CHAPTER 4

EFFECT OF N P K FERTILIZER TO GAMMA ORYZANOL CONTENT

4.1 Introduction

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 with out 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).

Indigenous nutrient supply, particularly P and K, showed important effects on rice yields and fertilizer recovery rates in the irrigated rice systems (Dobermann *et al.,* 1998; Haefele *et al*., 2003). Application of nitrogen and potassium fertilizers may variously affecting on some of key characters for determining contribution of husk and bran. Husk production has been increased by application of potassium fertilizer. Application of nitrogen and potassium has tremendously significant effects on bran production and also differences of bran production between genotypes (Bahmaniar, 2007). And the application of nitrogen and potassium fertilizers could markedly increase the yield and improve the quality of rice (Place *et al*., 1970).

However for characters qualitatively, its performance may severely affected by environment factors and procession techniques. In Mococa, the nitrogen dosage were positively correlated with protein in hybrid corn Gallo *et al* (1976). 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 nitrogen, phosphorus and potassium fertilizer to γ-oryzanol content in rice grain is still unknown.

4.2 Material and methods

In this chapter, effects of N P and K fertilizers on Gamma oryzonol content were evaluated. Outdoors pot experiments were conducted three experiments for each N, P and K were conducted separately. Soil which use in this experiment were analyzed by Department of Soil Science and Conservation, Faculty of Agriculture, CMU that present total N (0.011 g/100g), available P (1.44 mg/kg) and extract K (67.74 mg/kg) (Table 4.1).

Table 4.1 Nitrogen, phosphorus and potassium fertilizer in soil

Sample Designation	Total N (g/100g)	Available P (mg/kg)	Extract K (mg/kg)
Sample 1	0.013	1.72	73.52
Sample 2	0.008	0.96	63.02
Sample 3	0.014	1.06	70.24
Sample 4	0.012	1.57	65.64
Sample 5	0.010	1.91	66.30
Mean	0.011	1.44	67.74

Results were therefore presented in the separate data. Three varieties of purple glutinous rice (2 landrace: Kum 7677, Kum 88061 and 1 improved variety: Kum Doi Saket) were grown in the 30 cm diameter pots. A commercial white rice variety (RD6) was used as a comparison. The experiment was designed in a factorial design, with 3 replications.

In the first experiment, three rates of nitrogen fertilizer (ammonium sulfate: 21% N), 25 kg N/ha, 37.5 kg N/ha and 50 kg N/ha were test against the four rice varieties. To allow an optimum in plant growth, optimum basic macro-nutrients P (46%P_2O at 37.5 kg /ha) and K (50% K_2O at 25 kg/ha) were applied.

In the second experiment, three rates of phosphorus fertilizer (triple superphosphate: 46% P), 25 kg N/ha, 37.5 kg N/ha and 50 kg N/ha were test against the four rice varieties.

The third experiment was for testing potassium fertilizer. Three rates of Potash (50% K), 25 kg N/ha, 37.5 kg N/ha and 50 kg N/ha were test.

After harvesting, grain samples from each plot in each experiment were dried in the hot air oven at 60^0C for 48 hrs, then milled to purple rice grain (from purple rice) and brown rice (from the white rice) by SATAKE milling machine. The purple rice grains and brown rice grains were ground and stored in a cool room at 15^0C during the course of experimentation to retard quality changes due to aging effect and auto-oxidation.

The Xu and Godber method (1999) was applied in extraction crude oil by using hexane and ethyl acetate, extraction of Semi purification of γ-oryzanol using a Low-Pressure silica column and determine γ-oryzanol content by HPLC technique. The standard peak area obtained was in Figure 4.1. And the responded peak area graphs for N, P, K was (of RD6 as an example) in Figure 4.2, 4.3 and 4.4.

