INTRODUCTION

Rice (*Oryza sativa*), the principal cultivated species of rice is believed to have been domesticated nearly 10,000 years ago in an area that includes north eastern India, Bangladesh, Burma, Thailand, Laos, Vietnam, and southern China (John and David, 1995). Rice is an important staple cereal of a large fraction of the world's population. More than 90% of the world's rice is produced and consumed in Asia (John and David, 1995). In the North of Thailand, rice is the interesting source (Oka and Chang, 1963). There are high genetic variations in each ecology (Watabe, 1967). The variation of isozyme is found in lowland and upland rice which collected from the North of Thailand (Pan, 1997 and Panita, 1998). In addition, the genetic diversity is found in both within and between populations in grain, morphological and physiological characteristics and DNA level of the local rice varieties (Wichuta, 2008).

In Thailand, purple rice genotypes, rice with purple pigment in the husk (hull) and pericarp, are local races; the type is a glutinous *indica*, grown widely in different geographical areas across the kingdom and varied in their phenotype pigmentation. As the local ancient wisdom, the rice was considered to have an herbal property and has been used as a traditional Thai herb for various kinds of a medicinal treatment. Differing among the cultivated varieties depended on the pigmentation of purple color (anthocyanin) on the plant part, enriching the colorful agro-biodiversity of Thailand rice field. In addition, the purple rice is use as herbal crop in the ancient wisdom may notify a particular substance synthesized distinguishable from the white rice. Agriculture nutrition substances, that are responsible for the human health, are inquired (Andre, 2002).

In recent years, consideration on the natural antioxidant nutrition in food has been increasingly of interested in the prevention of oxidative stress-related diseases (Willcox *et al*., 2004). The first step to achieve this goal is to investigate the existing active nutrients having antioxidant capacity in vegetables and cereal crops consumed as a normal food diet.

Gamma oryzanol is a naturally component of rice bran, corn and barley oil which occurring mixture of plant chemicals called sterol which occurs in plants both in the free and esterified form, the latter esterified to other moieties, such as fatty acids, glucosidea and ferulic acid. Ferulic acid esters of phytosterols are commonly known as oryzanol. Rice bran oil (RBO) is rich in oryzanol and β-sitosterol (Itoh and Matsumoto, 1973). The content of γ-oryzanol differs with the source of RBO, ranging from 115 to 780 ppm depending on the degree and possibly the method of processing (Rogers *et al.,* 1993). In addition, Oryzanol has been reported to have diverse health effects, including a hypolipidemic effect, growth promotion, and stimulation of the hypothalamus (Seetharamiah and Chandrasekhara, 1990; Rukmini and Raghurum 1991; Nicolosi *et al*., 1991). Moreover, γ-oryzanol has been shown suitable as a natural UV filter in sun screen creams and to have antioxidant functionality (Duve and White, 1991; Tawatchai *et al.,* 2004).

It is primarily consumed after procession as polished rice. The bran or germ which comprises 10% of whole rice is removed during the polishing process. However, rice bran is an important source of rice oil and other phytochemicals which possess antioxidative and disease-fighting properties. Rice-bran products drawing much attention of biomedical researchers include: myo-inositol (a B vitamin), its phosphate-derivative inositol hexaphosphate (IP6 or phytate), rice-bran oil (RBO) and polyphenols with antioxidant function. IP6 is the major form of phosphorylated inositol present in foods, constituting 1-5% by weight of most cereals, nuts, oilseeds, legumes and grains. It occurs at 9.5-14.5% by weight in rice bran. Antioxidative polyphenols in rice bran include ferulic acid, its esterified derivatives (oryzanol), tocopherols and other phenolic compounds (Jariwalla, 2001). Moreover, rice bran oil is the principal source of γ-oryzanol, but it is also found in the bran of wheat and other grains, as well as various fruits, vegetables, and herbs.

Moreover, In Thai purple rice, it is found that the purple rice exhibited higher amount of γ-oryzanol than the white rice control variety. In addition, genetic variation of γ-oryzanol apparently exits in the population of purple rice cultivars as different amounts are found (Kalardee *et al.,* 2003). Moreover its anthocyanin (cyanidin-3-glucoside: C3G) is evidenced by its inhibition on the growth of Lewis lung carcinoma cells *in vivo* (Chen *et al*., 2005). However, these belief and reports are interesting. Since many medical evidences provided a support in a relationship between a dietary habit, particularly a special dietary components and a development of a certain health problem such as cholesterol and cancer. It means that the purple rice included in a dietary component could conceivably interrupt the pathogenic pathway of cancer. Moreover, variation in many phytochemical substances present in purple rice grains that believe to have a special biochemical and physiological properties act as an anti-cholesterol and anti-cancer can be used in researches aim to improve a rice processing of functional food. Therefore, investigation of issue should be performed before these purple rice varieties have disappeared forever from the paddy rice field of Thailand.

