CHAPTER 5

HERITABILITY OF GAMMA ORYZANOL

5.1 Introduction

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

$$\mathrm{h}^2 = V_G / V_P$$

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.

$$h^2 = (4/7)b_{(F4,F3)}$$

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

Qualitative traits differed from quantitative traits in which qualitative character controlled by a few genes which expressed major phenotypic effects. And their inheritance was referred to qualitative inheritance that was simply inherited, mostly by single genes with recognizable effects. Estimation of qualitative trait was therefore the phenotypes could be grouped in a small number of easily distinguished, discrete classes for any one trait. For example, black or white hulls and rough or smooth awns. In other hand, many traits important in plant breeding were not inherited in simple Mendelian terms. Their inheritance was dependent upon several to many genes at different loci, each gene contributing a small effect to the phenotypic expression of the quantitative character. And their inheritance was referred to as quantitative inheritance. The effect of a single gene contributing to the phenotype of a quantitative character was generally too small to be recognized; instead the sum of the effects of all genes contributing to the character was normally measured (John and David, 1995).

In the previous chapter, GE had mainly affected on γ-oryzanol content and conclusion was that inheritance characteristic for this character was polygenic inheritance.

So in this chapter, F2 generation and the derived generations F3 were cropped together to produce F3 and F4 derived generations respectively. Objective is to estimate heritability value of γ-oryzanol accumulation in rice grains using the regression of the derived F4 variation on F3 variation

5.2 Material and methods

Production of F_2 population was performed using the F_1 seed of crossing between parents having high and low content of γ-oryzanol from the first experiment (KDML 105 x Kum Doi Saket). Only the 10% of the highest and lowest contents of γ-oryzanol F_2 were selected and cropped to produce F_3 derived lines. Half of the seeds of each F_3 derived population were retrained in which another half seeds were further advanced as the F_4 derived population lines. Both F_3 retrained seeds and its F_4 derived population seeds were cropped together in the 2007 experiment. Determination for crude oil, semi-γ-oryzanol and γ-oryzanol contents in seeds were examined in both the selected F_3 and its F_4 derived populations using HPLC technique (Xu and Godber, 1999). Peak area of standard and of F_3 and F_4 were as follow:

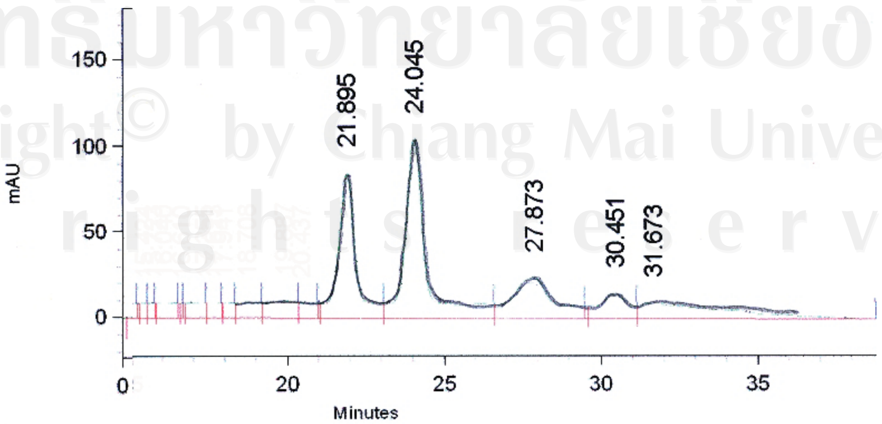

Figure 5.1 Chromatogram of γ-oryzanol standard in the analytical

reverse-phase HPLC

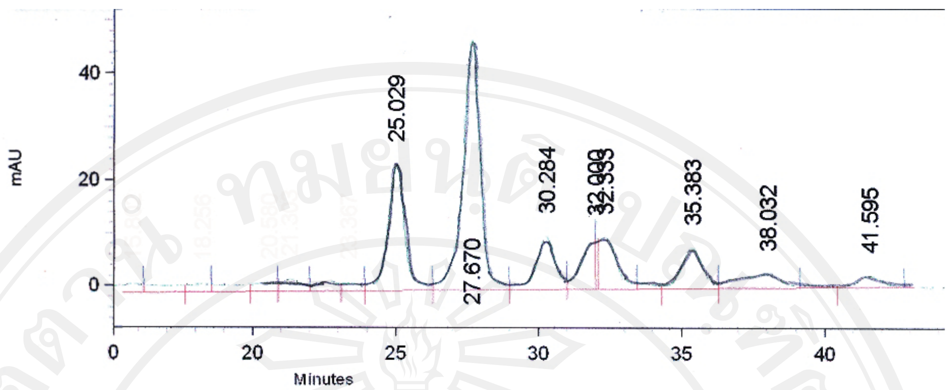

Figure 5.2 Chromatogram of γ-oryzanol in F4 generation in the analytical reverse-phase HPLC

Then estimation of the heritability was comparatively straightforward from the regression of F_3 γ-oryzanol content averaged from the derived populations on the contents of its correspondent F_2 population. Regression coefficient value was computed from the paired values (Smith and Kinman, 1965).

5.3 Result

5.3.1 Crude oil and semi purified γ-oryzanol content

Crude oil contents in seeds of F_3 populations and the derived F_4 were in table 5.1, it was found that crude oil content in F_3 varied from 1.40 – 2.80 g/100g grain. In F_4, crude oil content varied from 1.54 – 2.82 g/100g grain. There was found no correlation between crude oil in F_3 and F_4.

Table 5.2 showed the Semi purified γ-oryzanol contents examined from seeds of the F_3 and its derived F_4 populations. The contents in F_3 varied from 1.13 – 2.00 g/100g grain. And varied from 1.16 – 2.59 g/100g grain in F_4.

Crude oil and semi purified γ-oryzanol content in F_3 presented the highest in rice plant no.40 and presented the lowest in no.63. However, in their F_4 derived did not give the highest or the lowest in both content. It was found no correlation of both content between F_3 and its derived F_4.

Table 5.1 Crude oil content in seeds of F_3 populations and its derived F_4 populations

No.	Crude oil content (g/100g grain)	
	F_3	F_4
P40	2.80	2.10 2.82 1.54
P63	1.41	2.35 2.73 2.71
P80	1.94	1.83 1.70 2.40
P82	1.59	2.57 2.72 2.58
P93	2.19	2.10 2.78 2.00

Table 5.2 Semi-purified γ-oryzanol content in seeds of F_3 populations and its derived F_4 populations

No.	Semi-purified γ-oryzanol content (g/100g grain)	
	F_3	F_4
P40	2.00	1.98
		1.90
		1.16
P63	1.13	1.96
		2.50
		2.50
P80	1.46	1.38
		1.25
		1.44
P82	1.51	2.40
		2.59
		2.32
P93	1.75	1.95
		2.08
		1.70

5.3.2 Gamma oryzanol

The Gamma oryzanol contents of F_3 and F_4 were presented in table 5.3. It showed that in F_3, there was varied from 30.53 – 34.87 mg/100g grain. In F_4, the content varied from 24.18 – 32.75 mg/100g grain.

Response curve was manipulated as in Figure 5.3. Linear regression was observed with the coefficient of regression value (b) was 0.49. From the equation of

(Smith and Kinman, 1965), the heritability of γ-oryzanol content estimated from regression of F_4 on F_3 was 0.49.

Table 5.3 Gamma oryzanol contents in seeds of F_3 populations and its derived F_4 populations

No.	Crude oil content (g/100g grain)			
	F_3	**Peak area**	**F_4**	**Peak area**
P40	30.53	7892040	30.22	14242046
			29.00	1330540
			27.71	2318879
P63	33.40	5363454	32.92	4068488
			28.98	4531954
			32.72	6089442
P80	29.34	300947	23.13	435188
			28.55	758171
			24.18	434074
P82	34.32	143545	31.41	4087533
			31.76	19160438
			31.68	72887856
P93	34.87	1319966	32.75	1980749
			27.62	613165
			28.55	57566367

