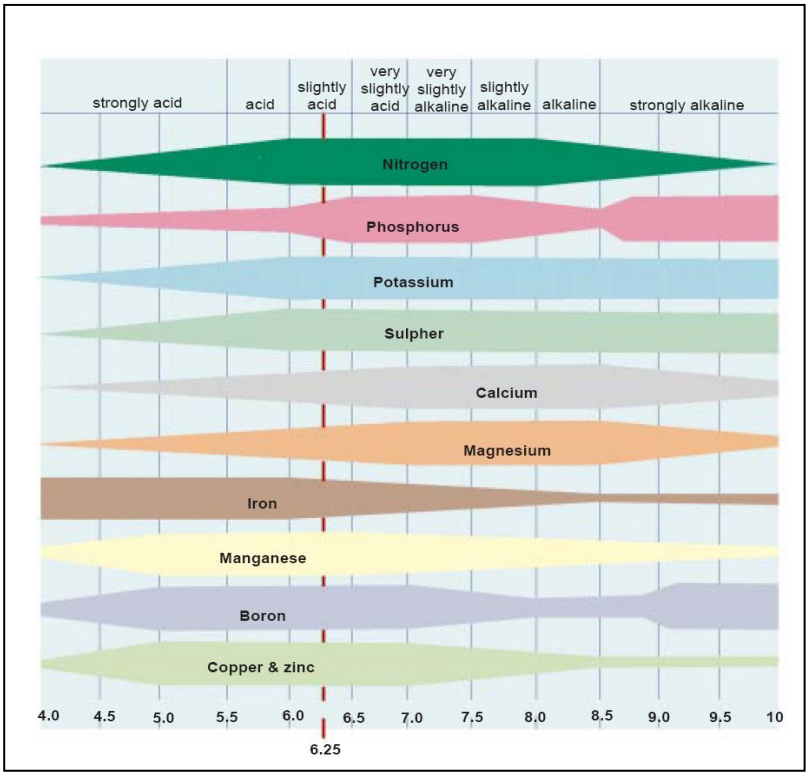

Figure 2 The availability of nutrient elements at different pH levels
Source: Department of Agriculture (2007)

7. Information of Khao Yoi District, Phetchaburi Province

Khao Yoi is a district which was established in 1897 and Luang Phromsan was assigned as the first governor. At first, it was named Huai Tha Chang. However, the name of the district was changed due to the move of the district office to Hua Saphan and Ban Noi until it finally became Khao Yoi. The general information of Khao Yoi District is described as following details (Table 1 and Figure 4). (1 rai = 1,600 m^2)

Table 1 General information of Khao Yoi District, Phetchaburi Province

Topics	Descriptions
Location	Northern part of Phetchaburi Province
Overall Area	191,030 rai
Agricultural Area	80,908 rai
Geographical Characteristic	Plain Area
Climate	Influencing from the southwest monsoon in rainy season and the northeast monsoon in winter
Season	Summer - Starts at February to May Rainy Season - Starts at May to October Winter - Starts at October to February
Type of Soil	Sandy Clay Loam
Occupation	Agriculture, Livestock, Handicraft, Industry, etc.

Source: Department of Provincial Administration (2007)

In 2006, the highest temperature is 35.6 °C (May 4, 2006 and August 24, 2006) and the lowest temperature is 16.0 °C (December 23, 24, 26, 2006). The yearly average temperature is 28.0 °C. The annual rainfall is 944.7 mm which the rainfall since 0.1 mm can be estimated totally 114 days. The record of rainfall during 1994 to 2006 showed that the mean rainy day is 102 days per year. At the period of 10 years (1997 to 2006), the annual average rainfall is 979.1 mm that the rainfall is highest during September to October (Department of Provincial Administration, 2007). From the data of the Public Health Office (2007) in Khao Yoi District, it presented that 16 % of all pregnant women had anemia as shown in Figure 3.

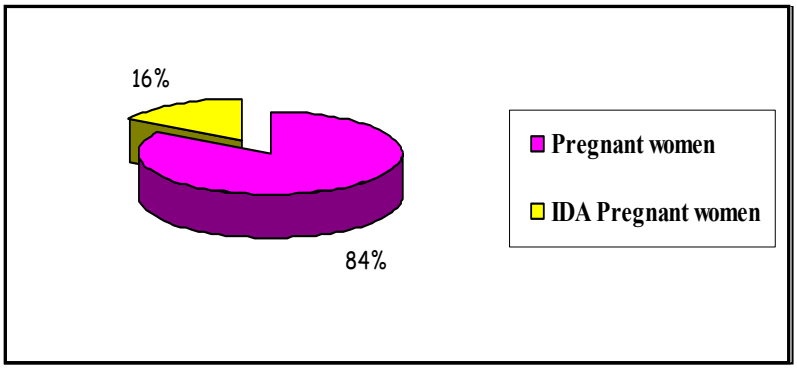

Figure 3 Data of anemia in pregnant women at Khao Yoi District, Phetchaburi Province
Source: The Public Health Office, Khao Yoi District (2007)

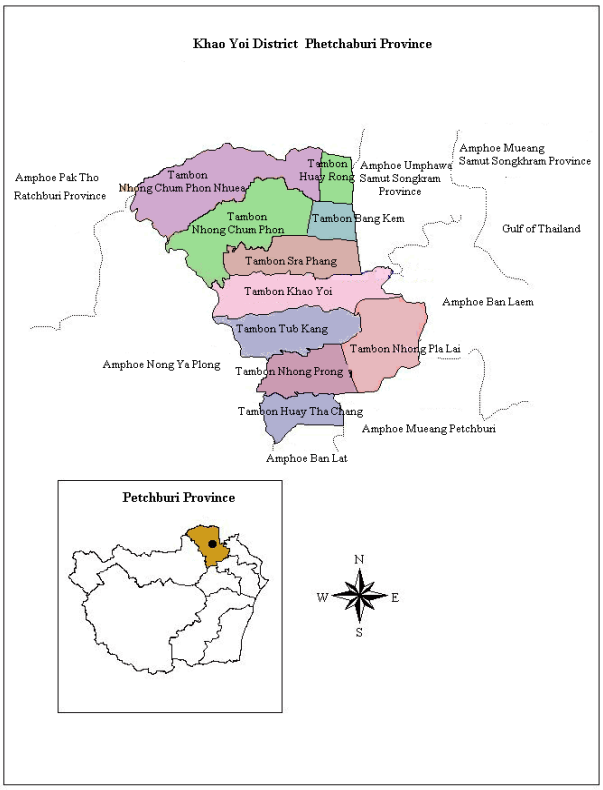

Figure 4 Map of Khao Yoi District, Phetchaburi Province
Source: Anonymous (2007)

The Khao Yoi district has an overall area of 191,030 rai. The rice cultivation area is 128,355.28 rai. Mostly, the soil series that the farmers in Khao Yoi district use for rice cultivation are Kamphang Saen, Phetchaburi and Samut Prakarn which their soil properties and area sizes are different as the Table 2 and 3.

Table 2 Soil properties used for rice cultivation in Khao Yoi District, Phetchaburi Province

Soil Series	Depth (cm)	OM (%)	CEC	%B.S.	Avail.P	Avail.K	pH
Phetchaburi (Pb)	0-25	medium	low	medium	low	high	5.5-6.5
Samut Prakarn (Sm)	0-25	medium	high	high	high	high	6.0-8.0
Kamphang Saen (Ks)	0-25	high	low	high	high	high	6.5-7.5

Source: Land Development Department (2007)

Table 3 Areas for rice cultivation in Khao Yoi District, Phetchaburi Province

Soil Series	Size of area (rai)	Area (%) in Khao Yoi District	Area for rice cultivation (%) in Khao Yoi District
Petchaburi (Pb)	70,032.5	38.9	35.4
Samut Prakarn (Sm)	55,196.8	27.9	27.9
Kamphang Saen (Ks)	3,125.9	1.7	1.6
Total area of Phetchaburi Province	428,152.0		
Area for cultivating rice	128,355.3		64.9

Source: Land Development Department (2007)

8. Hair as a Biomarker

Trace metals are ubiquitous elements. They can be found in the environment and in all living organisms. Some trace metals are essential to life because they are involved in multiple functions, such as enzymes structure, transport proteins, hormonal function and specific receptor sites, etc. On the contrary, some elements are not considered essential but their presence, even at very low levels, may result in impairment of biological functions (Apostoli, 2002). Previously, blood or serum/plasma or urine are the most common specimens used as biomarkers of internal dose to diagnose trace element deficiencies, monitor the nutrition status of essential elements and assess environmental or occupational exposure of individuals to toxic elements. However, the selection of appropriate specimen and measurement of biomarkers depend on several factors, such as time of appearance, convenience, invasiveness of the specimen collection procedure and potential for specimen contamination because they are critically important for health care management purposes, public health decision making and primary prevention activities (Rodrigues *et al.,* 2008). Thus, due to some limitations, hair becomes an alternative specimen that has attracted considerable attention as a marker of basic levels of metals in the human body (Amaral *et al.,* 2008). Hair analysis is considered as the potential diagnostic and preventive tool that can be used in the future for the clinical diagnosis (Austin and Soloway, 1992).

Hair is a biological specimen that is easily collected, inexpensive, painless and non-invasive. Samples can be kept in plastic bag, stored at room temperature in the dark and transported to the laboratory for analysis, including their composition does not change with time. Moreover, the advantages of using hair are also manifold. One is its ability to reflect the total body intake of certain elements better than more frequently used markers such as blood, serum and urine. Another one is the phenomenon of accumulation, which implied a higher concentration of metals in hair than in fluids. The affinity of metals for hair is primarily due to the relatively high presence of cystine in the keratin structure, as well as to follicular melanin, which is able to bind cations by ionic interactions. The level of metals in biological fluids represents in most cases a current status, while the distribution of elements in hair reflects more extended exposure, on account of growth (Forte *et al.,* 2005). These advantages have led to the widespread use

of trace element analysis of hair samples to assess wildlife and human exposure to different contaminants present in the environment or at the workplace (Ashraf *et al.,* 1994).

Senofonte *et al.* (2001) explored the suitability of trace element determination (Ca, Cu, Fe, Mg, Zn, Co, Cr, Mn, Mo and Ni) in human hair to verify whether extreme environmental conditions and significant changes in lifestyle can induce detectable effects on the participants in Antarctic expeditions. Sampling of hair of participants in the expeditions was carried out both prior to departure and at the end of the period spent at the bases. The results showed that there were significant differences between concentration values obtained before and after the expedition for Ca, Cr, Cu, Fe, Mg, Mn and Mo, whereas no relevant differences could be found for the remaining elements. The observed imbalances might be related to several factors, such as environmental impact on human adaptation, individual bio-rhythm, state of health, type of food, etc.

Forte *et al*. (2005) studied to determine the levels of Ca, Cu, Fe, Mg, Si and Zn in the hair of Parkinson's disease (PD) patients and compare them with those of control subjects in order to evaluate their possible implication in the pathology and assess whether hair could be reliable marker of possible changes. The findings in PD patients suggested a probable relationship between the pathology and a deficiency of Fe in the hair of patients. Ca levels decreased significantly with age. Variations in values of Cu, Si and Zn were insignificant. Differences were found for Ca and Mg depending on sex. Moreover, the levels of metals in hair were not affected by the duration or severity of the disease or by the type of pharmacological therapy. In conclusion, hair is only a partially useful biomarker of imbalances in the studied metals in PD.

Chojnacka *et al*. (2006) studied the content of elements in hair of individuals that lived together or were family related in an urban, industrialized (heavy industry dominates) area in Wroclaw city, Poland. The study was found that the differences resulted mainly from different living habits (Na, Si, Co, Fe, Mn and Zn) and local exposure (Pb, Cd and Al). It was also found that there were similar tendencies in the accumulation of the majority of elements by people that lived together.

Senofonte *et al.* (2000) analyzed hair samples of youngsters (3-15 years of age) from several urban areas of Rome to determine the content of 19 minor and trace elements with the aim of assessing Reference Values (RVs). Thirteen essential elements were taken into account, Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P. On the other hand, Al, As, Cd, Pb, Sr and Ti were also evaluated on the basis of non-essential elements. The result showed that the significant differences were found for certain elements depending on age and sex.

From the studies mentioned above, the findings can conclude that the level of elements in hair is affected not only by environmental exposure but also by many factors, including living habits (dietary intakes) and personal propensity (genetic abilities, hair color, sex, age, illness, ingestion of drugs). The seasonal variations and other variables also influence the level of elements in hair (Teresa *et al.,* 1997). Furthermore, the level of elements in hair can be also influenced by the level of other elements: synergistic and antagonistic effects were detected (Chojnacka *et al.,* 2006). However, hair analysis is subject to certain limitations, such as the occurrence of exogenous contamination. This contributes to a differential increase in the total contents of different contaminants (Bencze, 1990; Miekeley *et al.,* 1998; Frisch and Schwartz, 2002). The main sources of exogenous contaminants are deposits of sebum, sweat, polluted air residues or residues of cosmetic or pharmaceutical products. These constraints include the lack of scientific knowledge about the kinetics of trace element incorporation in hair and the insufficiency of pidemiological data to support predictions concerning the health effects, of a specific concentration of each element in hair (Rodrigues *et al.,* 2008).

MATERIALS AND METHODS

Materials

1. Materials for Soil Survey and Collection

1.1 Standard soil survey and sampling kit

1.2 Paper bags for keeping the samples

1.3 Labels for samples

1.4 Scissors

1.5 Permanent pens

1.6 Other necessary materials

2. Materials for Hydroponics Experiment

2.1 One-half strength Hoagland no.2 nutrient solutions

2.1.1 The base nutrient solution had the following composition: macronutrients; NH_4NO_3 1.5 mM, $CaCl_2$ 1 mM, $MgSO_4$ 1.6 mM, K_2SO_4 2 mM, KH_2PO_4 0.1 mM and micronutrients (in Micromolar concentrations); H_3BO_3 2μM, $MnSO_4$ 5 μM, $CuSO_4$ 1 μM, $(NH_4)_6MO_7O_{24}$ 0.5 μM, $ZnSO_4$ 1 μM.

2.2 Seed of rice cultivars

2.2.1 RD 23 cultivar

2.2.2 Chainat 1 cultivar

2.2.3 Pathumthani 1 cultivar

2.2.4 Suphanburi 1 cultivar

2.2.5 Suphanburi 3 cultivar

2.3 Material used during rice cultivation

2.3.1 Bucket
2.3.2 Water pump
2.3.3 Sponge foam sheet
2.3.4 Experimental labels

2.4 Materials used during the harvest

2.4.1 Knives for rice harvesting
2.4.2 Paper bags for keeping the rice samples
2.4.3 Permanent pens
2.4.4 Weighing scale for rice

3. Materials for Laboratory

3.1 Spectrophotometer (Spectronic 21)
3.2 Atomic Absorption Spectrophotometer
3.3 pH meter
3.4 Electrical conductivity meter
3.5 Micro Kjeldahl distillation apparatus
3.6 Digestion apparatus
3.7 Fume hood
3.8 Weighing scale of 3 positions
3.9 Temperature controlled oven
3.10 Grinder and 2 and 0.5 mm sieve
3.11 Other necessary materials

4. Materials for Phytic Acid Analysis

4.1 Vacuum
4.2 Whattman No. 1 filter paper
4.3 25 ml volumetric flask

4.4 Ion-exchange column (AG1-X4, 100-200 mesh, chloride form resin)

4.5 Spectrophotometer

4.6 Mixture of concentrated HNO_3/H_2SO_4

4.7 2.5% ammonium molybdate

4.8 0.7 M NaCl

Methods

1. Experiment 1: Survey the Information of Rice Fields and Population in Khao Yoi District, Phetchaburi Province

1.1 Sample Collection

Paddy soils at the depth of 0-15 cm, rice straws and brown rice from 60 rice fields of Khao Yoi District, Phetchaburi Province were collected for 10 samples per field which all composite samples, whereas the farmers' hair and the rice which is consumed by the farm owners were also collected. Moreover, the history of land management of sampling sites and nutrient condition of the farmers were also recorded.

1.2 Study on Soil Properties

The different soil series samples grown rice of Khao Yoi were collected. They were air-dried at room temperature, removed the plants scraps, ground to a fine state and sieved through a 2 mm mesh before the chemical and electrical analysis as follows:

1.2.1 Electrical Analysis

a. The pH values were determined with a pH-meter at the ratio of soil : water (1:1) (Attanandana and Chanchareonsook, 1999).

b. The electrical conductivity of soil solution which was determined from the water saturated soils was measured under a temperature of 25 °C with an electricity conductivity (Attanandana and Chanchareonsook, 1999).

1.2.2 Physical Properties

Soil texture was determined by hydrometer method (Day, 1965)

1.2.3 Chemical Analysis

a. The extractable potassium concentration was determined by extracting soil with 1N NH_4OAc (pH 7.0) and measured by using the atomic absorption spectrophotometer (Attanandana and Chanchareonsook, 1999).

b. The available phosphorus concentration was determined by Bray II method (Bray and Kurtz, 1945) and measured by the Colorimetric method (Attanandana and Chanchareonsook, 1999).

c. The extractable magnesium was extracted with 1N NH_4OAc (pH 7.0) and determined by using the atomic absorption spectrophotometer (Pratt, 1965).

d. The extractable calcium was extracted with 1N NH_4OAc (pH 7.0) and measured by using the atomic absorption spectrophotometer (Pratt, 1965).

e. The organic matter concentration was determined by the Walkley-Black rapid oxidation method (Walkley and Black, 1934; Attanandana and Chanchareonsook, 1999).

1.3 Total Fe and Mn Concentrations Analysis

The total Fe and Mn concentrations analysis in soil was conducted by digesting soil samples with the wet oxidation method. The soil samples were determined with HNO_3 and $HClO_4$ mixture at the ratio of 5:2 and determined by using the atomic absorption spectrophotometer (Attanandana and Chanchareonsook, 1999).

Moreover, the available iron and manganese analyse in soil were extracted from soils with DTPA (pH 7.3) and determined Fe and Mn concentrations by using the atomic absorption spectrophotometer (Attanandana and Chanchareonsook, 1999).

1.4 Plants Analysis

The plant samples were washed twice with deionized water. Then, the samples were oven-dried at 70 °C to constant weight, ground to a fine state and analyzed for metal concentration in rice plants and seeds. The total Fe and Mn concentration analyse in plant were conducted by digesting the plant samples with the concentrated HNO_3 : $HClO_4$ at the ratio of 5:2 followed by the analysis of Fe and Mn concentrations by using the atomic absorption spectrophotometer (Attanandana and Chanchareonsook, 1999).