In this study, objectives are set up in order to analyzing gamma oryzanol in clarifying its genetic variation and its relation to a purple color of anthocyanin and also to nitrogen and phosphorus fertilizers. Its genetic inheritance is also estimated. The results can indicate the diversity in γ-oryzanol content among local genotypes in Thailand rice germplasm. Moreover, the study γ-oryzanol content can be used for improve or increase a value of Thai rice. Agronomic characters such as leaf, stem and grain can provide understanding in genetic diversity and the purple color of the pericarp can also be applied as a genetic marker. And the Thai purple rice diversity could prove a priceless heritage of rice germplasm. Moreover, the results of heritability value could indicate the diversity in γ-oryzanol content among local genotypes in Thailand rice germplasm. And use this result for selection of parents for improves rice quality in breeding program. Moreover, it is to estimate the possibility of breeding program by heritability.

Objectives:

The main objectives included:

1. To classify the genetic variation among the Thai purple rice varieties.
2. To investigate the content of γ-oryzanol in unpolished grains of purple rice.
3. To estimate the effect of N, P and K fertilizer level on the increase of γ-oryzanol content in grains.
4. To calculate heritability value of γ-oryzanol accumulation in grains.

CHAPTER 1

LITERATURE REVIEW

Rice (*Oryza sativa*) is the most important cereal crop in the developing world and is the staple food of over half the world's population (Juliano, 1993). It belongs to the grass family, technically called Gramineae or Poaceae family and classified as *Oryza* genus. Rice grows well in the hot as well as warm climate, covering from 43 degree N latitude to 35 degree S latitude and can be grown on the fields ranging from mean sea level to high land approximately 2,500 meters (Jitrakorm, 2003). Because of its long history of cultivation and selection under diverse environments, *O. sativa* has acquired a broad range of adaptability and tolerance so that it can be grown in a wide range of water/soil regimens from deeply flooded land to dry hilly slopes (Lu and Chang, 1980).

Rice culture is classified according to source of water supply as rainfed or irrigated. Based on land and water management practices, rice lands are classified as:

- Lowland (wetland preparation of fields)
- Upland (dryland preparation of fields)

Then, according to water regime, rice lands have been classified as:

- Upland with no standing water
- Lowland, with 5-50 cm of standing water
- Deepwater, with >51 cm to 5-6 meters of standing water

Based on varietal type used, rice culture can be further classified into:

- Lowland rice, with plants of semidwarf to medium to tall (100 cm to 2 m) height
- Upland rice, with plants of medium to tall (130-150 cm) height
- Deepwater rice, with plants of medium to tall (120-150 cm tall without standing water, 2-3 m with rising water level) height

In Asia, the year is divided into fairly distinct wet and dry seasons. Lowland rice culture is the most predominant system followed (Surajit, 1981).

Origin, Species and Types of Rice

Oryza sativa, the principal cultivated species of rice is believed to have been domesticated nearly 10,000 years ago in an area that includes Northeaster India, Bangladesh, Burma, Thailand, Laos, Vietnam, and southern China (John and David, 1995). The greatest diversity of the primitive cultivated forms and their wild relatives have been found in this broad area. Rice is one of the oldest cultivated crops, having been cultivated in India and China for up to 8,000 years. Rice spread from its area of primary diversity throughout Southeast Asia and adjacent islands of the Pacific region. The only other cultivated species of rice, *O. glaberrima* Steud., is indigenous to the upper valley of the Niger river in West Africa. Although still cultivated in small areas of west tropical Africa, *O. glaberrima* is being replaced by improved cultivars of *O. sativa*. It has been suggested that *O. sativa* and *O. glaberrima* descended from a common progenitor in the distant past, but evolved through separate pathways following the fracture and drift of the great land masses that led to formation of the Asian and African continents.

The genus *Oryza* contains 20 species with a basic chromosome number of 12. The genus includes both diploid and tetraploid species with six genome groups, A, B, C, D, E, and F. The cultivated species, *O. sativa* (2n = 24) has the AA genome formula. *O. glaberrima* (2n = 24) does not pair well with *O. sativa* and has been given the genome formula A_g A_g. Six of the species of *Oryza* are annuals, the remainder perennial. Two diploid wild species that contain the AA genome, *O. nivara* and *O. rufipogon,* are widely distributed throughout Southeast Asia and hybridize freely with each other and with cultivated rice.

The species, *O. sativa*, has evolved into three types, generally characterized as follows:

- *Indica*: the tropical type, typically with tall plants, weak stems, long and droopy leaves, sensitive to low temperature and photoperiod, slender grains that shatter easily and remain dormant for long periods, and the source of dry-cooked rice.

- *Japonica*: the temperate type, typically with short leaves and stems, moderate tillering, resistant to low temperature, short rounded grains with low amylase content that makes the grain cohesive or sticky when cooked.

- *Javanica*: characteristically, tall with thick stems and broad stiff leaves, low tillering, long panicles, resistant to shattering, and large bold grains.