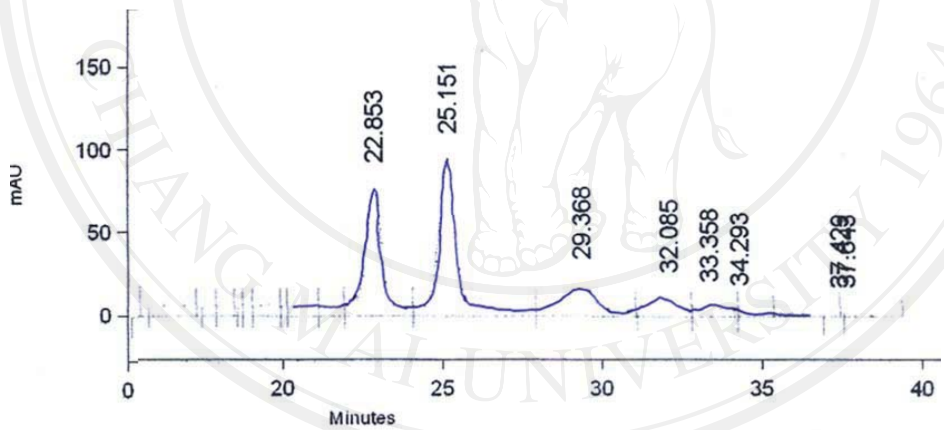

Figure 4.1 Chromatogram of γ-oryzanol standard in the analytical reverse-phase HPLC

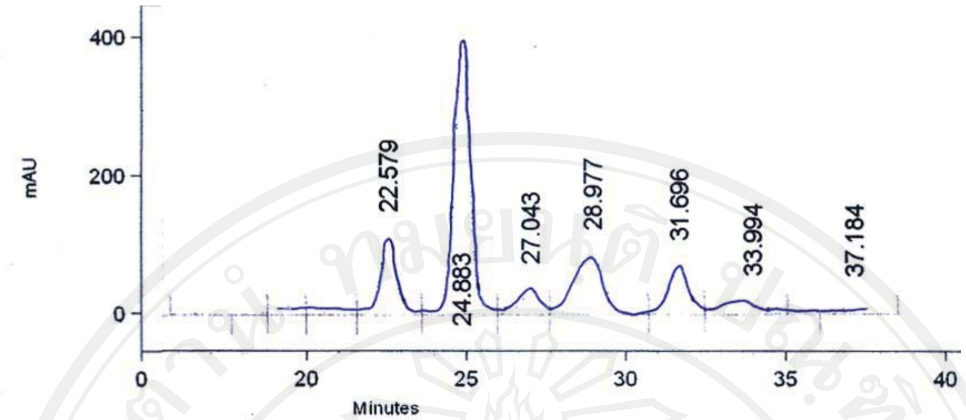

Figure 4.2 Chromatogram of γ-oryzanol of RD6 responsed to N-fertilizer in the analytical reverse-phase HPLC

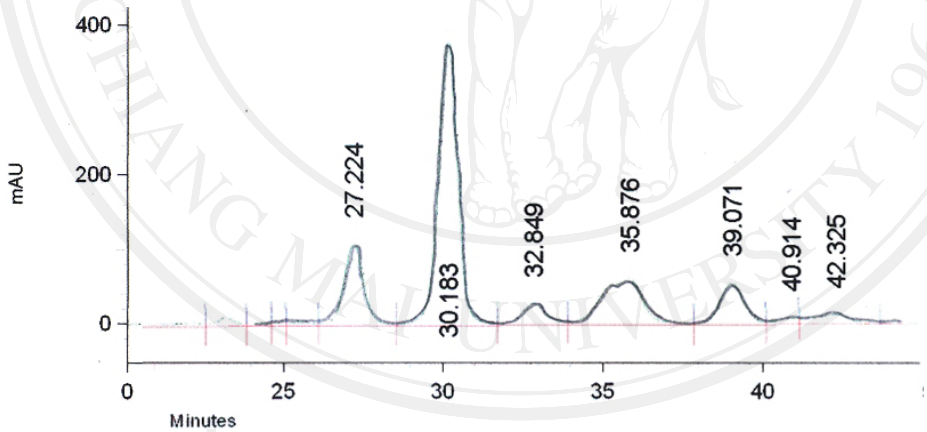

Figure 4.3 Chromatogram of γ-oryzanol of RD6 responsed to P-fertilizer in the analytical reverse-phase HPLC