1.5 Phytic Acid Analysis

1.5.1 Phytic Acid Measurement

The amount of phytic acid in original samples was determined by using ion-exchange method and calculated as hexaphosphate equivalents. Briefly, 2.00 g of dried and powdered sample was mixed with 40 ml HCl (2.4%) for 3 hours at room temperature. The solution was filtered with vacuum through Whatman No. 1 filter paper. The filtrate (1.0 ml) was pipetted into a 25 ml flask, 1.0 ml of NaOH (0.75 M)/Na_2EDTA (0.11 M) solution was added, and then diluted to 25 ml with distilled water. The solution was poured completely into the ion-exchange column. The column was made from AG1-X4, 100-200 mesh, chloride form resin (Bio-Rad Laboratories, Hercules, CA, USA). Phytic acid was washed with 15 ml of NaCl (0.7 M). Phosphorus in the resulting solution was released with a mixture of concentrated HNO_3/H_2SO_4 (3.0 and 0.5 ml, respectively), and then mixed with molybdate (2.5% ammonium molybdate in 1 N H_2SO_4) solution. Absorbance was read at 640 nm (Spectrophotometer model 320, Spectra UV, Sherwood Scientific Ltd., Cambridge, UK). Phosphorus concentration was calculated from phosphate standards (80, 240,

400 lg/ml) curve. Because 28.2% of phytic acid is phosphorus, so the amount of phytic acid was calculated using the following equation (Gargari *et al.*, 2007).

Phytic acid (mg/g of dried sample) = (mean k × A × 20) / (0.282 × 1000)

A	=	absorbance
k	=	standard phosphorus concentration (μg) / A
mean k	=	Σ k / n
n	=	number of standards (Lane, 1995)

1.6 Human Hair Analysis

Hair was cut close to the scalp about 1-2 cm. The hair samples were washed twice, first with acetone and then with double-distilled water. They were oven-dried below 70°C to constant weight and analyzed for total Fe and Mn concentrations by heating a gram of hair sample at 150 °C with 12 ml of 2:1 HNO_3-H_2O_2 acid mixture for 30 minutes. Concentrations of Fe and Mn were measured by using AAS (AA 240, Varian Australia Pty Ltd., Australia) (Harrison *et al*., 1969).

2. Experiment 2: Effect of Rice Cultivation and Fe Supply on Rice Growth: Grown in Hydroponics Experiment Filled with Fe-EDTA 28 Days

2.1 Experimental Plan

The experiment was designed on 5 × 4 of completely randomized design (CRD) in hydroponics system filled with Fe-EDTA at 28 days. The Deep Flow Technique (DFT) was used for this study which three replications were conducted. Two important factors of this experiment were composed of the five rice cultivars (Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3) and the nutrient solution that there were 4 levels of Fe concentration as follows:

2.1.1 Nutrient solution with Fe-EDTA 10 μM (Treatment 1 = T1)
2.1.2 Nutrient solution with Fe-EDTA 50 μM (Treatment 2 = T2)
2.1.3 Nutrient solution with Fe-EDTA 100 μM (Treatment 3 = T3)
2.1.4 Nutrient solution with Fe-EDTA 150 μM (Treatment 4 = T4)

2.2 Experimental Procedure

The seeds of five rice cultivars were washed in deionized water and allowed to germinate on moist filter paper for 48 hours. After that, the germinated seeds were planted in moist hydroponics sponge (2 seeds per a hole) grown for 4 days. The healthy seedlings were transferred to hydroponics pots containing the nutrient solutions with various Fe-EDTA levels as shown in Table 4 for 28 days. The height and appearance of plants were recorded every 7 days. After transplanting on 7th day, a rice seedling was removed from each hole. Moreover, the nutrient solution was changed every 3 days.

Table 4 Nutrient solution in experiment 2

Macronutrient	mmol L^{-1}	Micronutrient	μmol L^{-1}
NH_4NO_3	1.5	H_3BO_3	2
$CaCl_2$	1	$MnSO_4$	5
$MgSO_4$	1.6	$CuSO_4$	0.2
K_2SO_4	1	$(NH_4)_6Mo_7O_{24}$	0.05
KH_2PO_4	0.3	$ZnSO_4$	1
Fe-EDTA (μmol L^{-1})	10 (T1), 50 (T2), 100 (T3) and 150 (T4)		

Source: One-half strength no.2 nutrient solutions (Hoagland and Arnon, 1950)

2.3 Plant Analysis

After 28 days of transplanting, the plants were harvested, separated into leaves, straws, and roots, washed thoroughly once with tap water and twice with deionized water, and then the fresh weights were determined immediately. Plants were dried at 70 °C for 48 hours to constant weight and their dry weights were determined. Dried leaves, straws and roots were finely ground to analyze for Fe concentrations. The plant analysis was determined, digesting the plant samples with the concentrated HNO_3: $HClO_4$ at the ratio of 5:2 and analyzing for Fe concentration of plant with the Atomic Absorption Spectrophotometer (Attanandana and Chanchareonsook, 1999). The amount of Fe content (uptake and accumulation) was calculated using the following equation.

$$\text{Fe content} = \frac{\text{Fe concentration (mg kg}^{-1}) \times \text{Dry weight (g)}}{10^3}$$

2.4 Statistical Analysis

The statistical analysis was determined by analysis of variance using the IRRI and Statistica computer program.

3. Experiment 3: Experimental Cultivation of Five Rice Cultivars in Hydroponics Experiment Filled with Fe-EDTA 45 Days

3.1 Experimental Plan

The experiment was designed on 5 × 5 factorial of completely randomized design (CRD) which three replications were conducted. The Deep Flow Technique (DFT) was used for this study which three replications were conducted. Two important factors of this experiment were composed of the five rice cultivars (Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3) and the nutrient solution that there were 5 levels of Fe concentration as follows:

3.1.1 Nutrient solution with Fe-EDTA 50 µM (Treatment 1 = T1)
3.1.2 Nutrient solution with Fe-EDTA 75 µM (Treatment 2 = T2)
3.1.3 Nutrient solution with Fe-EDTA 100 µM (Treatment 3 = T3)
3.1.4 Nutrient solution with Fe-EDTA 125 µM (Treatment 4 = T4)
3.1.5 Nutrient solution with Fe-EDTA 150 µM (Treatment 5 = T5)

3.2 Experimental Procedure

The seeds of five rice cultivars were washed in deionized water and allowed to germinate on moist filter paper for 24 hours. After that, the germinated seeds were planted in moist hydroponics sponge (2 seeds per a hole) grown for 4 days. The healthy seedlings were transferred to hydroponics pots containing the nutrient solutions with various Fe-EDTA levels as shown in Table 5 for 45 days. The height and appearance of plants were recorded every 7 days. After transplanting on 7th day, a rice seedling was removed from each hole. Moreover, the nutrient solution was changed every 3 days.

Table 5 Nutrient solution in experiment 3

Macronutrient	mmol L^{-1}	Micronutrient	µmol L^{-1}
NH_4NO_3	1.5	H_3BO_3	2
$CaCl_2$	1	$MnSO_4$	5
$MgSO_4$	1.6	$CuSO_4$	0.2
K_2SO_4	1	$(NH_4)_6Mo_7O_{24}$	0.05
KH_2PO_4	0.3	$ZnSO_4$	1
Fe-EDTA (µmol L^{-1})	50 (T1), 75 (T2), 100 (T3), 125 (T4) and 150 (T5)		

Source: One-half strength no.2 nutrient solutions (Hoagland and Arnon, 1950)

3.3 Plant Analysis

The plants were harvested after transplanting at the period of 45 days, separated into leaves, straws, and roots, washed throughly once with tap water and twice with deionized water, and then the fresh weights were determined immediately. Plants were dried at 70 °C to constant weight and their dry weights were determined. Dried leaves, straws and roots were finely ground to analyze for Fe concentrations. The plant analysis was determined by digesting the plant samples, digesting the plant samples with the concentrated HNO_3 : $HClO_4$ at the ratio of 5:2 and analyzing for Fe concentrations of plant with the Atomic Absorption Spectrophotometer (Attanandana and Chanchareonsook, 1999). The amount of Fe content (uptake and accumulation) was calculated using the following equation.

$$\text{Fe content} = \frac{\text{Fe concentration (mg kg}^{-1}\text{)} \times \text{Dry weight (g)}}{10^3}$$

3.4 Statistical Analysis

The statistical analysis was determined by Analysis of Variance using the IRRI and Statistica computer program.

RESULTS AND DISCUSSION

1. Experiment 1: Survey The Information of Rice Fields and Population in Khao Yoi District, Phetchaburi Province

1.1 Iron dietary intake of rice consumed for populations in Khao Yoi District, Phetchaburi Province

The study on nutrition status of populations in Khao Yoi District, Phetchaburi Province was found that the populations were risk to anemia disease. Generally, human beings consume rice 300 g day^{-1} which get from cooking of polished rice 100 g (Asian Productivity Organization, 2000). Thus, if the populations in studied area consume the Suphanburi 1 and Chainat 1 rice cultivars from this area, they will take Fe at the level of 0.64 - 3.99 mg kg^{-1} and 0.48 - 5.77 mg kg^{-1}, respectively. The study of Kongjanthuek (2003) found that the consumers needed average energy at 2000 kcal per day which mostly came from carbohydrate. The given energy from rice consumption was at 60% of all energies which were daily demanded. As rice is staple food of world population, Beutler *et al.* (1995) reported that Fe absorption rate from food were different. Fe absorption rate from rice was about 1 - 2% of available Fe which affected to lower Fe intake than standard criteria. Therefore, if the population in Khao Yoi District consumed rice planted in studied areas as well as the poor nutritional consumption, they would take Fe from brown rice inadequate to Fe demanded in each day. Usually, the Fe demand of male and female is 11 and 16 mg day^{-1} (Ministry of Public Health, 2003), respectively as shown in Table 6.

Table 6 Iron dietary intake of populations in Khao Yoi District, Phetchaburi Province

Fe concentration (mg kg^{-1})	Rice cultivar		RDA for iron dietary[1/] (mg day^{-1})	
	Suphanburi 1	Chainat 1	Male	Female
Brown rice	6.42-39.98 ± 8.51	4.79-57.70 ± 13.12	11	16
Dietary intake[2/]	0.64-3.99 ± 0.85	0.48-5.77 ± 1.31		

[1/] Ministry of Public Health (2003)

[2/] Asian Productivity Organization (2000)

1.2 Ratio of Fe and phytic acid in the brown rice and polished rice of Chainat 1 and Suphanburi 1 planted in Khaoyoi District, Phetchaburi Province

Phytic acid is an ester which is mainly found in legumes, nuts and cereal grains (Harland and Oberleas, 1987; Gargari *et al.,* 2005), but the excess amount of phytic acid can decrease absorption of minerals such as zinc, iron, calcium and manganese which brings to malnutrition problem of world population. For this study, as being an important factor for determination of Fe bioavailability, phytic acid was measured to determine its content accumulated in brown rice. The brown rice of Chainat 1 and Suphanburi 1, which are commercial cultivars those were planted and consumed in Khao Yoi District, Phetchaburi Province. Forty samples of Chainat 1 and 20 samples of Suphanburi 1 were analyzed to investigate the content of phytic acid in the brown rice and polished rice as well as determine phytic acid and Fe mole ratio. The results are presented in Tables 7 and 8.

Table 7 Phytic acid, Fe content and Fe mole to phytic acid ratio in brown rice of Chainat 1 and Suphanburi 1 cultivated in Khao Yoi District, Phetchaburi Province

Rice cultivar	Fe (mg / 100g)	Phytic acid in brown rice (mg / 100g)	Fe mole to phytic acid ratio
Suphanburi1	2.37 ± 0.85 (0.64 - 3.99)	359.60 ± 43.42 (289 - 412)	1:15.2
Chainat1	2.63 ± 1.31 (0.48 - 5.77)	379.63 ± 57.17 (278 - 483)	1:15.6

The results in Table 7 show that the phytic acid in brown rice are 359.60 ± 43.42 and 379.63 ± 57.17 mg / 100g of Suphanburi 1 and Chainat 1, respectively. The Fe contents are 2.37 ± 0.85 and 2.63 ± 1.31 mg / 100g of dried weight, whereas Fe mole to phytic acid ratio is 15.2 and 15.6 of Suphanburi 1 and Chainat 1, respectively. Among two cultivars, Chainat 1 had greater amounts of phytic acid and Fe contents and Fe mole to phytic acid ratio was also higher than Suphanburi 1.

Table 8 Phytic acid, Fe content and Fe mole to phytic acid ratio in polished rice of Chainat 1 and Suphanburi 1 cultivated and consumed by population in Khao Yoi District, Phetchaburi Province

Rice cultivar	Fe (mg/100g)	Phytic acid in polished rice (mg/100g)	Fe mole to phytic acid ratio
Suphanburi1	0.98	227	1:2.4
Chainat1	1.50	321	1:1.8

The results in Table 8 show that the phytic acid contents are 227 and 321 of Suphanburi 1 and Chainat 1, respectively. The Fe contents are 0.98 and 1.50 of dried weight, whereas Fe mole to phytic acid ratio is 2.4 and 1.8 of Suphanburi 1 and Chainat 1, respectively. Between two rice cultivars, Chainat 1 had greater amounts of phytic acid and Fe contents but Fe mole to phytic acid ratio was lower than Suphanburi 1. According to the comparison between two rice cultivars of brown rice and polished rice, there was a significant decrease in phytic acid and Fe contents as

well as phytic acid to Fe mole ratio of Chainat 1 and Suphanburi 1 in polished rice during the milling process. It was suggested that the phytate content which located in the outer aleurone layer was reduced. Thus, the milling could enhance mineral bioavailability, although the content of minerals of the milled cereals was simultaneously reduced (Gibson *et al.,* 2006). Moreover, the result showed the higher efficiency of Fe uptake and transportation to rice grains of Chainat 1 as compared to Suphanburi 1, whereas phytic acid content of Chainat 1 was also higher than Suphanburi 1. The results can be suggested that the large variation in phytic acid content among rice cultivars indicated the possibility of developing the rice cultivars with low phytic acid content in rice grains. Great differences could be found among rice cultivars in phytic acid content.

The ratio of Fe mole to phytic acid shows that Chainat 1 has higher (1: 15.6) ratio in brown rice as compared to Suphanburi 1 (1: 15.2). On the other hand, phytic acid and Fe contents in polished rice of Chainat 1 were higher than Suphanburi 1 but Fe mole to phytic acid ratio was lower (1.82) than Suphanburi 1 (2.4) despite of the higher amounts of phytic acid. This was suggested that the cultivars played an important roles to reduce phytic acid. Usually, maximal inhibition of Fe uptake by phytic acid occurred at a 1: 10 ratio of Fe to phytic acid. Dialyzable Fe decreased with a low ratio of Fe to phytic acid but increased with Fe: phytic ratios greater than 1: 3 indicated that Fe was soluble at higher phytic acid levels. However, but less available (Glahn *et al.,* 2001). The supply of Fe decreased when consumed food had a low Fe content or when absorption of Fe was inhibited by the presence of phytic acid (Slingerland *et al.,* 2005). The phytic acid – Fe molar ratio was used as a proxy for Fe bioavailability in food. Amounts of Fe and phytic acid and their ratio in the rice grains differed among rice cultivars, which showed difference of Fe bioavailability in each rice cultivar. This result indicated that Chainat 1 had greater amount of total Fe in polished rice as compares to Suphanburi 1. Therefore, population in Khao Yoi area which consumed Chainat 1 would get Fe more than Suphanburi 1. At present, the researchers have tried to decrease phytic acid content which is called "biofortification" for Fe bioavailability. Biofortification is currently in practice to improve Fe supply.

1.3 Fe concentration in the hair samples of population in Khao Yoi District, Phetchaburi Province related to mulnutrition

The hair samples of 60 populations in Khao Yoi District, Phetchaburi Province were analyzed. The results of populations can be divided into 3 groups according to the Fe concentration in hair which are 0-140 mg kg^{-1}, 141-280 mg kg^{-1} and 281- 420 mg kg^{-1} respectively. The 43 populations, which are 21 male and 22 female, were formed into the first group averaged to 35.0% and 36.7%. The second group, there are 11 male which are averaged to 18.3%. Finally, the third group composes of 6 men that are averaged to 10.0%. According to the data mentioned above, the result shows that the a number of female is more than men of the first group that has the highest Fe concentration in hair at 140 mg kg^{-1} which is mean value of human hair (Spector, 1956; National Institute for Environmental Studies, 1996). A reason of this result may be due to the Fe loss in menstruation of reproductive female. Hence, female should take Fe more than male at the same age. Normally, male and non-menstruating female lose Fe about 1 mg day^{-1}. The menstruating female lose Fe from 0.6 to 2.5 percent day^{-1} (Killip *et al.*, 2007). Nevertheless, female have not been found in other groups which they have 11 and 6 male in second and third groups, respectively as shown in Figure 5.

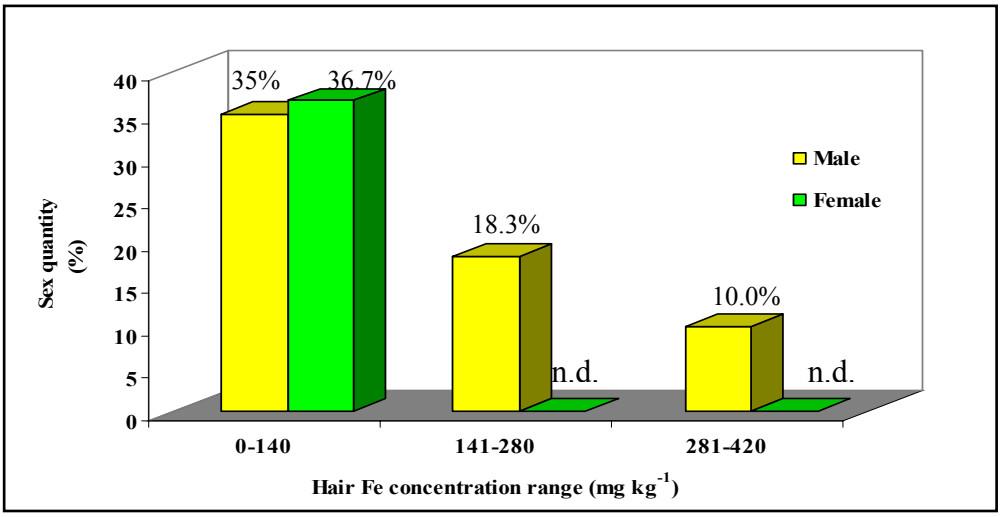

Figure 5 Fe concentration in human hair of population in Khao Yoi District, Phetchaburi Province

1.4 Physical and Chemical properties of three soil series which have been used for rice cultivation in Khao Yoi District, Phetchaburi Province

Based on soil series map (Land Development Department, 2007) and the Geographic Information System (GIS), it was found that the soil series which were mainly used for rice cultivation at Khao Yoi District were Petchaburi, Samut Prakan and Kamphaeng Saen series (Table 9).

Table 9 Soil series of rice cultivation area in Khao Yoi District, Phetchaburi Province

Soil Series	Areas (rai)	Area in Khao Yoi District (%)	Area for rice Cultivation in Khao Yoi District (%)
Petchaburi	70,032.50	38.9	35.4
Samut Prakan	55,196.80	27.9	27.9
Kamphaeng Saen	3,125.98	1.7	1.6

However, at present, the land use of Petchaburi series was used for other purpose such as housing, industry etc. Consequently, the area for rice cultivation of Petchaburi series was decreased. The 60 soil samples were collected from Khao Yoi District and divided into three soil series as follows: 22 samples of Petchaburi series, 33 samples of Samut Prakan series and 5 samples of Kamphaeng Saen series. The physical and chemical properties of three soil series are as following details.