In addition, IRRI can group rice grain size and shape type by use the following scale for size: extra long, > 7.5 mm; long, 6.61 to 7.50 mm; medium, 5.51 to 6.60 mm; and short, 4.50 mm. Grain shape is characterized based on length-to-width ratio:

slender, > 3.0; medium, 2.1 to 3.0; bold 1.1 to 2.0; and round, $\leq$ 1.0 (Juliano, 1993). In Thailand, rice was grown not less than 5,000 years. Rice has been important in the well-being and life of Thai people from the beginning until now. Both non-glutinous and glutinous rice have been the main food consumed daily by Thai people (Somrith, 1992). According to amylase content in grain, it can be separated in glutinous and non-glutinous rice. Non-glutinous rice had a translucent endosperm, whereas waxy (0 to 2 percent amylase) rice had an opaque endosperm because of the presence of pores between and within the starch granules. Thus, glutinous grain had about 95 to 98 percent the grain weight of non-glutinous grain (Juliano, 1993). The growing of non-glutinous rice had been increased while the long grain glutinous rice started to extinct during the 15th – 18th century. The increase of non-glutinous rice was due to the group of people who differed from the original group who liked glutinous rice. Another important reason that non-glutinous rice growing increased widely was that rice became export goods in the 18th century (Chitrakon and Somrith, 2003).

Genetic Diversity in Rice

There is great genetic diversity in rice. Natural mutants have occurred with a rather high frequency, enabling *O. sativa* to adapt to a wide range of agro climates. Tens of thousands of native varieties have evolved in the microclimates of traditional rice growing areas, and more have been added through rice breeding programs in many countries. Native varieties collected from farmers' fields are first grouped into ecological types based on culture and climate and into subpopulations based on visible characteristics such as pigmentation of the leaf sheaths, resistance to pathogen biotypes, gelatinization temperature of the grain, and many more including variations in quantitative traits.

Agriculture relies heavily on the genetic diversity of crop plants. Ever since the very beginning of agriculture (more than 10000 years ago), during the process of domestication and cultivation of crop plants, a wealth of genetic diversity has been utilized and partly preserved. It is estimated that not even 15 percent of the potential diversity has been utilized (FAO, 2002). Thousands of valuable allelic variations of traits of economic significance remain unutilized in nearly all crop plants. These can be discovered and effectively used to meet the existing and emerging challenges that threaten world food security. Sadly, this genetic wealth is being eroded due to neglect and over-exploitation. Developmental activities and exploitive land-use planning are destroying natural habitats, and modern varieties are replacing native species and landraces, resulting in a reduction of varietals diversity. Major crop species (rice, wheat and millet) suffered the most during the green revolution. In order to successfully meet future food requirements, it is necessary to manage the continuing genetic erosion and address the issues of genetic conservation and optimum utilization of what remains of the genetic diversity of important crop plants (FAO, 2002).

Rice is rich in genetic diversity, with thousands of varies grown throughout the world. In its natural unpolished state rice comes in many different colors, including grown, red, purple and even black. These colorful rice varieties are offer prized for their health properties. Unpolished rice has a higher nutrient content than milled or polished white rice (FAO, 2004).

Local Rice Thailand Genotypes

Thailand is one of the world's biggest rice producers, with paddy output of 27 million tones in 2003. Thailand is also the world's biggest rice exporter: annual shipments are worth more than $2,000 million and reached 7.5 million tons in 2003. Its main export markets are Indonesia, Nigeria, Iran, the United States and Singapore (FAO, 2004). Moreover, rice has been involved in Thai people's way of life. It is deeply embedded in the cultural heritage of their societies. There are several cultural activities related to rice. All of the related rice tradition, ceremonies, legends, beliefs, and relation systems are not only illustrative of the local community and people's way of life but they also reflect the benevolence between humans and nature, as well as among humans.

There is diversity of local Thailand rice genotype because the farmers have maintained and conserved these local rice genotypes following reasons (Bhundit and Areewan, 2006).

1. Specific local rice genotypes are very well adapted to the various rice ecosystems found in the area.
    a. *Flooded or deep water rice field.* The adapted rice varieties have the special characteristic that enable it the ability to elongate its stems as the water level increases. These rice genotypes are usually late maturing, and are harvested in December.
    b. *Lowland rice field.* The most adapted rice genotypes for lowland rice field are medium maturing genotypes that are usually harvested in November.
    c. *Swamp soil rice field.* The adapted rice species under this condition are late maturing genotypes that are harvested in December.

d. *Highland rice field.* The adapted rice varieties early – maturing – genotypes that are harvesting in October.

e. *Hill slopes or high paddy rice field.* The adapted rice genotypes in this condition are medium maturing genotypes.