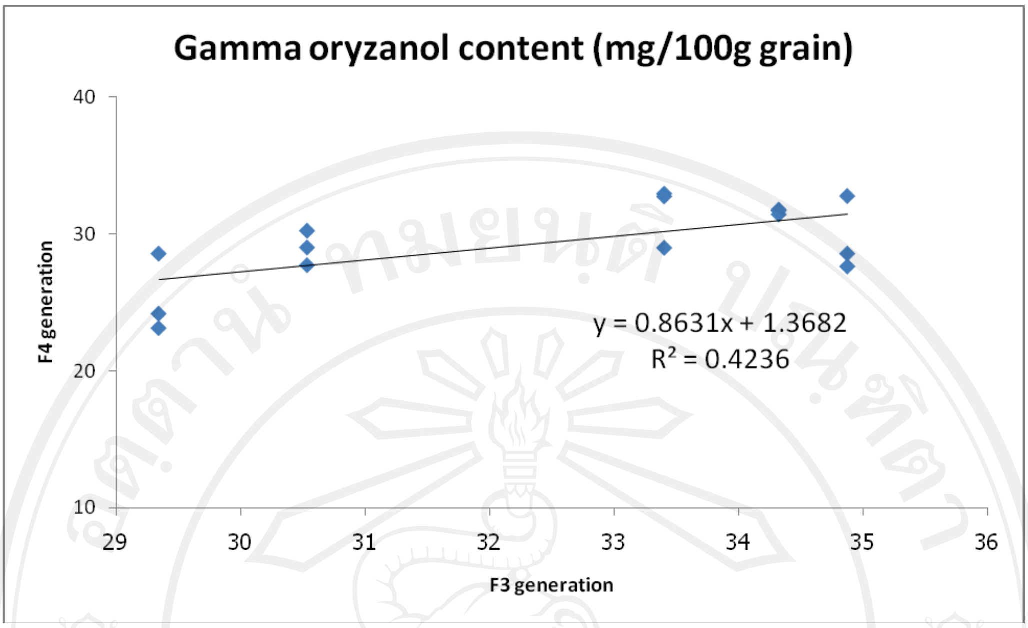

Figure 5.3 Regression of Gamma oryzanol contents of derived F4 lines on the its F3 generation lines

5.4 Discussion

The character of γ-oryzanol accumulation in the rice grain was proved to inherit in a quantitative manner in the previous chapter, means variation of the character in the population is a continuous distribution. Distinct classes of a major gene controlled were diminished. This is often phenomenon in many other nutritional traits in rice grain. Reported by Shi et al., (1996) on investigating *indica rice* was that, nutrient quality traits were controlled by cytoplasmic and maternal effects as well as by seed direct effects. Maternal effects for lysine content (LC), lysine index (LI), and the ratio of lysine content to protein content (RLP) were more important than seed direct effects, while protein content (PC) and protein index (PI) were mainly affected by seed direct effects. Cytoplasmic effects accounted for 2.41–20.80% of the total genetic variation and were significant for all nutrient quality traits. Eleni *et al.,*

(2008) found high heritability in oleate content which was important for the nutritional value and oxidative stability of soybean seed oil and was sufficiently high in early generation selection. In seed oil content of sunflowers, Fick (1975) found high estimated heritability by F_3 on F_2 parent-progeny regression method and indicated that the heritability values (0.52) were of sufficient magnitude to encourage selection for high oil content in early generations. Falconer (1981) pointed out that narrow-sense heritability, defined as the ratio of additive genetic variance, expresses the extent to which phenotypes are determined by the genes transmitted from the parents and the coefficient of parent-offspring regression gave a good estimate of narrow-sense heritability. Additive genetic effects were much more important than dominance effects for all of the traits studied, so that selection could be applied for these traits in early generations. It was supported by the equation of Nei and Shakudo (1958) indicated that with generation advancement the contribution of variance components due to additive and dominance effect to this regression coefficient decreases and thereby the regression coefficient increases.

The estimating heritability of γ-oryzanol content using parent-offspring F_3 on F_4 generation in this experiment showed a moderate narrow sense value ($h^2 = 0.49$), despite the effects of genotype x environment interactions in reducing heritability was found in the previous chapter. O'Brien and Quail (1986) working on grain quality of wheat showed that there were significant genotypic effects for all quality traits in each generation. Phenotypic correlations in F3 and in F7 showed positive correlations between protein content and sodium dodecyl sulfate sedimentation volume (SDS) and Pelshenke wheatmeal fermentation time (PEL). In their experiment, the realized heritability was high for PSI (77%), moderate for SDS (44%) and PEL (47%).