The Petchaburi series, the soil texture consists of sand 23.9 - 66.5 %, silt 6.2 - 32.2 %, clay 9.2 - 51.2 %. The pH value is in the range of 5.5 - 7.4. The electrical conductivity is 0.02 - 0.29 dSm^{-1}. The total organic matter is 2.6 - 1.3 %. The available phosphorus concentration is in the range of 1.1 - 30.4 mg P kg^{-1}. The extractable potassium concentration is 7.9 - 302.0 mg K kg^{-1}. The extractable calcium concentration is 83.3 - 365.1 mg kg^{-1}. The extractable magnesium concentration is 14.3 - 239.2 mg kg^{-1}. The total Fe concentration is 3842.0 - 77340.0 mg kg^{-1} and the available Fe concentration is 129.0 - 1027.0 mg kg^{-1}. The total Zn concentration is

18.2 - 56.1 mg kg^{-1} and the available Zn concentration is 0.4 - 1.7 mg kg^{-1}. The total Mn concentration is 76.0 - 3086.0 mg kg^{-1} and the available Mn concentration is 64.0 - 308.0 mg kg^{-1} as shown in Table 10.

For Samut Prakan series, the soil texture consists of sand 19.9 - 58.5 %, silt 34.2 - 8.2 %, clay 57.2 - 15.2 %. The pH value is in the range of 5.7 - 7.5. The electrical conductivity is 0.02 - 0.18 dSm^{-1}. The total organic matter is 0.9 - 3.1 %. The available phosphorus concentration is in the range of 1.6 - 40.1 mg P kg^{-1}. The extractable potassium concentration is 13.8 - 368.4 mg K kg^{-1}. The extractable calcium concentration is 157.5 - 640.3 mg kg^{-1}. The extractable magnesium concentration is 8.0 - 230.0 mg kg^{-1}. The total Fe concentration is 11565.0 - 64432.0 mg kg^{-1} and the available Fe concentration is 133.0 - 1441.0 mg kg^{-1}. The total Zn concentration is 13.5 - 66.6 mg kg^{-1} and the available Zn concentration is 0.3 - 1.7 mg kg^{-1}. The total Mn concentration is 235.0 - 6226.0 mg kg^{-1} and the available Mn concentration is 36.0 - 333.0 mg kg^{-1} as shown in Table 10.

For Kamphaeng Saen series, the soil texture consists of sand 52.8 - 56.9 %, silt 24.2 - 30.1 %, clay 16.9 - 21.7%. The pH value is in the range of 6.3 - 6.9. The electrical conductivity is 0.02 - 0.15 dSm^{-1}. The total organic matter is 1.2 - 3.3 %. The available phosphorus concentration is in the range of 15.4 - 41.9 mg P kg^{-1}. The extractable potassium concentration is 17.4 - 324.2 mg K kg^{-1}. The extractable calcium concentration is 267.0 - 435.1 mg kg^{-1}. The extractable magnesium concentration is 34.3 - 173.8 mg kg^{-1}. The total Fe concentration is 12972.0 - 50515.0 mg kg^{-1} and the available Fe concentration is 205.0 - 393.0 mg kg^{-1}. The total Zn concentration is 22.9 - 66.0 mg kg^{-1} and the available Zn concentration is 0.5 - 0.8 mg kg^{-1}. The total Mn concentration is 372.0 - 1462.0 mg kg^{-1} and the available Mn concentration is 93.0 - 369.0 mg kg^{-1} as shown in Table 10.

According to three soil series indicated that Kamphaeng Saen series was more fertility soil than Petchaburi and Samut Prakan series, whereas Petchaburi and Samut Prakan series had chemical soil properties which was quite equal to each other. The available Fe concentration in three soil series showed that the available Fe concentration was low as 129.0, 133.0 and 205.0 mg kg^{-1} of Petchaburi, Samut Prakan and Kamphaeng Saen series, respectively. This result indicated that probably Fe in three soil series was fixed in the form of unavailable.

Table 10 Physical and chemical properties of Petchaburi series, Samut Prakarn series and Kamphaeng Saen series in Khao Yoi District, Phetchaburi Province

Soil properties	Soil series		
	Petchaburi	Samut Prakan	Kamphaeng Saen
Sand (%)[1/]	23.9 - 66.5	19.9 - 58.5	52.8 - 56.9
Silt (%)[1/]	6.2 - 32.2	8.2 - 34.2	24.2 - 30.1
Clay (%)[1/]	9.2 - 51.2	15.2 - 57.2	16.9 - 21.7
pH[2/]	5.5 - 7.4	5.7 - 7.5	6.3 - 6.9
EC (dS m^{-1})[3/]	0.02 - 0.29	0.02 - 0.18	0.02 - 0.15
% O.M.[4/]	1.3 - 2.6	0.9 - 3.1	1.2 - 3.3
Available P (mg P kg^{-1})[5/]	1.1 - 30.4	1.6 - 40.1	15.4 - 41.9
Extractable K (mg K kg^{-1})[6/]	7.9 - 302.0	13.8 - 368.4	17.4 - 324.2
Extractable Ca (mg kg^{-1})[6/]	83.3 - 365.1	157.5 - 640.3	267.0 -435.1
Extractable Mg (mg kg^{-1})[6/]	14.3 - 239.2	8.0 - 230.0	34.3 - 173.8
Available Fe (mg kg^{-1})[7/]	129.0 - 1027.0	133.0 - 1441.0	205.0 - 393.0
Available Zn (mg kg^{-1})[7/]	0.4 - 1.7	0.3 - 1.7	0.5 - 0.8
Available Mn (mg kg^{-1})[7/]	308.0 - 64.0	36.0 - 333.0	93.0 - 369.0
Total Fe (mg kg^{-1})[8/]	3842.0 - 77340.0	11565.0 - 64432.0	12972.0 - 50515.0
Total Zn (mg kg^{-1})[8/]	18.2 - 56.1	13.5 - 66.6	22.9 - 66.0
Total Mn (mg kg^{-1})[8/]	76.0 - 3086.0	235.0 - 6226.0	372.0 - 1462.0

[1/] Hydrometer (Day, 1982)

[2/] pH meter (Soil : H_2O; 1:1)

[3/] Electric conductrometer 1:5 H_2O

[4/] Walkley and Black method (Walkley and Black, 1934)

[5/] Bray II method (Bray and Kurtz, 1945)

[6/] NH_4OAc pH 7.0 (Pratt, 1965)

[7/] 0.005 M DTPA pH 7.3 (Lindsay and Norvell, 1978)

[8/] Conc.HNO_3 : Conc.$HClO_4$ (5:2) acid mixture digestion

1.5 Distribution of available Fe concentration in soil and Fe concentration in brown rice, straws and leaves of Suphanburi 1 and Chainat 1

Scatter diagrams showing the relationship between Fe concentration in brown rice and Fe concentration in the soils, Fe concentration in brown rice and Fe concentration in straws, and Fe concentration in leaves and Fe concentration in the straws of both rice cultivars Suphanburi 1 and Chainat 1 are shown in Figures 6 - 8. The linear regression was set up as a procedure of evaluation of the correlation of those relationship as above mentioned.

With the exception of the relationship between Fe concentration in the straws and Fe concentration in the leaves of rice Chainat 1, the linear regression model of evaluation of the correlation of the other relationship were very poor. That because the model were not closely fit the data (r^2) over the whole data set (50% or $r^2 < 0.5$). Whereas, the relationship between Fe concentration in leaves and Fe concentration in the straws of Chainat 1, the model are given fit the data (r^2) nearly 80% ($r^2 = 0.77$) as shown in Figures 6 - 8.

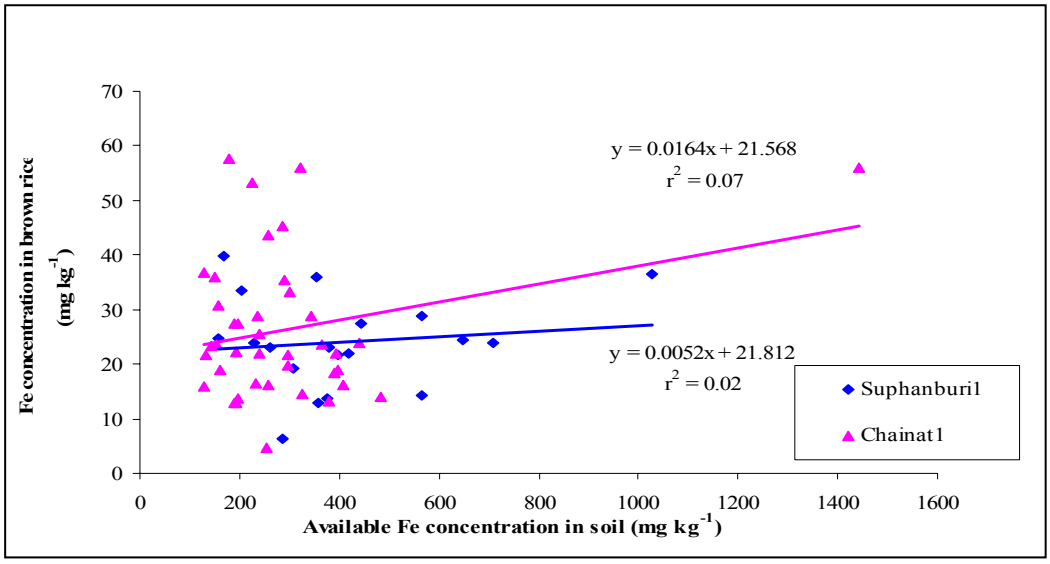

Figure 6 Relationship between available Fe concentration in soils and Fe concentration in brown rice

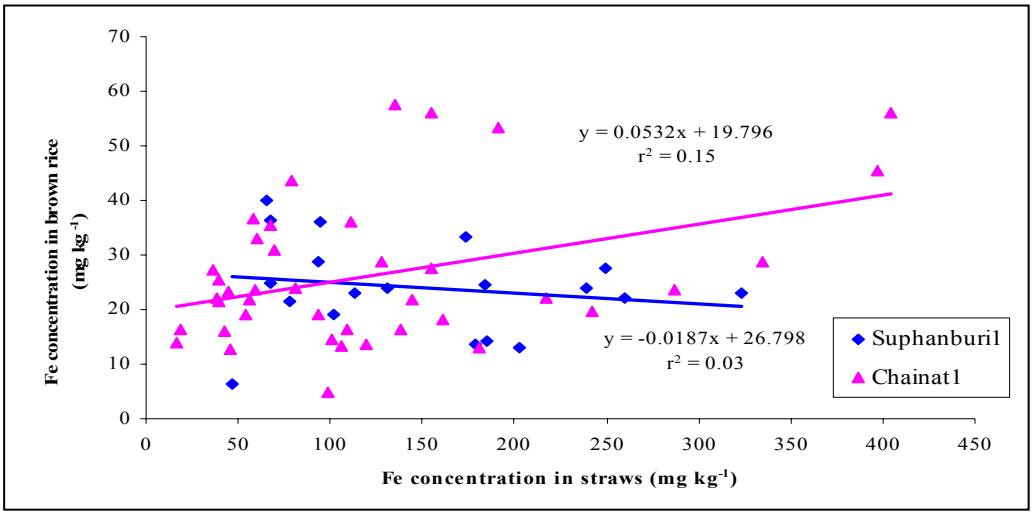

Figure 7 Relationship between Fe concentration in brown rice and Fe concentration in straws

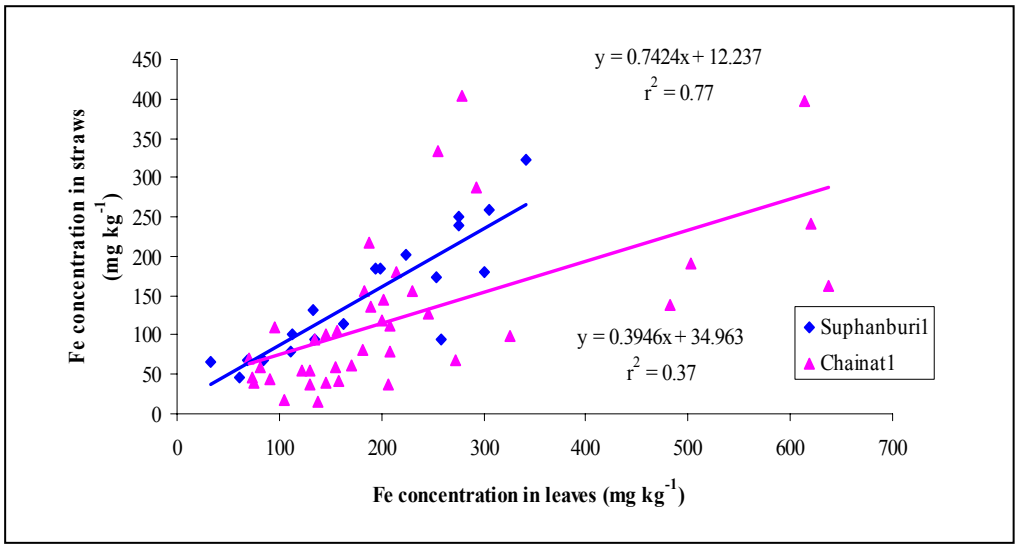

Figure 8 Relationship between Fe concentration in leaves and Fe concentration in straws

By best matching, the study showed that there were no relationship between Fe concentration in the soils and in the brown rice, suggesting that this plant part is not suitable for Fe diagnosis in both rice cultivars. Like Fe concentration in the soils , there was no relationship between Fe concentration in brown rice and Fe

concentration in the straws. The results confirm that Fe within rice part hardly mobiled from the soils to straws to brown rice.

The analysis on relationship of Fe and other elements which inhibited available Fe of the Suphanburi 1 cultivar found that Mn, Zn and P had antagonistic relationship with Fe as shown in Appendix Tables 1 and 2 which agreed to Kabata-Pendias and Pendias (2001) that Mn decreased absorption and translocation of Fe as well as decreased chlorophyll content due to the effect of Fe in producing chloroplast (Osotsapar, 2003). Boardman and McGuire (1990) found that Zn concentration in plant was high then, the symptom of Fe deficiency would appear in young leaves. Owing to similar hydrate ion of Fe and Zn, the relationship between two elements was antagonism for uptake of roots which plant would uptake lower Fe. Moreover, Havlin *et al.* (2005) reported that Fe was also antagonism to Zn translocation in plant. Troeh and Thompson (2005) reported that high phosphorus levels in neutral could similarly reduce iron availability and contribute to iron deficiency. The study found that Fe concentration of Suphanburi 1 was higher in leaves than Chainat1, suggesting that the ability of phloem sap loading and unloading in Suphanburi 1 might be higher than Chainat 1. This might involve with transportation ability in the xylem and phloem of Suphanburi 1 which was higher than Chainat 1. Kaweeta *et al. (*2006) reported that some ions were translocated to leaves in high content and the excess amount would be accumulated in vacuole of leaves such as Fe precipitation as ferric phosphate.

The results as shown above might cause from the different rice cultivars (Türemiş *et al.,* 1997) which agreed to Marschner *et al.* (1986) that the difference of Fe concentration in leaves among the rice cultivars might be caused by different Fe absorption capacities through leaves and roots. These situations were partly related to the localization and binding properties of Fe in leaves. A portion of Fe might be precipitated in the apoplasm of leaves and not be physiologically active (Mengel and Geurtzen, 1988). Due to Fe precipitation in leaves, Fe in rice plants was unable to distribute to rice grains. Therefore, this study found that Chainat 1 had less Fe precipitation in leaves which affected to Fe distribution to rice grains more than Suphanburi 1.

The different Fe concentration in soils may cause equal Fe concentration in brown rice of same rice cultivar. That is because Fe level in soils may be high enough to be toxic. Plant can buffering internal Fe using mechanisms such as ferritin (Briat and Lobreaux, 1997). The amount of Fe buffering capacity must be limited and, at high concentrations, Fe could be detoxified by precipitation in the apoplasm (Becker *et al.*, 1995). It is unlikely that internally precipitated Fe could be remobilized and this may lead to the apparent non - remobilization. The amount of Fe that is remobilized would be the Fe stored in normal physiological forms, whilst that not remobilized would present the precipitated Fe.

Moreover, the different soil series is a factor that cause different Fe concentration in brown rice of same rice cultivar as shown in Figure 9. The study found that rice grown in Samut Prakan series had higher Fe concentration in brown rice than Kamphaeng Saen and Petchaburi series, respectively. It can be suggested that the different physical and chemical soil properties could be synergistic and antagonistic to Fe concentration in brown rice as shown in Appendix Tables 3-5.

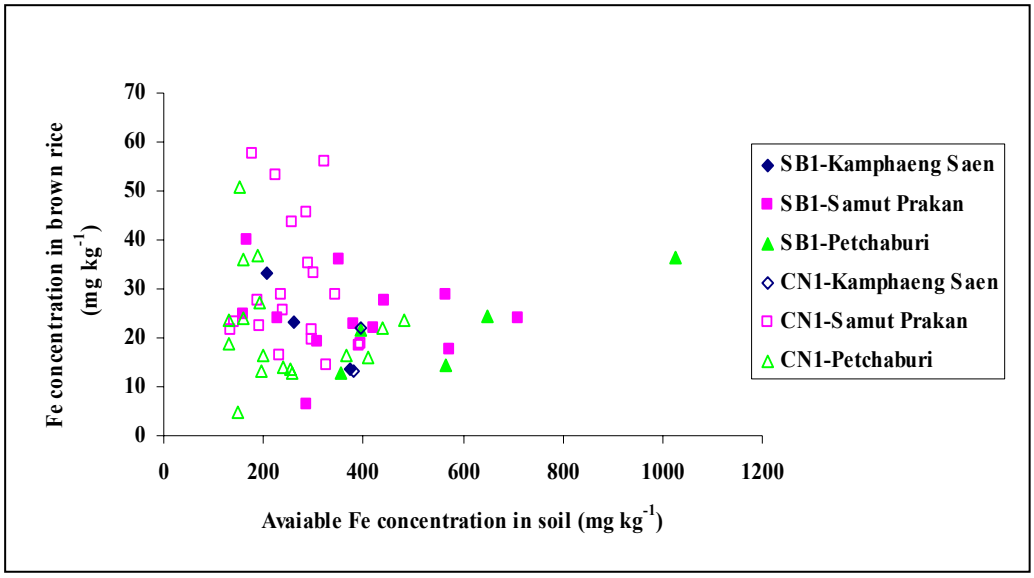

Figure 9 Relationship between available Fe concentration in Samut Prakan series, Kamphaeng Saen series and Petchaburi series and Fe concentration in brown rice of Chainat 1 and Suphanburi 1

1.6 Relationship between available Fe and available Mn concentrations in Petchaburi, Samut Prakan and Kamphaeng Saen series

The samples of three soil series, which consisted of Petchaburi, Samut Prakan and Kamphaeng Saen series, were collected from sampling area in Khao Yoi District, Phetchaburi Province to investigate available Fe and available Mn concentrations. The result shows that there is an interaction between Fe concentration and Mn concentration in three soil series. For Petchaburi series, the analysis of soil series samples indicates that Fe concentration in soil is higher than Mn concentration. The ratio of Fe and Mn is in the range of 0.6 - 8.7. For Samut Prakan series, the result shows that Fe concentration is higher than Mn concentration. The ratio of Fe and Mn is in the range of 1.08 - 5.69. For Kamphaeng Saen series, the result is similar to Petchaburi and Samut Prakan series that Fe concentration in soil is higher than Mn concentration. The ratio of Fe and Mn is in the range of 1.01 - 2.14.

