CHAPTER 1

Introduction

1.1 Historical background

Rice is the staple food for over half of the world's population (Khush, 2005). In recent years, rice production has been increasing as the result of high yielding varieties development, improving of production conditions and cultural practices. Thailand is the world's top exporter and the fifth largest cultivator of rice in the world, However, based on USDA Statistic data (USDA, 2012), Thailand's rough rice yield is only 2.87 t/ha that much lower than other rice exporter's counties such as China, Vietnam and India which rice yield are about 6.69, 5.65 and 3.42 t/ha, respectively. Therefore, a unique characteristic of rice production in Thailand and trade is the emphasis placed on grain quality. This has brought benefits to farmers, rice traders and country's export earnings in higher prices and the capture of a major share of the world market in high quality rice.

Rice grain quality can be defined in many aspects including physical (appearance and milling), physico-chemical (cooking and eating) and nutritional qualities (Webb, 1991). Physical quality includes all of external and integral characteristic such as its appearances (grain size, shape, colour), weigh, whiteness and translucency, hardness, volume, thermal properties and chalkiness (Bhattacharya, 2011). The physicochemical quality includes gelatinization temperature, gel consistency, amylose content, while nutritional quality is defined by content of nutritional compounds such as proteins, vitamins and metabolites and minerals such as zinc and iron.

Rice grain qualities are determined by both genetic control and environment conditions. Rice breeders continuously trying to maintain and/or improve grain quality characteristic by manipulation of the genetics. This is for the new varieties with high grain quality to meet the market standards and requirement of consumers. Breeding for high quality has so far been based partly on the known such as amylose content, gelatinization temperature and the other obvious and measurable qualities such as grain size and shape. The control of many other key quality characteristics such as head rice yield, grain chalkiness, grain translucency, grain whiteness, the gloss on the pericarp and polished grain, are less well described. On the other hand, environmental factors controlling rice grain qualities are difficult to control or predict, so it can only be partially manipulated both in short and long terms. The timely important question, what is the risk of rice quality is posed by environments such as climate change, flood, drought, and farmers' management.

Previous studies have investigated factors affecting rice grain yield and quality. High temperature during growth, grain filling and ripening stages decreased grain weight and increased number of chalky grain (Cooper *et al.*, 2008; Yoshida and Hara, 1977; Yoshida *et al.*, 1981). Moreover, variable high night temperature had a major effect on rice physicochemical properties (Cooper *et al.*, 2008) and milling quality (Cooper *et al.*, 2006). While low temperature induced male sterility in 80% of spikelets (Satake and Hayase, 1970) reduced grain growth rate, extended duration of grain filling period, delay maturation and causes abnormal grain (Yoshida, 1981). Soil water condition, especially during the grain filling period, had influence grain quality of rice (Dingkuhn and Le Gal, 1996). There was reported that soil water levels can improve rice grain qualities especially grain chalkiness characteristic (Hayashi *et al.*, 2011) and rice grain reserve partitioning (Yang *et al.*, 2001). Fertilizer applications had influence on head rice yield, protein fraction and nutrient content in rice grain. (Borrell *et al*., 1999; Islam *et al*., 1996; Leesawatwong *et al*., 2005; Ning *et al*., 2009; Perez *et al*., 1996)

Unlike other cereals such as wheat and corn, which are processed to yield flours, rice is generally marketed and consumed as whole milled grain. Nutritional value of rice is lowered by the removal of nutrient-rich aleurone layers and embryo by milling process to produce white rice from brown rice. Therefore, poor people in developed and developing countries who consumed rice for the staple food are found to have nutrients deficiency, especially in Fe and Zn. However, there are many efforts to increased Fe and Zn in rice grain to solve the previous problem of nutrients deficiency such as breeding program (Graham *et al.*, 1999; Welch and Graham, 2004), biofortification (Vasconcelos *et al.*, 2003; Zheng *et al.*, 2010), and fortification (Prom-u-thai *et al.*, 2008; Prom-u-thai *et al.*, 2010). However, there are few reports about increasing the concentration of Fe and Zn in white rice together with increasing grain quality.

To achieve uniformity in the grain quality characteristics of rice, it is necessary to understand factors affecting variations in key quality characteristics. Understanding how key quality characteristics vary among Thailand's genetically diverse rice germplasm will (a) enhance the country's capacity in the production of high quality rice, directly and by enabling the national rice breeding programs to select for high quality with the more precision, and (b) develop strategies to maintain rice quality standards under difficult environment of climate variations.

1.2 Literature reviews

1.2.1 Grain structure and storage compounds

1) Structure of rice grain

The rice seed or grain comprises an outer protective covering, the hull, and the caryopsis or brown rice (Figure 1). The hull composes of the big lemma, which covers the dorsal side and the palea which covers the ventral side. The lemma and palea are joined together longitudinally by hook-like structure. Hull is high in crude fiber, silica but low in protein, lipids and starch (Marshall and Wadsworth, 1994). Brown rice comprised outer layers of pericarp, seed coat, nucellus, embryo and endosperm. The endosperm comprise the aluerone layer, sub-aluerone layer and the starchy endosperm (Juliano and Bechtel, 1985). The hull constitutes about 16-20% of rough rice weight while 1-2%nt in weight of the brown rice is pericarp, 4-6% is aluerone layer, nucellus and seed code, 1% embryo and 90-91% endosperm (Juliano, 1972). The aluerone layer varies from one to five cell layers and is thicker at the dorsal side than at the ventral side and is thicker in short grain than in long grain rice (Del Rosario *et al*., 1968; Tanaka *et al*., 1973). The aleurone layer and embryo have high concentration of protein bodies, phytic acid and lipid (Tanaka *et al*., 1973). The endosperm cells are thin walled and packed with amyloplasts which contain starch granules.