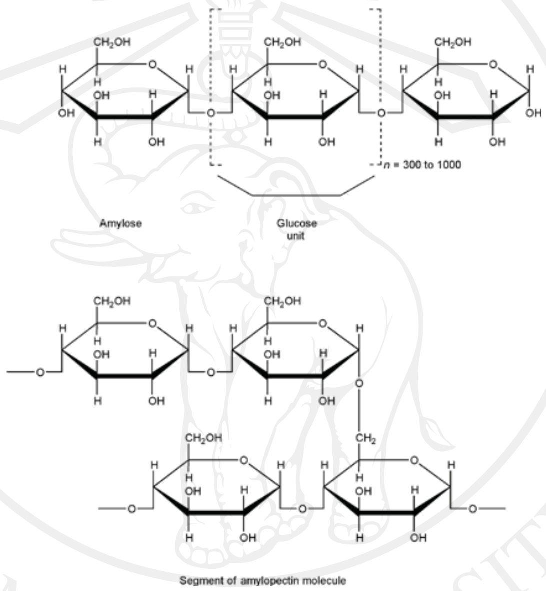
2. The rice eating culture and preferences of people. Different areas within the region have different culture in eating rice.
3. Medical beliefs. Many people believe that eating some rice genotypes is good for health.
4. The special rice genotypes for cooking specific food or dessert in festival or traditional ceremony. These rice genotypes usually ripe and ready to be harvested for festival or traditional ceremony periods.
5. Food security and labor allocation. Many farmers grow different rice genotypes with different maturity periods, for example early-, medium- and late-maturing genotypes, to ensure that they have rice supply all throughout the year. Before the main harvest season and when in storage is almost empty, the farmers would grow an early maturing genotypes because of the urgent need for rice. In addition, the planting of different rice genotypes with different maturity periods enables the farmers to manage and allocate family labor more wisely.
6. Market demand. The price of some local rice genotypes in the market is higher than others. Thus, farmers tend to grow those genotypes in demand. Consequently, the available rice for family or community consumption decreases. The market price condition is a significant factor that affects farmer decision-making.

7. Reduce cost of production. Local rice genotypes are strong, insect or disease resistant, and already adjusted to the local environment. Some local rice genotypes are high yield but do not require pesticide or chemical fertilizer.
8. Conservation spirituality, some farmers are conscious that all local rice genotypes are descended from their ancestors who used to grow them. These rice genotypes therefore, are not supposed to be lost from their rice field.

Thailand is situated in the diversity center of both wild and cultivated rice. There are 5 kinds of wild rice cultivated that are the ancestry of cultivated Asian rice. Apart from the different types of rice, Thailand also has large number of rice varieties. There are approximately 3,500 names with different characters. The obvious different characters are those of non-glutinous, glutinous rice with early or medium and late maturity. Within each character there are further distinctive endosperm type varieties, such as, having different amylase contents that make cooked rice soft, hard or sticky. From the genetic diversity found in wild rice that is the ancestry of cultivated rice as well as that of cultivated rice found in Thailand, it has been acceptable that Thailand is the origin and distributor of Asian rice (Chitrakon and Somrith, 2003).

There are two types of rice: glutinous (waxy) and non-glutinous (non-waxy). The difference between glutinous and non-glutinous rice is well know due to the separation in cooking methods for their usage as food. It corresponds to the difference in relative ratios of amylase and amylopectin which vary in terms of the chemical structure of molecules in the reserve starch (Figure 1.1). This starch is contained in endosperms of grains or pollens. The reserve starch in non-glutinous rice comprises approximately 20% of amylase and 80% of amylopectin in a mixed form.

Conversely, the starch in glutinous rice comprises 100% of amylopectin (Sakamoto, 1993). It can be easily distinguished from the common starch of the latter by using the iodine color reaction test. All the reserve starch contained in embryos, pre-fertilized ovaries, culms, leaves, glumes and roots is stained blue in iodostarch reaction (Watabe and Umekage, 1958).



Source: http://www.medicinescomplete.com

Figure 1.1 Structure of amylase and amylopectin in rice

It has been observed that even endosperm with red or black-colored pigment on its seed coat, produces similar changes inside. The distribution of glutinous rice cultivation in Southeast Asia appears to center in Northern and Northeastern Thailand and Laos, spreading to the surrounding regions of Mynma, Yunnan, Viet Nam and Cambodia – a wide distribution sphere ranging from Latitude 15° to 25° N. Glutinous rice is the principal staple food in its central area of cultivation and seems to be coming into use for production of confectioneries and alcoholic drinks. Thai farmers have little comprehension of varietal distinctions. They sometimes give entirely different names to the same variety and on the contrary lump different varieties under the same name. Thus, any rice which was called in the similar name, it may be different varieties. However, if classified only according to their main morphological characteristics, there would still be perhaps several hundred varieties (Watabe, 1967).

Morphological variations

Among the traditional rice cultivars, some rare-colored variants that are caused by anthocyan-origin pigments are existed. Purple rice plants are colored deep purple, including the glumes, leaves, nodes, internodes, awns and stigmas (Kitano *et al.,* 1993).