The result could conclude that the available Fe concentration of three soil series was higher than the available Mn concentration. The study of three soil series found that their pH values were moderately acid to slightly alkaline salt which it affected to the solubility and availability of Fe in soil as Troeh and Thompson (2005) found that most of Fe contained in igneous rocks was in the ferrous (Fe^{2+}) form. Fe in waterlogged soils tended to remain in this form and contributed to the bluish-gray colors that indicated wetness. Many compounds of ferrous Fe compounds were even less soluble. The solubility of ferric and ferrous Fe was much lower at high pH than at low pH. While Foth and Ellis (1996) found that the soil conditions which led to Fe deficiency in plants included pH above 7.0. Moreover, the available phosphorus value was during low to high which could affect to the Fe availability in soil as Brown and Jones (1975) found that the high phosphorus levels in neutral or alkaline soils could similarly reduce Fe availability and contributed to Fe deficiency. For Mn availability, since Mn solubility was related to oxidation - reduction reactions in the soil, so the availability of Mn was closely related to weather. Cool temperatures might slow down the mineralization of organic Mn. Thus, high levels of available Fe in organic soils or high levels of organic matter in sand might lead to Mn deficiency because of the high ratio of Fe to Mn within the plants. This ratio was particularly

important since certain chelated Mn carriers will actually make the situation worse rather than correcting Mn deficiency (Foth and Ellis, 1996). With consideration of Fe / Mn ratio, it indicated that Petchaburi series had the lowest ratio as 0.6. Subsequently, this soil series would rather be risk on Fe deficient than Samut Prakan and Kamphaeng Saen series. The reason was probably supported by Kabata - Pendias and Pendias (2001) reported that Mn - Fe antagonism was widely known and observed mainly in acidic soils that contained large amounts of available Mn. In general, Fe and Mn were interrelated in their metabolic functions, and their appropriate proportion (the Fe / Mn ratio should range from 1.5 to 2.5). Below this range, the symptoms of Mn toxicity and Fe deficiency might occur and above 2.5, toxic effects of Fe, associated with Mn deficiency, would be observed.

1.7 Relationship between Fe and Mn concentrations in each plant part of Suphanburi 1 and Chainat 1

The 60 samples of rice, which were Suphanburi 1 and Chainat 1, were collected from the same sampling area of soil in Khao Yoi District, Phetchaburi Province. All samples were separated into brown rice, leaves and straws for investigating Fe and Mn concentrations. The Fe and Mn concentrations of the Suphanburi 1 and Chainat 1 cultivars are shown as Table 11.

Table 11 Fe and Mn concentrations in brown rice, leaves and straws of Suphanburi 1 and Chainat1

Plant part	Suphanburi 1		Chainat 1	
	concentration (mg kg^{-1})		concentration (mg kg^{-1})	
	Fe	Mn	Fe	Mn
brown rice	6.42 - 39.88	24.58 - 86.20	4.79 - 57.70	18.79 - 74.50
Leaves	32.88 - 670.83	97.75 - 550.80	70.17 - 637.63	25.88 - 566.04
Straws	10.81 - 323.25	195.56 - 577.06	16.44 - 404.19	206.75 - 533.00

For Suphanburi 1, the result indicates that Mn concentration is higher than Fe concentration in all plant parts. The order of Mn concentration from high to low is straws, leaves and brown rice as 195.56 - 577.06, 97.75 - 550.80 and 24.58 - 86.20 mg kg^{-1}, respectively. Fe concentration is in order of leaves, straws and brown rice as 32.88 - 670.83, 10.81 - 323.25 and 6.42 - 39.88 mg kg^{-1}, respectively. For Chainat 1, the result shows that Mn concentration tends to be higher than Fe concentration in plant parts especially in brown rice and straws, whereas Fe concentration is higher than Mn concentration in leaves. The order of Mn concentration from high to low is straws, leaves and brown rice as 206.75 - 533.00, 25.88 - 566.04 and 18.79 - 74.50 mg kg^{-1}, respectively. Fe concentration is in order of leaves, straws and brown rice as 70.17 - 637.63, 16.44 - 404.19 and 4.79 - 57.70 mg kg^{-1}, respectively. Among two rice cultivars, the analysis presents that Fe and Mn concentrations are quite high in leaves and straws; on the contrary, brown rice are found to have lowest Fe and Mn concentrations.

From the result of Fe concentration in plant parts as shown in Table 9, the finding might be due to Fe stored up in leaves. Normally, 80 % of Fe in green leaves was in chloroplast whether the plant had sufficient Fe or Fe deficiency. Fe stored up in plastid of stroma was namely phytoferritin that Fe could be kept in ferric form or ferric phosphate. However, the phytoferritin could not be found in chloroplast when the plant appeared Fe deficiency, but the plant would suddenly store Fe when Fe was given to leaves (Lobreaux *et al.,* 1992). Rice appeared to be very inefficient in transporting Fe to grains because there were only 4 % of total shoot Fe which was found in the grains (Marr *et al.,* 1995). This could indicate that rice had poor Fe remobilization. According to the high accumulation of Fe in leaves as mentioned above, the leaves might be used as an index in indicating the sufficient, deficient or toxic range by depend on the period of plant growth. Due to being the immobile element of Fe, the symptoms of toxicity or deficiency mostly appeared at young leaves (Osotsapar, 2003). In general, the optimum range of tillering stage was during 100 - 150 mg kg^{-1} and the critical range was higher than 300 - 500 mg kg^{-1} (Dobermann and Fairhurst, 2000). For Fe concentration in grains, the result showed that Fe concentration was lowest as compared to other plant parts. The amount of Fe concentration in grains would depend on the amount taken up by the root during

grains development and the amount redistributed to the grains from vegetative tissue via phloem. Fe was described as having intermediate phloem mobility (Kochian, 1991).

For Mn concentration, Mn was mostly accumulated in the leaves, though a small fraction was incorporated into proteins with superoxide dismutase activity (Lidon, 2001). Moreover, most of grains content at maturity could be accounted by Mn which entered the shoots after anthesis. Whether this was from uptake to plant or remobilization from root, normally, the optimum range of leaf at tillering stage was during 40 - 700 mg kg^{-1} (Dobermann and Fairhurst, 2000). The Mn toxic symptoms were associated with brown spots on mature leaves (Wissemeier and Horst, 1992) interveinal chlorosis and necrosis, deformation of young leaves, growth retardation (Foy *et al.,* 1978, 1988).

Moreover, the study found that there was an interaction between Fe and Mn concentrations in all plant parts of two rice cultivars. The result showed that Mn concentration of Suphanburi 1 and Chainat 1 was higher than Fe concentration in straws, leaves and rice grains, respectively. This higher Mn concentration could reduce Fe accumulation and availability in all plant parts (Tiffin, 1967) and also interfere with the transportation of Fe from the roots to the shoots (Epstein and Stout, 1951). Consequently, the plants were grown with chlorosis symptoms due to Fe deficiency. In general, the chlorotic plants were found in acidic soil that contained large amounts of available Mn (Somers & Shive, 1942). However, this interaction actually suggested that Mn might compete with Fe at the absorption step which was significantly correlated with rice cultivar and soil properties. The result of analysis was observed that Fe concentration of Chainat 1 was higher than Mn concentration in leaves, whereas this result was not found in Suphanburi 1. That could indicate that rice cultivar was one of significant factors for determined Fe and Mn concentrations in plants.

2. Experiment 2: Effect of Rice Cultivation and Fe Supply on Rice Growth: Grown in Hydroponics Experiment Filled with Fe-EDTA at 28 days

The five rice cultivars, which composed of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3, were selected to investigate the effect of rice cultivation in hydroponics system that was applied with Fe-EDTA. As being the main rice cultivars planted in Khaoyoi District, Phetchaburi Province, Chainat 1 and Suphanburi 1 were also selected for this study, whereas other rice cultivars, Pathumthani 1, RD 23 and Suphanburi 3, were selected because they has been promoted for cultivation by Department of Agricultural Extension. This hydroponics experiment was divided into 4 treatments according to different levels of Fe-EDTA supply which were 10 μM (T1), 50 μM (T2), 100 μM (T3) and 150 μM (T4). The results of the experiment are presented as following details.

2.1 Growth of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 after transplanting at 28 days

2.1.1 Height of rice

The statistical analysis showed that the rice cultivars affected to the height of rice which the values showed the highly significant difference ($P \leq 0.05$). It is evident that the Chainat 1 cultivar gives the highest height as 62.00 cm whereas the Suphanburi 1 cultivar gives the lowest height as 53.30 cm. The average height of other rice cultivars from high to low is RD 23, Suphanburi 3 and Pathumthani 1 as 61.40, 61.20 and 57.80 cm, respectively.

The Fe concentration supply seemed to have the effect on the height of rice. However, the height of rice in all five rice cultivars was not significantly different (Figure 10). The average of the height of rice plants is 56.20, 61.10, 59.90 and 59.30 at the Fe supply as 10 μM (T1), 50 μM (T2), 100 μM (T3) and 150 μM (T4) of Fe-EDTA, respectively (Appendix Table 6).

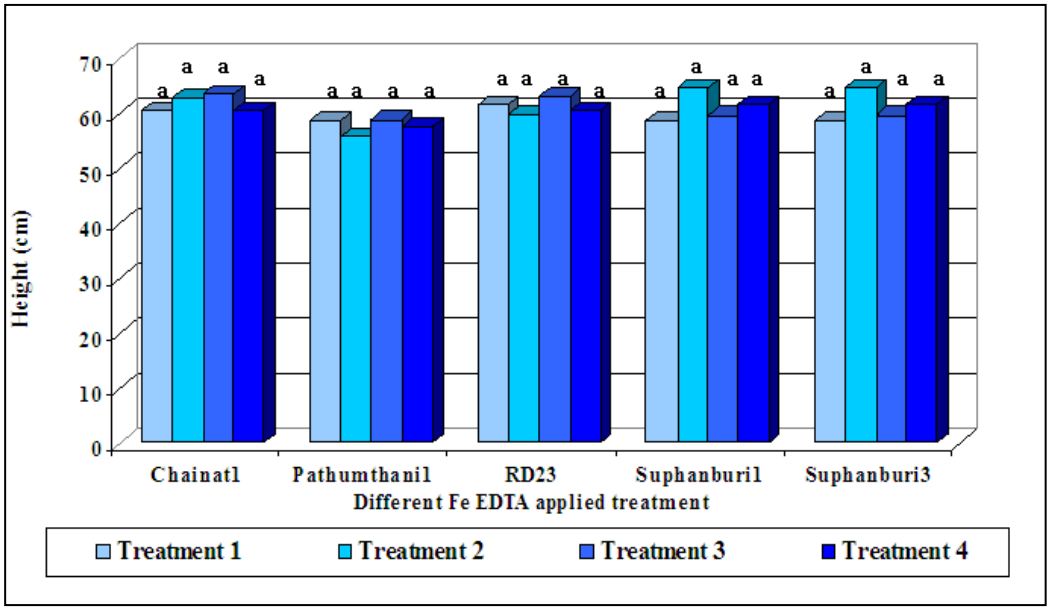

Figure 10 Height of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe-EDTA applied treatment at 28 days after transplanting

2.1.2 Dry weight of leaves, straws and roots

a. Dry weight of rice leaves

The statistic analysis showed that the rice cultivars affected to the dry weight of rice leaves which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 28 days found that the rice leaves of RD 23 have the highest average dry weight as 1.39 g whereas the rice leaves of Suphanburi 1 have the lowest average dry weight as 0.66 g. The average dry weight of other rice cultivars from high to low is Chainat 1, Suphanburi 3 and Pathumthani 1 as 1.25, 0.89 and 0.82 g, respectively.

According to the height of all rice cultivars, the Fe concentration supply tended to increase the dry weight of rice leaves but the values were not significantly different (Figure 11). The average dry weight of leave at 10 µM (T1), 50 µM (T2), 100 µM (T3) and 150 µM (T4) of Fe-EDTA is 0.87, 1.09, 1.05 and 1.00 g, respectively (Appendix Table 7).

b. Dry weight of rice straws

The statistic analysis showed that the rice cultivars affected to the dry weight of rice straws which the values showed the significant difference ($P \leq 0.05$). The analysis after transplanting at 28 days found that the rice straws of RD 23 have the highest average dry weight as 0.85 g whereas the rice straws of Suphanburi 1 have the lowest average dry weight as 0.48 g. The average dry weight of other rice cultivars from high to low is Chainat 1, Suphanburi 3 and Pathumthani 1 as 0.81, 0.63 and 0.59 g, respectively.

According to the height of all rice cultivars, the Fe concentration supply tended to increase the dry weight of rice straws but the values were not significantly different (Figure 11). At 10 μM of Fe-EDTA supply, the average of dry weight of rice straws is lowest as 0.57 g. As the Fe-EDTA supply from 50 to 150 μM, the average of dry weight of rice is nearly equal as 0.72, 0.71 and 0.70 g at 50 μM (T2), 100 μM (T3) and 150 μM (T4) of Fe-EDTA, respectively (Appendix Table 8).

c. Dry weight of rice roots

The statistic analysis showed that the rice cultivars affected to the dry weight of rice roots which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 28 days found that the rice straws of RD 23 have the highest average dry weight as 0.29 g whereas the rice straws of Suphanburi 1 have the lowest average dry weight as 0.11 g. The average dry weight of other rice cultivars from high to low is Chainat 1, Suphanburi 3 and Pathumthani 1 as 0.22, 0.14 and 0.13 g, respectively.

According to the height of all rice cultivars, the Fe concentration supply tended to increase the dry weight of rice roots but the values was not significantly different (Figure 11). The average dry weight of roots at 10 μM (T1), 50 μM (T2), 100 μM (T3) and 150 μM (T4) of Fe-EDTA was about 0.15, 0.18, 0.16 and 0.22 g, respectively (Appendix Table 9).

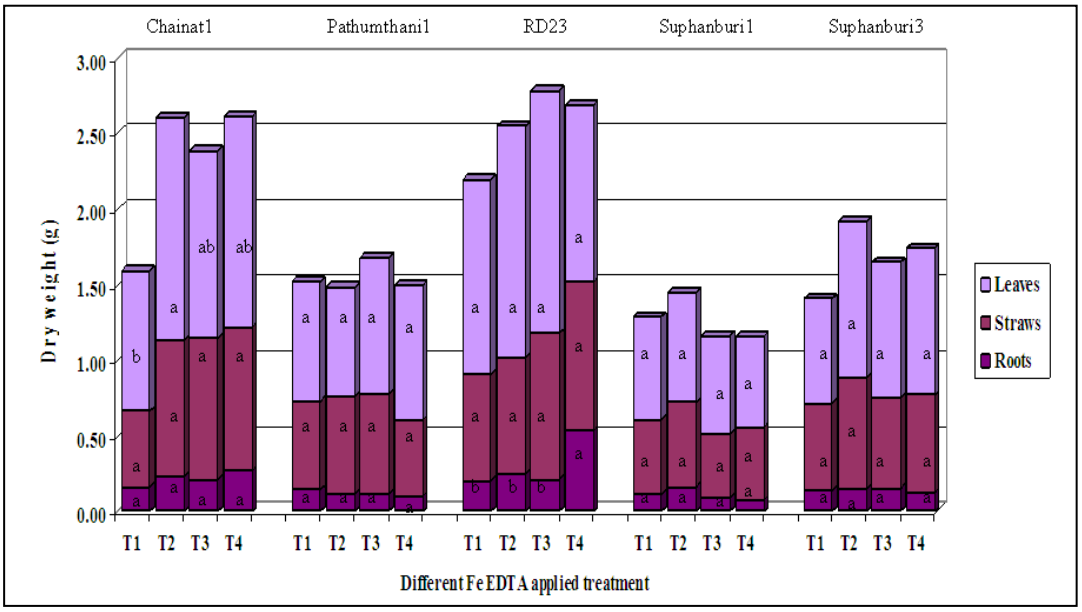

Figure 11 Dry weight of leaves, straws and roots of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe-EDTA applied treatment at 28 days after transplanting

2.1.3 Chlorosis and toxic symptoms of five rice cultivars

As shown in Table 12, it presented the chlorosis symptom of rice growth period being effected from the application of Fe-EDTA solution at four levels consisting of 10 µM (T1), 50 µM (T2) 100 µM (T3) and 150 µM (T4). The appearance of symptoms in rice was observed in every 7 days of 28 days after transplanting. The chlorosis and toxic symptom of rice in all five cultivars were made by scoring as shown in Table 12. In all five rice cultivars applied with Fe-EDTA at the rate of 10 µM, the chlorosis symptom is appeared at level 3. At the application of Fe-EDTA as 50 µM, the chlorosis symptom of Suphanburi 3 is appeared at level 2, whereas other rice cultivars are at level 1. As the Fe-EDTA supply up to 100 µM, most of rice cultivars do not show the chlorosis symptom with the exception of RD 23 appearing the toxic symptom. At the highest Fe-EDTA supply (150 µM), all rice cultivars are toxic. The leaves of the rice are typically blast that the small brown spots are appeared on the lower leaves starting at the tips. After that, the lower leaves turn brown and die as shown on Figure 12.

Table 12 Chlorosis and toxic symptoms of five rice cultivars applied with the different Fe-EDTA levels at 28 days after transplanting

Treatment	Chlorosis and Toxic symptoms				
	Chainat1	Pathumthani1	RD23	Suphanburi1	Suphanburi3
Fe 10µM	3	3	3	3	3
Fe 50µM	1	1	1	1	2
Fe 100µM	0	0	blast	0	0
Fe 150µM	blast	blast	blast	blast	blast

score	level of chlorosis	Blast = toxic
0	non chlorosis	
1	mild	
2	moderate	
3	severe	

Figure 12 Toxicity symptom of rice leave

2.2 Fe concentration in leaves, straws and roots of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 at 28 days after transplanting

2.2.1 Fe concentration in rice leaves

The statistic analysis showed that the rice cultivars did not affect to Fe concentration in rice leaves because the values did not present the significant difference. The average Fe concentration of five rice cultivars from high to low is RD

23, Pathumthani 1, Chainat 1, Suphanburi 1 and Suphanburi 3 as 209.90, 183.50, 181.50, 173.10 and 168.30 mg kg^{-1}, respectively.

The Fe supply affected to Fe concentration in rice leaves which the values showed the highly significant difference ($P \leq 0.01$) as shown in Figure 13. The analysis after transplanting at 28 days found that the rice leaves have the highest average Fe concentration as 234.69 mg kg^{-1} with the Fe supply at 150 μM (T4), whereas the rice leaves have the lowest average Fe concentration as 106.56 mg kg^{-1} with the Fe supply at 10 μM (T1). The average Fe concentration of other treatments from high to low is 100 μM (T3) and 50 μM (T2) as 222.76 and 162.29 mg kg^{-1}, respectively.

2.2.2 Fe concentration in rice straws

The statistic analysis showed that the rice cultivars affected to Fe concentration in rice straws which the values showed the highly significant difference ($P \leq 0.05$). The analysis after transplanting at 28 days found that Suphanburi 3 has the highest average Fe concentration in rice straws as 202.20 mg kg^{-1} whereas Pathumthani 1 has the lowest average Fe concentration as 104.29 mg kg^{-1}. The average Fe concentration of other rice cultivars from high to low is Chainat 1, Suphanburi 1 and RD 23 as 111.53, 111.34 and 105.03 mg kg^{-1}, respectively.

