

Responses of Landrace Rice to Organic Fertilizer for Physiological Traits, Grain Yield and Total Phenolic Content

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Abstract

Organic fertilizers are used as a main source of plant nutrients in organic food production. A better understanding on the responses for the physiological traits of rice to the application of organic fertilizers is essential for organic rice production. The aim of this study was to determine the effect of organic fertilizer on physiological traits, yield components and total phenolic content of two landrace rice varieties, i. e Leam Phu and Hom Dong. A 2×3 factorial experiment in randomized complete block design with three replications was undertaken under field condition during July 2018 to December 2018. Two rice varieties were assigned as factor A and three fertilizers were assigned as factor B. Data were collected for crop growth rate (CGR) at tillering to panicle initiation stage. Leaf area index (LAI) was collected at 60, 90 and 120 days after transplanting (DAT). Agronomic traits including number of tiller per plant, plant height, number of panicle per plant, one thousand seed weight, grain yield and total phenolic content were collected at appropriate times. Fertilizers showed significantly different effect on CGR and LAI. Although chemical fertilizer gave the highest grain yield (2,572.6 kg/ha), it was not significantly higher than organic fertilizer (2,100 kg/ha). Leam Phu gave higher total phenolic content than Hom Dong at all fertilizers applied without significant difference.

Keywords: fertilizer types, crop growth rate, polyphenol, yield components
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1. Introduction

Rice is an important staple food crop of the world and it is source of carbohydrate and protein. Rice is also rich in many important phytochemicals including flavonoids, niacin, riboflavin and phenolic compounds [1]. Phenolic compounds with strong antioxidant activity are the secondary metabolites found in many plant species. Daily consumption of 500- 1,000 mg of phenolic compounds can reduce the risk of heart disease, have anti-cancer and anti-inflammatory effects, and reduce blood cholesterol [2].

Rice productivity has increased continually due largely to the use of high yielding varieties and the application of chemical fertilizers. The application of chemical fertilizers is more important means for yield increase of rice [3]. However, the application of chemical fertilizers at high rates and for long term in recent years causes a serious concern about the harmful effects on soil and environment [4]. Therefore, the application of fertilizers from organic sources might help reduce the application of chemical fertilizers.

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Previous studies have been reported on the effects of organic fertilizers on phytochemical traits of many crops. In pepper, application of organic manures represented a suitable alternative means for food production with nutraceutical quality [5]. Organic fertilizer was also found to increase phenolic compounds and antioxidant activity in tomato [6]. Total phenolic content was found to be lower in rice treated with control and organic fertilizer than rice treated with inorganic fertilizer [7]. Phenolic compound in japonica rice grown under organic and conventional farming systems showed the same total phenolic content but the antioxidant capacity (DPPH) was significantly higher in organic farming treatment [8].

The effects of organic fertilizers on phenolic compounds are still not conclusive. The effects seemed to be dependent on plant species and genotype. The responses to organic fertilizer in rice on physiological traits, grain yield and phenolic content have not been clearly studied in indigenous rice, which might be different from high yielding varieties. The objective of this study was to determine the effects of organic fertilizer on physiological traits, grain yield, yield components and total phenolic content in rice. The information will be used for planning organic rice production in the future.

2. Materials and Methods

2.1 Location and experimental design

A 2×3 factorial experiment was undertaken under field condition to study the effects of organic fertilizer on growth, yield and phenolic content of two indigenous rice varieties. Two rice varieties which was representative of rice with black and white pericarp including Leam Phu and Hom Dong were assigned as factor A. Leam Phu is a black sticky landrace rice in Thailand. Hom Dong is a fragrant landrace variety with white pericarp. Three fertilizer applications consisting of no fertilizer (NO), chemical fertilizer (CF) and organic fertilizer (OF) were assigned as factor B. and the six treatment combinations were arranged in a randomized complete block design with three replications at the Research Station of Plant Production Technology Department, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The organic fertilizer used in this study was well-decomposed livestock manure and pH, EC, organic matter, total N, total P and total K were 8.3, 27.3 dS/m, 44.3%, 1.85%, 1.15% and 1.89%, respectively. The chemical fertilizers consisted of N-P-K (16-20-0 and 46-0-0).