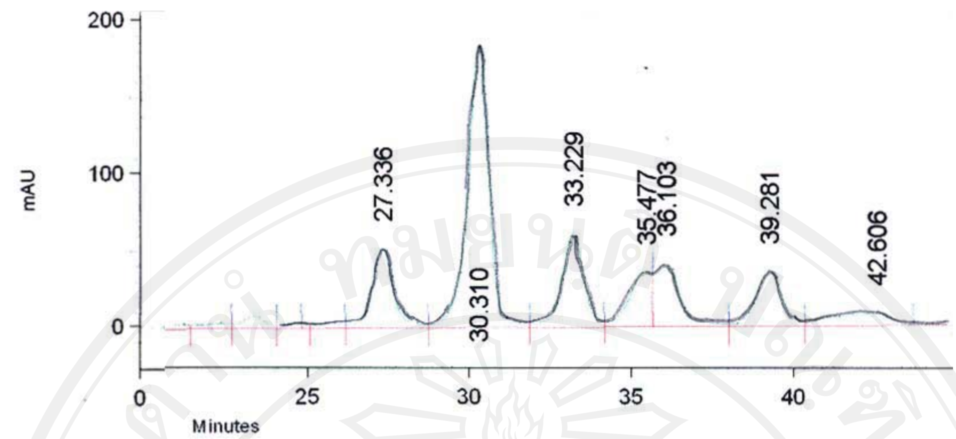

Figure. 4.4 Chromatogram of γ-oryzanol of RD6 responsed to P-fertilizer in the analytical reverse-phase HPLC

4.3 Result

4.3.1 Effect of Nitrogen fertilizer

4.3.1.1 On Gamma oryzanol Content

Differences response among the varieties, levels of N fertilizer and the interaction were found significant statistically (Table 4.2). The tested varieties responded in accumulating Gamma oryzanol differently to the N fertilizer rates. While Kum Doi Saket gave a higher responded of the content (72.90 mg/100gm grain) at the lower rate of N (25 kg/ha), Kum 88061 and Kum 7677 needed a higher N rate (37.5 and 50 kg/ha respectively) to achieve the high responses. The white rice; RD6 was also similar to Kum Doi Saket, needed only a low level of N (25 kg/ha) to achieve a high response but its ability to synthesize Gamma oryzonol was less than the purple rice varieties. RD6's Gamma oryzonol production was averagely 33.11 mg/100g grain lower than the lowest variety of the purple rice: Kum 88061 (48.83 mg/100g grain).

4.3.1.2 On crude oil and semi purified gamma oryzanol content

Crude oil content and Semi purified gamma oryzanol interacted significantly to N fertilizer rates in similar fashion as to γ-oryzanol (Table 4.3 and 4.4). Rice varieties interacted differently to N- rates. While Kum Doi Saket and Kum 7677 needed a lower rate to reach the maximum crude oil content, other varieties needed the higher rates (Table 4.3). In addition, semi purified γ-oryzanol content in Kum Doi Saket and RD6 needed a lower rate to reach the maximum content (Table 4.4).