The Fe supply did not have the effect on Fe concentration in rice straws of all five rice cultivars because the values did not show the significant difference. The average of the Fe concentration in rice straws from high to low is 158.57, 136.17, 126.95 and 85.82 mg kg^{-1} at the Fe supply as 150 μM (T4), 100 μM (T3), 10 μM (T1) and 50 μM (T2) of Fe-EDTA, respectively.

There is a highly significant interaction ($P \leq 0.05$) between rice cultivars and Fe supply on Fe concentration in rice straws as shown in Figure 13. For the Chainat 1, Pathumthani 1 and RD 23 cultivars, Fe concentration in straws increases with the increasing Fe supply. That is, Fe concentration in straws is highest when the Fe supply at 10 μM (T1) is given and the lowest is shown with the Fe supply

is given at 150 μM (T4). On the contrary, Fe concentration in straws of Suphanburi 1 and Suphanburi 3 is highest when the Fe supply at 10 μM (T1) is given and the lowest is shown with the Fe supply is given at 50 μM (T2) as shown in Figure 13.

2.2.3 Fe concentration in rice roots

The statistic analysis showed that the rice cultivars affected to the Fe concentration in rice roots which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 28 days found that RD 23 has the highest average Fe concentration in rice roots as 577.04 mg kg^{-1} whereas Suphanburi 3 has the lowest average Fe concentration as 332.71 mg kg^{-1}. The average Fe concentration of other rice cultivars from high to low is Pathumthani 1, Chainat 1 and Suphanburi 1 as 427.83, 394.33 and 361.29 mg kg^{-1}, respectively.

The Fe supply also affected to Fe concentration in rice roots which the values showed the highly significant difference ($P \leq 0.01$) as shown in Figure 13. The analysis after transplanting at 28 days found that the rice roots have the highest average Fe concentration as 651.70 mg kg^{-1} with the Fe supply at 100 μM (T3), whereas the rice roots have the lowest average Fe concentration as 123.27 mg kg^{-1} with the Fe supply at 10 μM (T1). The average Fe concentration of other treatments from high to low is 150 μM (T4) and 50 μM (T2) as 629.27 and 270.33 mg kg^{-1}, respectively.

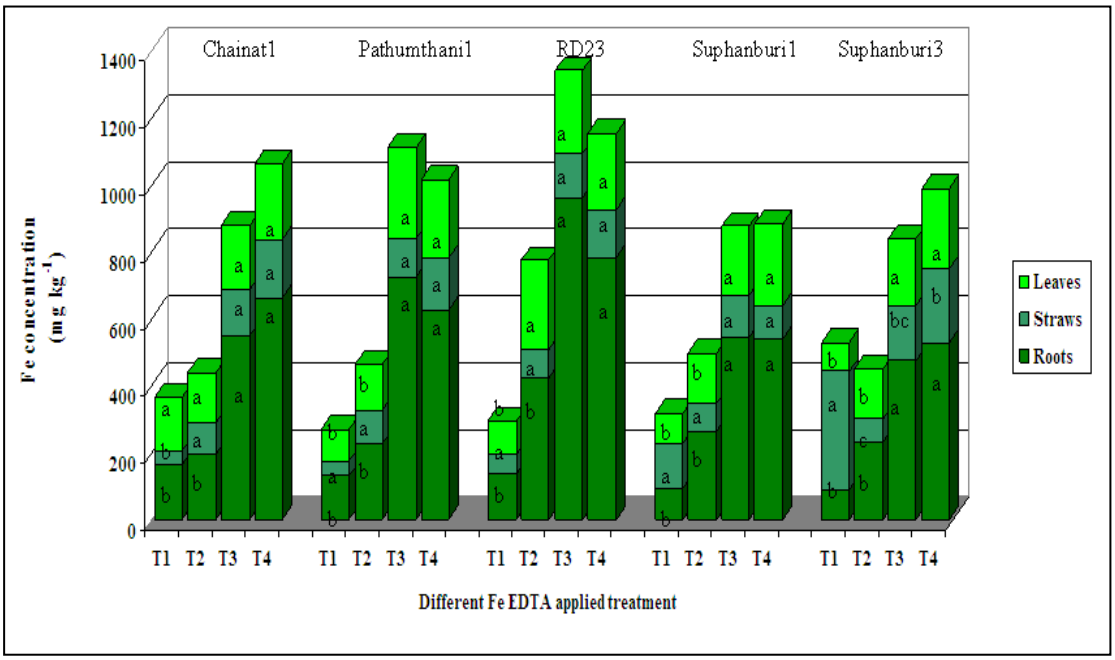

Figure 13 Fe concentration in leaves, straws and roots of 5 rice cultivars grown in nutrient solution as affected by Fe-EDTA 10 µM (T1) Fe-EDTA 50 µM (T2), Fe-EDTA 100 µM (T3) and Fe-EDTA 150 µM (T4) at 28 days after transplanting

According to the data of Fe concentration in leaves, straws and roots of five rice cultivars as mentioned above, Fe concentration in leaves tissue of tested cultivars is ranged from 87.95 mg kg^{-1} to 162 mg kg^{-1}, 135.20 mg kg^{-1} to 266.79 mg kg^{-1}, 189.45 mg kg^{-1} to 272.41 mg kg^{-1} and 225.20 mg kg^{-1} to 265 mg kg^{-1} in the treatment of Fe-EDTA supply at 10, 50, 100, 150 µM, respectively. Fe concentration of straw tissue is ranged from 41 mg kg^{-1} to 353.12 mg kg^{-1}, 68.25 mg kg^{-1} to 102.08 mg kg^{-1}, 126.66 mg kg^{-1} to 167.62 mg kg^{-1} and 100.45 mg kg^{-1} to 219.79 mg kg^{-1} in the treatment of Fe-EDTA supply at 10, 50, 100, 150 µM, respectively. Fe concentration of roots tissue is ranged from 90.16 mg kg^{-1} to 164.83 mg kg^{-1}, 169.16 mg kg^{-1} to 425.33 mg kg^{-1}, 474.67 mg kg^{-1} to 963.33 mg kg^{-1} and 530.16 mg kg^{-1} to 783.50 mg kg^{-1} in the treatment of Fe-EDTA supply at 10, 50, 100, 150 µM, respectively.

Fe concentration in leaves, straws and roots of Chainat 1 significantly increased with increasing Fe level in nutrient solution. For Suphanburi 1 and Suphanburi 3, Fe concentration in leaves and straws significantly increased with increasing Fe level, whereas Fe concentration of Pathumthani 1 and RD 23 increased significantly only in straws. The RD 23 cultivar had the highest Fe concentration in roots at Fe level of 100 μM (963.33 mg kg^{-1}) when compared to all rice cultivars and treatments. In contrary, Chainat 1 had the lowest Fe concentration in straws at Fe level of 10 μM (41.00 mg kg^{-1}) when compared to all rice cultivars and treatments. On the average, Fe concentration in roots of all rice cultivars was higher than in leaves and straws.

2.3 Fe uptake and accumulation in leaves, straws and roots of Chainat1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 at 28 days after transplanting

2.3.1 Fe uptake and accumulation in rice leaves

The statistic analysis showed that the rice cultivars affected to Fe uptake and accumulation in rice leaves which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 28 days found that RD 23 has the highest average Fe uptake and accumulation in rice leaves as 0.2851 mg dw whereas Suphanburi 1 has the lowest average Fe uptake and accumulation as 0.0002 mg dw. The average Fe uptake and accumulation of cultivars from high to low is Chainat 1, Suphanburi 3 and Pathumthani 1 as 0.0011, 0.0005 and 0.0004 mg dw, respectively.

The Fe supply also affected to Fe uptake and accumulation in rice leaves which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 28 days found that the rice leaves have the highest average Fe uptake and accumulation as 0.2374 mg dw with the Fe supply at 100 μM (T3), whereas the rice leaves have the lowest average Fe concentration as 0.0985 mg dw with the Fe supply at 10 μM (T1). The average Fe concentration of other treatments from high to low is 150 μM (T4) and 50 μM (T2) as 0.2317 and 0.1923 mg dw, respectively.

There is a highly significant interaction ($P \leq 0.05$) between rice cultivars and Fe supply on Fe uptake and accumulation in rice leaves as shown in Figure 14. The Fe content in leaves of Chainat 1, Suphanburi 1 and Suphanburi 3 increases as the Fe-EDTA supply increases. That is, the Fe content of three rice cultivars is highest when the Fe supply is given at 150 μM (T4) and the lowest is shown when the Fe supply is given at 10 μM (T1). However, for Pathumthani 1 and RD 23, the Fe content in leaves is lowest at the Fe supply as 10 μM (T1) but the Fe content decreases with the Fe supply at 150 μM (T4) for Pathumthani 1, whereas the Fe content of RD 23 decreases with the Fe supply at 100 μM (T3).

2.3.2 Fe uptake and accumulation in rice straws

The statistic analysis showed that the rice cultivars affected to Fe uptake and accumulation in rice straws which the values showed the highly significant difference ($P \leq 0.01$) as shown in Figure 14 . The analysis after transplanting at 28 days found that Suphanburi 3 has the highest average Fe uptake and accumulation in rice straws as 0.1263 mg dw whereas Suphanburi 1 has the lowest average Fe uptake and accumulation as 0.0487 mg dw. The average Fe uptake and accumulation of other rice cultivars from high to low is RD 23, Chainat 1 and Pathumthani 1 as 0.0931, 0.0877 and 0.0629 mg dw, respectively.

On the contrary, the Fe supply did not have the effect on Fe uptake and accumulation in rice straws because the values did not show the significant difference. The average of Fe uptake and accumulation in rice straws from high to low is 0.10565 0.0946, 0.0701 and 0.0647 mg dw at the Fe supply as 150 μM (T4), 100 μM (T3), 10 μM (T1) and 50 μM (T2) of Fe-EDTA, respectively.

2.3.3 Fe uptake and accumulation in rice roots

The statistic analysis showed that the rice cultivars affected to Fe uptake and accumulation in rice roots which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 28 days found that RD 23 has the highest average Fe uptake and accumulation in rice roots as 0.1856 mg dw

whereas Suphanburi 1 has the lowest average Fe uptake and accumulation as 0.0391 mg dw. The average Fe uptake and accumulation of other rice cultivars from high to low is Chainat 1, Pathumthani 1 and Suphanburi 3 as 0.0920, 0.0596 and 0.0456 mg dw, respectively.

Moreover, the Fe supply also affected to Fe uptake and accumulation in rice roots which the values showed the highly significant difference ($P \leq 0.01$) as shown in Figure 14. The analysis after transplanting at 28 days found that the rice roots have the highest average Fe uptake and accumulation as 0.1542 mg dw with the Fe supply at 150 µM (T4), whereas the rice leaves have the lowest average Fe concentration as 0.0193 mg dw with the Fe supply at 10 µM (T1). The average Fe concentration of other treatments from high to low is 100 µM (T3) and 50 µM (T2) as 0.1145 and 0.0193 mg dw, respectively.

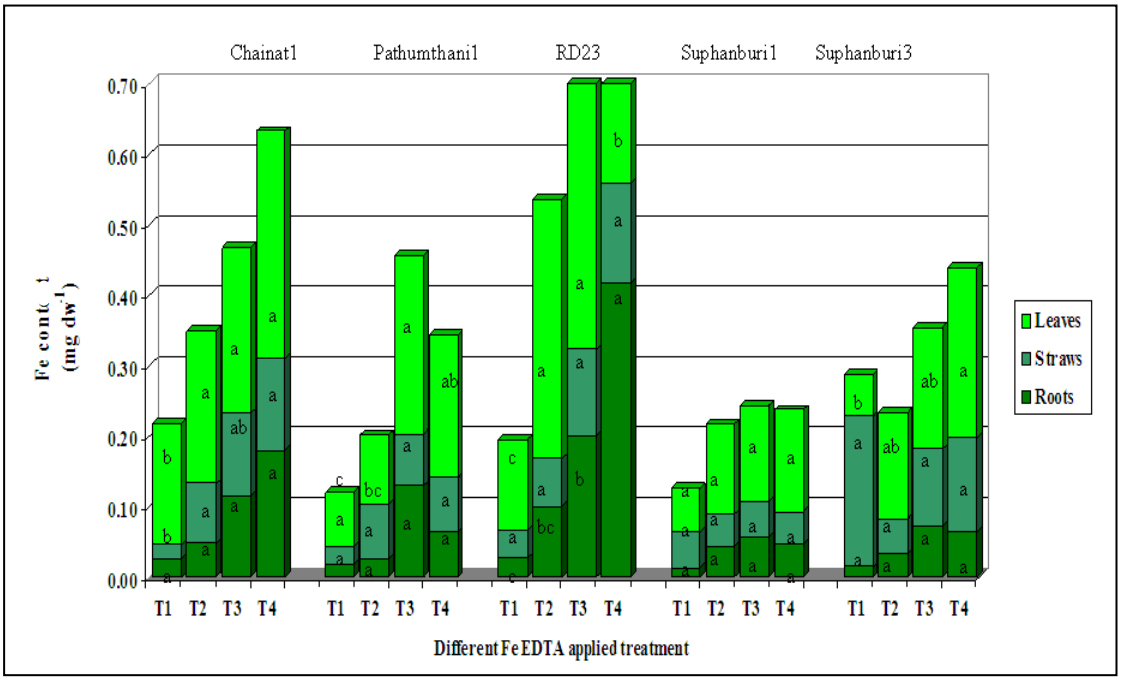

Figure 14 Fe uptake and accumulation in leaves, straws and roots of 5 rice cultivars grown in nutrient solution as affected by Fe-EDTA 10 µM (T1) Fe-EDTA 50 µM (T2), Fe-EDTA 100 µM (T3) and Fe-EDTA 150 µM (T4) at 28 days after transplanting

From the result of analysis to investigate Fe uptake and accumulation in plant parts of the five rice cultivars which were applied with the different Fe-EDTA levels, the study could conclude that Fe uptake and accumulation of each parts of rice were different according to rice cultivars and levels of Fe-EDTA application. For the Chainat 1, RD 23 and Suphanburi 3 cultivars, Fe uptake and accumulation increased continuously from treatment 1 to treatment 4, whereas Fe uptake and accumulation of the Pathumthani 1 and Suphanburi 1 cultivars tended to decrease in treatment 4. As shown in Figure 13, it was observed distinctly that Fe uptake and accumulation of RD 23 was rather high in all treatments as compared to other rice cultivars. Moreover, Fe was mostly uptake and accumulated in leaves of all five rice cultivars at all treatments as compared to straws and roots except the treatment 4 of RD 23 which Fe was highly uptake and accumulated in roots. Therefore, when considering from the statistic analysis, the rice cultivars was an important factor which affected to Fe uptake and accumulation of rice leaves, straws and roots.

In this study, Fe concentration and uptake of five rice cultivars grown in hydroponics solution was significantly changed according to the condition of variable cultivars at all treatments. From the comparison of Fe concentration in leaves, straws and roots, the result showed the highest Fe concentration in roots was due to the ability of roots to reduce Fe^{3+} to Fe^{2+}. At normal soil pH levels, Fe organic complexes apparently played an important role in plant nutrition. With Fe deficiencies, roots of cereal plants released mugineic acid that were effective in mobilizing Fe and other trace metals, even from calcareous soils (Furr *et al.,* 1976; Osotsapar, 2003). The separation of chelated Fe prior to the absorption step appeared to require reduction of Fe^{3+} to Fe^{2+} at the surface of the root.

From considering Fe content of whole rice among five rice cultivars, the result of this experiment can conclude as following details.

The Chainat 1 cultivar has the highest Fe content in all plant parts with Fe-EDTA supply at 150 (T4) μM. The Fe content tends to increase when Fe-EDTA is applied at 10 (T1) μM, 50 (T2) μM, 100 (T3) μM and 150 (T4) μM,

respectively. However, the Fe chlorosis symptom appears at level 3 and level 1 with the application of Fe-EDTA at 10 (T1) μM and 50 (T2) μM, respectively, whereas the Fe toxicity appears when Fe-EDTA is applied at 150 (T4) μM. Therefore, from the consideration of height, dry weight, chlorosis symptom, toxicity and Fe content, the appropriate Fe-EDTA level is 100 (T3) μM.

The Pathumthani 1 cultivar has the highest Fe content in all plant parts with Fe-EDTA supply at 100 (T3) μM. The Fe content tends to increase when Fe-EDTA is applied at 10 (T1) μM, 50 (T2) μM and 100 (T3) μM, respectively, but the Fe content decreases with Fe-EDTA supply at 150 (T4) μM. Moreover, the Fe chlorosis symptom appears at level 3 and 1 with the application of Fe-EDTA at 10 (T1) μM and 50 (T2) μM, respectively, whereas the Fe toxic symptom is observed when Fe-EDTA is applied at 150 (T4) μM. Therefore, from the consideration of height, dry weight, chlorosis symptom, toxicity and Fe content, the appropriate Fe-EDTA level is 100 (T3) μM.

The RD 23 cultivar has the highest Fe content at all plant parts with Fe-EDTA supply at 100 (T3) μM and 150 (T4) μM. The Fe content tends to increase when Fe-EDTA is applied at 10 (T1) μM, 50 (T2) μM, 100 (T3) μM and 150 (T4) μM, respectively. However, the Fe chlorosis symptom appears at level 3 and 1 with the application of Fe-EDTA at 10 (T1) μM and 50 (T2) μM, respectively, whereas the Fe toxicity appears with Fe-EDTA supply at 100 (T3) μM and 150 (T4) μM. Therefore, from the consideration of height, dry weight, chlorosis symptom, toxicity and Fe content, the appropriate Fe-EDTA level is in the range of 50 μM to less than 100 μM.

The Suphanburi 1 cultivar has the highest Fe content at all plant parts with Fe-EDTA supply at 100 (T3) μM. The Fe content tends to increase when Fe-EDTA is applied at 10 (T1) μM, 50 (T2) μM and 100 (T3) μM, respectively but the Fe content decreases with Fe-EDTA supply at 150 (T4) μM. Moreover, the Fe chlorosis symptom is observed at level 3 and 1 with the application of Fe-EDTA at 10 (T1) μM and 50 (T2) μM, whereas the Fe toxic symptom appears when Fe-EDTA is applied at 150 (T4) μM. Therefore, from the consideration of height, dry weight,

chlorosis symptom, toxicity and Fe content, the appropriate Fe-EDTA level is in the range of 50 μM to less than 100 μM.

The Suphanburi 3 cultivar has the highest Fe content of all plant parts with Fe-EDTA supply at 150 (T4) μM. The Fe content decreases when Fe-EDTA is applied at 50 (T2) μM and tends to increase when Fe-EDTA is applied at 100 (T3) μM and 150 (T4) μM, respectively. Moreover, the Fe chlorosis symptom appears at level 3 and 2 with the application at Fe-EDTA at 10 (T1) μM and 50 (T2) μM, respectively, whereas the Fe toxicity appears with Fe-EDTA supply at 150 (T4) μM. Therefore, from the consideration of height, dry weight, chlorosis symptom, toxicity and Fe content, the appropriate Fe-EDTA level is in the range of 50 μM to less than 100 μM.