In seeds: the variations on grain shape of rice cultivars exist close relationships between their grain shapes and ecotypes. Short grains correspond to *japonica*, long grains to *indica* and large grains to *javanica*. Based on these relations, the ratios of length to width of husked grains are used as one of the criteria for classifying rice ecotypes (Kitano *et al.,* 1993).

In grain color: A wide range of variations are recognized among the traditional cultivars in the colors of husked grains. The variations in grain color are due to the presence of anthocyan-origin pigments in a pericarp of kernels. With the absence of this pigment, the kernel color is as white as usual. In cases where the kernels contain pigments of red or purple in pericarps, the cultivars producing those kernels are called red rice (Arashi, 1974; Kalardee *et al.,* 2007).

Local Purple Rice Thailand Genotypes

Rice has been the source of variety of traditions, both directly related to rice and indirectly related to rice which originated from the accumulation of experiences, knowledge and local heritage, for survival and in order toset up rules and regulations for the future generations (Jitrakorn, 2003). Rice which has purple pigment in the husk (hull) and pericarp as a unique characteristic was called "Kaow Kum". The word "Kaow" means rice and "Kum" means dark red (Kalardee *et al.,* 2007). Therefore, Kaow Kum means the dark red rice which is "purple rice" (Kalardee *et al.,* 2007). In Thailand, purple rice genotypes are local races; the type is a glutinous *Indica*, grown widely in different geographical areas across the kingdom and varied in their phenotype pigmentation. The pigment is anthocyanin (Hayashi and Abe, 1952). As the local ancient wisdom, the farmer always sowed purple rice at the starter point of water coming in irrigating to the paddy rice field. They believe purple rice is the king of rice which can protect all of their rice from diseases or insects. Moreover, the rice was considered to have an herbal property and has been used as a traditional Thai herb for various kinds of a medicinal treatment. Uteri inertia and diarrhea treatment therapies were treated by the medicinal property of purple rice (Kalardee *et al.,* 2007). At present, it has been seen that gamma oryzanol (γ-oryzanol), an extraction from rice crude oil is an effective medicinal substance in reducing plasma cholesterol (Lichenstein *et al.,* 1994), cholesterol absorption and decreasing early atherosclerosis (Rong *et al.,* 1997) and is used in treatment for nerve imbalance and disorders of menopause (Nakayama *et al.,* 1987). Moreover, its anthocyanin (cyanidin-3-glucoside) is evidenced by its inhibition on the growth of Lewis lung carcinoma cells *in vivo* (Chen *et al*., 2005)

Cytology of Purple Rice

Sunarin *et al.* (2000) found two white rice varieties and three purple rice genotypes shows an equal chromosome number of 2n = 24. Chromosome size and characteristic were specific and varied among the varieties. The 'purple rice' presented larger size of chromosomes than those of the 'white rice'.

Environmental effect

Grain quality is an economically important trait in rice. Rice grain quality includes the milling, appearance, cooking and nutritional qualities that people pay more attention to the quality (Huang *et al.,* 1998). The soil nutrition is one of factor that effected to grain quality. Nutrition is the supply and absorption of those nutrient chemical elements required by an organism. For rice, nitrogen, phosphorus and potassium are essential elements must be used in optimum amounts and in forms usable by rice plants. Nitrogen is found in both inorganic and organic form in the plant and combines with C, H, O and sometimes S to form amino acids, amino enzymes, mucleic acids, chlorophyll, alkaloids and purine bases. Although inorganic N can accumulate in the plant, primarily in stems and conductive tissue in the nitrate (NO_3) form, organic N predominates as high molecular weight proteins in plant. Phosphorus is a component of certain enzymes and proteins, adenosine triphosphate (ATP), ribonucleic acids (RNA), deoxyribonucleic acids (DNA) and phytin. ATP is involved in various energy transfer reactions and RNA and DNA are components of genetic information. Potassium in involved in maintaining the water status of the plant, the turgor pressure of its cells and the opening and closing of its stomata. Potassium is required for the accumulation and translocation of newly formed carbohydrate (Jones, 1998). Nitrogen is the key element to increased yield of rice.

The paddy plant depends mainly for its nitrogen upon the decomposition of organic matter under anaerobic conditions, and in the early stages of growth takes up nitrogen in the form of ammonia. Many experiments have shown that the application of nitrogen in the form of nitrates in the early stages of growth is without effect or is even deleterious to the plant, owing to its conversion to nitrites. In later stages of growth, fertilizing with nitrates has sometimes proved to be satisfactory (Grist, 1986).