2.2 Experimental details and crop management

Two rice varieties were planted for 25 days and the seedlings were transplanted at the rate of one plant per hill and a spacing of 25 cm between plants and 25 cm between rows in a plot size of 1.5×1.5 m. The rice bunds with 1 m in width were constructed as the alleys between blocks. The water level was maintained at 5 cm above the soil surface throughout the experiment. Chemical fertilizer with 16-20-0 of N-P-K at the rate of 156.25 kg/ha was applied at 15 days after transplanting (DAT) and chemical fertilizer (46-0-0) at the rate of 156.25 kg/ha was pre-heading stage. Organic fertilizer at the rate of 2,000 kg/rai (12,500 kg/ha) was applied at 15 DAT and pre-heading stage. Therefore, total chemical fertilizer applied to the crop was 312.50 kg/ha and total organic fertilizer was 25,000 kg/ha. Pesticide and herbicide were applied as needed.

2.3 Data collection

1) Physiological traits: Leaf Area Index (LAI) was recorded at 60, 90 and 120 days after transplanting. LAI was calculated as a formula;

$$\text{LAI} = \text{leaf area (cm}^2\text{)}/\text{ground area (GA)}.$$

Crop growth rate (CGR) was recorded at tillering to panicle initiation stages. Two plants from each plot were cut into small pieces and oven-dried at 80 °C for 72 h or until the weight was constant. Crop growth rate ($\text{g m}^{-2} \text{day}^{-1}$) during tillering to panicle initiation stages was calculated by using the formula;

$$\text{CGR} = \text{W2-W1}/\text{T2-T1},$$

where W2 = plant dry weight at panicle initiation stage and W1 = plant dry weight at tillering stage, T1 = Time unit at first harvest, T2 = Time unit at next harvest.

2) Yield components: At flowering stage, five plants of each subplot were randomly chosen and the data were recorded for plant height in the field. The same plants in each plot were also used for recording number of tillers per plant and number of panicles per plant. Seed yield was measured at harvest time (120 DAT). The seeds were sundried at approximately 13% moisture content and weighed. One thousand seed were randomly chosen and weighted to determine 1000-seed weight.

3) Total phenolic content: Husked rice (with husk removal) was ground using a grinding machine and subsample of 2 g for each plot was used for extraction of phenolic compounds. The ground rice powder was then extracted in 10 ml of methanol for 24 h at room temperature, filtered through a 0.45 μm of nylon membrane filter and stored at 4 °C for further analysis.

Total phenolic content was determined by Folin-Ciocalteu's assay [9]. The crude extract (50 μl) was diluted to 3.0 ml with distilled water. Folin-Ciocalteu reagent at the concentration of 250 μl was added to the sample and stirred thoroughly. The sample was further added with 20% sodium carbonate solution at the volume of 750 μl and the mixture was allowed to stand for 2 h. The mixture solution was measured at 765 nm using a Thermo Spectrophotometer. The phenolic content was demonstrated as Gallic acid equivalent (mg GAE/100 g dry weight of rice seed).

2.4 Data analysis

Data were analyzed statistically according to a factorial experiment in a randomized complete block design using STATISTIX 8 [10]. Least significance difference (LSD) was used to compare means [11]. The correlations between grain yield and LAI and total phenolic content were analyzed using regression method.

3. Results and Discussion

3.1 Physiological traits

Varieties were not significantly different for CGR at tillering to panicle initial stage whereas fertilizers were significantly different ($P \leq 0.01$) (Table 1). Chemical fertilizer produced the highest

Table 1. Crop growth rate (CGR) during tillering to panicle initiation stage and leaf area index (LAI) at 60, 90 and 120 days after transplanting (DAT) of two landrace rice varieties treated with different fertilizer applications.

Treatment	CGR (g day ⁻¹ cm ⁻¹)	LAI at 60 DAT	LAI at 90 DAT	LAI at 120 DAT
Variety (V)				
Leam Phu (LP)	26.39	1.79 ^b	6.57 ^a	6.88
Hom Dong (HD)	24.47	2.35 ^a	5.46 ^b	4.28
Fertilizer (F)				
Chemical fertilizer (CF)	37.46 ^a	0.98 ^b	8.42 ^a	9.20
Organic Fertilizer (OF)	22.20 ^a	4.23 ^a	5.95 ^b	5.36
Non-fertilizer (NF)	16.63 ^b	1.00 ^b	3.76 ^c	1.88
Variety × Fertilizer				
LP × CF	36.29	0.93	8.93	12.18 ^a
LP × OF	21.85	3.74	7.34	5.80 ^b
LP × NF	15.28	0.67	3.41	2.04 ^c
HD × CF	38.63	1.01	7.91	5.80 ^b
HD × OF	22.54	4.71	4.54	4.92 ^b
HD × NF	17.99	1.33	3.92	1.71 ^c
F – Test				
V	ns	*	**	**
F	**	**	*	**
V × F	ns	ns	ns	*
C.V. (%)	8.72	13.10	17.52	24.80