The outer layer of the endosperm or subaleurone layer has higher protein and lipid but low in amyloplasts than inner endosperm.

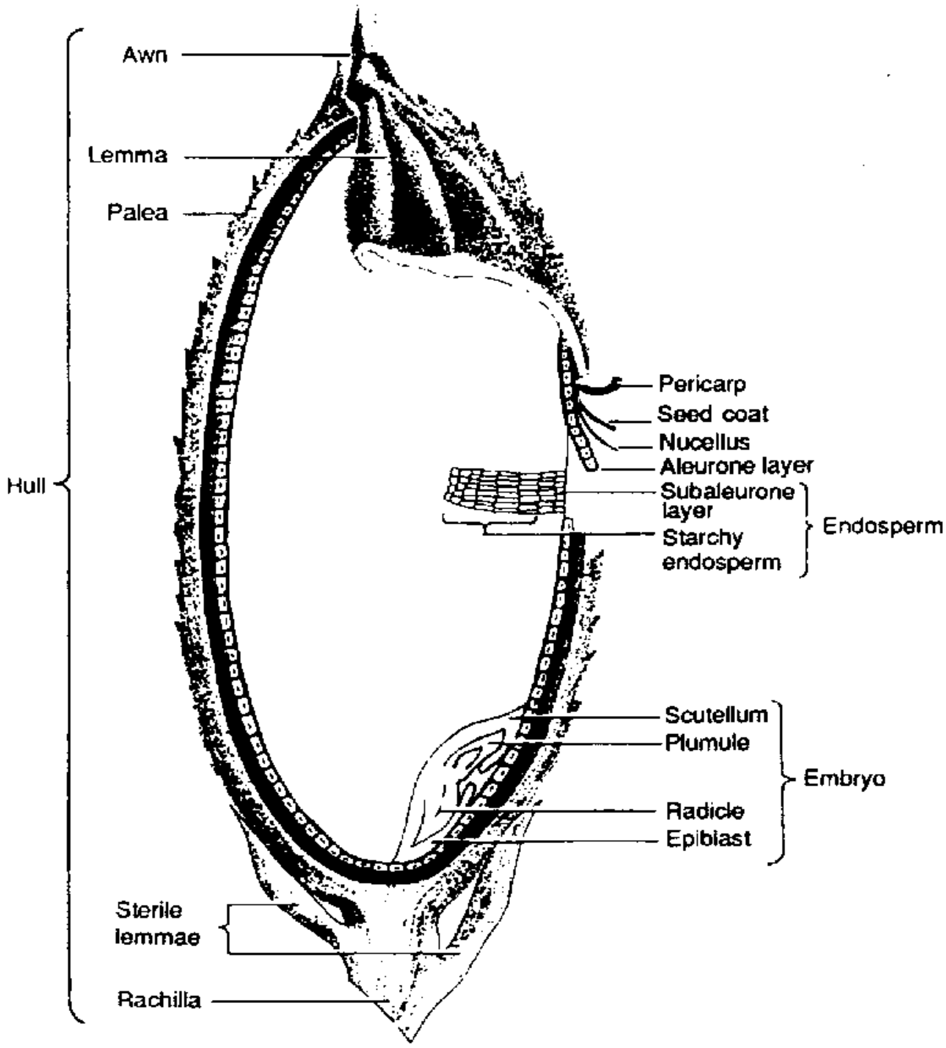

Figure 1.1 Structure of rice grain (Juliano and Bechtel, 1985)

2) Storage compounds in rice grain

2.1 Starch

Starch is the major constituent of the rice endosperm with more than 90% of dry matter. Rice granule is the smallest of rice grain starch; its shape is polyhedral and average 3-9 µm in size (Del Rosario *et al.*, 1968). Granule compound can have the diameter up to 150 µm form cluster containing between 20-60 granules in amyloplast (Juliano and Bechtel, 1985). Starch comprises polymer of D-glucose linked α-(1-4) and usually consists of the linear fraction, amylose and amylopectin, a branched fraction linked α-(1-6) (Juliano *et al.*, 1993). The main variation in starch composition is caused by the relation proportion of the two fractions together with the change length distribution, the frequency and space of branching point of the amylopectin molecule (Lu *et al.*, 1997). In glutinous rice, amylose content range from 1-2% only while amylose in ordinary or non-glutinous rice range from 10-33% (Juliano, 1980). Rice amylose is the mixture of branched and linear chain with degree of polymerization (DP_n) of 1100 to 1700 and 700 to 900, respectively. The branch fraction constituted 25-50% by number and 30-60% by mass of amylose. Rice amylopectin chain length of 19-22 glucose units, DP_n of 5000 to 15000 glucose units with 220 to 700 chains per molecules.

2.2) Protein

Protein is the second most abundant consistent in rice grain, following starch, which is most abundant in subaleurone layer and also present in aleurone cells (Azhakanandam *et al.*, 2000). Rice protein could be classified based on their solubility to prolamine (alcohol soluble), albumin (water soluble), globulin (salt soluble), and glutelin (alkaki soluble). Seed storage proteins are localized mainly in a proteinaceous organelle or protein body (PB). Protein body presents in two types; spherical type (PB-I) and irregular shape (PB-II). The PB-I contain glutelin and globulin while PB-II contain prolamin. In cereal grain, PB presents in embryo, aleurone layer and endosperm serve as the main accumulation site of storage protein and the protein that deposited in PBs are mainly alcohol soluble protein prolamin. In rice, however, glutelin accounts for 80% of total protein in starchy endosperm whereas prolamin is a minor storage protein (Juliano, 1972). Proteins in the endosperm are form of large spherical protein bodies with sized 0.5-4 µm distributed throughout the endosperm while crystalline protein bodies that rich in prolamin and small sized spherical protein bodies are located mainly in the subaleurone layer that rich in glutelin. Protein is most abundant in the subaleurone layer and also present in aleurone cells (Azhakanandam *et al.*, 2000). It has been reported that the endosperm comprises 7-18% albumin plus globulin, 5-12% prolamin and the rest is glutelin (Padhye and Salunkhe, 1979). Ogawa *et al*. (1987) estimated that endosperm storage proteins were composed of 60–65% PB-II proteins, 20–25% PB-I proteins.

