CHARACTERIZATION OF LOW PHYTIC ACID IN PURPLE RICE GRAINS (*Oryza sativa* L.)

INTRODUCTION

Humans require a diverse, well-balanced diet containing a complex mixture of both macronutrients and micronutrients in order to maintain optimal health. Macronutrients consist of carbohydrate, lipid and protein. These macronutrients are foodstuff which is primarily used as an energy source. Micronutrients are not used for energy supplies but are nonetheless needed for good health. Large numbers of people in developing countries rely on simple diets composed primarily of staple foods (e.g. cassava, wheat, corn and rice) that are poor sources of micronutrients and some macronutrients. Lacking in diversity of food consumption often leads to micro-nutrient deficiencies such as iron, zinc, iodine, vitamin A etc. Inadequate micro-nutrient can promote risk of disease such as anemia, premature death and impaired cognitive abilities. Since micronutrient concentrations are often low in staple food crops, improving of nutrient content and micronutrient bioavailability in staple food crops is a good approach for alleviating malnutrition of population, particularly the poor population.

Plants contain “promoter” compounds that stimulate the absorption of essential mineral elements by the gut, such as certain organic compound and amino acids. In addition, plants (especially seeds) contain anti-nutrient such as phytic acid or myo-inositol hexakis-phosphate ($InsP_6$) that can limit absorption of minerals (White and Broadley, 2005). Thus, the major concern of staple plant foods is poor sources of micronutrients. The nutrient deficiency in staple crop may cause by inadequate amounts of the nutrient in plant or the "anti-nutrients" absorption molecule

in plant which can interfere with absorption and utilization (bioavailability) of micronutrients into the human body.

Phytic acid is one of an anti-nutrient compound in plant and widely found in plant tissue. However, the amount of phytic acid is concentrated in grains since it is the primary storage form of phosphorus (P) in seeds, accounting for up to 85% of total P. The negatively charged phosphates in phytic acid strongly bind to metallic cations (e.g. K, Mg, Mn, Fe, Ca and Zn) to form a mixed salt called phytin or phytate that accumulates in seed protein bodies either dispersed throughout the bodies or in dense inclusions called globoids (Lott *et al*., 1995). Phytic acid also plays an important role in animal and human nutrition because it is important a factor hindering the uptake of several minerals. The phytate is very poorly absorbed by the gastrointestinal tract. The forming mixed salts of phytic acid is largely excreted by humans and other non-ruminants animals such as poultry, swine and fish because they have no or limited phytase activity in their digestive tract. Excretion of a large fraction of phytic acid can be accumulated in soil and water which leads to the pollution of waterways. Subsequently, It becomes a problem in agricultural ecosystems.

The amount of phytic acid in plant food is depended on both genetic and environmental factors and subsequently, affecting the bioavailability of dietary non-heme Fe, Zn and other nutrients to humans (Welch and Graham, 2004). Thus, decreasing level of phytic acid content in seeds is desired goal for genetic improvement in several crops. The development of low phytic acid in seed genotypes may help to reduce human malnutrition and reduce animal waste phosphorus.

There are several reports revealed low phytic acid (lpa) mutant that have been produced by non-transgenic techniques in rice (Larson *et a*l., 2000), maize (Raboy *et*

al , 2000; *Shi* et al, 2003), wheat (Guttieri *et al.*, 2003) and soybean (Wilcox *et al.*, 2000). These reports concluded that the phytic acid content of lpa seeds is reduced by 50-80% compared with non-mutant seeds. Plants having the lpa characteristic produce seeds that have normal levels of total P but greatly reduced levels of phytic acid. The lpa mutations block the ability of a seed to synthesize P into phytic acid because enzyme in the part of phytic acid biosynthesis are impaired by disruption of gene which create enzyme concerning phytic acid pathway (e.g. myo-inositol phosphate synthase (MIPS), inositol phosphate kinase (IPK).

Rice is one of the most important staple crops worldwide and Thai's people consume rice as a major food. Investigation of phytic acid content in rice found that phytin is located in the aleurone layer of rice grain. The reduction of phytic acid in rice may elevate the level of minerals such as iron, zinc in the central endosperm (Raboy, 2000). Thus, decreasing level of phytic acid content in the rice seed is likely to increase bioavailability of micronutrients.

In 2000, team working of Larson identified low phytic acid mutant of rice (*Oryza sativa L.*). The mutant was induced by γ radiation of the Arkansas rice cultivar "Kaybonnet" (KBNT). The phytic acid portion of seed phosphorus (P) in KBNT lpa1-1 is reduced from 71 to 39% and the inorganic portion of seed P is increased from 5 to 32%, with little effect on total seed P. The KBNT lpa1-1 received registration in Reg. no. GP-86, PI 632282.

Low phytic acid mutants are isolated by screening for high inorganic P (HIP) characteristics. This method is suitable for screening the seeds of a large mutagenic population. The high-performance thin layer chromatography (HP-TLC) method is high sensitivity for detection of small amount of sample. This method confirmed that

phytic acid level is decreased in low phytic acid mutant. This method may help investigation of the enzyme involved in the phytic acid pathway that is alternative for identification of gene concerned about phytic acid biosynthesis. (Hatzack and Rasmussen, 1999).

Jao Hom Nin (BT) rice is superb of grain micronutrient composition especially high in iron. Thus, reduction of phytic acid content in Jao Hom Nin grain may be promoting a high nutritional value for human consumption. Using mutational breeding approach for reducing phytic acid in Jao Hom Nin grain appears to be a prominent approach. The HIP and HP-TLC system for screening low phytic acid of Jao Hom Nin mutagenic can be a good tool to identify low phytic acid rice varieties.

OBJECTIVES

1. To isolate low phytic acid mutant from Jao Hom Nin mutagenic rice population by HIP screening.

2. To analyze qualitative of low phytic acid mutant by HP-TLC for divide phenotype characteristic of low phytic acid mutant.

3. To study characterization of low phytic acid mutant in rice grains.