Table 4.2 Response of Gamma oryzanol content (mg/100g grain) in purple rice and white rice to the different nitrogen fertilizer levels

Varieties	Gamma Oryzanol content (mg/100g grain)			
	25 kg N/ha	37.5 kg N/ha	50 kg N/ha	mean
Kum Doi Saket	72.90 a	66.87 b	63.43 c	67.73 A
Kum 88061	45.44 f	45.62 f	55.44 e	48.83 C
Kum 7677	54.63 e	67.08 b	60.39 d	60.70 B
RD6	39.06 g	27.55 i	32.72 h	33.11 D
mean	53.01 Y	51.78 Z	53.00 Y	
	Nitrogen (N)	Varieties (V)	N*V	
LSD (0.05)	0.67**	0.78**	1.35**	

% CV = 1.51

Note ** = significant at 1% level CV = coefficient of variance

Table 4.3 Crude oil content (g/100g grain) in purple and white rice at different nitrogen fertilizer level

Varieties	Crude oil content (g/100g grain)			mean
	25 kg N/ha	37.5 kg N/ha	50 kg N/ha	
Kum Doi Saket	1.91 bc	2.04 a	1.87 cd	1.94 A
Kum 88061	1.84 de	1.79 ef	1.91 bc	1.85 B
Kum 7677	1.51 h	1.79 ef	1.56 gh	1.62 D
RD6	1.77 f	1.59 g	1.93 b	1.76 C
mean	1.76 Z	1.80 Y	1.82 Y	
	Nitrogen (N)	Varieties (V)	N*V	
LSD (0.05)	0.03**	0.03**	0.05**	

% CV = 1.69

Note ** = significant at 1% level CV = coefficient of variance

Table 4.4 Semi purified gamma oryzanol content (g/100g grain) of purple rice and white rice at different nitrogen fertilizer level

Varieties	Semi purified gamma oryzanol content (g/100g grain)			mean
	25 kg N/ha	37.5 kgN/ha	50 kgN/ha	
Kum Doi Saket	1.56 c	1.00 i	1.49 d	1.35 A
Kum 88061	1.10 g	1.08 gh	1.70 a	1.29 C
Kum 7677	1.07 gh	1.49 d	1.40 e	1.32 B
RD6	1.60 b	1.05 h	1.33 f	1.33 AB
mean	1.33 Y	1.16 X	1.48 Z	
	Nitrogen (N)	Varieties (V)	N*V	
LSD (0.05)	0.02**	0.02**	0.04**	

% CV = 1.58

Note ** = significant at 1% level CV = coefficient of variance

4.3.2 Effect of Phosphorus fertilizer

4.3.2.1 On gamma oryzanol content

The response to Phosphorus fertilizer was differed in rice genotypes. However, mostly the maximum content was at 37.5 kg P/ha except Kum 88061 needed higher. In the same level of Phosphorus fertilizer, γ-oryzanol content of purple rice genotypes were higher than white rice. Kum Doi Saket still, showed as a better variety in accumulation the substance (54.33 mg/100g grain). Also, the content in the white rice RD6 was lower (21.64 mg/100g grain) than in the purple rice (Table 4.5).

Table 4.5 Gamma oryzanol content in purple rice and white rice at different phosphorus fertilizer level

Varieties	Gamma Oryzanol content (mg/100g grain)			
	25 kg P/ha	37.5 kg P/ha	50 kg P/ha	mean
Kum Doi Saket	46.19 e	65.34 a	51.45 c	54.33 A
Kum 88061	38.17 g	46.56 e	48.87 d	44.53 C
Kum 7677	41.86 f	59.93 b	50.34 cd	50.71 B
RD6	15.82 i	33.64 h	15.35 i	21.60 D
mean	35.51 C	51.37 A	41.50 B	
	Phosphorus(P)	Varieties (V)	P*V	
LSD (0.05)	1.07**	1.24**	2.15**	

% CV = 2.96

Note ** = significant at 1% level CV = coefficient of variance

4.3.2.2 On crude oil and Semi purified gamma oryzanol content

Crude oil content in Kum Doi Saket, Kum 88061, Kum 7677 and RD6 responded to Phosphorus fertilizer in different way. Kum Doi Saket and Kum 88061 had the highest average of crude oil content (2.09 and 2.12 g/100 g grain, respectively). Kum 7677 and RD6 presented the lowest of crude oil content in average (1.71 and 1.73 g/100g grain, respectively) (Table 4.6).