2.3) Lipid

Rice lipids are presented in aleurone layer and bran are form of lipid bodied or spherosomes (Juliano and Bechtel, 1985). Most of lipids in endosperm are associated with protein bodies and starch granules as bound lipids. Based on cellular distribution and its association, rice lipids are generally separated as starch lipids, which associated with starch granules (Choudhury and Juliano, 1980) and lipids that non-starch lipids that distributed throughout the grain. The nonstarch-lipids are located in the aleurone, sucaleurone and embryo that accounted about 86-91% of neutral lipids, 2-5% of glycolipids and 7-9% of phospholipids. However, these proportions are variable by different milling degree. Non-starch lipid accounted 22-25% palmitic, 37-41% oleic acid and 37-41% linoleic acid. In brown rice, the non-starch lipids locate in embryo 14-18%, in bran 39-41%, in endosperm. Starch lipid consists of lysophospholipid, tricylglycerols, and free fatty acid (FFA). Linoleic, oleic and palmitic acid are the major fatty acid in lipid. The starch lipid was reported lowest in waxy rice (0.2 %), slightly lower in high-amylose rice and was highest in intermediate-amylose rice (1.0 %) (Choudhury and Juliano, 1980). While waxy milled rice have more non-starch lipids than non-waxy rice.

1.2.2 Rice grain characteristic definitions and standards

1) International markets standard,

This standard specification approved by the FAO Inter-Governmental Group on rice (Tuaňo *et al.*, 2011) and The Codex Alimentarius Commission committee considering the draft standard for rice proposed the following classification of milled rice (Codex Alimentarius Commission, 1990). The meaning of the terminology in Rice Standards as follows;

Rice means whole and broken kernels obtained from the species *Oryza sativa* L.

Paddy rice means rice which has retained its husk after threshing.

Husked rice (brown rice or cargo rice) means paddy rice from which the husk only has been removed. The process of husking and handling may result in some loss of bran.

Milled rice (white rice) means husked rice from which all or part of the bran and germ have been removed by milling.

Parboiled rice means rice may be husked or milled rice processed from paddy or husked rice that has been soaked in water and subjected to a heat treatment so that the starch is fully gelatinized, followed by a drying process.

Glutinous rice; waxy rice: Kernels of special varieties of rice which have a white and opaque appearance. The starch of glutinous rice consists almost entirely of amylopectin. It has a tendency to stick together after cooking.

Whole kernel is a kernel without any broken part.

Head rice is a kernel, the length of which is equal to or greater than three quarters of the average length of the corresponding whole kernel.

Broken kernels can be classified into 4 groups as follow:

1. Large Broken Kernels are fragments of kernel, the length of which is less than three-quarters but greater than one-half of the average length of a corresponding whole kernel.
2. Medium Broken Kernels are fragments of kernel, the length of which is equal to or less than one-half but greater than one-quarter of the average length of a corresponding whole kernel.
3. Small Broken Kernel are fragments of kernel, the length of which is equal to or less than one-quarter of the average length of a corresponding whole kernel but which does not pass through a metal sieve with round perforation 1.4 mm in diameter.
4. Chips are fragments of kernel which pass through a metal sieve with round perforations 1.4 mm in diameter.

Milling degree means the extent in which the bran layers and germ have been removed; it can be used for classify white rice into 3 groups as follow:

1. Under-milled rice is obtained by milling husked rice but not to the degree necessary to meet the requirements of well-milled rice.
2. Well-milled rice is obtained by milling husked rice in such a way that some of the germ and all the external layers and most of the internal layers of the bran have been removed.
3. Extra-well-milled rice is obtained by milling husked rice in such a way that almost all of the germs, all of the external layers and the largest part of the internal layers of the bran, and some of the endosperm have been removed.

Chalky Kernels are whole or broken kernels except for glutinous rice, of which at least three-quarters of the surface has an opaque and floury appearance.

Grain size rice kernel is classified accordance with one of the following specifications, indicated which classification option is chosen.

By kernel length/width ratio

1. Long grain rice
   - Husked rice or parboiled husked rice with a length/width ratio of 3.1 or more.
   - Milled rice or parboiled milled rice with a length/width ratio of 3.0 or more.
2. Medium grain rice
   - Husked rice or parboiled husked rice with a length/width ratio of 2.1–3.0.
   - Milled rice or parboiled milled rice with a length/width ratio of 2.0–2.9.
3. Short grain rice
   - Husked rice or parboiled rice with a length/width ratio of 2.0 or less.
   - Milled rice or parboiled milled rice with a length/width ratio of 1.9 or less.

By the kernel length

1. Long grain rice has a kernel length of 6.6 mm or more.
2. Medium grain rice has a kernel length of 6.2 mm or more but less than 6.6 mm.
3. Short grain rice has a kernel length of less than 6.2 mm.

By a combination of the kernel length and the length/width ratio

1. Long grain rice has either a kernel length of more than 6.0 mm and with a length/width ratio of more than 2 but less than 3, or a kernel length of more than 6.0 mm and with a length/width ratio of 3 or more.
2. Medium grain rice has a kernel length of more than 5.2 mm but not more than 6.0 mm and a length/width ratio of less than 3.
3. Short grain rice has a kernel length of 5.2 mm or less and a length/width ratio of less than 2.