3. Experiment 3: Effect of Rice Cultivation and Fe Supply on Rice Growth: Grown in Hydroponics Experiment Filled with Fe-EDTA at 45 days

Due to the reproductive phase of rice, this experiment was designed to investigate the effect of rice cultivation at 45 days in hydroponics system that was applied with the different levels of Fe-EDTA solution. The five rice cultivars as planted in experiment 2 were selected and analyzed. There were five treatments according to the different levels of Fe-EDTA solution which consisted of 50 μM (T1), 75 μM (T2), 100 μM (T3), 125 μM (T4) and 150 μM (T5), which were appropriate levels from the experiment 2. The results are presented as following details.

3.1 Growth of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 after transplanting at 45 days

3.1.1 Height of rice

The statistical analysis showed that the rice cultivars affected to the height of rice which the values presented the highly significant difference ($P \leq 0.01$). It is evident that the Chainat 1 cultivar gives the highest height as 65.1 cm whereas the Suphanburi 3 cultivar gives the lowest height as 55.90 cm. The average height of

other rice cultivars from high to low is Pathumthani 1, RD 23 and Suphanburi 1 as 59.0, 59.0 and 56.30 cm, respectively.

The Fe supply had also the effect on the height of all five cultivars and the values caused the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice cultivars have the highest average height as 61.70 cm with the Fe supply at 100 µM (T3), whereas the rice cultivars have the lowest average height as 57.00 cm with the Fe supply at 150 µM (T5). The average height with the Fe supply at other treatments from high to low is 50 µM (T1), 125 µM (T4) and 75 µM (T2) as 60.60, 59.10 and 57.70 cm of Fe-EDTA, respectively.

There is a highly significant interaction ($P \leq 0.01$) between rice cultivars and Fe supply on the height of rice at 45 days after transplanting as shown in Figure 15 (Appendix Table 10).

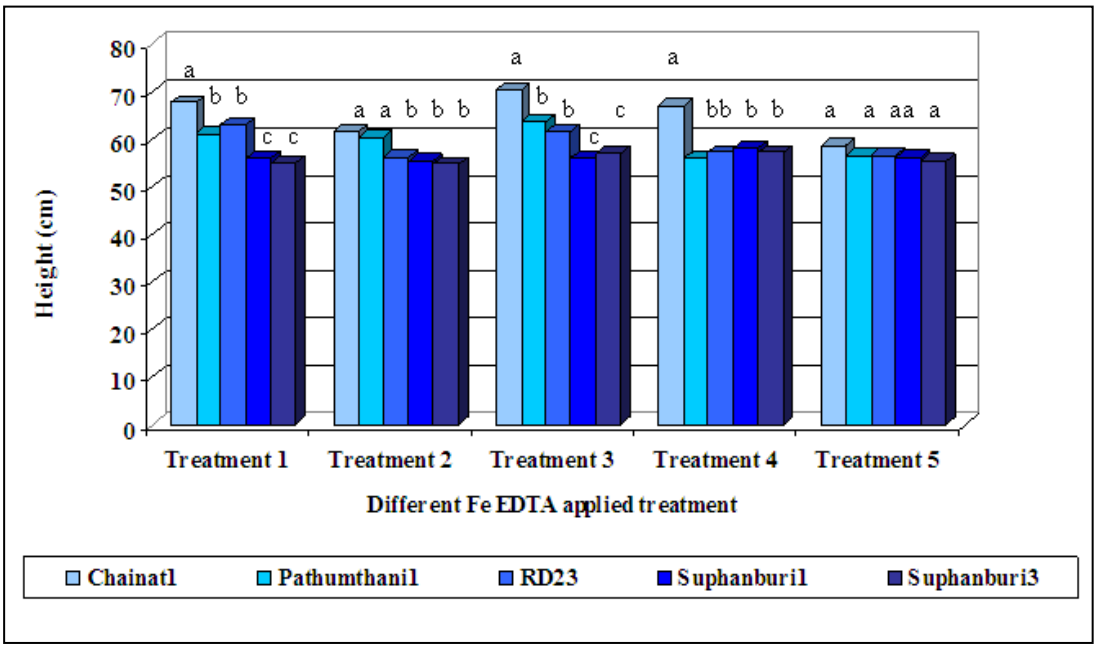

Figure 15 Height of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe-EDTA applied treatment at 45 days after transplanting

3.1.2 Dry weight of leaves, straws and roots at 45 days after transplanting

a. Dry weight of rice leaves

The statistic analysis showed that the rice cultivars affected to the dry weight of rice leaves which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice leaves of Chainat 1 have the highest average dry weight as 3.14 g whereas the rice leaves of Pathumthani 1 have the lowest average dry weight as 2.14 g. The average dry weight of other rice cultivars from high to low is Suphanburi 1, Suphanburi 3 and RD 23 as 2.84, 2.65 and 2.16 g, respectively.

The Fe supply had also the effect on the dry weight of rice leaves. The Fe supply tended to increase the dry weight of rice leaves with the increasing Fe supply which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice leaves have the highest average dry weight as 3.09 g with the Fe supply at 150 μM (T5) whereas the rice leaves have the lowest average dry weight as 2.15 g with the Fe supply at 50 μM (T1). The average dry weight with the Fe supply at other treatments from high to low is 125 μM (T4), 100 μM (T3) and 75 μM (T2) of Fe-EDTA as 2.84, 2.55 and 2.31 g, respectively.

There is no interaction between rice cultivars and Fe supply on the dry weight of rice leaves at 45 days after transplanting as shown in Figure 16 (Appendix Table 11).

b. Dry weight of rice straws

The statistic analysis showed that the rice cultivars affected to the dry weight of rice straws which the values presented the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice straws of Chainat 1 have the highest average dry weight as 3.27 g whereas the rice leaves of Pathumthani 1 have the lowest average dry weight as 2.13 g. The average dry weight

of other rice cultivars from high to low is Suphanburi 1, Suphanburi 3 and RD 23 as 2.83, 2.63 and 2.20 g, respectively.

The Fe supply had also the effect on the dry weight of rice straws. The Fe supply tended to increase the dry weight of rice straws with the increasing Fe supply which the values presented the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice straws have the highest average dry weight as 2.93 g with the Fe supply at 150 μM (T5) whereas the rice straws have the lowest average dry weight as 2.26 g with the Fe supply at 50 μM (T1). The average dry weight with the Fe supply at other treatments from high to low is 125 μM (T4), 100 μM (T3) and 75 μM (T2) of Fe-EDTA as 2.85, 2.61 and 2.41 g, respectively.

There is a highly significant interaction ($P \leq 0.01$) between rice cultivars and Fe supply on the dry weight of rice straws at 45 days after transplanting as shown in Figure 16 (Appendix Table 12).

c. Dry weight of rice roots

The statistic analysis showed that the rice cultivars affected to the dry weight of rice roots which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice roots of Chainat 1 have the highest average dry weight as 1.07 g whereas the rice leaves of RD 23 have the lowest average dry weight as 0.56 g. The average dry weight of other rice cultivars from high to low is Suphanburi 3, Suphanburi 1 and Pathumthani 1 as 0.71, 0.69 and 0.57 g, respectively.

The Fe supply had also the effect on the dry weight of rice roots. The Fe supply tended to increase the dry weight of rice roots with the increasing Fe supply which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice roots have the highest average dry weight as 0.95 g with the Fe supply at 150 μM (T5) whereas the rice roots have the lowest average dry weight as 0.51 g with the Fe supply at 50 μM (T1). The

average dry weight with the Fe supply at other treatments from high to low is 125 µM (T4), 100 µM (T3) and 75 µM (T2) of Fe-EDTA as 0.86, 0.72 and 0.57 g, respectively.

There is a highly significant interaction ($P \leq 0.05$) between rice cultivars and Fe supply on the dry weight of rice roots at 45 days after transplanting as shown in Figure 16 (Appendix Table 13).

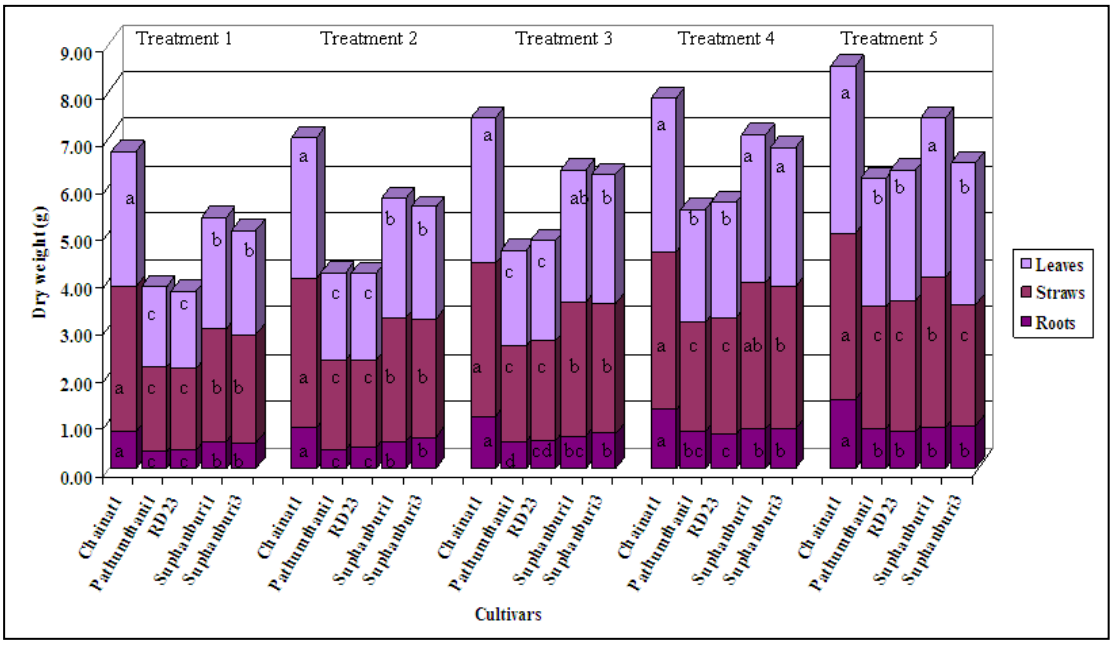

Figure 16 Dry weight of leaves, straws and roots of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe-EDTA applied treatment at 45 days after transplanting

3.1.3 Chlorosis and toxic symptoms of five rice cultivars

By observation, Fe deficiency in all rice cultivars did not occur. However, all five rice cultivars showed Fe toxicity at 125 and 150 µM of Fe-EDTA application. Among the rice cultivars, the severity of Fe toxicity of the Suphanburi 1 and Suphanburi 3 cultivars performs as level 1 when Fe-EDTA at 75 and 100 µM are supplied. In particular, the severity level of the Suphanburi 3 cultivar increases up to level 2 as Fe-EDTA supply increases at 100 µM. At 125 and 150 µM of Fe-EDTA

application, the severity of Fe toxicity at level 1 is performed in the Chainat 1, Pathumthani 1 and RD23 cultivars, whereas the severity of Fe toxic symptom of Suphanburi 1 and Suphanburi 3 increases up to level 2 when Fe-EDTA at 125 and 150 µM is applied as shown in Figure 17.

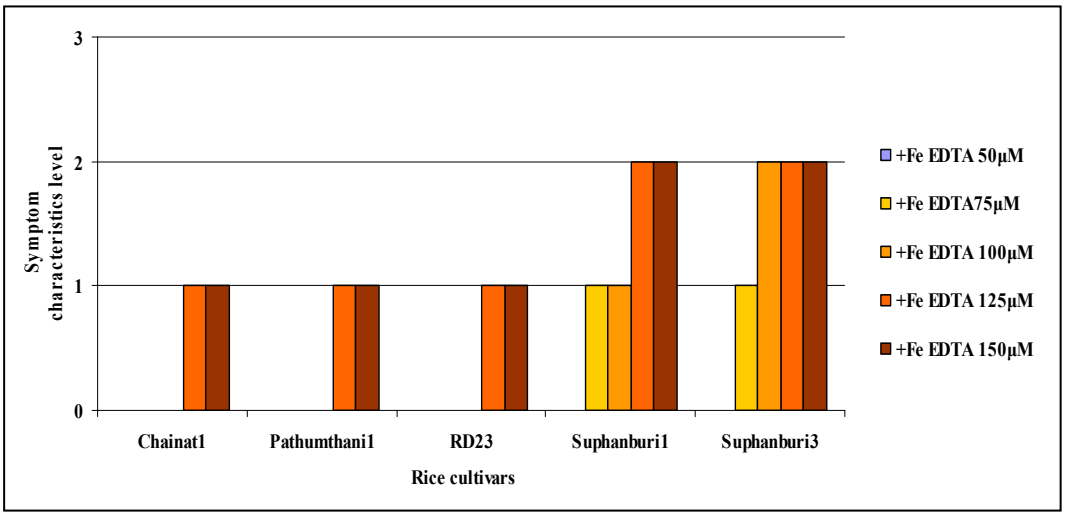

Figure 17 Toxicity score of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 an Suphanburi 3 cultivated with 50, 75, 100, 125, 150 µM of Fe-EDTA within 45 days

3.2 Fe concentration in leaves, straws and roots of Chainat1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 at 45 days after transplanting

3.2.1 Fe concentration in rice leaves

The statistic analysis showed that the rice cultivars affected to Fe concentration in rice leaves which the values presented the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that Chainat 1 has the highest average Fe concentration in rice leaves as 383.90 mg kg^{-1} whereas Pathumthani 1 has the lowest average Fe concentration as 225.50 mg kg^{-1}. The average Fe concentration of other rice cultivars from high to low is Suphanburi 1, RD 23 and Suphanburi 3 as 288.00, 274.10 and 242.50 mg kg^{-1}, respectively.

The Fe supply had also the effect on Fe concentration in rice leaves which the values were the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice leaves have the highest average Fe concentration as 414.90 mg kg^{-1} with the Fe supply at 125 μM (T4), whereas the rice leaves have the lowest average Fe concentration as 211.50 mg kg^{-1} with the Fe supply at 50 μM (T1). The average Fe concentration of other treatments from high to low is 100 μM (T3), 150 μM (T5) and 75 μM (T2) as 283.30, 268.80 and 235.60 mg kg^{-1}, respectively.

There is a highly significant interaction ($P \leq 0.01$) between rice cultivars and Fe supply on Fe concentration in rice leaves as shown in Figure 18.

3.2.2 Fe concentration in rice straws

The statistic analysis showed that the rice cultivars affected to Fe concentration in rice straws which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that Chainat 1 has the highest average Fe concentration in rice straws as 358.00 mg kg^{-1} whereas Suphanburi 3 has the lowest average Fe concentration as 96.00 mg kg^{-1}. The average Fe concentration of other rice cultivars from high to low is RD 23, Suphanburi 1 and Pathumthani 1 as 255.60, 120.90 and 98.90 mg kg^{-1}, respectively.

The Fe supply had also the effect on Fe concentration in rice straws which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice straws have the highest average Fe concentration as 236.70 mg kg^{-1} with the Fe supply at 100 μM (T3), whereas the rice straws have the lowest average Fe concentration as 117.50 mg kg^{-1} with the Fe supply at 50 μM (T1). The average Fe concentration of other treatments from high to low is 150 μM (T5), 75 μM (T2) and 125 μM (T4) as 208.50, 183.70 and 182.90 mg kg^{-1}, respectively.

There is a highly significant interaction ($P \leq 0.01$) between rice cultivars and Fe supply on Fe concentration in rice straws as shown in Figure 18.

3.2.3 Fe concentration in rice roots

The statistic analysis showed that the rice cultivars affected to Fe concentration in rice roots which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that Chainat 1 has the highest average Fe concentration in rice roots as 5057.40 mg kg^{-1} whereas Suphanburi 1 has the lowest average Fe concentration as 3429.10 mg kg^{-1}. The average Fe concentration of other rice cultivars from high to low is RD 23, Suphanburi 3 and Pathumthani 1 as 4239.80, 3999.70 and 3902.40 mg kg^{-1}, respectively.

The Fe supply had also the effect on Fe concentration in rice roots. The Fe supply tended to increase the Fe concentration in rice leaves with the increasing Fe supply which the values were the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice roots have the highest average Fe concentration as 5982.90 mg kg^{-1} with the Fe supply at 150 μM (T5), whereas the rice roots has the lowest average Fe concentration as 1985.10 mg kg^{-1} with the Fe supply at 50 μM (T1). The average Fe concentration of other treatments from high to low is 125 μM (T4), 100 μM (T3) and 75 μM (T2) as 5874.70, 3795.70 and 2989.90 mg kg^{-1}, respectively.

There is a highly significant interaction ($P \leq 0.01$) between rice cultivars and Fe supply on Fe concentration in rice roots as shown in Figure 18.

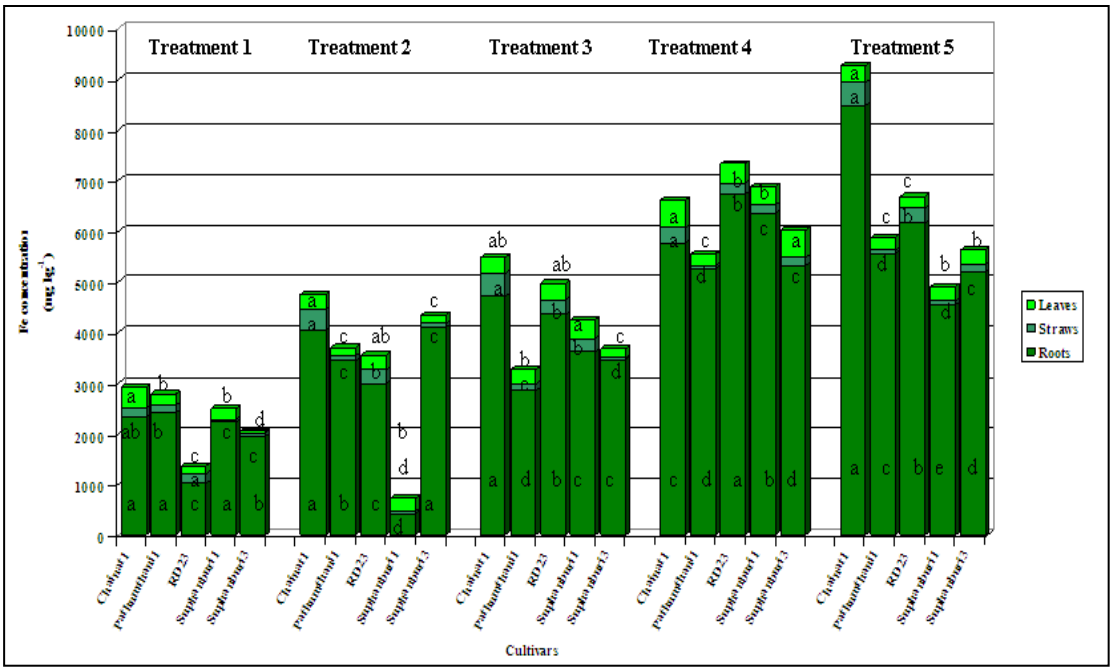

Figure 18 Fe concentration in leaves, straws and roots of 5 rice cultivars grown in nutrient solution as affected by Fe-EDTA 50 µM (T1), Fe-EDTA 75 µM (T2), Fe-EDTA 100 µM (T3), Fe-EDTA 125µM (T4) and Fe-EDTA 150 µM (T4) at 45 days after transplanting

3.3 Fe uptake and accumulation in rice leaves, straws and roots of Chainat1, Pathumthani 1, RD23, Suphanburi 1 and Suphanburi 3 after transplanting at 45 days

3.3.1 Fe uptake and accumulation in rice leaves

The statistic analysis showed that the rice cultivars affected to Fe uptake and accumulation in rice leaves which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that Chainat 1 has the highest average Fe uptake and accumulation in rice leaves as 1.2067 mg dw whereas Pathumthani 1 has the lowest average Fe uptake and accumulation as 0.4867 mg dw. The average Fe uptake and accumulation of other rice cultivars from high to low is Suphanburi 1, Suphanburi 3 and RD 23 as 0.8453, 0.6891 and 0.6035 mg dw, respectively.