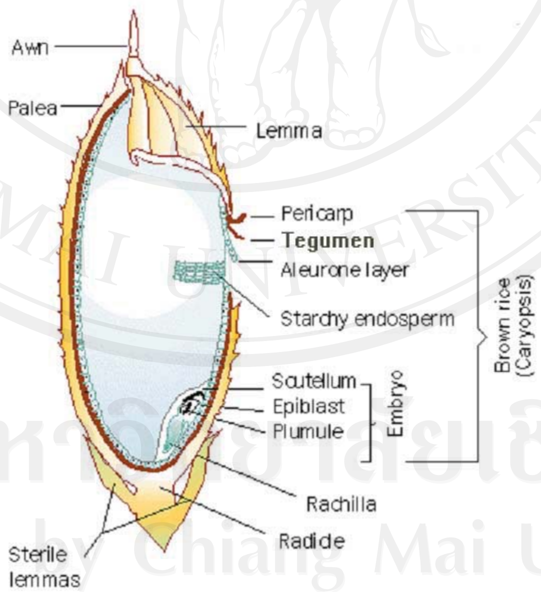
The composition of rice differs with the variety, the nature of the soil, environmental conditions and the fertilizer applied (Juliano *et al.* 1964). Nitrogen is an essential element often found limiting for optimum grain production by rice which has a relatively high N requirement (Brandon *et al.,* 1986) especially at the seedling stage (Wells *et al.,* 1960). Chanseok *et al* (2003) found the amount of protein in rice grains depended on the variable rate N fertilizer application. Nitrogen fertilizer application at flowering resulted in 30 – 60% increase in head-rice protein yield which correlated between brown-rice weight, milled rice protein and translucency (Consuelo *et al.,* 1996). Jung *et al* (2003) found the functional components of pigmented rice were different according to the cultivation conditions and their total production per unit area depended on the brown rice yield. Moreover, the functional components of pigmented rice were significantly increased according to the level of N-fertilizer. But the relation of N fertilizer and γ-oryzanol content in rice grain is still unknown.

Rice Nutrition

Structure of rice grain

The rice grain (rough rice or paddy) consists of an outer protective covering, the hull, and the rice caryopsis or fruit (brown, cargo, dehulled or dehusked rice) (Juliano and Bechtel, 1985) (Figure 1.2).

The brown rice grain is wrapped by a palea on the adaxial side and by a lemma on the abaxial side. The lemma has an awn on its head, which has been degenerated in many present cultivars. Both palea and lemma are tied to a short rachilla having a piece of glume, each of which is attached to the adaxial and abaxial sides located underneath the lemma. Any parts other than the brown rice grains are called hull or husk.



Source : Juliano, 1985

Figure 1.2 Longitudinal section of rice grain

Brown rice consists of the outer layers of pericarp, seed coat and nucellus; the germ or embryo; and the endosperm. The endosperm consists of the aleurone layer and the endosperm proper, consisting of the subaleurone layer and inner endosperm.

The aleurone layer encloses the embryo. Pigment is confined to the pericarp (Juliano and Bechtel, 1985). The aleurone and embryo cells are rich in protein bodies, containing globoids or phytate bodies, and in lipid bodies (Tanaka *et al.,* 1973; Tanaka *et al.,* 1977). The endosperm cells are thin-walled and packed with amyloplasts containing compound starch granules. The subaleurone layer are rich in protein and lipid and have smaller amyloplasts and compound starch granules than inner endosperm (del Rosario *et al.,* 1968; Bechtel and Pomeranz, 1978).

Rice is the second most widely consumed cereal in the world next to wheat. It is the staple food for two-thirds of the world's population. Over 2 billion people in Asia alone derive 80% of their energy needs from rice, which contains 80% carbohydrates, 7-8% protein, 3% fat and 3% fiber (Juliano, 1985). In the ancient, rice-growing in Asian countries such as Thailand, China and India had attributed some medicinal properties to rice, in addition to it being the mainstay as food. Rice is the only cereal that is eaten as a whole grain that is more easily digested than flour (Ahuja *et al.,* 1995). It is considered the best as food among all cereals and whole brown rice is mentioned as the perfect food (Ahuja *et al.,* 2008). At present, rice is the staple food and primary source of carbohydrate or starch. Its mineral content, starch quality, glycemic index, and antioxidant activity has made rice unique among cereals. In comparison with other sources (wheat, potato and maize), rice starch is nearly completely absorbed by the human body (Strocchi and Levitt, 1991). Positive qualities of high digestibility of starch, high biological value of amino acids, high content of essential fatty acids, selenium and anti-hypertension effect. Rice can therefore be described now as a functional food (Ahuja *et al.,* 2008). Rice is the least allergic food and is recommended for people afflicted with the irritable bowel syndrome. Colored rice (red and black) have been extensively studied and their anthocyanins or colored pigment and flavonoids are associated with antioxidant properties (Zhang *et al.,* 2005). Red and black rice are considered more nutritious, have been found to be rich in iron (Fe), zinc (Zn), minerals and antioxidant properties. These rice reduced atherosclerotic plaque by 50% more than white rice in rabbits (Ling *et al.,* 2001).