1.2.3 Specific markets: Case studies in Thailand

Thai rice standard definitions adapted from Notification of Ministry of Commerce, Thailand, Subject: Rice Standard, B.E. 2540 (Thai Rice Exporters association, 1997). The meaning of the terminology in Thai Rice Standards as follows

Rice standards means the minimum specifications for rice of each type and grade for domestic trade and international trade.

Rice means any form of non-glutinous and glutinous rice (*Oryza Sativa* L.)

Paddy rice means rice that is not yet dehusked.

Cargo rice (Loonzain rice, Brown rice, Husked rice) means rice that has only been dehusked.

White rice means rice that is obtained by removing bran from cargo non-glutinous rice.

White glutinous rice means rice that is obtained by removing bran from cargo glutinous rice.

Parboiled rice means non-glutinous rice that has passed through the parboiling process and has its bran removed.

Parts of rice kernels mean each part of the whole kernel that is divided lengthwise into 10 equal parts.

Whole kernels mean rice kernels that are in whole condition without any broken part, including the kernels that have the length as from 9 parts or more.

Head rice mean broken kernels whose lengths are more than those of brokens but have not reached the length of whole kernels. This includes spilt kernels that retain the area as from 80% of the whole kernel.

Broken rice mean broken kernels that have the length as from 2.5 parts but have not reached the length of head rice. This includes split kernels that retain the area less than 80% of the whole kernel.

Chalky kernels mean non-glutinous rice kernels that have an opaque area like chalk covering the kernels as from 50% or more.

Milling degree means the degree to which the rice is milled. Milling degree is divided into 4 groups as follows:

1. Extra well milled is the removal of bran entirely to the extent that the rice kernel has a specially beautiful appearance.
2. Reasonably well milled is the removal of a large amount of bran to the extent that the rice kernel has a reasonably beautiful appearance.
3. Ordinarily milled is the removal of some portions of bran only.

Undermilled kernels mean milled rice kernels that have the milling degree below that specified for each grade of rice.

Classes of rice kernels mean classes of rice kernels which are classified in accordance *with* the length of the whole kernel. Classes of rice kernels are divided into 4 classes as follows

1. Long grain Class 1 is whole kernel having the length exceeding 7.0 mm.
2. Long grain Class 2 is whole kernel having the length exceeding 6.6 mm. upto 7.0 mm.
3. Long grain Class 3 is whole kernel having the length exceeding 6.2 mm. upto 6.6 mm.
4. Short grain is whole kernel having the length not exceeding 6.2 mm.

1.2.4 Standard for Thai Hom Mali Rice

Modified from Notification of the Ministry of Commerce Re: Prescribing Thai Hom Mali Rice as a standardized commodity and the standard of Thai Hom Mali Rice, B.E. 2544 (2001) (Thai Rice Exporters association, 2001). and No .2, B.E. 2545 (2002) (Thai Rice Exporters association, 2002).

Thai Hom Mali Rice (or Thai jasmine rice or Thai fragrant rice, or any other manes that have the same meaning, whether or not they contain a word that means "Thai") means cargo rice and white rice derived from paddy non- glutinous rice of fragrant rice varieties which are sensitive to photo period and cultivated as a main crop in Thailand, and which are certified by the Department of Agriculture, Ministry of Agriculture and Cooperatives as being Kao Dok Mali 105 variety and RD 15 variety with a natural fragrant aroma depending on its age, and when cooked, such rice kernels shall have a tender texture. Thai Hom Mali Rice Standard shall be divides into 2 types as white rice and cargo rice and standardized Thai Hom Mali Rice shall have the standard as follow

1. Containing not less than 92.0% Thai Hom Mali Rice
2. Having a moisture content not exceeding 14.0 %.
3. Having the general characteristics of a long grain rice with naturally little chalk
4. Not having any live insect
5. Having the kernel's sizes as follows
   - The average length of the whole kernels without any broken part shall not be less than 7.0 mm.
   - The ratio of the average length to the average width of the whole kernel without any broken part shall not be less than 3.2:1.
6. Having the chemical properties as follows
   - Amylose content shall not be less than 13.0% and shall not exceed 18.0% at the moisture content of 14.0%.
   - Alkali spading value of white rice kernel at levels 6-7.

1.3 Nutritional quality of rice grain

1.3.1 Energy

Rice contains starch as the principle component, like other cereal grain, it has low fat content so rice grain has a low energy density (approximate 450 kcal/100 g). However, rice accounts for 21% of global energy, provides 715 kcal/person/day. In Asia country where rice is the staple food, rice is supplied more than 50% of dietary energy, accounted about 777 kcal/person/day. Moreover, in Southeast Asia, it rise to 1256 kcal/person/day and the average rice consumption per day is about 350 g (FAOSTAT, 2009). In Thailand, the average rice consumption is about 365 g/day provided 1323 kcal/person/day.

1.3.2 Protein

Protein nutritional quality is determined by the proportions of essential amino acids, which cannot be synthesized by animals and hence must be provided in the diet. Ten amino acids are strictly essential including lysine, isoleucine, leucine, phenylalanine, tyrosine, threonine, tryptophan, valine, histidine and methionine. The aleurone and embryo tissues of grains do contain higher contents of essential amino acids but these are often not available for human nutrition as they are removed by milling process. Lysine is the first limiting essential amino acid cereal grain protein, but it has been reported that lysine content in brown rice was about 8% that was higher compared to other cereal grain such as wheat, millet, maize, and sorghum (Juliano *et al.*, 1993). Among protein fractions, albumin has the highest lysine while prolamin has the lowest therefore; the higher lysine in rice than other cereals comes from its lower prolamin and higher in glutelin. (Juliano *et al.*, 1973),). It has been reported that, the proportion of albumin and globulin are highest in the outer layer of rice grain and decreased inward the center while glutelin have inversed distribution (Houston and Mohammad, 1970). Therefore, lysine in milled rice is presented too low to meet the nutritional equipment in human.