This signified that a rather large proportion (49%) varied in the variation of the phenotype ($V_P = V_A + V_D + V_I + V_E$) was contributed to the effect of additive genes (V_A), indicated a large genetic effect controlling the character and that response to early generation selection can be expected. As additive genetic variance and hence narrow sense heritability, generally declined over generation of selection, even in a constant environment (Brown and Caligari, 2008). Horie *et al.* (1964) found the heritability of some agronomic traits in rice from parent-offspring regression for two F_2 population varied from four experimental sites. Chang (1974) reported that the heritability for rice grain length estimated from regression of F_3 on F_2 was 0.594 in one population and 0.721 in the other. Hof *et al.,* (1999) found differences in response and heritability of oil content in *Dimorphotherca pluvialis* (oilseed crop) that can be attributed to different genetic constitutions of the populations used. Though heritabilities were specific for populations, selection procedures and environmental circumstances. And indicated that a substantial part of the phenotypic variance was additive genetic variance. Therefore, selection for a level of γ-oryzanol content could performe efficiently in F_3 (or even in F_2: an early generation). Shi et al., (1996) suggested in correspondent that, the selection for the nutrient quality traits in *indica rice* could be applied in the early generation.

DISCUSSION

Genetic Resources Consideration

However, In any plant breeding program, genetic diversity is essential, by providing the raw material for hybridization. Without this material diversity, crop improvement is almost impossible. The reduction in biodiversity often increases in vulnerability to stress changes such as climate and pathogens. Further, may raise a risk for individual farmer and can undermine the stability of agriculture (WRI, 2002). The wide range of diversity means a better choice for a breeder to select for the appropriate kind of variants and that a better chance for his success.

In my study, 13 accessions of purple glutinous rice were collected in the north and north eastern Thailand in which the rice was produced to meet a household need rather than for sale. It was found that the color of leaf sheath and leaf blade varied from green, purple rim to purple. Moreover, there was no variation within color in both leaf sheath and leaf blade. In addition, the range of flag leaf length was 24.1 to 48.7 cm and flag leaf width was 1.4 10 2.2 cm. The result was the same in modern oriental rice cultivars in U.S. which were a wide range of variation in the two flag characteristics (Kuo and Li, 1994). Moreover, it was found the variation of seed length and seed width in both whole grain and unpolished grain. The length varied from 8.2 – 10.1 mm, the width varied from 3.2 – 3.6 mm in whole grain. In unpolished grain, the length varied from 5.7 – 6.6 mm and the width varied from 2.6 – 3.1 mm. The variation of seed length and width in this result was supported by

Khampheng Mounmeuangxam (2003) that evaluated rice varieties collected in Houaphanh province in Lao PRD and found a grain length and width ranged from 6.41 to 9.92 mm and 2.83 to 4.06 mm, respectively.

Morphological features of leaf ligules were used as a key identification index of taxonomy in grasses. For example cereal crops such as rice maize and wheat, usually possessed a ligule while barnyard grasses do not (Josef, 1972). Although most rice varieties contained ligules, different varieties usually contained various ligule lengths or shapes. The range of ligule length of double haploid rice population varied from 5.8 mm to 17.3 mm (Dali *et al.,* 2007). In this study, the legule length differed according to variety. The range of ligule length of purple rice in this collection was 15 mm to 30 mm. Furthermore, the ligule of the 13 collections was only purple in color, and the color of an auricle exhibited either purple color or colorless.

Stigma color was either purple or colorless. These findings agreed with the morphology studied conducted by Elizabeth *et al* (2008) indicating that, stigma color varied among and within *O. glumaepatula* populations which present white and purple stigma. In addition, Syed *et al* (2007) found the variation of stigma color in rice CMS lines that varied from white, lower half white to purple. It may indicate that purple rice was land race which had wild rice ancestor and had been cultivated that are adapted to varied environmental condition.

In this study, it was found the variation of many characters in purple rice varieties. Thus, the wide range of diversity means a better choice for a breeder to select for the appropriate kind of variants and that a better chance for success. Moreover, the pericarp color of purple could also be applied as a genetic marker and

Thai local purple rice diversity could prove a priceless heritage of rice genetic resources for Thailand rice breeding programs.