Means in the same column followed by the same letter were not significantly different at $P \leq 0.05$ by LSD.

ns, * and ** = non-significant, significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.

CGR of 37.46 g day⁻¹cm⁻¹ followed by organic fertilizer (22.20 g day⁻¹cm⁻¹) whereas control (non-fertilizer) control showed significantly lowest CGR. The interaction between rice variety and fertilizer was not significant.

CGR is a simple and important index for evaluating agricultural productivity based on the rate of dry matter production. In this study, fertilizer types were significantly different for CGR. According to Reddy and Reddi [12], application of nitrogen significantly affected CGR but it did not significantly affect plant height of rice. In this study, both chemical and organic fertilizers could increase GGR. Higher GCR for these treatments would be possibly due to higher tillers and higher vegetative growth. According to Hasanuzzaman *et al.* [13], wetland rice treated with poultry manure had the highest crop growth rate (CGR) and relative growth rate (RGR) because of the higher number of tillers per plant. The interaction between rice variety and fertilizer was not significant, indicating that the indigenous rice varieties responses to fertilizer application in a similar pattern.

The differences between rice varieties were significant for LAI at all growth stages (Table 1). At final harvest (120 DAT), Leam Phu had higher LAI than Hom Dong. The results indicated that Leam Phu had slower leaf senescence than did Hom Dong. Organic fertilizer produced the highest LAI at 60 DAT whereas chemical fertilizer produced the highest LAI at 120 DAT. The interaction between rice variety and fertilizer for LAI was significant at 120 DAT but not at 60 and 90 DAT. It was shown that Leam Phu treated with chemical fertilizer gave the highest LAI of 12.18

with significant difference. The results were similar to earlier report by Ko *et al.* [14] who reported that LAI in rice had the highest under inorganic fertilizer treatment. Kumar *et al.* [15] also reported that chemical fertilizer influence LAI by increasing leaf size and tiller number.

3.2 Growth and yield components

Rice varieties and fertilizers were not significantly different for plant height and the interaction between rice variety and fertilizer was not significant (Table 2). The lack of variation for plant height would possibly due to the fact that both rice varieties are indigenous varieties with tall type.

Rice varieties were not significantly different for tiller number but fertilizers was significantly different for this trait. Chemical fertilizer had the highest tillers (9.23) followed by organic fertilizer (8.06) whereas non-fertilizer had the lowest tillers (5.78). There is no significant difference on the interaction between rice variety and fertilizer which indicated that rice varieties responded to fertilizers in the similar fashion.

Rice varieties were not significantly different for number of seeds per panicle but fertilizers was significantly different for this trait. Chemical fertilizer produced the highest the number of seeds per panicle (326.13 seeds) followed by organic fertilizer (291.73 seeds) and non-fertilizer (234.03 seeds), respectively. The non significant interaction between rice variety and fertilizer indicated that the responses of rice varieties were similar.

Varieties and fertilizers were significantly different for 1,000 seed weight but the interaction between variety and fertilizer was not significant (Table 2). Hom Dong had large seeds (28.72 g) than did Leam Pua (20.71 g). Chemical fertilizer and organic fertilizer produced higher 1,000 seed weight than did non-fertilizer control. The non significant interaction between rice variety and fertilizer showed that rice varieties responded similarly for this trait.

Rice varieties were not significantly different for grain yield but the differences among fertilizers were significant. Chemical fertilizer had the highest grain yield per hectare (2,572.6 kg/ha) followed by organic fertilizer (2,100.4 kg/ha) and no fertilizer control (1,711.0 kg/ha), respectively.

The results were similar to those reported in previous studies. Sudarsono *et al.* [16] found that application of cattle manure increased plant height, number of tillers per plant and grain yield. However, the application of organic fertilizer was still lower than the application of chemical fertilizer for these traits. Non-significant interaction between rice variety and fertilizer also indicated the similar responses of rice varieties for grain yield.