1.3.3 Lipids

Almost all of rice oil content is located in outer layer of the grain which is removed in milling process. Rice lipids or rice oils are characterized by a high nutritional value due to its high proportion of unsaturated fatty acid that accounted up to 80% of total lipid in rice grain. Lipids are rich in the core of protein bodies (Choudhury and Juliano, 1980; Tanaka *et al.*, 1978). The major fatty acids of these lipids are linoleic,oleic and palmitic acids (Hemavathy and Prabhakar, 1987; Taira *et al.*, 1988). However, the essential fatty acids in rice are is linoleic that accounted about 29 to 42 percent and 0.8 to 1.0 percent linolenic (Jaiswal, 1983) which play an important role in physiological process such as developing and functioning of the nervous system, but cannot synthesized in the human body.

1.3.4 Vitamins

Rice grain has no vitamin A, vitamin D or vitamin C but vitamin B and E are concentrated in the bran layers (Juliano *et al.*, 1993). The vocational gradient in the whole rice grain is steeper for thiamine (B1) than for riboflavin (B2) and niacin (B3), resulting in a lower percent retention of thiamine in white rice. The B1 is concentrated in scutellum accounted about 50% of total grain. While B3 vitamin is in the pericarp and aleurone layer 80 to 85 percent (Hinton and Shaw, 1954 cited by Juliano *et al*., 1993). Vitamin E was found in the embryo accounts for more than 95 percent of total grain E (Gopala Krishna *et al.*, 1984).

1.3.5 Minerals

The minerals in rice grain are concentrated in bran fraction. It has been reported that 90% of phosphorus in bran present in phytin form and 40% in white rice while potassium and magnesium are the principle salts of phytin. Phytin or phytic acid is an antinutritional factor because it can be complex with proteins and some nutritionally important micronutrients such as Fe and Zn, resulting in a reduction in the bioavailability of these nutrients (Raboy, 2001). In addition, typsin inhibitor, oryzacystein and hememagglutonin-lecyin are also reported to be an antinutritional in rice (Juliano *et al.*, 1993). These antinutritional factors also found concentrate in rice bran fraction.

1.4 Genotype x Environment interactions affecting rice grain quality

Rice grain quality has been found to be very widely among rice varieties and environment. Several previous studies demonstrated grain quality traits are expressed in all environments tested, with some evidences of significant genotypic by environment interaction. Milling quality, cooking and eating quality and nutritional quality are affected differently by rice genotype and the environment they are also valued differently by farmers, traders and consumers. The effect of genotype by environment interactions will now be considered separately for milling quality, cooking and eating quality and nutritional quality.

1.4.1 Effect of genotype x environment on milling quality

In the rice milling process, the hull of rice grain is removed to yield brown rice then the embryo and bran are removed from brown rice to produce milled rice (or white rice or polished rice) which includes head rice and broken rice, plus the polishing or bran as by-product. Head rice is defined differently by different market standards. In Thailand, head rice includes spilt kernels that retain the area as from 80% of the whole kernel while in the U.S market and world rice trade define as unbroken milled kernel which are at least ¾ of length of whole grain after milling (Webb, 1991). Rice breakage during milling causes a significant economic loss since broken rice often has only 30-50% of the value of head rice. Many reports suggested that head rice yield is affected by environment factors and production managements.

Temperature is the major factor affecting on both rice yield and milling quality (Cooper *et al.*, 2008; Wada *et al.*, 2010). Cooper *et al*. (2006) reported high nighttime temperature during grain development correlated with decrease in head rice yield. High temperature accelerates the grain filling rate, but correspondingly shortens its duration, thus resulting in increasing the proportion of loosely packed starch granules which results in grain chalkiness, increased abnormal grain and reduced grain weight (Lisle *et al.*, 2000; Resurreccion *et al.*, 1977; Tashiro and Wardlaw, 1991; Yamakawa *et al.*, 2007; Yoshida and Hara, 1977). Yoshida and Hara (1977) reported that grain dimensions decreased with increasing nighttime temperature which is supported by Sun and Siebenmorgan (1993) and Siebenmorgen *et al*. (2006) who indicated that head rice yield is influence by the thickness distribution pattern of rice grain.

Chalky grain has negative impact on milling quality as the grain tend to break off during milling process resulting in low head rice yield. In addition, consumers' objection lowers the value and price of chalky grain in some markets. Chalky grains are categorized into white-core, milky-white, white-back, white-based and white-belly types depending on the position of the chalky part in the grain (Tashiro and Wardlaw, 1991). The formation of chalkiness is under genetic control, but it can also be influenced by the environment, especially high temperature during certain stages of grain development (Cooper *et al.*, 2008; Funaba *et al.*, 2006; Ishimaru *et al.*, 2009). Lisle *et al*. (2000) reported that rice grew in high temperature (38/12 ºC) had more chalky grain than grew in low temperature (26/15 ºC) and grains at inferior position had more chalky grain than in superior position. Cooper *et al*. (2008) tested effect of nighttime temperature at 18, 22, 26 and 30 ºC from 12 pm to 5 am on grain qualities and found that increasing nighttime temperature, the head rice yield and grain dimension were significantly decreased while grain chalkiness was increased. Wakamatsu *et al*. (2007) reported that milky-white, white-back and white-based types are frequency found when the average temperature during 20 days after heading exceeds 27ºC.