Semi purified gamma oryzanol content varied from rice genotypes. In addition, the response to Phosphorus fertilizer level was varied from rice genotypes. The average of semi purified gamma oryzanol content was the highest (1.50 g/100g grain). Kum Doi Saket, Kum 88061 and Kum 7677 presented lower than RD6 (1.45, 1.43 and 1.07 g/100g grain, respectively) (Table 4.7).

Table 4.6 Crude oil content in purple rice and white rice at different phosphorus fertilizer level

Varieties	Crude oil content (g/100g grain)			mean
	25 kg P/ha	37.5 kg P/ha	50 kg P/ha	
Kum Doi Saket	1.92 e	2.27 a	2.07 cd	2.09 A
Kum 88061	2.17 b	2.08 bcd	2.11 bc	2.12 A
Kum 7677	1.77 f	1.69 fg	1.66 g	1.71 B
RD6	1.53 h	1.99 de	1.68 fg	1.73 B
mean	1.85 Z	2.01 Y	1.88 Z	
	Phosphorus(P)	Varieties (V)	P*V	
LSD (0.05)	0.05**	0.06*	0.1**	

% CV = 3.07

Note *= significant at 5% level, ** = significant at 1% level CV = coefficient of variance

Table 4.7 Semi purified gamma oryzanol content in purple rice and white rice at different phosphorus fertilizer level

Varieties	Semi purified gamma oryzanol content (g/100g grain)			mean
	25 kg P/ha	37.5 kg P/ha	50 kg P/ha	
Kum Doi Saket	1.80 a	1.24 c	1.30 c	1.45 B
Kum 88061	1.27 c	1.77 a	1.25 c	1.43 B
Kum 7677	1.06 e	1.04 e	1.10 de	1.07 C
RD6	1.51 b	1.83 a	1.16 d	1.50 A
mean	1.41 Y	1.47 X	1.20 Z	
	Phosphorus(P)	Varieties (V)	P*V	
LSD (0.05)	0.04**	0.04**	0.07**	

% CV = 3.21

Note ** = significant at 1% level CV = coefficient of variance

4.3.3. Effect of potassium fertilizer to gamma oryzanol content

4.3.3.1 On gamma oryzanol content

An effect of K-fertilizer on γ-oryzanol content seemed to differing from N and P. The interaction was significant but at lower rates, rice varieties showed the better response and at 37.5 kg/ha, every variety achieved an optimum level (Table 4.8). Kum Doi Saket still was the highest response content (65.72 mg/100g grain) with in contrast, RD6 was the lowest (30.73 mg/100g grain).

Table 4.8 Gamma oryzanol content in purple rice and white rice at different potassium fertilizer level

Varieties	Gamma Oryzanol content (mg/100g grain)			
	25 kg K/ha	37.5 kg K/ha	50 kg K/ha	mean
Kum Doi Saket	65.44 a	65.72 a	63.10 b	64.75 A
Kum 88061	49.35 f	52.45 e	44.55 g	48.78 C
Kum 7677	57.83 d	40.83 c	52.85 e	50.50 B
RD6	21.99 j	30.73 h	28.45 i	27.06 D
mean	48.65 X	44.69 Y	47.24 Z	
	Potassium (K)	Varieties (V)	K*V	
LSD (0.05)	0.84**	0.97**	1.69**	

% CV = 2.01

Note ** = significant at 1% level CV = coefficient of variance

4.3.3.2 On crude oil and Semi purified gamma oryzanol content

The contents of crude oil and semi purified γ-oryzanol responded to K-fertilizer as similar as to γ-oryzanol content. The rate of 37.5 kg/ha also indicated as the high response. A disagreeable to γ-oryzanol content was, RD6 was better in accumulating the nutrients than either Kum 88061 or Kum 7677 (Table 4.9 and 4.10). Therefore, in accumulating crude oil and semi purified γ-oryzanol content, the white rice (RD6) content was within the variation the purple rice contents.