3.3 Phenolic content

Significant difference between rice varieties was observed for phenolic content and Leam Phu gave higher phenolic content than Hom Dong (Figure 1). Fertilizers were not significantly different for phenolic content and the interaction between rice variety and fertilizer was not significant.

Phenolic compounds are known to have antioxidant activity. In this study, fertilizers were not significantly different for phenolic content. The results were in agreement with those reported in previous studies. Kessarwani *et al.* [8] found that rice crops grown under organic and conventional farming systems were not significantly different for phenolic content. In many plant species such as raspberry [17], pepper [5] and tomatoes [6], organic management increased total phenolic content better than did conventional management. The results indicated that plant species respond differently to organic fertilizer for phenolic content.

In this study, Leam Phu had total phenolic contents four times higher than did Hom Dong (Figure 1). This is because Leam Phu is black rice and Hom Dong is normal white rice. Muntana

Table 2. Plant height (cm), number of tillers per plant (Till./Plant), number of seeds per panicle (Seed/Pa), 1,000 seed weight (1,000 SW) and grain yield of two landrace rice varieties treated with different fertilizer applications.

Sources of variation	Height (cm)	Till./Plant	Seed/Pa (seeds)	1,000 SW (g)	Grain yield (kg/ha)
Variety (V)					
Leum Pua (LP)	164.71	7.51	290.56	20.71 ^b	1,880.5
Hom Dong (HD)	186.74	7.87	277.38	28.72 ^a	2,376.1
Fertilizer (F)					
Chemical fertilizer (CF)	179.05	9.23 ^a	326.13 ^a	25.27 ^a	2,572.6 ^a
Organic Fertilizer (OF)	182.00	8.06 ^a	291.73 ^b	24.79 ^{ab}	2,100.4 ^{ab}
No-fertilizer (NF)	166.13	5.78 ^b	234.03 ^c	24.08 ^b	1,711.0 ^b
Variety × Fertilizer					
LP × CF	173.44	8.96	337.47	21.25	2,543.1
LP × OF	184.66	8.06	314.80	20.84	2,137.8
LP × NF	176.22	5.50	300.40	20.03	1,617.9
HD × CF	187.77	9.50	283.07	29.29	3,369.7
HD × OF	144.48	8.06	233.80	28.74	2,052.1
HD × NF	187.78	6.06	234.27	28.13	1,482.1
F – Test					
V	ns	ns	ns	*	ns
F	ns	*	**	**	*
V × F	ns	ns	ns	ns	ns
C.V. (%)	13.93	6.79	8.85	2.56	23.98

Means in the same column followed by the same letter were not significantly different at $P \leq 0.05$ by LSD.

ns, * and ** = non-significant, significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.

and Prasong [18] reported that total phenolic content in colored rice was higher rather than in white rice.

The range of phenolic contents in this study was from 14.79 to 97.89 mg GAE/100 g seeds. When the results were compared with those in other studies, the range found in this study was considered intermediate. Tuaño *et al.* [7] reported that total phenolic compounds ranged from 30.01 to 37.59 mg GAE/100 g wet seeds and Kesarwani *et al.* [8] also reported that the range of phenolic content was from 162.7 to 167.4 mg GAE/100 g fresh seeds. The differences in the ranges of phenolic content in different studies would be due to the differences in rice varieties used and the differences in seed growth stages (mature, immature or dry seeds).

Correlation between grain yield and phenolic content, CGR, leaf area index at 60 DAT, 90 DAT was presented in Figure 2. The results indicated that the correlation between grain yield and LAI 90 ($R^2 = 0.5789$) was highest followed by CGR, phenolic content and LAI 60. A better crop growth rate and high leaf area index at 90 DAT may be used as selection criteria for high grain yield and indicators for crop responses to fertilizer application.