Low temperature also affects grain quality, by extending the flowering periods, delayed heading, incomplete panicle exertion, may cause male sterility in up to 80% of spikelet (Satake and Hayase, 1970), reduced grain growth rate, extended duration of grain filling period, delay maturation and causes abnormal grain (Yoshida, 1981).

Nitrogen (N) fertilization has been reported to increased head rice yield and reduced grain brakeage by modifying distribution of proteins in rice grain (Leesawatwong *et al.*, 2005; Perez *et al.*, 1996). Topdressing N at flowering stage increased rough rice yield and grain proteins (Perez *et al*., 1996). Leesawatwong *et al*. (2005) noted that rice grain had high N concentration and more storage protein accumulated in the lateral regions resulting from N application at flowering stage. The percentage of unbroken grain was positively correlated with relative abundance of storage protein in the lateral region of the endosperm of rice grain.

1.4.2 Effect of genotype x environment on cooking quality

Rice cooking quality is affected by factors such as variety, amylose content, gelatinization temperature and cooking method (Juliano and Perez, 1983; Mundo *et al.*, 1989). Rice with similar chemical properties may differ in its texture. The ratio of amylose to amylopectin in rice largely determines the texture of cooked rice. Rice with higher percentage of amylopectin is the greater water absorption and lower starch gelatinization temperature. Amylose content directly affects water absorption and volume expansion during cooking. Tenderness and stickiness of cooked rice is inversely correlated with amylose content. Cooked rice with high amylose content are relatively dry, individual grains remaining separate, and are less tender and become hard upon cooling. Low amylose rice become soft and sticky (Ramesh *et al.*, 1999). However, many cultivars with similar amylose contents have shown different pasting and texture properties. Champagne *et al*. (1999) that suggested that components other than amylose also affected the cooking properties of rice. Gel consistency is developed as an index to measure cooked rice hardness among high amylose rice (Cagampang *et al.*, 1973). Rice of similar amylose content can be differentiated according to tenderness. Within the same amylose group, cooked rice with softer gel consistency is tenderer.

High temperature at grain ripening stage has been reported decrease amylose content and aberrant amylopectin structure in non-waxy rice (Yamakawa *et al*., 2007). Zhong *et al*. (2005) investigated effect of high temperature on cooking quality of indica varieties with different amylose contents. The results showed that high temperature affected on amylose content and gel consistency in different varieties differently. By contrast, the gelatinization temperature of all varieties was increased. Pantiadol and Wang (2002) studied the fine structure and physicochemical properties of rice starch from translucent and chalky grain in six rice varieties and found starch from chalky grain had lower amylose content than translucent grain.

Nitrogen application has been reported to increase protein content in rice grain (Leesawatwong *et al.*, 2005; Ning *et al.*, 2009; Ning *et al.*, 2010). Protein plays an important role in determining the cooking properties (Martin and Fitzgerald, 2002). It has been reported that protein affected the amount of water the rice absorbs in early cooking, and the availability of water in early cooking will determine the hydration of the protein and the concentration of the dispersed and viscous phases of the starch, which will determine the texture of the cooked rice. In addition, Furukawa *et al*. (2006) noted that protein fractions such as prolamin increased the hardness of cooked rice while glutelin degraded the appearance of cooked rice.

1.4.3 Effect of genotype x environment on nutritional quality

Nitrogen application has a marked effect on protein content of both brown and white rice. Adequate nitrogen fertilizer application at panicle initiation can increase grain protein concentration (De Datta et al., 1972). However, the responses of grain protein to nitrogen application depend on rice cultivars (Islam *et al.*, 1996) and grain protein concentrations (Borrell *et al.*, 1999; Leesawatwong *et al.*, 2005). Ning (2009) reported that nitrogen application reduced phytic acid concentration and increased ratio of glutelin to total protein in rice grain, indicating that nitrogen has a benefit on rice nutritional quality. Hao *et al*. (2007) found that applying nitrogen fertilizer increased the concentration of Fe, Mn, Cu and Zn in brown rice which reached the highest concentrations at 160 kg N/ha. Jin *et al*. (2008) reported that foliar Fe and B complex after rice anthesis increased Fe concentration and 16 amino acids such as lysine, threonine and argentine. However, micronutrients in white rice especially Fe and Zn which are the most prevalent deficiency in human did not increase or increased only marginally because most of them were removed during milling process. It is important to find out how to increase Fe and Zn in white rice which is the form of rice most commonly consumed if the Fe and Zn intake of poor rice eaters can be increased and their health improved.

1.5 Nutrients transport into rice plant

1.5.1 Nutrient uptake

In general, plant nutrient solutes in soil solution are generally moves to root surface driven by diffusion and mass flow (Marschner, 1995). Root cell wall consists of cellulose, hemicellulose and pectin that have different size of pores but large enough to allow ions and water move into root. The movement of the ion via this route is called apoplastic. Then ions pass through the plasma membrane or plasmolemma to the xylem symplastically by move through the cytoplasm of the cell to another cell. In plasma membrane, proteins were embedded and act as a transporter for the ions which is specific for nutrient element.