In the US, 60% of domestic rice consumption went into direct food use, 11% into processed food, and 29% into beer production around 1975 (Rutger, 1981). Rice bran contains 15–17% oil, and is a source of vitamin B, used as a preventative and cure of beriberi. The seeds are used in folk medicine for breast cancers, stomach indurations, other tumors, and warts (Hartwell, 1971). Reported to be antidotal, aperitif, astringent, demulcent, diuretic, excipient, larvicidal, refrigerant, stomachic, tonic, and vermifuge, rice is a folk remedy for abdominal ailments, beriberi, bowels, burns, diarrhea, dysentery, dyspepsia, epistaxis, fever, filariasis, flux, hematemesis, inflammations, jaundice, nausea, ophthalmia, paralysis, piles, psoriasis, skin ailments, sores, splenosis, stomach ailments, and swellings (Duke and Wain, 1981). The flowers are dried as cosmetic and dentifrice in China, awns are used for jaundice in China. The stem is used for bilious conditions; ash for discharges and wounds, sapraemia in Malaya; infusion of straw for dysentery, gout, and rheumatism (Duke and Ayensu, 1984). The husk is used for dysentery and considered tonic in China. In China, rice cakes are fried in camel's fat for hemorrhoids; rice water is used for fluxes and ulcers and applied externally for gout with pepper in Malaya. Boiled rice is used for carbuncles in Malaya and poulticed onto purulent tumors in the East Indies. The root is considered astringent, anhidrotic, and is decocted for anuria. Sprouts are used for poor appetite, dyspepsia, fullness of abdomen and chest, and weak spleen and stomach in China. The lye of charred stems is used as a hair wash and used internally as an abortifacient. In the Philippine Islands, an extract, rich in antineuritic B_1 vitamin, made of rice polishings, is used in treatment of infantile beriberi and for malnutrition in adults. In Java, the vitamins are extracted and supplied as lozenges (Reed, 1976). In addition to being a rich source of dietary energy, rice is a good source of thiamine, riboflavin (FAO, 2004), trocopherol (vitamin E) and γ-oryzanol (Bergman and Xu, 2003).

Brown rice protein contains in g/16g N: 4.6 g isoleucine, 7.9 g leucine, 3.6 g lysine, 5.1 g phenylalanine, 4.7 g tyrosine, 5.3 g total sulfur amino acids, 2.8 g methionine, 3.6 g threonine, 1.4 g tryptophane, and 6.4 g valine (Rutger, 1981). Miller (1958) reports that the straw contains 88.0–93.4% DM (mean 91.5) and, on a zero moisture basis contains 2.8–6.2 % CP (mean 4–2), 0.7–2.3% EE (mean 1.4), 27.6–38.3% CF (mean 35.1), 14.0–20.1% ash (mean 16.9), and 36.6–48.1% NFE (mean 42.4). Han and Anderson (1974) found 0.19 mcal/100 g, 4.5% CP, 1.5% EE, 35% CF, 4.5% lignin, 34.0% cellulose, 42.0% NFE, 16.5% ash, 14.0% silica, 0.19% Ca, 1.2% K, 0.4% Mg, 0.10% P, and 0.10% S.

Gamma oryzanol

Rice is cultivated primarily for the grain which forms an important part of the diet in many countries, especially in Asia. Grains are quite nutritious when not polished. Brown rice protein contains in g/16g N: 4.6 g isoleucine, 7.9 g leucine, 3.6 g lysine, 5.1 g phenylalanine, 4.7 g tyrosine, 5.3 g total sulfur amino acids, 2.8 g methionine, 3.6 g threonine, 1.4 g tryptophane, and 6.4 g valine (Rutger, 1981). Han and Anderson (1974) found 0.19 mcal/100 g, 4.5% CP, 1.5% EE, 35% CF, 4.5% lignin, 34.0% cellulose, 42.0% NFE, 16.5% ash, 14.0% silica, 0.19% Ca, 1.2% K, 0.4% Mg, 0.10% P, and 0.10% S in rice grains.). In addition to being a rich source of dietary energy, rice is a good source of thiamine, riboflavin (FAO, 2004), trocopherol (vitamin E) and γ-oryzanol (Bergman and Xu, 2003).