Consideration Concerning Healthy Nutrition Sources

Rice genotypes with pigmented either red or purple/black bran layer have been cultivated for a long time in Asia. Although rice is one of the most important cereal crops in the world, pigmented rice varieties are cultivated only in restricted areas of the globe where they are appreciated because of a long lasting tradition (Ahuja et al., 2007). Notwithstanding their low defussion, in the recent years pigmented rice varieties have received increased attention because of their antioxidant properties (Abdel-Aal et al., 2006; Ahuja et al., 2007; Chung and Shin, 2007; Finocchiaro et al., 2007; Jang and Xu, 2009; Nam et al., 2006; Shen et al., 2009; Zhang et al., 2006).

Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C, and thus might contribute significantly to the protective effects *in vivo* (Lin and Weng, 2006; Ling et al., 2001; Toyokuni et al., 2002; Xia et al., 2003). It is now possible to establish the antioxidant activities of plant-derived flavonoids in the aqueous and lipophilic phases, and to assess the extent to which the total antioxidant potentials of wine and tea can be accounted for by the activities of individual polyphenols.

Such healthy properties have been related to several classes of antioxidant compound present in rice. These include gamma-oryzanol, tocotrienols, tocopherols, squalene and phenolic compounds (Finocchiaro et al., 2007; Ling et al., 2001; Sugano and Tsuji, 1997; Xu et al., 2001).

Gamma oryzanol (γ-oryzanol) is a mixture of phytosteryl ferulates which occur in rice bran oil (Scavariello and Arellano, 1998). Moreover, it had been proposed as a natural antioxidant to improve the stability of foods (Nanua *et al.,* 2000; Kim and Godber, 2001).

Gamma Oryzanol (γ-Oryzanol) is found in rice bran oil and other plants. Gamma oryzanol has antioxidant activity and may be helpful in maintaining healthy cholesterol levels. Furthermore, rice bran oil and gamma-oryzanol could be used in the treatment of hyperlipoproteinaemias and other conditions

Gamma Oryzanol also is the active ingredient in strengthening human mussel so that sportsmen such as Athletic and Body Builder could use as a supplement diet.

In this study, the γ-Oryzanol contents found in many varieties of purple rice indicated the better performance of the varieties in accumulating γ-Oryzanol over the white rice varieties (KDML 105 and RD6). Finocchiaro et al., (2009) studied of biodiversity of flavonoid content in the rice caryopsis, reported also that the pigmented rice genotypes showed a higher polyphenol content than the white. In particular the black rice cv Artemide characterized the highest polyphenol content. They suggested that the pigmented rice varieties could be sources in breeding for a high poly phenol level. Results in my experiments therefore, indicated that landrace purple rice varieties cultivated in various parts of Thailand were wealthy sources for breeding program purposed in enhancing the healthy nutrition of γ-Oryzanol in rice grain.

Although, γ-oryzanol is one of the minor components of crude oil, its value for health is high because of its advantageous in health properties. Therefore, a rice genotype producing high levels of γ-oryzanol would be commercially valuable. In this result, the amount of extract crude oil, semi- purified γ-oryzanol and of γ-oryzanol content however, varied among the white rice and the purple rice. The average of γ-oryzanol content in purple was higher than in white rice check. It was indicated that accumulation of γ-oryzanol in rice is under genotypic determination and its genetic diversity exists naturally in this collected accessions. Miller *et al* (2003) also found variation of γ-oryzanol to be associated with genotype and environment. A higher or a lower γ-oryzanol means a higher or a lower of others health substances such as lipids and alpha-tocopherol. Apparently, the higher amount of this substance showed in the particular purple rice genotypes than in the white rice check genotypes, suggested the purple rice genotype with high gamma oryzanol would also had a higher amount of others health substances.

Thus, based on the benefit of healthy oil activities, diversity of the purple rice varieties is worth to be conserve as germplasm for the future breeding program, in particular the program for improving the functional rice food product.

Heritability and Consideration on Selection for High Gamma Oryzanol Genotypes

The interaction of genotype and N, P, K fertilizer levels (GE) indicated a different response in synthesizing γ-Oryzanol of a purple rice variety to a level of fertilizer applied. A significant interaction of GE means that genotypes differ in its stability over environments and as such suitable environment needed for high stability of any genotype.