In case of Fe uptake, under Fe deficiency condition, plant has regulated mechanism for acquiring Fe from soil and can be group into 2 strategies (Marschner, 1995). Non-graminaceous plants take up Fe by a reduction-base strategy I which consists of three process, first, proton extrusion by proton-ATPases in order to acidify the rhizosphere and increase Fe^{3+} solubility then solubilized Fe^{3+} is reduced by membrane-bound ferric reductases and finally, Fe^{3+} is absorbed into root by transmenbrane Fe transporter (Sperotto *et al.*, 2012). In contrast, graminaceous plant uses a cheletion-based strategy II that release phytosiderophores (PS) mugenic acid (MA) family that have high affinity for Fe^{3+} into rhizosphere. The Fe^{3+}-PS complexes are taken up into root cells by the yellow-stripe (YS) family (Bashir *et al.*, 2010; Ishimaru *et al.*, 2010; Kim and Guerinot, 2007). Inoue *et al*. (2009) noted that *OsYSL15* has been identified as an Fe-MA transporter from the rhizosphere to the root and is involved in internal Fe homeostasis. In plant, Fe is transported symplastically until it is loading in the xylem. The mechanism of xylem unloading to symplast and reabsorbed to apoplast is unclear however it is believed that component of strategy I uptake play a role when Fe moved across the plasma membrane of the leaf cells (Kim and Guerinot, 2007). Recently research has been suggested that rice can be utilizing Fe by both strategies. Beside secreting MA, it also absorb Fe^{2+} which is more abundant under submerged conditions which rice is well adapted (Ishimaru *et al.*, 2006)

In low Zn availability soil, plant roots released PS to complex with Zn then transported to the outer face of the root cell plasma membrane. However this did not occur in rice. Rice plant exudes low molecular weight organic, such as citrate that is reported to be effective transported Zn compound to bound with Zn then taken up by plant roots as Zn^{2+} or $Zn(OH)_2$ into root cell and transported in plant either as Zn^{2+} or bound to transporter proteins. These proteins are known as zinc regulated in the iron regulated-like protein gene family or ZIPs. In rice plants, *OsZIP1*, *OsZIP3*, *OsZIP-4* and *OsZIP5* were the Zn transporters induced by Zn deficiency. While OsZIP1, *OsZIP3* and *OsZIP-4* were expressed in the vascular bundles and epidermal and cells in rice roots and in the vascular bundles in rice shoot. In addition, *OsZIP4* and *OsZIP5* are found to be the main protein involved in Zn translocation within rice plant (Ishimaru *et al.*, 2005; Lee *et al.*, 2010).

1.5.2 Nutrients mobilization

Once taken up to the root, nutrient ions are transported to different plant parts via two transports system, the xylem and the phloem. The transport of ions in the xylem is driven by the gradient in hydrostatic pressure (root pressure) and the gradient in the water that created by the loss of water from leaf surface by evapotranspiration. The movement of ions in the xylem is directed to the highest transpiration sites that might not be the highest nutrient requirement sites (Marcherner, 1995). The transports in the phloem take place in sieve tubes. It is evident that the entry of ions into phloem due to osmotic potential that creates a positive internal pressure. This pressure induces a mass flow in the phloem from higher to lower pressure sites that causes removal ion or solutes from the phloem. The retranslocation of nutrients take occurs in the phloem because of bidirectional transport property.

Radial transport of Fe from root epidermis to xylem vessel is assumed as the complex with the chelator such as nicotiananide (NA) as Fe^{2+}-NA on the symplastic route. The release of the Fe to the xylem vassels is the transfer from the symplast to the apoplast. Nutrient ions has loaded into non-living xylem vessel for transport with transpiration steam to plant shoot. In xylem vessel, Fe is oxidized then transported as Fe^{3+-}citrate complex

(Hell and Stephan, 2003) while Zn complexes with citrate or malate. Nutrients loading into phloem is very important for nutrient accumulation in grain because the taken up nutrient by xylem is unidirectional therefore nutrients cannot transports to the entire demand organs. The nutrient ions in xylem vessels move symplascally to the phloem. However, Fe requires specific protein transporters to move across cell to reach the phloem. *OsYSL2* has been identified as and Fe transporter in rice. For Zn, *OsZIP4* has detected exclusively for Zn translocation within rice (Ishimaru *et al.*, 2011; Ishimaru *et al.*, 2005). In phloem, a hydrostatic pressure gradient form the source tissue induces a mass flow in the phloem to the site of lower pressure caused removal of nutrient from the phloem to sink (Marschner, 1995; Waters and Sankaran, 2011). In addition, the xylem to phloem transfer is importance for mineral nutrient in plant because the xylem transport is directly to the highest transpiration organ which is not the site of highest demand of mineral nutrient (Marschner, 1995) such as young tissue and grain.

Grain consists of embryo, endosperm and aleurone layer, enclosed by maternal tissues (seed coat). The developing grain is connected to the maternal plant by a single vascular bundle (Zhang *et al.*, 2007). This vascular bundle ends at the seed coat and is not connect to the endosperm or embryo. It has been suggested that nutrients are delivered to the maternal tissues surrounding the seed and eventually effluxes appoplastically into the grain tissue (Waters and Sankaran, 2011). In rice, Krishnan and Dayanandan (2003) suggested that the transports of the mineral nutrients to the endosperm is moving appoplastically exists vascular trace, chalaza, nucellar projection and nucellar epidermis through the alrurone cells into the endosperm.

Nutrients may remobilize from the reserved organ such as root, stem and leaf to seed. In rice, flag leaf is the major source of photo-assimilates for developing seed and believed to be one of the major sources of remobilized metals to seeds (Sperotto *et al.*, 2010). It has been reported that Zn concentration in rice flag leaf decreased during grain reproductive stage in high seed Zn varieties. Whereas Fe concentration in flag leaf of high seed Fe decreased during reproductive stage while in low seed Fe varieties, the Fe concentration in rice flag leaf show high level of Zn residual in flag leaf (Sperotto *et al.*, 2009). Jiang *et al*. (2008) reported The Zn accumulated in rice grain is uptake by root but not from remobilization from leaves after rice flowering. However, Wissuwa *et al*. (2008) and Phattarakul *et al*. (2012) reported that foliar Zn fertilizer could increase grain Zn in rice which suggested that Zn density in rice grain might from the Zn retranslocation in rice plant.