The Fe supply had also the effect on Fe uptake and accumulation in rice roots which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice leaves have the highest average Fe uptake and accumulation as 1.2105 mg dw with the Fe supply at 125 µM (T4), whereas the rice roots have the lowest average Fe uptake and accumulation as 0.4866 mg dw with the Fe supply at 50 µM (T1). The average Fe uptake and accumulation of other treatments from high to low is 150 µM (T5), 100 µM (T3) and 75 µM (T2) as 0.8397, 0.7373 and 0.5572 mg dw, respectively.

There is a highly significant interaction ($P \leq 0.01$) between rice cultivars and Fe supply on Fe uptake and accumulation in rice leaves as shown in Figure 19.

3.3.2 Fe uptake and accumulation in rice straws

The statistic analysis showed that the rice cultivars affected to Fe uptake and accumulation in rice straws which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that Chainat 1 has the highest average Fe uptake and accumulation in rice straws as 1.1820 mg dw whereas Pathumthani 1 has the lowest average Fe uptake and accumulation as 0.2029 mg dw. The average Fe uptake and accumulation of other rice cultivars from high to low is RD 23, Suphanburi 1 and Suphanburi 3 as 0.5696, 0.3547 and 0.2585 mg dw, respectively.

The Fe supply had also the effect on Fe uptake and accumulation in rice straws which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that the rice straws have the highest average Fe uptake and accumulation as 0.6485 mg dw with the Fe supply at 100 µM (T3), whereas the rice straws have the lowest average Fe uptake and accumulation as 0.2611 mg dw with the Fe supply at 50 µM (T1). The average Fe uptake and accumulation of other treatments from high to low is 150 µM (T5), 125 µM (T4) and 75 µM (T2) as 0.6464, 0.5413 and 0.4703 mg dw^{-} respectively.

There is a highly significant interaction ($P \leq 0.01$) between rice cultivars and Fe supply on Fe uptake and accumulation in rice straws as shown in Figure 19.

3.3.3 Fe uptake and accumulation in rice roots

The statistic analysis showed that the rice cultivars affected to Fe uptake and accumulation in rice roots which the values showed the highly significant difference ($P \leq 0.01$). The analysis after transplanting at 45 days found that Chainat 1 has the highest average Fe uptake and accumulation in rice roots as 5.9313 mg dw whereas Pathumthani 1 has the lowest average Fe uptake and accumulation as 2.4607 mg dw. The average Fe uptake and accumulation of other rice cultivars from high to low is Suphanburi 3, RD 23 and Suphanburi 1 as 3.0100, 2.6953 and 2.6507 mg dw, respectively.

The Fe supply had also the effect on Fe uptake and accumulation in rice roots which the values showed the highly significant difference ($P \leq 0.01$). The Fe supply tended to increase Fe uptake and accumulation with the increasing Fe supply which the values showed the significant difference. The analysis after transplanting at 45 days found that the rice roots have the highest average Fe uptake and accumulation as 6.0293 mg dw with the Fe supply at 150 μM (T5), whereas the rice straws have the lowest average Fe uptake and accumulation as 1.0420 mg dw with the Fe supply at 50 μM (T1). The average Fe uptake and accumulation of other treatments from high to low is 125 μM (T4), 100 μM (T3) and 75 μM (T2) as 5.0720, 2.8240 and 1.7807 mg dw, respectively.

There is a highly significant interaction ($P \leq 0.01$) between rice cultivars and Fe supply on Fe uptake and accumulation in rice roots as shown in Figure 19.

From considering Fe content of whole rice among five rice cultivars, the result of this study can conclude as following details.

The Chainat 1 cultivar has the highest Fe content in all plant parts with Fe-EDTA supply at 150 (T5) µM. The Fe content tends to increase when Fe-EDTA is applied at 50 (T1), 75 (T2), 100 (T3), 125 (T4) and 150 (T5) µM, respectively. However, the Fe toxic symptom occurs at level 1 with the application of Fe-EDTA at 125 (T4) and 150 (T5) µM.

The Pathumthani 1 cultivar has the highest Fe content in all plant parts with Fe-EDTA supply at 150 (T5) µM. The Fe content tends to increase when Fe-EDTA is applied at 50 (T1), 75 (T2), 100 (T3), 125 (T4) and 150 (T5) µM, respectively. However, the Fe toxic symptom occurs at level 1 with the application of Fe-EDTA at 125 (T4) and 150 (T5) µM.

The RD23 cultivar has the highest Fe content in all plant parts with Fe-EDTA supply at 100 (T3) µM. The Fe content tends to increase when Fe-EDTA is applied at 50 (T1), 75 (T2), 100 (T3) and 125 (T4) µM, respectively whereas the Fe content decreases with Fe-EDTA supply at 150 (T5) µM. Moreover, the Fe toxicity occurs at level 1 with the application of Fe-EDTA at 125 (T4) and 150 (T5) µM.

The Suphanburi 1 has the highest Fe content in all plant parts with Fe-EDTA supply at 125 (T3) µM. The Fe content tends to increase when Fe-EDTA is applied at 50 (T1), 75 (T2), 100 (T3) and 125 (T4) µM, respectively whereas the Fe content decreases with Fe-EDTA supply at 150 (T5) µM. Moreover, the Fe toxicity at level 1 is observed with the application of Fe-EDTA at 50 (T1) and 75 (T2) µM and the Fe toxicity at level 2 occurs with the application of Fe-EDTA at 125 (T4) and 150 (T5) µM, respectively.

The Suphanburi 3 has the highest Fe content in all plant parts with Fe-EDTA supply at 125 (T3) µM. The Fe content tends to increase when Fe-EDTA is applied at 50 (T1), 75 (T2), 100 (T3) and 125 (T4) µM, respectively whereas the Fe content decreases with Fe-EDTA supply at 150 (T5) µM. Moreover, the Fe toxicity

at level 1 is observed with the application of Fe-EDTA at 75 (T2) µM and the Fe toxicity at level 2 occurs with the application of Fe-EDTA at 100 (T3), 125 (T4) and 150 (T5) µM, respectively.

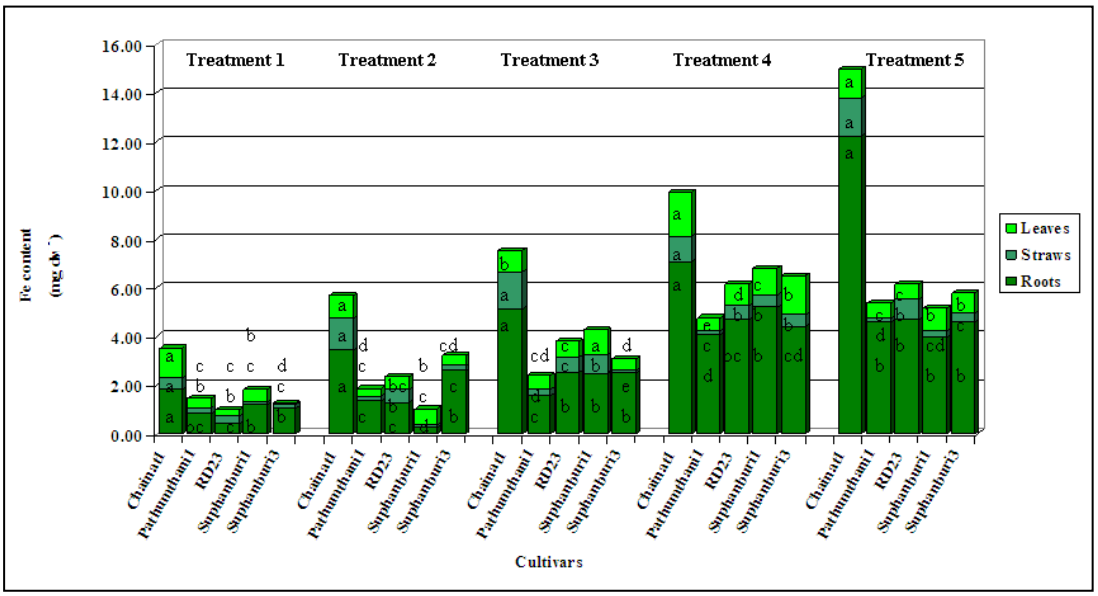

Figure 19 Fe uptake and accumulation in leaves, straws and roots of 5 rice cultivars grown in nutrient solution as affected by Fe-EDTA 50 µM (T1) Fe-EDTA 75 µM (T2), Fe-EDTA 100 µM (T3), Fe-EDTA 125 µM (T4) and Fe-EDTA 150 µM (T5) at 45 days after transplanting

The micronutrients are one of important and necessary factors to plant growth. The accumulation of micronutrients such as Fe and Mn is due to rice genotypes which have ability in different Fe uptake (Christ, 1974). The different uptake showed the ability of absorption, translocation and distribution in whole plant (Hao *et al.,* 2007). The reduction in growth of low Fe plant was indicative of the very low Fe level in the solution. Based on the reduced yield, it was postulated that the low Fe plant were just above that level when Fe deficiency induced deficiency symptom. Fe toxicity is one of the symptoms most frequently found in nutrient solution culture. In this experiment, the five rice cultivars showed the different toxicity level following the varied Fe application. Suphanburi 1 and Suphanburi 3 show the toxicity symptoms when they are applied with Fe-EDTA at 75 µM, whereas Chainat 1, Pathumthani 1 and RD 23 show the toxicity symptoms when Fe-EDTA at

125 µM is given. This data indicated that the toxicity appearance did not occur from Fe application but was due to rice cultivars (Albano and Miller, 1996). Furthermore, all rice cultivars were observed to show the toxicity symptoms after transplanting at 45 days when they were applied with Fe-EDTA at 125 µM. This result could explain that the rice cultivars had different Fe sensitivity and the excessive Fe application could affect to plant growth (Prade and Ottow, nd).

Fe concentration of five rice cultivars in roots, straws and leaves were investigated in this study. Each part of rice cultivars responded differently to the application of varied Fe quantities. From Fe application at 45 days after transplanting, the data showed that Fe concentration in root of all rice cultivars at all treatments was higher than in leaves and straws, respectively. Therefore, the significant difference of Fe concentration in plant parts was not found between the treatments. On the contrary, the result showed the significant difference of Fe concentration in roots between the rice cultivars. Obviously, Fe concentration of Chainat 1 was higher than other cultivars. When comparing between cultivars, Fe concentration in root of Chainat 1 continuously increased when Fe application increased especially after transplanting 45 days. Moreover, after Fe treatments, Fe concentration in leaves increased and reached optimal levels in some cultivars. The difference of Fe concentration in leaves among the cultivars might be caused by different Fe absorption capacities through the leaves and roots. Due to the duration of 45 days is reproductive phase of rice, which rice uptake nutrient from adventitious root. Consequently, rice uptake external Fe supply. Moreover, Fe-EDTA are chromophores that absorb strongly in the ultraviolet (UV) and blue regions of the spectrum. Absorption of this energy causes the destruction of the chelate complex into ferrous Fe that precipitates as Fe oxides, glyoxylic acid, formaldehyde, CO_2 and amine residue. This results related to Albano and Miller (2001) reported that plant tissue will have poor growth when they grown in Fe-EDTA solution which was irradiated, indicating that any soluble toxic by product of Fe-EDTA photodegradation.

Generally, the critical level of Fe deficiency in rice leave is 70 mg kg^{-1} and Fe toxicity is 300 mg kg^{-1}. Neither the tissue Fe concentration nor plant growth differed group were low Fe and high Fe plant. It was assumed that all Fe^{2+}

added would have been oxidized to Fe^{3+} and precipitated in the apoplasm within a few days (Mengel and Geurtzen, 1988). Fe appeared to be phloem immobile and there was no apparent reproductive mobilization with Fe concentration being highest in dead tissue (Hocking, 1994; Miller, 1994).

CONCLUSION

1. Experiment 1: Survey the Information of Rice Fields and Population in Khao Yoi District, Phetchaburi Province

1.1 Fe concentration in populations' hair samples was lower than mean value of human hair.

1.2 Fe deficiency was found in the populations more than 70% especially in female.

1.3 Available Fe in soil did not relate to available Fe in brown rice due to being antagonism between Fe and other elements such as available P, Mn, Zn, etc.

1.4 Rice cultivar is a factor that influence to Fe uptake and accumulation of plant.

1.5 Chainat 1 had total Fe in brown rice more than Suphanburi 1.

2. Experiment 2: Effect of Rice Cultivation and Fe Supply on Rice Growth: Grown in Hydroponics Experiment Filled with Fe-EDTA 28 Days

2.1 Chlorosis symptom appeared in all five rice cultivars when Fe-EDTA was applied at 10 and 50 μM.

2.2 All five rice cultivars appeared toxic symptom when Fe-EDTA was applied at 150 μM.

2.3 Fe-EDTA at 100 μM was an appropriate Fe level for Chainat 1 and Pathumthani 1.

2.4 Fe-EDTA at more than 75 to less than 100 μM was an appropriate Fe level for RD 23, Suphanburi 1 and Suphanburi 3.

3. Experiment 3: Experimental Cultivation of Five Rice Cultivars in Hydroponics Experiment Filled with Fe-EDTA 45 Days

3.1 Chainat 1, Pathumthani 1 and RD 23 had proper growth when Fe-EDTA at 100 μM was applied and the chlorosis and toxic symptoms of rice were not found at this level.

3.2 For Suphanburi 1 and Suphanburi 3, the toxicity symptom appeared at level 1 and 2, respectively when Fe-EDTA at 100 μM was applied.

3.3 Chainat 1 cultivar had better capacity in Fe uptake and accumulation as compared to other rice cultivars.

3.4 It was suggested that the selecting rice cultivar which contributed higher Fe in rice was suitable for biofortification.

RECOMMENDATION

From the study of researched area in Khao Yoi District, Phetchabuti Province and the rice cultivation experiment with hydroponics experiment in Greenhouse, Kasetsart University, the findings are only primary and fundamental information for the consideration of nutrition situation and Fe uptake and accumulation of different rice cultivars. The results of all experiments indicated that Chainat 1 is popular for cultivation in researched area. It has better capacity in Fe uptake and accumulation than other rice cultivars. Therefore, an additional study, such as rice cultivation in soil, should be set up for investigating the supporting information. Moreover, the rice cultivation and consumption of Chainat 1 should be also promoted to the agriculturists in researched area because it may lead to biofortification which can reduce the malnutrition problem.

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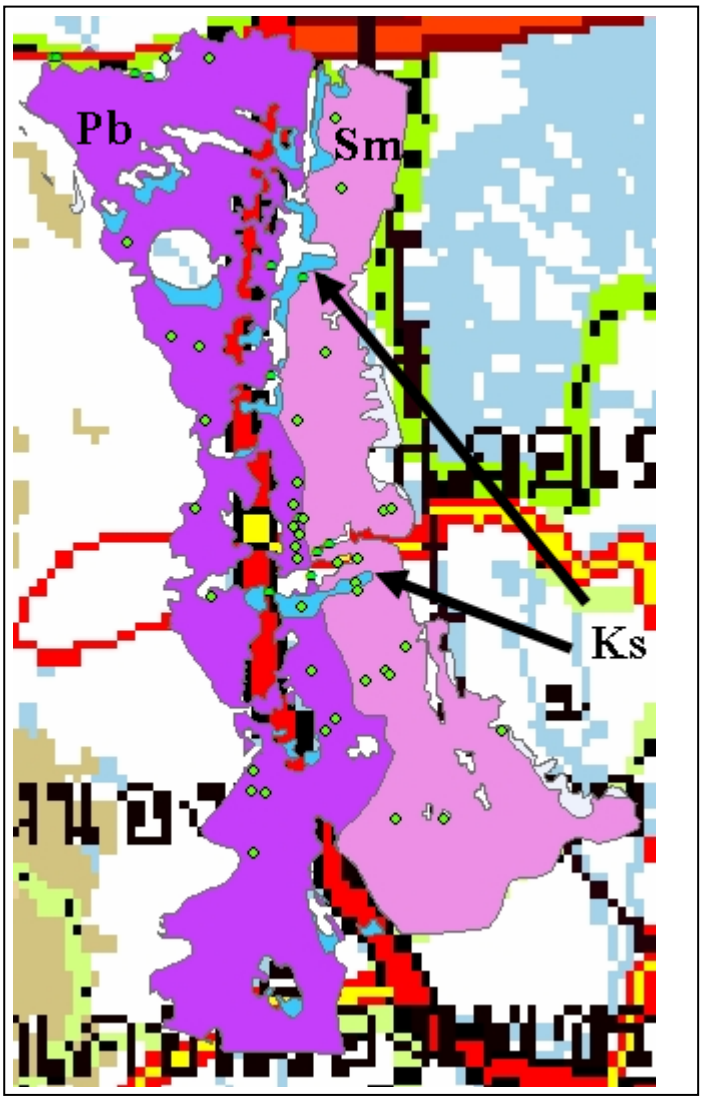
APPENDIX

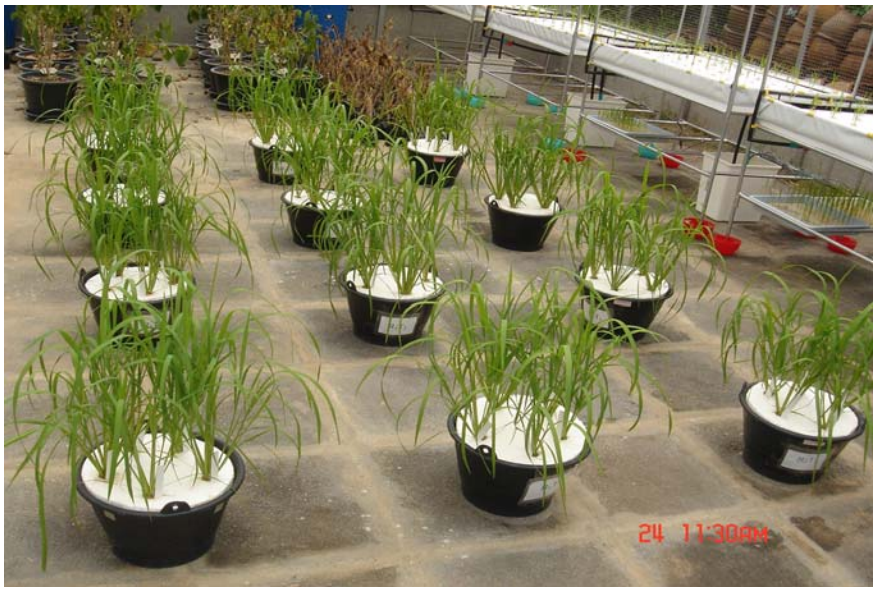


Appendix Figure 1 Soil sampling map of Petchaburi series (Pb), Samut Prakan series (Sm) and Kamphaeng Saen series (Ks) of Khao Yoi District, Phetchaburi Province