Table 4.9 Crude oil content in purple rice and white rice at different potassium fertilizer level

Varieties	Crude oil content (g/100g grain)			mean
	25 kg K/ha	37.5 kg K/ha	50 kg K/ha	
Kum Doi Saket	2.18 b	2.32 a	2.22 b	2.24 A
Kum 88061	1.90 e	2.09 c	1.81 f	1.93 B
Kum 7677	1.14 g	1.79 f	1.78 f	1.57 C
RD6	1.90 e	2.01 d	1.81 f	1.91 B
mean	1.78 Z	2.05 X	1.91 Y	
	Potassium (K)	Varieties (V)	K*V	
LSD (0.05)	0.03**	0.04**	0.07**	

% CV = 2.17

Note ** = significant at 1% level CV = coefficient of variance

Table 4.10 Semi purified gamma oryzanol content in purple rice at different potassium fertilizer level

Varieties	Semi purified gamma oryzanol content (g/100g grain)			mean
	25 kg K/ha	37.5 kg K/ha	50 kg K/ha	
Kum Doi Saket	1.13 f	1.40 d	1.45 c	1.33 B
Kum 88061	0.99 h	1.09 fg	1.65 b	1.24 C
Kum 7677	1.06 g	1.08 g	1.09 fg	1.08 D
RD6	1.32 e	1.73 a	1.28 e	1.44 A
mean	1.13 Z	1.33 Y	1.37 X	
	Potassium (K)	Varieties (V)	K*V	
LSD (0.05)	0.02**	0.03**	0.05**	

% CV = 2.24

Note ** = significant at 1% level CV = coefficient of variance

4.4 Discussion

Interaction found 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.

However, as nitrogen was a major component of chlorophyll, a higher amount of N-fertilizer applied may promote photosynthesis activity. In consequence, it also promoted the accumulation of γ-oryzanol. Therefore, a significant interaction between rice varieties and N-levels was detected. Phi (2009) found that a higher rate of N application could enhance an accumulation of chlorophyll in purple rice leaves at flowering time and also of C_3G in purple rice grain. Furthermore, the present of the purple pigment (anthocyanidin) could enhance the content of γ-oryzanol in purple rice varieties. Velioglu (1998) reported the relationship between purple pigment and γ-oryzanol in blue berry. Kähkönen (1999) also found positive relation in blue berry, between purple pigment and phenolic compound for antioxidant activities. Jaakola (2002) demonstrated a **cor**relation between anthocyanin accumulation and expression of the flavonoid pathway genes during the ripening of berries. At the early stages of berry development, procyanidins and quercetin were the major flavonoids, but the levels decreased dramatically during the progress of ripening. During the later stages of ripening, the content of anthocyanin**s** increased strongly and they were the major flavonoids in the ripe berry.

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.

In this research, suitable fertilizer recommend from soil analysis for P and K fertilizer were 37.5 kg/ha. Crude oil content was lower when K-fertilizer rate was higher. This result was similar in cotton seeds, potassium applied at all concentrations resulted in a decrease in the total saturated fatty acids in seed oil (Sawan *et al.,* 2007).

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, Anwar *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.

In this results, the comparison white rice variety (RD6) showed its crude oil content in between the variation of the purple rice crude oil contents, but its γ-oryzanol content was lower than the 3 varieties of purple rice. Explanation could be that RD6 had been in a modern process of hybridization and selection in which to optimize mainly on the quantitative yields, and not to nutritional value. The diversity of such favorable nutritional characteristics did not represent in the most widespread HYVs currently prevailing in Asian rice cultivation (Frei and Becker, 2004). Qualitative traits, γ-oryzanol content in particular was therefore artificially left out, resulting in a lower the content than the content in the purple rice varieties which were landrace varieties and have not been exploited seriously by any modern breeding program.