1.5.3 Fe and Zn distribution in rice grain

Previous studies have reported a wide variation Fe and Zn concentration in brown rice among different varieties from 4 to 24 mg Fe/kg and 13.5-58.4 mg Zn/ kg (Gregorio, 2002). However, Fe and Zn in white rice is generally much lower at 2 to 11 mg Fe/ kg (Prom-u-thai *et al*., 2007) and 9.7–26.5 mg Zn/kg (Prom-u-thai *et al*., 2010; Sellappan *et al*., 2009) due to the removal during the milling process.

Rice grain has high concentration of Fe and Zn in bran fraction which consists of pericarp, seed coat, nucellus, aluerone layer and embryo which are removed in milling process (Prom-u-thai *et al*., 2007). Therefore, 25-85% of the nutrient concentrations can be lowered after milling (Prom-u-thai *et al*., 2007; Sellappan *et al*., 2009) depending on degree of milling (Yeshwant *et al*., 1979), grain morphology (Prom-u-thai *et al*., 2007) and cultivars (Prom-u-tha *et al*., 2007). In addition, the bran fraction of rice grain are concentrated in inhibitory substances, such as certain polyphenols, flavonoid such as proanthocyanidins known as tannin and phytic acid, that can reduce nutrient availability.

In cereal grain, Fe and Zn are preferentially stored together with phytate which is a strong chelator of divalent cations and significantly reduces mineral bioavailability. The complex of Fe- or Zn-phytate is stored in membrane enclosed globoids in the protein storage vacuoles. Phytic acid or *myo*-inositol 1,2,3,4,5,6-hexa*kis*phosphate (Ins*P*6) is the known storage of phosphorous (P) in seed which typically represents about 65-85% of seed total grain phosphorus (Raboy, 1997). In rice grain, Fe and Zn are present highest concentrations in the aleurone and embryo, whereas there is very low concentration in endosperm or white rice which is the most preferable consuming form.

However, cereal grains also contain endogenous phytase, an enzyme that can be decreasing or eliminating the anti-nutritional effect of phytic acid (Gobbetti *et al.*, 2005). These enzymes act by hydrolyzing phytic acid into lower myo-inositol phosphate esters with a lower capacity to bind minerals (Leenhardt *et al.*, 2005). Furthermore, it has been reported that milling is the critical process that affects the contents of both phytic acid and nutrients in milled rice. Milling removed a substantial proportion of nutrient inhibitors such as phenol and phytate, however, nutrient concentrations such as Fe and Zn in the white rice were also substantially reduced, due to the loss of Fe and Zn in the bran fraction (Prom-u-thai *et al.*, 2006; Wang *et al.*, 2011)

1.5.4 Increasing Fe and Zn in rice grain

Micronutrient malnutrition is currently list as a major risk factor for human health and affecting more than 3 billion people worldwide (Hotz and Brown, 2004; Welch and Graham, 2004). Among micronutrient deficiencies, Fe and Zn deficiencies are the most prevalent one especially in developing countries where a large proportion of dietary Fe and Zn intake is derived from consumption of cereal grain such as rice.

Increasing Fe and Zn concentration in rice grain is a challenge to alleviate Fe and Zn deficiency health problems in people in developing countries who consumed rice as a stable food. The efforts to increase Fe and Zn concentrations in rice grain are expected to promote their intake by rice consumers and decreased Fe and Zn deficiency in developing countries where consumed rice as a staple food. The emphasis of this effort has been on agronomic management, selecting and breeding crop for high Fe concentration (Graham *et al.*, 1999; Welch and Graham, 2004), biotechnology (Vasconcelos *et al*., 2003; Zheng *et al*., 2010) and nutrients fortification (Prom-u-thai *et al*., 2008; Prom-u-thai *et al*., 2010). Iron and Zn fortification in parboiled rice has been reported significantly increase Fe and Zn concentrations in white rice as high as 140 mg Fe/kg and 13.2-44.1 mg Zn/kg. In addition, this Fe enriching process does not show adverse effect on cooking quality and sensory attributes (Prom-u-thai *et al*., 2008; Prom-u-thai *et al*., 2009). Moreover, it had high potential Zn bioavailability of human intake. However, this method is not already successful for rice parboiled manufactory because of high cost.

Nutrients bio-fortification could be provided Fe and Zn in rice grain through different nutrients application methods such as soil application, seed priming and foliar application. Foliar application is considered as a short time tool for bio-fortification of plant such as foliar Zn in wheat because soil application of most Zn sources is generally ineffective because of the rapid conversion of soluble from into unavailable form. Foliar application can be enriching micronutrients in crop grain by penetrated of the cuticle or via the stomata pathway. Many studied reported that foliar application is currently used to alleviate micronutrient deficiencies with the effectiveness and low-cost strategy. Recently, it was reported that Fe and Zn concentration in cereal grain can be increased by foliar Fe and Zn fertilizers. For examples, in rice, Zhang *et al*. (2008) reported that rice grain Fe could be increased about 88% when sprayed with the complex of 0.1% (v/w) Fe(II)amino and compound amino acid by 88% compared to the control. Yuan (2012) sprayed Fe combined with amino acid and 1% (v/w) nicotianamine acid (NA) in rice once after anthesis and the results shown Fe concentration in brown rice of most cultivars increased by 32.5% while the combined Zn and Fe-AA solution can improved Zn in rice grain by 42.4%.

1.6 Objectives

From literature reviews, a number of studying rice quality have focus on effected of environment on milling quality or nutritional quality, separately and most of work have been studied on japonica rice and quite limited studied on milling quality along with nutritional quality on indica rice especially in Thai rice varieties. The work reported in this thesis based on the following objectives:

1. To determine effects of genotype and environment on selected grain quality characteristics of main rice varieties.
2. To identify rice genotypes with consistently high quality characteristics.
3. To identify likely environmental threats to rice quality and possible methods for control.