Appendix Figure 2 Plant samples of experiment 1

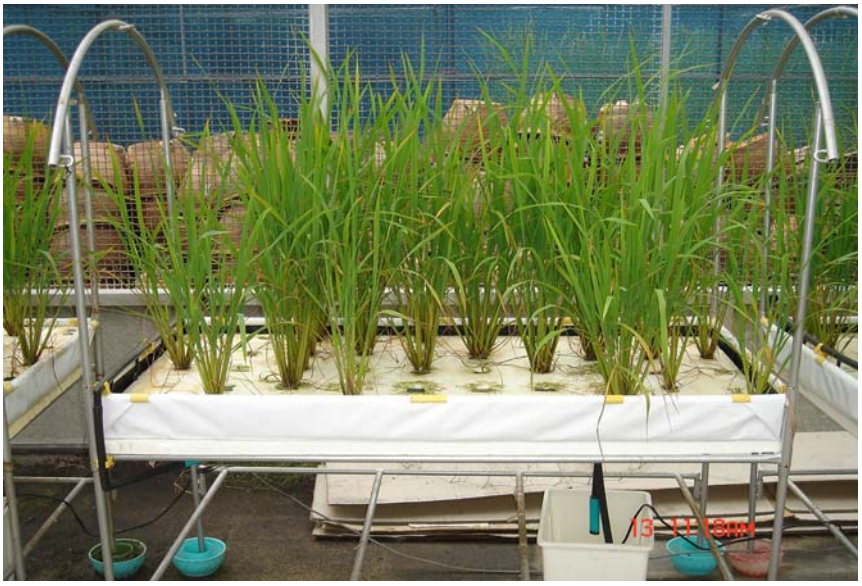
Appendix Figure 3 Soil samples of experiment 1

Appendix Figure 4 Five rice cultivars after transplanting on hydroponics of experiment 2



Appendix Figure 5 Five rice cultivars at 28 days after transplanting on hydroponics of experiment 2

Appendix Figure 6 Five rice cultivars after transplanting on hydroponics of experiment 3



Appendix Figure 7 Five rice cultivars at 45 days after transplanting on hydroponics of experiment 3

Appendix Table 1 Relationship between chemical soil properties and total Fe in brown rice, leaves and straws of Suphanburi 1, which harvested from Khao Yoi District, Phetchaburi Province

	Total Fe in Brown rice	Total Fe in Leaves	Total Fe in Straws	Avail. Mn	Avail. Zn	Avail. P
Total Fe in Brown rice	1.00					
Total Fe in Leaves	-0.23	1.00				
Total Fe in Straws	-0.10	0.24	1.00			
Available Mn	-0.21	-0.06	0.17	1.00		
Available Zn	-0.19	0.46	-0.07	0.39	1.00	
Available P	-0.16	0.05	-0.18	0.00	-0.08	1.00

Appendix Table 2 Relationship between chemical soil properties and total Fe in brown rice, leaves and straws of Chainat 1, which harvested from Khao Yoi District, Phetchaburi Province

Total Fe	Total Fe			
	Brown rice	Leaves	Straws	Soils
Brown rice	1.00			
Leaves	0.17	1.00		
Straws	0.39	0.60*	1.00	
Soils	-0.40	0.10	-0.22	1.00

Remark * = significant at 95% of confidence

Appendix Table 3 Relationship between physical and chemical soil properties of Samut Prakan series and total Fe in brown rice of Chainat 1 and Suphanburi 1, which harvested from Khao Yoi District, Phetchaburi Province

	Fe concentration in brown rice	pH	Sand (%)	Silt (%)	Clay (%)	EC	% O.M.	Avail. P	Extrac. K	Total Zn	Avail. Zn	Extrac. Ca	Extrac. Mg	Total Mn	Avail. Mn	Total Fe	Avail. Fe
Fe concentration in brown rice	1.00																
pH	0.90	1.00															
Sand (%)	-0.60	-0.89	1.00														
Silt (%)	-	-	-	-													
Clay (%)	0.60	0.89	-1.00	-	1.00												
EC	0.40	0.76	-0.97	-	0.97	1.00											
% O.M.	-0.95	-0.99	0.81	-	-0.81	-0.66	1.00										
Avail. P	0.59	0.18	0.29	-	-0.29	-0.50	-0.32	1.00									
Extrac. K	0.81	0.98	-0.96	-	0.96	0.86	-0.95	0.01	1.00								
Total Zn	-0.51	-0.09	-0.37	-	0.37	0.58	0.23	-1.00	0.08	1.00							
Avail. Zn	-0.70	-0.32	-0.15	-	0.15	0.38	0.45	-0.99	-0.15	0.97	1.00						
Extrac. Ca	-0.90	-1.00	0.89	-	-0.89	-0.77	0.99	-0.17	-0.99	0.08	0.31	1.00					
Extrac. Mg	0.61	0.89	-1.00	-	1.00	0.97	-0.82	-0.28	0.96	0.37	0.14	-0.90	1.00				
Total Mn	0.08	0.50	-0.84	-	0.84	0.94	-0.37	-0.76	0.64	0.82	0.66	-0.51	0.84	1.00			
Avail. Mn	-0.74	-0.96	0.98	-	-0.98	-0.92	0.90	0.11	-0.99	-0.20	0.03	0.96	-0.99	-0.73	1.00		
Total Fe	0.45	0.79	-0.98	-	0.98	1.00	-0.69	-0.46	0.88	0.54	0.33	-0.80	0.98	0.93	-0.93	1.00	
Avail. Fe	-0.60	-0.89	1.00	-	-1.00	-0.97	0.81	0.29	-0.96	-0.37	-0.15	0.89	-1.00	-0.84	0.98	-0.98	1.00

Appendix Table 4 Relationship between physical and chemical soil properties of Kamphaeng Saen series and total Fe in brown rice of Chainat 1 and Suphanburi 1, which harvested from Khao Yoi District, Phetchaburi Province

	Fe concentration in brown rice	pH	Sand (%)	Silt (%)	Clay (%)	EC	% O.M.	Avail. P	Extrac. K	Total Zn	Avail. Zn	Extrac. Ca	Extrac. Mg	Total Mn	Avail. Mn	Total Fe	Avail. Fe
Fe concentration in brown rice	1.00																
pH	0.21	1.00															
Sand (%)	0.90	0.54	1.00														
Silt (%)	-0.46	-0.89	-0.76	1.00													
Clay (%)	-0.11	0.85	0.24	-0.81	1.00												
EC	0.36	-0.29	0.38	0.07	-0.45	1.00											
% O.M.	0.74	-0.01	0.74	-0.23	-0.33	0.88	1.00										
Avail. P	-0.18	0.41	-0.19	-0.23	0.51	-0.98	-0.77	1.00									
Extrac. K	0.50	-0.55	0.31	0.33	-0.77	0.87	0.83	-0.85	1.00								
Total Zn	0.77	-0.25	0.58	0.08	-0.64	0.71	0.88	-0.61	0.89	1.00							
Avail. Zn	-0.21	-0.42	-0.14	0.37	-0.43	0.83	0.49	-0.92	0.63	0.31	1.00						
Extrac. Ca	0.73	0.04	0.70	-0.14	-0.41	0.75	0.93	-0.65	0.78	0.93	0.40	1.00					
Extrac. Mg	0.17	-0.83	-0.12	0.72	-0.96	0.67	0.51	-0.72	0.89	0.71	0.62	0.52	1.00				
Total Mn	0.69	0.20	0.79	-0.36	-0.16	0.83	0.97	-0.71	0.70	0.79	0.47	0.94	0.35	1.00			
Avail. Mn	0.17	-0.25	0.25	0.10	-0.38	0.98	0.78	-0.98	0.78	0.57	0.92	0.67	0.60	0.77	1.00		
Total Fe	0.81	0.04	0.81	-0.37	-0.17	0.80	0.95	-0.67	0.74	0.78	0.34	0.80	0.37	0.90	0.67	1.00	
Avail. Fe	-0.84	-0.54	-0.96	0.66	-0.13	-0.47	-0.80	0.30	-0.39	-0.67	-0.02	-0.83	0.02	-0.88	-0.38	-0.79	1.00

Appendix Table 5 Relationship between physical and chemical soil properties of Petchaburi series and total Fe in brown rice of Chainat 1 and Suphanburi 1, which harvested from Khao Yoi District, Phetchaburi Province

	Fe concentration in brown rice	pH	Sand (%)	Silt (%)	Clay (%)	EC	% O.M.	Avail. P	Extrac. K	Total Zn	Avail. Zn	Extrac. Ca	Extrac. Mg	Total Mn	Avail. Mn	Total Fe	Avail. Fe
Fe concentration in brown rice	1.00																
pH	-0.52	1.00															
Sand (%)	-0.99	0.61	1.00														
Silt (%)	0.64	-0.99	-0.72	1.00													
Clay (%)	-0.14	0.92	0.24	-0.85	1.00												
EC	0.16	0.76	-0.06	-0.65	0.95	1.00											
% O.M.	0.94	-0.19	-0.89	0.33	0.22	0.50	1.00										
Avail. P	-0.75	0.96	0.82	-0.99	0.76	0.53	-0.47	1.00									
Extrac. K	-0.57	1.00	0.66	-1.00	0.89	0.72	-0.25	0.97	1.00								
Total Zn	-0.01	0.86	0.12	-0.78	0.99	0.98	0.34	0.67	0.83	1.00							
Avail. Zn	-0.43	-0.55	0.33	0.43	-0.84	-0.96	-0.72	-0.28	-0.50	-0.90	1.00						
Extrac. Ca	-0.47	1.00	0.56	-0.98	0.94	0.80	-0.13	0.94	0.99	0.89	-0.60	1.00					
Extrac. Mg	-0.09	0.90	0.19	-0.82	1.00	0.97	0.27	0.73	0.87	1.00	-0.86	0.92	1.00				
Total Mn	0.50	-1.00	-0.59	0.99	-0.93	-0.77	0.16	-0.95	-1.00	-0.87	0.57	-1.00	-0.91	1.00			
Avail. Mn	0.41	-0.99	-0.51	0.96	-0.96	-0.83	0.06	-0.91	-0.98	-0.92	0.65	-1.00	-0.94	0.99	1.00		
Total Fe	0.49	0.49	-0.39	-0.36	0.80	0.94	0.76	0.22	0.44	0.87	-1.00	0.55	0.83	-0.51	-0.60	1.00	
Avail. Fe	0.33	-0.98	-0.43	0.94	-0.98	-0.88	-0.02	-0.87	-0.96	-0.95	0.71	-0.99	-0.97	0.98	1.00	-0.66	1.00

Appendix Table 6 Average height of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe supply at 28 days after transplanting

Treatment	Average height of rice (cm)					Mean
	Chainat1	Pathumthani1	RD23	Suphanburi1	Suphanburi3	
Fe EDTA 10 μM (T1)	60.70a	58.70a	61.70a	58.70a	58.70a	56.20a
Fe EDTA 50 μM (T2)	63.00a	55.70a	60.00a	64.70a	64.70a	61.10a
Fe EDTA 100 μM (T3)	63.70a	59.00a	63.30a	59.70a	59.70a	59.90a
Fe EDTA 150 μM (T4)	60.70a	57.70a	60.70a	61.70a	61.70a	59.30a
Mean	62.00a	57.80a	61.40a	53.30b	61.20a	59.10
CV (%)			12.9			
F-test (rice cultivars)			*			
F-test (treatment)			ns			
F-test (treatment x cultivars)			ns			

Remark - ns = non significant

- * = significant at 95% of confidence

- Means in each column followed by the same letter are not significant by DMRT at 95% of confidence

Appendix Table 7 Average dry weight of rice leaves of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe supply at 28 days after transplanting

Treatment	Average dry weight of rice leaves (g)					Mean
	Chainat1	Pathumthani1	RD23	Suphanburi1	Suphanburi3	
Fe EDTA 10 µM (T1)	0.93b	0.79a	1.28a	0.68a	0.69a	0.87a
Fe EDTA 50 µM (T2)	1.46a	0.71a	1.53a	0.72a	1.03a	1.09a
Fe EDTA 100 µM (T3)	1.23ab	0.90a	1.60a	0.65a	0.89a	1.05a
Fe EDTA 150 µM (T4)	1.39ab	0.89a	1.15a	0.60a	0.97a	1.00a
Mean	1.25a	0.82bc	1.39a	0.66c	0.89b	1.00
CV (%)			26.9			
F-test (rice cultivars)			**			
F-test (treatment)			ns			
F-test (treatment x cultivars)			ns			

Remark - ns = non significant

- ** = significant at 99% of confidence

- Means in each column followed by the same letter are not significant by DMRT at 95% of confidence

Appendix Table 8 Average dry weight of rice straws of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe supply at 28 days after transplanting

Treatment	Average dry weight of rice straws (g)					Mean
	Chainat1	Pathumthani1	RD23	Suphanburi1	Suphanburi3	
Fe EDTA 10 µM (T1)	0.49a	0.58a	0.71a	0.48a	0.57a	0.57a
Fe EDTA 50 µM (T2)	0.89a	0.65a	0.76a	0.56a	0.73a	0.72a
Fe EDTA 100 µM (T3)	0.94a	0.65a	0.96a	0.41a	0.59a	0.71a
Fe EDTA 150 µM (T4)	0.94a	0.50a	0.99a	0.47a	0.63a	0.70a
Mean	0.81ab	0.59b	0.85a	0.48c	0.63abc	0.70
CV (%)			41.2			
F-test (rice cultivars)			*			
F-test (treatment)			ns			
F-test (treatment x cultivars)			ns			

Remark - ns = non significant

- ** = significant at 99% of confidence

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Appendix Table 9 Average dry weight of rice roots of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe supply at 28 days after transplanting

Treatment	Average dry weight of rice roots (g)					Mean
	Chainat1	Pathumthani1	RD23	Suphanburi1	Suphanburi3	
Fe EDTA 10 μM (T1)	0.16a	0.14a	0.19b	0.11a	0.14a	0.15a
Fe EDTA 50 μM (T2)	0.23a	0.11a	0.24b	0.16a	0.15a	0.18a
Fe EDTA 100 μM (T3)	0.21a	0.11a	0.21b	0.09a	0.15a	0.16a
Fe EDTA 150 μM (T4)	0.27a	0.10a	0.53a	0.08a	0.13a	0.22a
Mean	0.22ab	0.13bc	0.29a	0.11c	0.14bc	0.18
CV (%)			68.9			
F-test (rice cultivars)			**			
F-test (treatment)			ns			
F-test (treatment x cultivars)			ns			

Remark - ns = non significant

- ** = significant at 99% of confidence

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Appendix Table 10 Average height of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe supply at 45 days after transplanting

Treatment	Average height of rice (cm)					Mean
	Chainat1	Pathumthani1	RD23	Suphanburi1	Suphanburi3	
Fe EDTA 50 µM (T1)	67.70a	61.30b	63.00b	56.00c	55.00c	60.60ab
Fe EDTA 75 µM (T2)	61.70a	60.30a	56.30b	55.30b	54.70b	57.70bc
Fe EDTA 100 µM (T3)	70.30a	63.70b	61.70b	56.00c	57.00c	61.70a
Fe EDTA 125 µM (T4)	67.00a	56.00b	57.30b	58.00b	57.30b	59.10abc
Fe EDTA 150 µM (T5)	58.70a	56.70a	56.70a	56.30a	55.30a	57.00c
Mean	65.10a	59.00b	59.00b	56.30b	55.90b	59.20
CV (%)			3.3			
F-test (rice cultivars)			**			
F-test (treatment)			**			
F-test (treatment x cultivars)			**			

Remark - ns = non significant

- ** = significant at 99% of confidence

- Means in each column followed by the same letter are not significant by DMRT at 95% of confidence

Appendix Table 11 Average dry weight of rice leaves of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe supply at 45 days after transplanting

Treatment	Average dry weight of rice leaves (g)					Mean
	Chainat1	Pathumthani1	RD23	Suphanburi1	Suphanburi3	
Fe EDTA 50 µM (T1)	2.85a	1.69c	1.65c	2.36b	2.19b	2.15e
Fe EDTA 75 µM (T2)	2.99a	1.85c	1.83c	2.53b	2.38b	2.31d
Fe EDTA 100 µM (T3)	3.08a	2.04c	2.11c	2.80ab	2.72b	2.55c
Fe EDTA 125 µM (T4)	3.27a	2.39b	2.45b	3.12a	2.96a	2.84b
Fe EDTA 150 µM (T5)	3.53a	2.73b	2.76b	3.39a	3.03b	3.09a
Mean	3.14a	2.14d	2.16d	2.84b	2.65c	2.59
CV (%)			7.1			
F-test (rice cultivars)			**			
F-test (treatment)			**			
F-test (treatment x cultivars)			ns			

Remark - ** = significant at 99% of confidence

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Appendix Table 12 Average dry weight of rice straws of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe supply at 45 days after transplanting

Treatment	Average dry weight of rice straws (g)					Mean
	Chainat1	Pathumthani1	RD23	Suphanburi1	Suphanburi3	
Fe EDTA 50 µM (T1)	3.07a	1.81c	1.73c	2.40b	2.31b	2.26d
Fe EDTA 75 µM (T2)	3.16a	1.90c	1.86c	2.62b	2.50b	2.41cd
Fe EDTA 100 µM (T3)	3.25a	2.04c	2.14c	2.84b	2.77b	2.61bc
Fe EDTA 125 µM (T4)	3.34a	2.31c	2.48c	3.12ab	3.01b	2.85ab
Fe EDTA 150 µM (T5)	3.54a	2.59c	2.79c	3.17b	2.57c	2.93a
Mean	3.27a	2.13c	2.20c	2.83b	2.63b	2.61
CV (%)			6.5			
F-test (rice cultivars)			**			
F-test (treatment)			**			
F-test (treatment x cultivars)			**			

Remark - ** = significant at 99% of confidence

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Appendix Table 13 Average dry weight of rice roots of Chainat 1, Pathumthani 1, RD 23, Suphanburi 1 and Suphanburi 3 with the different Fe supply at 45 days after transplanting

Treatment	Average dry weight of rice roots (g)					Mean
	Chainat1	Pathumthani1	RD23	Suphanburi1	Suphanburi3	
Fe EDTA 50 µM (T1)	0.77a	0.33c	0.38c	0.54b	0.52b	0.51c
Fe EDTA 75 µM (T2)	0.85a	0.39c	0.42c	0.56b	0.63b	0.57c
Fe EDTA 100 µM (T3)	1.08a	0.55d	0.57cd	0.68bc	0.72b	0.72b
Fe EDTA 125 µM (T4)	1.23a	0.77bc	0.69c	0.81b	0.82b	0.86a
Fe EDTA 150 µM (T5)	1.44a	0.81b	0.75b	0.86b	0.87b	0.95a
Mean	1.07a	0.57c	0.56c	0.69b	0.71b	0.72
CV (%)			9.8			
F-test (rice cultivars)			**			
F-test (treatment)			**			
F-test (treatment x cultivars)			*			

Remark
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