Rice exhibits the highest level of steryl ferulates which have antioxidant, antimutagenic, anticancer and other positive effects as well as play an important role in maintaining health than corn, wheat, rye, barley and wild rice (Moreau *et al.,* 1998). The mixture of steryl ferulates found in rice is termed gamma oryzanol (γ-oryzanol) which occur in rice bran oil (Scavariello and Arellano, 1998; Nystrom, 2007). Ten γ-oryzanol identified in rice bran consist of ferulic acid and triterpene derived compounds, which are combined by an ester bond. Cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesteryl ferulate are the three major components of γ-oryzanol in rice bran (Xu *et al.,* 1999; Fang *et al.,* 2003). Furthermore, These have been identified as the major components which were found to have antioxidant activity 10 times greater than the major tocopherol and tocotrienol components of vitamin E. Ishihara *et al.* (1982) reported that γ-oryzanol supplementation is beneficial in the treatment of menopausal symptoms (Murase and Iishima, 1963; Ishihara *et al*., 1982), elevated cholesterol (Nakayama *et al*, 1987; Sakamoto *et al*., 1987; Seetharamaiah *et al*., 1990; Scavariello *et al*., 1998), and numerous gastrointestinal conditions (Mizuta *et al*., 1978; Ichimaru *et al*, 1984). Many body builders believe the steroid nature of the ingredients in gamma oryzanol has activity in the body similar to anabolic steroids. This activity includes increased release of growth hormone and increased production and release of testosterone.

Studies in animals and humans have shown that γ-oryzanol may help lower elevated cholesterol levels.(Scavariello *et al.,* 1998; Nakayama *et al*., 1987; Berger *et al*., 2004). This benefit is apparently the result of a combination of effects including reduced cholesterol absorption, increased conversion of cholesterol to bile acids, and an increased excretion of those bile acids. (Sakamoto *et al*., 1987; Seetharamaiah *et al.,* 1990). Moreover, it can reduce of total plasma cholesterol and increase of HDL cholesterol levels, inhibition of the platelet aggregation (Cicero and Gaddi, 2001). Kim *et al.,* (1995) reported that γ-oryzanol exhibits antioxidant properties in *in vitro* systems, such as pyrogallol autoxidation, lipid peroxidation and induced in porcine retinal homogenate by ferric ion (Hiramitsu and Armstrong, 1991) and cholesterol oxidation accelerated by 2-methylpropionamidine (Xu and Godber*,* 2001).

Gamma oryzanol has been proposed as a natural antioxidant to improve the stability of foods (Nanua *et al.,* 2000; Kim and Godber, 2001). Moreover, it has been proposed as a UV-A filter in sunscreen cosmetics (Coppini *et al.,* 2001). It seems reasonable to assume that γ-oryzanol can also be used as antioxidant for pharmaceutucal purposes.

In purple, the pigment presenting the color is anthocyanin (Hayashi and Abe, 1952). Moreover, anthocyanin (cyanidin-3-glucoside) was found to inhibit growth of Lewis lung carcinoma cells *in vivo* (Chen *et al.,* 2005). Gamma oryzanol and anthocyanin (cyanidin-3-glucoside) are believed to be responsible for the effects. Previous researches on γ-oryzanol have been concentrated on the content in the rice bran (Xu and Godber, 1999; Bergman *et al.,* 2003), which is not useful in human diet.

Heritability

Heritability (h^2) is the proportion of the total variance that is attributable to differences of breeding values, and this is what determines the degree of resemblance between relatives (Falconer and Mackay, 1996). Variation among individuals may be due to genetic and environmental factors. Heritability analyses estimate the relative contributions of differences in genetic and non-genetic factors to the total phenotypic variance in a population. There are two types of heritability.

1. Broad-sense heritability

$$h^2 = V_G / V_P$$

The h^2 is the broad-sense heritability and reflects all possible genetic contributions to a population's phenotypic variance. Included are effects due to allelic variation (additive variance) and dominance variation.

2. Narrow-sense heritability

$$h^2 = V_A / V_P$$

The second type of heritability is more important because it provides a measure of the breeding value of a population which is due to the additive effects of genes in a specific population (Michael and Kearsey, 1996).

Estimation of heritability

The heritability is estimated from the degree of resemblance between relatives. From the formula, $h^2 = b_{AP}$, the regression or correlation expressed in terms of the heritability is

$$h^2 = b / r$$

Where r is the coefficient of the additive variance in the covariance.

Thus, when expressed in terms of the correlation (or regression) between relatives, the heritability is the observed correlation as a proportion of the correlation that would be found if the character were completely inherited, i.e., if all the variance were additive genetic (Falconer and Mackay, 1996).

Offspring-parent regression

Bias in the estimate of the heritability is usually a more important consideration than precision. It is introduced by environmental sources of covariance and, in the case of full sibs, by dominance. In the half-sib correlation and the regression of offspring on father are the most reliable from this point of view. The regression of offspring on mother is sometimes liable to give too high an estimate on account of maternal effects.

The estimation of the heritability from the regression of offspring on parents is comparatively straightforward. The data are obtained in the form of measurements of parents- one or the mean of both – and the mean of their offspring. The covariance is then computed from the cross-products of the paired values (Falconer and Mackay, 1996).

F_4 on F_3 regression

The estimation of the heritability from the regression of F_4 on F_3 is comparatively straightforward. The data are obtained from the mean of F_4 and F_3. The covariance is computed from the paired values.

$$h^2 = (4/7)b_{(F3,F2)}$$

Where b is the regression of F_4 on F_3 (Smith and Kinman, 1965).