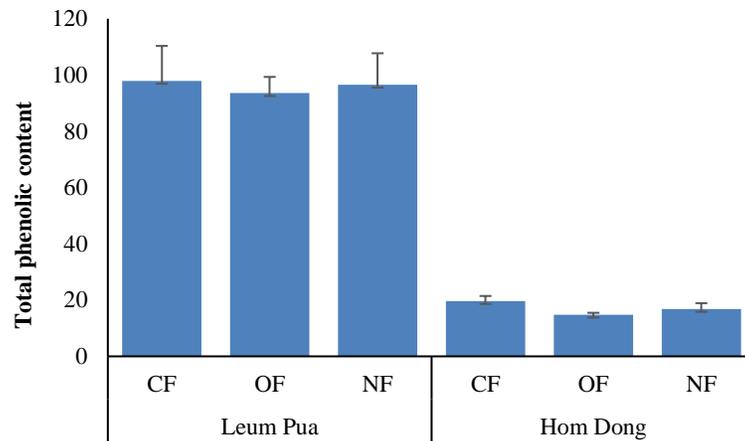


Figure 1. Total phenolic content (mg/GAE/100g seeds) in two landrace rice varieties treated with different fertilizer applications, CF = chemical fertilizer, OF =organic fertilizer and NF = no fertilizer.

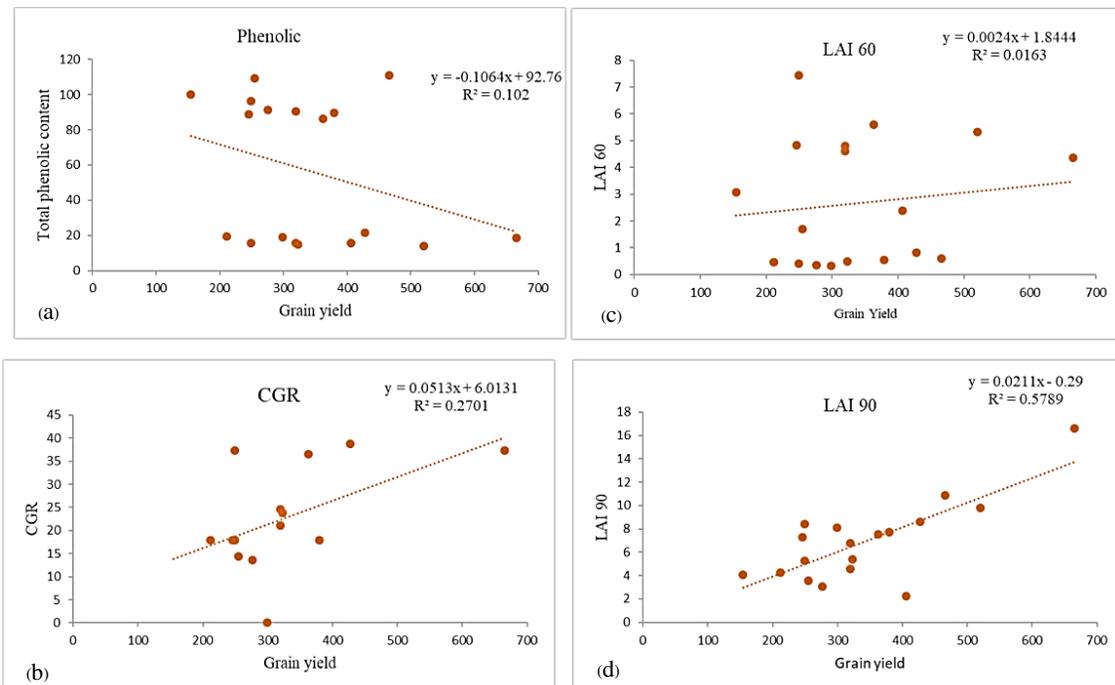


Figure 2. Correlation coefficients between grain yield and total phenolic content (a), grain yield and crop growth rate (b), grain yield and leaf area index at 60 DAT (c) and grain yield and leaf area index at 90 DAT (d) of two rice varieties treated with different fertilizer applications.

4. Conclusions

Organic fertilizer as well as chemical fertilizer increased crop growth rate, leaf area index, yield components and grain yield of two landrace rice varieties. Organic fertilizer gave lower grain yield than chemical fertilizer but not statistically significant. The increase in grain yield was due to the increases in tiller number, grain number and grain weight but not due to plant height. Most interactions between rice variety and fertilizer were not significant, indicating the similar responses of rice varieties to fertilizer application. Leam Phu gave higher total phenolic content than Hom Dong but fertilizer application did not significantly affect the total phenolic content. Although this experiment found that organic fertilizer did not affect phenolic compounds but it was not decreased. The effect of organic fertilizer on individual phenolic acids and antioxidant capacity in landrace rice should further be examined.

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