There was variation of γ-oryzanol content in purple rice varieties and found the interaction between the rice varieties and N, P, K fertilizer rates indicated that the constitution of the controlling genes and the environments had the strong effects of the phenotypes. The genetic effect for the expression of γ-oryzanol content was therefore controlled mainly by genetic main effects, but also influenced by GE interaction effects. The result signified that phenotypic characteristic of γ-oryzanol content that varied in a degree could be attributed to the interaction between the controlling genes and their environments. Shi et al., (1996) reported that the heterosis of cooking quality showed that these traits were influenced by genetic heterosis and GE interaction heterosis especially for amylose content trait in indica rice. So, this means that the inheritance of γ-oryzanol content was a polygenic inheritance and inherits quantitatively. Genetic variation found for γ-oryzanol signified that, to obtain rice genotypes with a high level of γ-oryzanol, new cultivars would needed to be produced through hybridization and selection. Furthermore the GE interaction also signified that growing conditions had a great effect. Bergman and Xu (2003) suggested that rice breeders selecting genotypes with optimum levels of vitamine E and γ-oryzanol will need to grow their breeding material in multi-environments and years.

Crude oil content also was found having minimum affected by nitrogen fertilizer. As nitrogen is a major component of protein rather than of oil, applying more amount of nitrogen fertilizer was therefore could not enhance crude oil content. Tunctuck and Yildirim (2004) reported that in safflower, different in N-fertilizer doses had affected on seed yield and crude oil yield but crude oil content. Rathke *et al.,* (2005) found in winter oilseed rape (*Brassica napus* L.) that under high N rates, the lowest oil contents (43.8 – 44.1%) were observed. In contrast, highest oil concentrations were found for the unfertilized plots (46.8 – 47.7%).

Increasing in P fertilizer limited γ-oryzanol accumulation. A higher or a lower P rate caused a deteriorate in the content. In contrast to N response, response to P fertilizer was similar feature among the rice varieties, this means in consequence, an accumulation of γ-oryzanol content in rice is mainly under a genetic constitution of the rice variety. This result agreed with Geleta *et al.* (1997) that application P did not influence sunflower seed yield and oil content. Residual nitrate level did not significantly influence either the seed yield or the seed oil content. The lack of response to N or P fertilizers regardless of the soil N and P levels, indicate that other growth factors such as types of sunflower cultivars are the possible sunflower yield-controlling factors for the region. In addition, Seguin and Zheng (2006) found that isoflavones (positive impacts on human health) and oil in soybean seeds did not response to potassium and phosphorus fertilizer.

However, Crude oil and semi purified γ-oryzanol content was no relate to γ-oryzanol content which was the same result in chapter 3. In addition, Farooq *et al.,* (2005) found the same correlation between the contents of γ-oryzanol and oil of the rice bran from Pakistan. This indicates that γ-oryzanol is a major component of crude oil thus, may affect the oil concentration.

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Suggestion for Further Researches

Further researches concerning purple rice landrace varieties in Thailand must promptly be considerate are:

1. Intensive programs on conservation and evaluation.

As, the rice is cultivated scattering though out the country, a wide range of genetic diversity is phenomenon. This diversity needed to be collected and evaluated before seriously diminished. A long term conservation of favorable genotypes has to be effectively retained for further improvement rice programs.

2. Chromosomal DNA analysis for sib-relation among cultivars

As mentioned earlier that landraces of purple rice in Thaialnd have been identified its name as either Kao Kum or Kao Neang Dum, sib-related among genotypes is in doubt. Bio-technology for DNA analysis could proof the relationship. Mapping of genes controlling Gamma oryzanol is also worth considering.

3. Breeding programs for high Gamma oryzanol genotypes

As proved in this study that inherit of Gamma oryzanol is effective in early generations, Pedigree method of selection with appropriate environment controlled conditions could be applied. Also relation with anthocyanin must take in evaluation and selection for high Gamma Oryzanol with high anthocyanidin geneotypes could be taken in to account.

4. Benefit properties as functional food product

Through the use of selective refining and fractionation techniques, rice bran oil and concentrates may be produced to optimize composition for specific applications. Examples include:

- Refined rice bran oil that retains much of its naturally-occurring Gamma Oryzanol and anthocyanidin activities
- Concentrated natural antioxidant to provide health benefits associated with dietary intake of antioxidants